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JOURNAL

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VOL. 71, NO. 1

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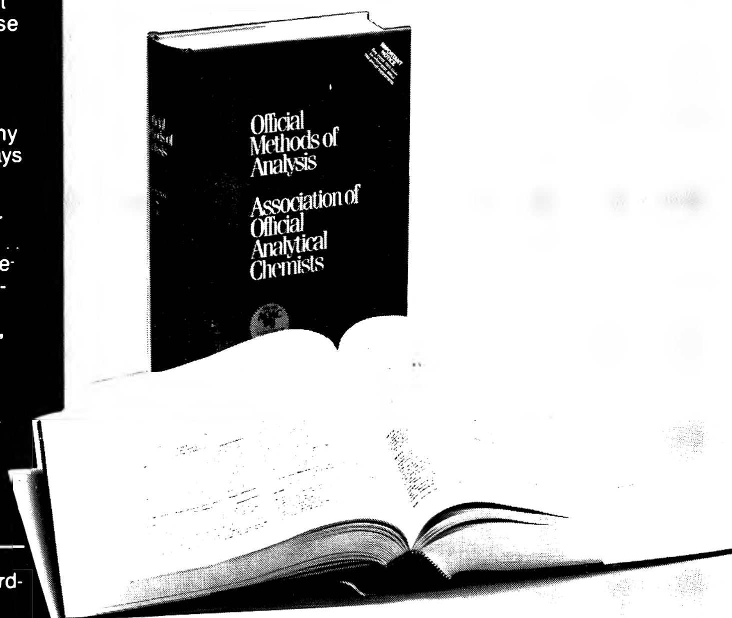

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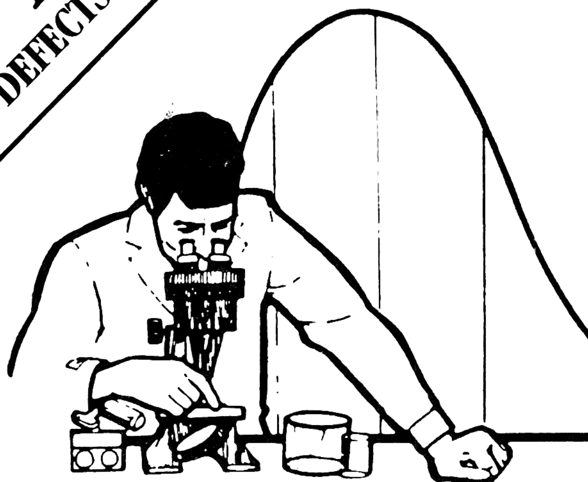

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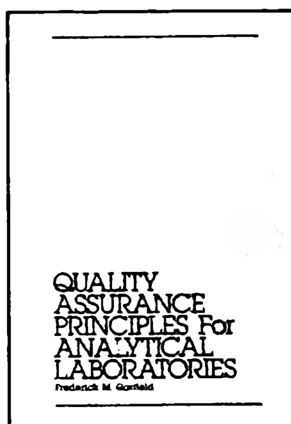

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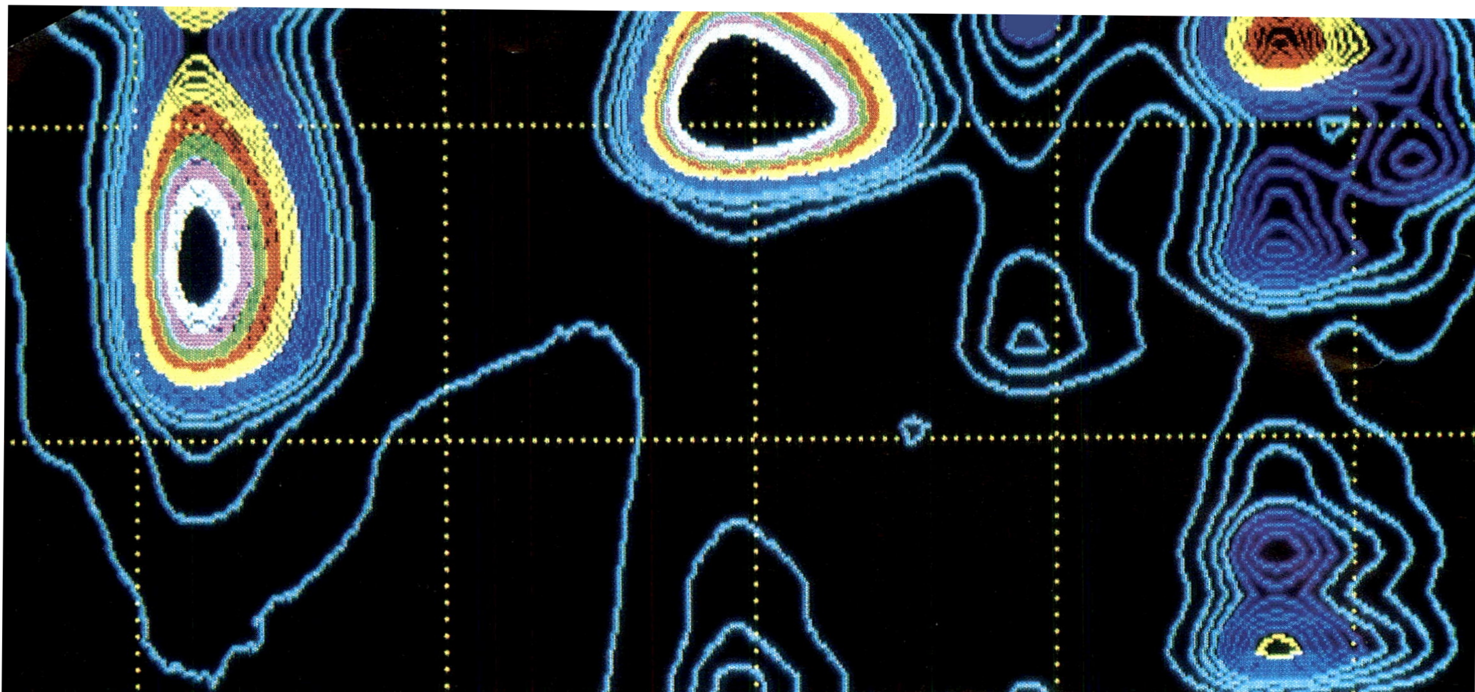

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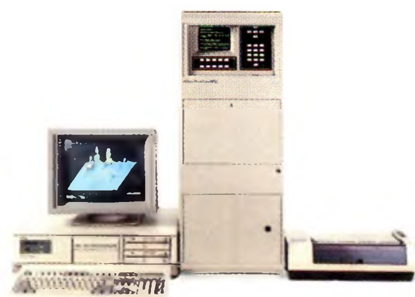

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JANUARY/FEBRUARY 1988
VOL. 71, NO. 1
JANCA 71(1)1-240 (1988)

9A *New Products*
13A *For Your Information*
19A *Thanks to Reviewers of Journal Manuscripts*
21A *Instructions to Authors*
22A *Information for Subscribers, Contributors, and Advertisers*
23A *Books in Brief*
24A *Authors in This Issue*

Wiley Award Address

1 Improving and Developing Analytical Methods . . . A Continuing Challenge
Walter Holak

President's Address

7 "Association"—"Official"—"Analytical Chemist"
Frank J. Johnson

Disinfectants

9 Bacterial Numbers on Penicylinders Used in Disinfectant Testing: Use of 24 Hour Adjusted Broth Cultures
Eugene C. Cole and William A. Rutala

Vitamins and Other Nutrients

12 Disposable Cartridge Extraction of Retinol and Alpha-Tocopherol from Fatty Samples
Claude Fernand Bourgeois and Nadine Ciba

16 Comparison of Paired-Ion Liquid Chromatographic Method with AOAC Fluorometric and Microbiological Methods for Riboflavin Determination in Selected Foods
Erlinda S. P. Reyes, Kathleen M. Norris, Cheryl Taylor, and Donald Potts

19 Liquid Chromatographic Determination of Vitamin D in Fortified Milk
Vipin K. Agarwal

Pesticide Formulations

23 Liquid Chromatographic Method for Determination of Anilazine in Formulated Products: Collaborative Study
Stephen C. Slahck

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26 Liquid Chromatographic Method for Determination of Cyhexatin in Technical and Formulated Products: Collaborative Study
Stephen Lupan

Drugs

29 Determination of Ammonium Ion in Povidone by Derivatization and Square Wave Voltammetry
Robert M. Ianniello

31 Determination of Aztreonam and L-Arginine Combination in Parenteral Formulations
Abdel-Aziz M. Wahbi, Mohammed A. Abounassif, El-Rasheed A. Gad-Kariem, Mahmoud E. Ibrahim, and Hassan Y. Aboul-Enein

33 Liquid Chromatographic Determination of Flucytosine in Capsules: Collaborative Study
Donald Shostak and Clifford Klein

Cosmetics

36 Stability-Indicating Liquid Chromatographic Determination of Alpha-Ionone in Toothpaste
Ramesh J. Trivedi

Fish and Other Marine Products

38 Manual Determination of Minced Fish Flesh in Mixed Fillet-Minced Cod Blocks: Collaborative Study
J. Perry Lane and Robert J. Learson

Mycotoxins

41 Use of Ten Gram Samples of Corn for Determination of Mycotoxins
Octave J. Francis, Jr, George M. Ware, Allen S. Carman, Gary P. Kirschenheuter, and Shia S. Kuan

43 Rapid Determination of Aflatoxins in Raw Peanuts by Liquid Chromatography with Postcolumn Iodination and Modified Minicolumn Cleanup
Joe W. Dorner and Richard J. Cole

Drug Residues in Animal Tissue

48 Simultaneous Liquid Chromatographic Screening of Five Coccidiostats in Chicken Liver
Keith D. Gallicano, Hyun Park, John Yee, Larry M. Young, and Peter W. Saschenbrecker

Technical Communications

51 Spectrophotometric Method for the Microdetermination of Bendiocarb Standard Residues in Water
Swadesh K. Handa

53 Liquid Chromatographic Method for Determination of Piperine in *Piper nigrum* (Black and White Pepper)
Kenneth M. Weaver, Michael E. Neale, and Ann Laneville

General Referee Reports: Committee on Pesticide Formulations and Disinfectants

56 Disinfectants—*Aram Beloian*

56 Pesticide Formulations: Fungicides and Disinfectants—*Peter D. Bland*

57 Pesticide Formulations: Herbicides I; Other Organophosphorus Insecticides; Rodenticides and Miscellaneous Pesticides—*G. Marshall Gentry*

57 Pesticide Formulations: Herbicides II—*Arthur H. Hofberg*

57 Pesticide Formulations: Herbicides III—*Peter D. Bland*

58 Pesticide Formulations: Organohalogen Insecticides; Other Insecticides, Synergists, and Insect Repellants—*James E. Launer*

General Referee Reports: Committee on Drugs and Related Topics

59 Cosmetics—*Ronald L. Yates*

59 Diagnostics and Tests Kits—*Richard A. Baldwin*

59 Drug Residues in Animal Tissues—*Charlie J. Barnes*
60 Drugs I—*James W. Fitzgerald*
60 Drugs II—*Edward Smith*
61 Drugs III—*Martin J. Finkelson*
62 Drugs IV—*Linda L. Ng*
62 Drugs V—*Thomas G. Alexander*
63 Immunochemistry—*Dennis M. Hinton*

General Referee Reports: Committee on Foods I

64 Coffee and Tea—*Robert H. Dick*
64 Dairy Chemistry—*Gary H. Richardson*
65 Decomposition and Filth in Foods (Chemical Methods)—*Walter F. Staruszkiewicz, Jr*
66 Fish and Other Marine Products—*Louis L. Gershman*
66 Food Additives—*Thomas Fazio*
68 Meat, Poultry, and Meat and Poultry Products—*Jon E. McNeal*
70 Mycotoxins—*Peter M. Scott*
76 Oils and Fats—*David Firestone*
79 Plant Toxins—*Samuel W. Page*
81 Seafood Toxins—*Edward P. Ragelis*

General Referee Reports: Committee on Foods II

84 Alcoholic Beverages—*Randolph H. Dyer*
84 Cereals and Cereal Products—*Ralph H. Lane*
85 Chocolate and Cacao Products—*Henk J. Vos*
85 Color Additives—*Sandra J. Bell*
86 Flavors—*Kurt L. Schoen*
86 Fruits and Fruit Products—*Frederick E. Boland*
87 Nonalcoholic Beverages—*John M. Newton*
87 Preservatives and Artificial Sweeteners—*James J. Nelson*
88 Processed Vegetable Products—*Thomas R. Mulvaney*
88 Spices and Other Condiments—*James E. Woodbury*
89 Sugars and Sugar Products—*Margaret A. Clarke*
91 Vitamins and Other Nutrients—*Mike J. Deutsch*

General Referee Reports: Committee on Residues

92 Metals and Other Elements—*Stephen G. Capar*
93 Multiresidue Methods (Interlaboratory Studies)—*Leon D. Sawyer*
94 Organohalogen Pesticides—*Bernadette M. McMahon*
97 Organonitrogen Pesticides—*W. Harvey Newsome*
98 Organophosphorus Pesticide Residues—*Gail Abbott Parker*
99 Radioactivity—*Edmond J. Baratta*

General Referee Reports: Committee on Microbiology

100 Analytical Mycology of Foods and Drugs—*Stanley M. Cichowicz*
101 Extraneous Materials in Foods and Drugs—*Jack L. Boese*
102 Dairy Microbiology—*James W. Messer*
102 Drug and Device Related Microbiology—*Gordon S. Oxborrow*
103 Food Microbiology (Nondairy)—*Wallace H. Andrews*

Committee on Feeds, Fertilizers, and Related Materials

106 Antibiotics—*Stanley E. Katz*
106 Drugs in Feeds—*Robert L. Smallidge*
107 Feeds—*Joel M. Padmore*
108 Fertilizers and Agricultural Liming Materials—*Frank J. Johnson*
108 Plants—*Robert A. Isaac*
109 Tobacco—*Ray F. Severson*
109 Veterinary Analytical Toxicology—*P. Frank Ross*

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

112 Soils and Sediments—*James Dragun*
112 Waste Materials—*David Friedman*

116 Committee on Pesticide Formulations and Disinfectants: Recommendations for Official Methods
Thomas L. Jensen, Alan R. Hanks, James J. Karr, James P. Minyard, Jr, Fran C. Porter, H. Hedegaard Povlsen, Stephen C. Slahck, Richard S. Wayne, and Richard H. Collier

119 Committee on Drugs and Related Topics: Recommendations for Official Methods
Eric B. Sheinin, Kaiser J. Aziz, Ronald C. Backer, Charles Bibart, Charles C. Clark, Sal Fusari, Ted M. Hopes, Thomas P. Layloff, Robert Livingston, John O'Rangers, John E. Zarembo, and William W. Wright

122 Committee on Foods I: Recommendations for Official Methods
Henry B. S. Conacher, Richard L. Ellis, Elmer George, Michael E. Knowles, John D. McKinney, Arthur E. Waltking, Douglas Engebretson, Douglas L. Park, and Michael W. O'Donnell, Jr

124 Committee on Foods II: Recommendations for Official Methods
Laura L. Zaika, Harry G. Lento, Roger Wood, Adeline A. Peake, Earl F. Richter, George R. Tichelaar, Patricia Bulhack, Robert A. Martin, and John Gilbert Phillips

128 Committee on Residues: Recommendations for Official Methods
Richard Schmitt, William A. Steller, Kenneth W. Boyer, Paul E. Corneliussen, William G. Fong, Gerald R. Myrdal, Edgar R. Elkins, Keith A. McCully, and Richard H. Albert

132 Committee on Microbiology: Recommendations for Official Methods
Michael H. Brodsky, Phillip Alioto, John S. Gecan, Donald E. Lake, Khalil Rayman, Robert M. Twedt, Donald A. Mastrorocco, Jr, Foster D. McClure, and Richard E. Young

135 Committee on Feeds, Fertilizers, and Related Materials: Recommendations for Official Methods
Rodney J. Noel, Howard Casper, Louis W. Ferarra, David W. Fink, Gayle Lancette, Alexander MacDonald, Wilbert Shimoda, Harold Thompson, Carolyn Geisler, Billy M. Colvin, Paul R. Rexroad, and Daniel H. Mowrey

137 Committee on Hazardous Substances in Water and the Environment: Recommendations for Official Methods
Douglas J. Dube, Nile Frawley, Robert Graves, Erika E. Hargesheimer, Ross J. Norstrom, Laszlo Torma, Mark F. Marcus, and Forest C. Garner

138 Executive Director's Report
146 Secretary/Treasurer and the Finance Committee
147 Official Methods Board
150 Editorial Board
151 Archives Committee
151 Committee on the Constitution
159 Committee on Instrumental Methods and Data Handling
160 Committee on Interlaboratory Studies
172 Committee on International Coordination
173 Intersociety Committee on Methods of Air Sampling and Analysis
174 Joint AOAC-AOCS-AACC-IUPAC Mycotoxin Committee
175 Committee on Laboratory Quality Assurance
176 Long Range Planning Committee
177 Committee on Meetings, Symposia, and Educational Programs
177 Committee on Membership
178 Committee on Regional Sections
179 Committee on Safety
179 Committee on Ways and Means
181 AOAC Officers and Committees: 1988

Changes in Official Methods of Analysis

199 Fourth Supplement
231 Index

Helium Shutoff Valve
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J&W Scientific recently introduced the GS-Q column, the company's first gas-solid, open-tubular megabore column. This column is designed to analyze light hydrocarbons, Freons, esters, sulfides, ketones, and water up to 10 times faster than the conventional packed columns which it replaces. No cryogenic cooling is necessary. J&W Scientific.
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Liquid Chromatography Methods Development Software
Optim 3 applications software package is designed for automated liquid chromatography methods development. Using a logical and empirical approach, Optim 3 adjusts the conditions of analyses to result in the best chromatographic separation and resolution in the least amount of time. The program is resident on a plug-in chip for the Spectra-Physics SP4270 computing integrator. Spectra-Physics Autolab Division.
Circle No. 319 on reader service card.

Database for U. S. Federal Chemical Regulations
A new online database, Suspect, that deals with U. S. federal regulations on chemicals is available through the Chemical Information System. Suspect permits users to find data about all regulations applying to a given chemical simply by entering its name or registry number. Alternatively, one can enter a regulatory code to find data about all substances covered by a given regulation. Chemical Information Systems.
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Structure-Building Software
With Concord structure-building software, the user enters a simple chemical line notation and within seconds, a high-quality, approximate 3-D structure is generated. Concord runs on any DEC Vax computer running VMS. Evans & Sutherland Molecular Science Group.
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THE 102nd AOAC ANNUAL INTERNATIONAL MEETING AND EXPOSITION

AT THE BREAKERS, PALM BEACH, FLORIDA
AUGUST 29 - SEPTEMBER 1, 1988

Program Includes:
The Spotlight Symposium on **BIOTECHNOLOGY** - Chairman: D.M. Hinton

Symposia on:
Laboratory Information Management Systems (LIMS) - Chairmen: J.J. Karr and H. Morris
Fertilizer Phosphate Evaluation and Analysis - Chairmen: F. Johnson and J.R. Trimm
Drug Residues in Foods of Animal Origin - Chairmen: W.A. Moats and B. Shaikh
Pesticides in Foods - Chairman: P. Corneliussen

Over 200 **technical poster presentations** on topics such as: Pesticides Formulations and Disinfectants; Foods; Residues; Microbiology; Feeds, Fertilizers and Related Topics; Drugs and Related Topics; Hazardous Substances in Waste and the Environment.

Open Forum
Regulatory Roundtable: Safety - The 1986-87 OSHA Regs
Laboratory Quality Assurance Short Course
Laboratory Equipment and Supplies Exposition

For further information, contact: Administrative Manager, AOAC, 1111 N. 19th St., Ste. 210, Arlington, VA 22209, or call (703) 522-3032.

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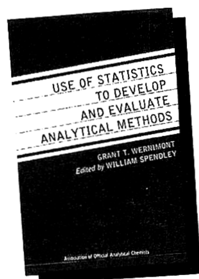
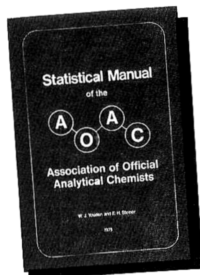
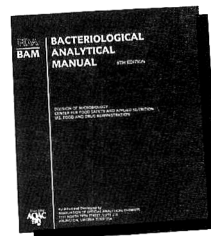


☐ **Use of Statistics to Develop and Evaluate Analytical Methods**
By G.T. Wernimont. Ed. by W. Spendley. 1985. 199 pp. Index. Figures. Tables. Glossary. Softbound. ISBN 0-935584-31-5.
Members: $47.55 in U.S., $50.55 outside U.S.
Nonmembers: $52.50 in U.S., $55.50 outside U.S.
A basic reference for evaluating collaborative studies and a natural extension to the *Statistical Manual of the AOAC*.



☐ **Statistical Manual of the AOAC**
By W.J. Youden and E.H. Steiner. 1975. 96 pp. Softbound. Illustrations. ISBN 0-935584-15-3.
Members: $18.55 in U.S., $19.55 outside U.S.
Nonmembers: $20.50 in U.S., $21.50 outside U.S.
A do-it-yourself manual for statistical analysis of interlaboratory collaborative tests.



☐ **FDA Bacteriological Analytical Manual, 6th Ed.**
1984. 448 pp. Looseleaf. ISBN 0-935584-29-3.
Members: $44.85 in U.S., $47.85 outside U.S.
Nonmembers: $49.50 in U.S., $52.50 outside U.S.
Provides regulatory and industry laboratories with methods for detection of micro-organisms. Includes one *Classification of Visible Can Defects* poster, 24" × 36", in color, with photographs.

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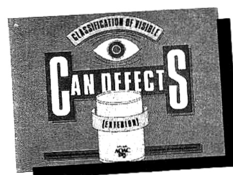
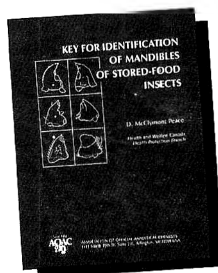


☐ **Classification of Visible Can Defects—poster/pamphlet**
1984. 24" × 36" chart. In color with photographs. Minimum order, 1 package of 10 charts, $40.00 + $3.00 postage; 2nd package of 10, $30.00 + $3.00 postage; each additional package, $25.00 + $3.00 postage.

☐ **Principles of Food Analysis for Filth, Decomposition, and Foreign Matter— FDA Technical Bulletin No. 1**
1981. 286 pp. 2nd printing 1985. Illustrated. Softbound.
Members: $42.60 in U.S., $45.60 outside U.S.
Nonmembers: $47.00 in U.S., $50.00 outside U.S.
Comprehensive laboratory manual/text on basic concepts of food sanitation analysis.



☐ **Key for Identification of Mandibles of Stored-Food Insects**
1985. vi + 166 pages. Illustrated. 125 photographs. Softbound. ISBN 0-935584-32-3.
Members: $42.00 in U.S., $43.50 outside U.S.
Nonmembers: $46.50 in U.S., $48.00 outside U.S.
Enables food sanitation analysts to identify species from all major stored-food insect pest groups.

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FOR YOUR INFORMATION

Meetings

February 11–15, 1988: American Association for the Advancement of Science, 154th national meeting, Sheraton Boston and Hynes Convention Center, Boston, MA. Contact: AAAS Meetings Office, 1333 H St, NW, Washington, DC 20005, telephone 202/326-6448.

April 10–15, 1988: RIS 88 Symposium, "Resonance Ionization Spectroscopy and Its Applications," National Bureau of Standards, Gaithersburg, MD. Sponsored by the U. S. Department of Energy, University of Tennessee, National Bureau of Standards, International Union of Pure and Applied Physics, EG&G, and Battelle Northwest Laboratories. Contact: Thomas B. Lucatorto, A243 Physics Building, National Bureau of Standards, Gaithersburg, MD 20899, telephone 301/975-3734.

April 26–28, 1988: AOAC Infant Formula Conference II, Radisson Francis Marion Hotel, Charleston, SC. Contact: James Tanner, Food and Drug Administration, HFF-266, 200 C St, SW, Washington, DC 20204, telephone 202/472-5364.

May 16–19, 1988: 9th International Symposium on Capillary Chromatography, Conference Center, Monterey, CA. Contact: P. Sandra, Research Institute for Chromatography, PO Box 91, B-8610 Wevelgem, Belgium.

May 17–20, 1988: 2nd Intensive 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.

May 19, 1988: AOAC New York/New Jersey Regional Section Meeting, Fort Hamilton Officers' Club, Brooklyn, NY. Contact: Arthur Waltking, Best Foods Research and Engineering Center, CPC International, Inc., 1120 Commerce Ave, Union, NJ 07083, telephone 201/688-9000; Anthony Gross, Nabisco Brands Inc., 100 Deforest Ave, East Hanover, NJ 07936, telephone 201/884-3404.

June 20–22, 1988: AOAC Northeast Regional Section Meeting, Lowell University, Lowell, MA. Contact: Edmond Baratta or James Fitzgerald, Food and Drug Administration, Winchester Engineering and Analytical Center, 109 Holton St, Winchester, MA 01890, telephone 617/729-5700.

June 20–22, 1988: AOAC Midwest Regional Section Meeting, Holiday Inn West, Columbia, MO. Contact: George Rottinghaus, University of Missouri, Columbia, Veterinary Medicine Diagnostic Laboratory, Columbia, MO 65211, telephone 314/882-6811.

June 23–24, 1988: AOAC Pacific Northwest Regional Section Meeting, Evergreen College, Olympia, WA. Contact: H. Michael Wehr, Oregon Department of Agriculture, 635 Capitol St, NE, Salem, OR 97310, telephone 503/378-3793.

June 25–30, 1988: National Environmental Health Association, 52nd Annual Educational Conference, Cleveland, OH. Contact: Mel Monkelis, Conference Program Coordinator, NEHA, 720 S Colorado Blvd, Rm 970, South Tower, Denver, CO 80222, telephone 303/756-9090.

June 1988: AOAC Southeast Regional Section Meeting, location to be announced. Contact: Frank Allen, Environmental Protection Agency, Reg. 4 ESD, Athens, GA 30613, telephone 404/546-3387.

July 19–22, 1988: Fine Particle Society, 19th Annual Meeting and 1988 Particulate/Powder Technology, Microcontamination and Biotechnology Exhibition, Santa Clara Convention Center, Santa Clara, CA. Contact: Fine Particle Society, L-117 Keplinger Hall, University of Tulsa, Tulsa, OK.

July 18–22, 1988: European Chemoreception Research Organization, Eighth International Biennial Congress, University of Warwick, Coventry, UK. Contact: S. Van Toller, Warwick Olfaction Research Group, Department of Psychology, University of Warwick, Coventry CV4 7AL, UK, telephone UK 0203 523613, telex 31406.

August 23–26, 1988: 7th International Symposium on Mass Spectrometry in Life Sciences, State University of Ghent, Belgium. Contact: A. De Leenheer, Laboratoria voor Medische Biochemie en voor Klinische Analyse, Harelbekestraat 72, B-9000 Ghent, Belgium, telephone (091) 21-89-51, ext. 324.

August 29–September 1, 1988: 102nd AOAC Annual International Meeting and Exposition, spotlight on "Biotechnology," The Breakers, Palm Beach, FL. Contact: Margaret Ridgell, AOAC, 1111 N 19th St, Suite 210, Arlington, VA 22209, telephone 703/522-3032.

September 25–30, 1988: 17th International Symposium on Chromatography, Hofburg, Vienna, Austria. Contact: Gerhard Schomburg, Max-Planck-Institut fur Kohlenforschung, PO Box 10 1353, D-4330 Mulheim/Ruhr, FRG, telephone (0208) 30 64 30.

October 2–7, 1988: A world congress on vegetable protein utilization in human foods and animal feedstuffs, organized by American Oil Chemists' Society and cosponsored by American Soybean Association, Westin Stamford/Plaza Hotel, Raffles City, Singapore. Contact: Meetings Manager, American Oil Chemists' Society, PO Box 3489, Champaign, IL 61821, telephone 217/359-2344, telex, 493 8651 AOCS UI, fax, 217/351-8091.

October 3–7, 1988: Midwestern Association of Forensic Scientists fall meeting, hosted by Minnesota Forensic Science Laboratory, Sheraton Park Place Hotel, Minneapolis, MN. Contact: Laura Nelson or Dan Bergman, BCA Forensic Science Laboratory, 1246 University Ave, St. Paul, MN 55104, telephone 612/642-0700.

May 23–26, 1989: IIIrd International Symposium on Quantitative Luminescence Spectrometry in Biomedical Sciences, State University of Ghent, Faculty of Pharmaceutical Sciences, Ghent, Belgium. Contact: Willy R. G. Baeyens, State University of Ghent, Pharmaceutical Institute, Laboratory of Pharmaceutical Chemistry and Drug Quality Control, Harelbekestraat 72, B-9000 Ghent, Belgium, telephone 091/21-89-51, ext. 254.

September 25–28, 1989: 103rd AOAC 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.

August 26–31, 1990: Euroanalysis VII, organized by Austrian Society for Microchemistry and Analytical Chemistry, Vienna, Austria. Contact: Interconvention, PO Box 80, A-1107 Wien, Austria, telephone +43-222-2369-640, telex 11 18 03, telefax +43-222-2369-648.

September 10–13, 1990: 104th AOAC 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.

1988 AOAC Short Courses

In addition to its popular quality assurance short courses—2 day sessions that teach how to plan, design, and manage a laboratory quality assurance

program—AOAC in 1988 will also offer a short course on field and laboratory sampling of food, drugs, and agricultural commodities. Details of the sampling course will be published at a later date. Dates and locations for the QA courses are: April 13–14 and July 12–13, Westpark Hotel, Arlington, VA, and August 27–28 at the Breakers Hotel, Palm Beach, FL. The sampling course will be taught November 30–December 1, Westpark Hotel, Arlington, VA.

Cost for each course is $475 to AOAC members and $525 to nonmembers. Interested parties should contact Margaret Ridgell, AOAC, 1111 N 19th St, Suite 210, Arlington, VA 22209.

Youden Award Nominations

AOAC was justly proud to have been the publisher of the 1987 winner of the W. J. Youden Award in Interlaboratory Testing, *Use of Statistics to Develop and Evaluate Analytical Methods,* written by the late Grant T. Wernimont and edited by William Spendley.

The award's sponsor, the American Statistical Association, has announced that the final date for receipt of nominations for its 1988 W. J. Youden Award is March 1, 1988. The award, which recognizes a publication that makes an outstanding contribution to the design or analysis of interlaboratory tests or that describes ingenious applications to the planning and evaluation of data from such tests, consists of $1000 and a suitable citation. Eligible publications must have appeared in professionally refereed journals or monograph series in 1986–1987.

Nominations, along with 6 copies of the publication, should be sent to the chairperson of the award committee, Mary G. Natrella, 25 N Floyd St, Alexandria, VA 22304.

Second AOAC Conference on Infant Formula

An AOAC scientific conference on the production, regulation, and analysis of infant formula will be held April 26–28, 1988, at the Radisson Francis Marion Hotel in Charleston, SC.

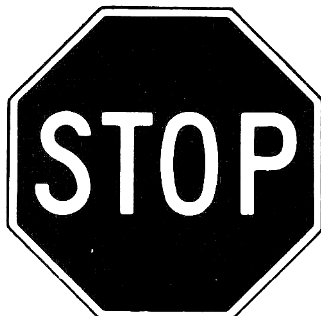
Invited lectures and contributed papers will focus on such topics as analysis of infant formula; accuracy, precision, and standards in analysis; regulatory concerns with respect to infant formula; medical aspects of infant formula; labeling of infant formula; and quality assurance.

For further information, contact: James Tanner, Food and Drug Administration, HFF-266, 200 C St, SW, Washington, DC 20204, telephone 202/472-5364.

Journal of the AOAC To Go On Line with STN International in 1988

The Board of Directors of AOAC has approved the recommendation of the Editorial Board to mount the full text (minus tables and figures) of the *Journal of the AOAC* on Chemical Journals Online (CJO), a database available on the Scientific and Technical Information Network (STN International). STN, jointly operated by the American Chemical Society, Fachinformationszentrum Energie, Physik, Mathematik GmbH, and Japan Association for International Chemical Information (see item following), is rapidly becoming the predominant chemical information database in the world.

STN International was established in 1984 as a cooperative undertaking of the American Chemical Society, Columbus, OH, and Fachinformationszentrum Energie, Physik, Mathematik GmbH, Karlsruhe, FRG, to share resources and to provide convenient on-



IMPORTANT DEADLINE! NOTE IT ON YOUR 1988 CALENDAR.

For the 102nd AOAC Annual International Meeting, Palm Beach, Florida, August29-September 1, 1988.

Abstracts **April 20, 1988**

For more information, contact: Administrative Manager, AOAC, 1111 N. 19th Street, Suite 210, Arlington, VA 22209 or call 703-522-3032.

Quality Assurance Means Never Having to Say You're Sorry

Attend the AOAC QUALITY ASSURANCE SHORT COURSE

Take this intensive comprehensive two-day course on *planning, designing, and managing* a laboratory quality assurance (QA) program.

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- **How to organize and document a QA program**
- **Why you should commit the necessary resources**
- **What it will cost**

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I QA — Planning and Management
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III Analytical Control Charting
IV Personnel Management — Role in QA
V Equipment and Supplies Management
VI General Discussion and Review

Second Day

VII QA in Sampling
VIII QA in Sample Analysis
IX Records and Reporting
X Proficiency Testing — Inter- and Intralaboratory
XI Audit Procedures for QA
XII General Discussion and Review

COURSE SCHEDULE

Wednesday and Thursday, April 13–14, 1988
Westpark Hotel, Arlington, Virginia

Tuesday and Wednesday, July 12–13, 1988
Westpark Hotel, Arlington, Virginia

Saturday and Sunday, August 27–28, 1988
AOAC 102nd Annual International Meeting and Exposition
The Breakers, Palm Beach, Florida

The popular QA manual, *Quality Assurance for Analytical Laboratories*, is provided FREE to attendees.
Registration Fee: $475 for AOAC members, $525 for nonmembers.

REGISTER NOW! COURSE IS LIMITED TO 45 PARTICIPANTS!

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Please sign me up for the short course

QA for Analytical Laboratories, ☐ April 13–14, 1988, Arlington, Virginia
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☐ August 27–28, 1988, Palm Beach, Florida

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Payment Must Accompany Registration

Return with payment to: AOAC QA Short Course, 1111 N. 19th Street, Suite 210-J, Arlington, Virginia 22209
(AOAC reserves the right to cancel courses at any time.)

For additional information, contact Margaret Ridgell at AOAC, (703) 522-3032.

line access in Europe and North America to a wide range of national and international scientific and technical databases. Under the STN network arrangement, a particular database is loaded at one site, eliminating duplication of file storage and updating costs. A searcher accesses the nearest host computer and is linked to whichever computer in the network stores the database to be searched. All computers in the network use "Messenger" software, developed for STN by Chemical Abstracts Service, so that databases at any location can be searched using the same commands.

Other journals so far mounted on CJO/STN include all the journals of ACS, the 5 polymer journals of John Wiley & Sons, and the 10 primary journals of the Royal Society of Chemistry. Also to be added in 1988 is VCH's *Angewande Chemie.*

It is hoped that the *Journal of the AOAC* will be on line by mid-1988, beginning with volume 70 (1987). Readers will be kept apprised of developments as they occur over the next few months.

Japan Information Center of Science and Technology Goes On Line with STN International

Fulfilling plans announced earlier in 1987, computers at the Japan Information Center of Science and Technology (JICST) in Tokyo were linked on November 30 to computers at Chemical Abstracts Service in Columbus, OH, and at Fachinformationszentrum Energie, Physik, Mathematik in Karlsruhe, FRG. JICST is the central organization for scientific and technical information in Japan. STN users in North America and Europe can access the JICST-E file, JICST's comprehensive English-language database on Japanese science, technology, and medicine.

The JICST-E file, updated monthly from 4500 Japanese journals and serials, contains citations and abstracts for more than 400 000 Japanese publications on chemistry, engineering, pharmacology, and the life and medical sciences from 1985 to date.

STN International currently provides access to more than 45 databases in chemistry. physics, mathematics, and engineering through the Columbus, Karlsruhe, and Tokyo service centers.

STN Tutorials Available

Four new STN International Tutorials are available. Of particular interest to *Journal of the AOAC* readers, in view of AOAC's decision to mount the *Journal* on line, is "STN Mentor: Introduction to Chemical Journals Online." The tutorial describes the chemical Journals Online family of files on STN and illustrates how material such as experimental data can be located in and retrieved from these files. As an adjunct, "STN Mentor: Spotlight on Authors I" is an introduction to author searching in all STN files that contain author information. It teaches how STN's Messenger software can be used to the greatest advantage in cross-file searching for documents by a specific author.

STN Mentor tutorials cost $19.50 each. For more information, call Chemical Abstracts Service customer service, 800/848-6538, or write STN-Columbus, % Chemical Abstracts Service, PO Box 02228, Columbus, OH 43202.

COMING IN THE NEXT ISSUE

FOCUS ON SAMPLING

- Sampling and Preparation of Sample for Chemical Examination—*W. Horwitz*
- Statistical Sampling Approaches—*J. A. Springer and F. D. McClure*

ANTIBIOTICS

- In vitro Analytical System for Determining the Ability of Antibiotics at Residue Levels to Select for Resistance in Bacteria—*M. S. Brady, R. J. Strobel, and S. E. Katz*
- Method to Determine Effect of Residue Levels of Antibiotics on R-factor Transfer—*M. S. Brady and S. E. Katz*

PLANTS

- Gas Chromatographic Determination of the Monosaccharide Composition of Plant Cell Wall Preparations—*P. J. Harris, A. B. Blakeney, R. J. Henry, and B. A. Stone*

New Standard Reference Material from NBS

A new standard reference material (SRM) from the National Bureau of Standards (NBS) was developed primarily for use in calibrating the chromatographic instruments used to measure nitrated polycyclic hydrocarbons (N-PAHs). Besides functioning as a calibration tool, the new SRM also can be used to spike laboratory samples with known amounts of N-PAHs for research purposes. The new material, called Dinitropyrene Isomers and 1-Nitropyrene in Methylene Chloride (SRM 1596), is packaged in a kit containing 5 vials of the certified solution and is sold for $281 by the Office of Standard Reference Materials, National Bureau of Standards, B311 Chemistry Building, Gaithersburg, MD, telephone 301/975-6776.

Accuracy in Drug Testing Possible

Proper operating procedures are the key to accurate testing for drugs of abuse, according to a recent article in *Clinical Chemistry* (1987, **33**, 1683-1686). In "Status of Drugs-of-Abuse Testing in Urine: An AACC Study" by C. S. Frings, R. M. White, and D. J. Battaglia, the authors demonstrate that testing accuracy of nearly 100% is an achievable goal and that clients should realistically expect reliable, accurate service from a contract laboratory if it is staffed with certified professionals using appropriate quality assurance techniques.

Reprints of the article can be obtained from Christopher S. Frings, 633 Winwood Dr, Birmingham, AL 35226.

Two New Journals Announced

Premiere issues of 2 new journals will appear in January 1988: *Chemical Research in Toxicology,* published by the American Chemical Society, and *Japan Technology,* published by University Microfilms International.

Chemical Research in Toxicology, a bimonthly journal edited by Lawrence J. Marnett, Wayne State University, will cover, among other areas, (1) structure elucidation of novel toxic agents, (2) chemical and physical studies on chemical agents that provide insight into their mode or mechanism of action, (3) experimental and theoretical investigations of the interaction of toxic chemicals with biological macromolecules and other biological targets. It will impose rigorous standards for characterization of compounds and the description of methods used. Toxic effects will be broadly defined to include toxicity, mutagenicity, carcinogenicity, teratogenicity, neurotoxicity, immunotoxicity, and other related areas.

To order, write American Chemical Society, Marketing Communications Department, 1155 Sixteenth St, NW, Washington, DC 20036.

Japan Technology is actually a series of 10 specialized editions, each of which will contain 400–500 new article summaries per month of the latest business and technical developments in Japan. The 10 editions will include: Biosciences, Business, Chemistry, Computers, Electronics, Energy, Manufacturing, Materials, Telecommunications, and Transportation. For more information, call toll free 800/521-0600; Michigan and Alaska call collect 313/761-4700; Canada call 800/343-5299; or write Linda Stott, University Microfilms International, 300 N Zeeb Rd, Ann Arbor, MI 48106.

ISO Technical Report Available

The following technical report has been published by the International Organization for Standardization, Technical Committee 134—Fertilizers and Soil Conditioners. The report and price information are available from American National Standards Institute, Inc., 1430 Broadway, New York, NY 10018, telephone 212/354-3300.

ISO/TC 7553: Fertilizers—Sampling—Minimum mass of increment to be taken to be representative of the total sampling unit.

New Members

AOAC's newest government supporting agency is: Massachusetts Department of Food and Agriculture, Boston, MA.

New sustaining members include: American Crystal Sugar Co., Moorhead, MN; California Almond Growers Exchange, Sacramento, CA; Kemira OY, Helsinki, Finland.

AACC Holds Annual Meeting in Nashville

Opryland Hotel, Nashville, TN, was the site of the 72nd Annual Meeting of the American Association of Cereal Chemists, November 1–5, 1987.

Named Fellows were: Robert R. Matsuo, Canadian Grain Commission; Joseph G. Ponte, Jr, Kansas State University; and Gerald Reed, Universal Foods Corp. The William F. Geddes Memorial Award went to James W. Doty, Doty Laboratories. Incoming officers of AACC are: Lamartine F. Hood, Pennsylvania State University, president; Bruce R. Stillings, Nabisco Brands, Inc., secretary; and Paul A. Seib, Kansas State University, and Mary K. Stiedemann, General Mills, Inc., directors.

Honors outside AOAC

The Takeru Higuchi Research Prize, awarded by the American Pharmaceutical Association to recognize outstanding contributions to pharmaceutical research, was awarded to William I. Higuchi, University of Utah, at the opening session of the joint Japan–United States Congress of Pharmaceutical Sciences, December 2, 1987, in Honolulu, HI. Higuchi, brother of the man for whom the prize is named, received a bronze medal and a stipend of $10 000.

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 and will be submitted for adoption official first action at the 102nd AOAC Annual International Meeting, Aug. 29–Sept. 1, 1988, at Palm Beach, FL: by the Methods Committee on Foods I—(*1*) Modification of AOAC Infrared Spectroscopic Method for Fat, Lactose, Protein, and Solids in Milk, submitted by D. A. Biggs (Guelph, Ontario, Canada) and (*2*) Modification of AOAC Babcock Method for Fat in Milk and Modified Mojonnier Ether Extraction Method for Fat in Milk, submitted by D. M. Barbano and J. L. Clark (Cornell University, Dept of Food Science, Ithaca, NY) and C. E. Dunham (Texas Milk Market, Carrollton TX; by the Methods Committee on Microbiology—(*1*) MICRO-ID System for Identification of *Salmonella, Escherichia coli,* and Other Enterobacteriaceae in Foods, submitted by S. L. Keelan and R. S. Flowers (Silliker Laboratories, Chicago Heights, IL) and B.J. Robison (Organon Teknika Corp., Durham, NC) and (*2*) Visual Immunoassay for Detection of *Salmonella* in Foods, submitted by R. S. Flowers, M. J. Klatt, and S. L. Keelan (Silliker Laboratories, Chicago Heights, IL).

Copies of the methods are available from the AOAC office.

The JOURNAL OF THE ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS

EDITORIAL PROFILE

The *Journal of the AOAC* is the official bimonthly journal of the Association of Official Analytical Chemists (AOAC). Published for analytical scientists who develop and use analytical methodology, it is used by its subscribers in their day-to-day work in the fields of food composition and contamination, feeds, pharmaceuticals, cosmetics, agricultural and household chemicals, water analysis, and environmental control. Users include analytical chemists, biologists, microbiologists, biochemists, toxicologists, spectroscopists, statisticians, forensic and other scientists in laboratory, administrative, and top management positions in industry, government, and universities in the United States and more than 90 other countries.

The *Journal of the AOAC* is a principal forum for exchange of information among methods researchers in the regulatory agencies and regulated industries.

The *Journal of the AOAC* has an average paid circulation of about 4,000 subscribers and a referenced readership of more than 25,000 purchasers and specifiers of laboratory equipment and supplies, testing apparatus, instrumentation, and related products and services. Subscribers order the *Journal* because it is professionally necessary to do so.

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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
4. **Figure captions** (separate sheet(s), **double spaced**): Designate all illustrations, including schemes, as figures and include caption for every one. Identify curves (See **Figures**) and include all supplementary information in caption rather than on face of figure. Spell out word Figure.
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1/88

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The *Journal of the Association of Official Analytical Chemists* (ISSN 0004-5756) is published bimonthly by AOAC, 1111 N 19th St, Suite 210, Arlington, VA 22209. Each volume (one calendar year) will contain about 1200 pages. The scope of the *Journal* encompasses the development and validation of analytical procedures pertaining to the physical and biological sciences related to foods, drugs, agriculture, and the environment. Emphasis is on research and development of precise, accurate, and sensitive methods for the analysis of foods, food additives and supplements, contaminants, cosmetics, drugs, toxins, hazardous substances, pesticides, feeds, fertilizers, and the environment.

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1/88

BOOKS IN BRIEF

Reactions of Acids and Bases in Analytical Chemistry. By Adam Hulanicki. Translated by Mary R. Masson. Published by John Wiley & Sons, Inc., One Wiley Dr, Somerset, NJ 08873, 1987. 308 pp. Price: $122.95. ISBN 0-470-20246-7.

This book presents acid-base reactions on the principle of the Brønsted-Lowry theory. It is also a guide to other acid-base theories and to more complex acid-base systems and is suitable as a text for advanced analytical chemistry students.

Modern Methods of Polymer Characterization. Compiled by T. P. Lodge. Special issue of *Anal. Chim. Acta* 189(1). Published by Elsevier Science Publishers, PO Box 211, 1000 AE Amsterdam, The Netherlands, 1986. 216 pp. Price: United States $75.00; Dfl. 155.00.

Fourteen original research papers are published in this special issue of *Anal. Chim. Acta*. The papers for the issue were selected with the purpose of fostering increased communication among scientists working in chemical analysis or polymers. They illustrate the types of issues which are of interest in polymer characterization today.

Chemometrics: A Textbook. By D. L. Massart, B. G. M. Vandeginste, S. N. Deming, Y. Michotte, and L. Kaufman. Published by Elsevier Science Publishers, PO Box 1663, Grand Central Station, New York, NY 10163, 1987. 464 pp. Price: United States $85.25; Dfl. 175.00. ISBN 0-444-42660-4.

Emphasis in this book is on time series, correlation, and transformation methods, filtering, smoothing, etc., in the science of chemometrics.

The Agrochemicals Handbook, Second Edition. Published by the Royal Society of Chemistry; available through the American Chemical Society, 1155 Sixteenth St, NW, Washington, DC 20036, 1987. 1300 pp. Price: $341.00. ISBN 0-85186-416-3.

This completely revised edition of *The Agrochemicals Handbook* contains chemical, physical, analytical, toxicity, and usage data for over 500 pesticide active ingredients used worldwide. The price one pays includes updates, issued about every 6 months, through 1990.

Detection in Analytical Chemistry. By Lloyd A. Currie. *ACS Symposium Series No. 361.* Published by American Chemical Society, 1155 Sixteenth St, NW, Washington, DC 20036, 1987. 338 pp. Price: United States and Canada $64.95; export $77.95. ISBN 0-8412-1445-X.

The meaning of detection in chemical measurement science from both fundamental and practical perspectives is explored and the issue of detection limits is addressed. Among other information, the editor presents discussions of real world problems and needs for meaningful detection both in the laboratory and in the regulatory environment.

The Impact of Chemistry on Biotechnology. Edited by Marshall Phillips, Sharon P. Shoemaker, Roger D. Middlekauff, and Raphael M. Ottenbrite. *ACS Symposium Series No. 362.* Published by American Chemical Society, 1155 Sixteenth St, NW, Washington, DC 20036, 1987. 398 pp. Price: United States and Canada $79.95; export $95.95. ISBN 0-8412-1446-8.

This volume offers information to all chemists and chemical engineers interested in research results, application information, analytical uses, or other benefits of biotechnology. Its 8 sections include: overview, microbial and biochemical technology, polymer, analytical, agrochemical, agricultural and food chemistry, chemical marketing, and chemical information.

Detection and Data Analysis in Size Exclusion Chromatography. Edited by Theodore Provder. *ACS Symposium Series 352.* Published by American Chemical Society, 1155 Sixteenth St, NW, Washington, DC 20036, 1987. 307 pp. Price: United States and Canada $69.95; export $83.95. ISBN 0-8412-1429-8.

Discussions in this 27-chapter volume focus on the advantages and disadvantages of various size exclusion chromatography detectors and techniques for data analysis. The book was developed from a symposium sponsored by the Division of Polymeric Materials Science and Engineering at the 191st Meeting of the American Chemical Society, New York, NY, April 13–18, 1986.

Chromatographic Chiral Separations. Edited by Morris Zief and Laura J. Crane. *Chromatographic Science Series/40.* Published by Marcel Dekker, Inc., 270 Madison Ave, New York, NY 10016, 1987. 432 pp. Price: United States and Canada $99.75; all other countries $119.50. ISBN 0-8247-7786-7.

This book explains direct separation of enantiomers by available chiral stationary phases synthesized from chiral amino acids as well as synthetic and natural chiral polymers. It also covers indirect separation by the formation of diastereomers.

Food Toxicology. Part A, Principles and Concepts; Part B, Contaminants and Additives. By Jose M. Concon. *Food Science and Technology Series/26.* Published by Marcel Dekker, Inc., 270 Madison Ave, New York, NY 10016, 1987. 1440 pp. Price (sold only as a set): United States and Canada $250.00; all other countries $300.00. ISBN Part A, 0-8247-7736-0; Part B, 0-8247-7737-9.

This 2-volume treatise features over 9100 bibliographic citations of the literature and more than 400 diagrams, photographs, and tables. In addition, the work synthesizes results of interdisciplinary investigations in a single source; includes legal, regulatory, and safety concerns; discusses micro- and macrobiological contaminants; covers manmade chemical contaminants—exploring "incidental" food additives as well as naturally occurring radionuclides; considers "intentional" food additives and derived toxicants—designating toxic compounds from food processing, preparation, and storage.

Reviews in Biochemical Toxicology, Volume 8. Edited by Ernest Hodgson, John R. Bend, and Richard M. Philpot. Published by Elsevier Science Publishing Co., Inc., PO Box 1663, Grand Central Station, New York, NY 10163, 1987. 296 pp. Price: United States and Canada $65.00; outside North America Dfl. 225.00. ISBN 0-444-01169-2.

For toxicologists, biochemists, and professionals in related sciences, these volumes cover the most recent advances in the field, with an emphasis on detoxication and intoxication mechanisms and modes of toxic action.

Irradiation of Dry Food Ingredients. Edited by Josef Farkas. Published by CRC Press, Inc., 2000 Corporate Blvd, NW, Boca Raton, FL 33431, due in 1988. 176 pp. Price: United States $99.00; outside United States $120.00. ISBN 0-8493-6686-0.

Presented for the first time is a survey of world literature on the scientific, technological, engineering, economic, and regulatory aspects, advantages, and limitations of irradiation of dry food ingredients as compared to conventional methods of decontamination.

Graphics for Chemical Structures. Edited by Wendy A. Warr. *ACS Symposium Series No. 341.* Published by American Chemical Society, 1155 Sixteenth St, NW, Washington, DC 20036, 1987. 176 pp. Price: United States and Canada $44.95; export $53.95. ISBN 0-8412-1401-8.

The latest information from recognized experts in the field of chemical structure handling on microcomputers is included in this book, as are the newest developments in related systems on both mini- and mainframe computers.

Hydrocarbons. Volume I: Gas Chromatography. Edited by Walter L. Zielinski, Jr. Published by CRC Press, Inc., 2000 Corporate Blvd, NW, Boca Raton, FL 33431, 1987. Price: Prepublication, United States $175.00; outside United States $205.00. ISBN 0-8493-3041-6.

This volume covers the world's hydrocarbon literature between 1970 and 1980 and addresses the wide diversity of hydrocarbons structures—the basic operating components of the gas chromatographic system—and their control for optimizing separations.

Pentachlorophenol. Environmental Health Criteria, No. 71. Published by World Health Organization, 1211 Geneva 27, Switzerland, 1987. 236 pp. Price: United States $10.80; Sw. fr. 18. ISBN 92 4 154271 3. French edition in preparation.

Because of their broad pesticidal efficiency spectrum and low cost, pentachlorophenol (PCP) and its salts have been widely used as algicides, bactericides, fungicides, herbicides, and molluscicides. Though some of these uses have been banned or restricted in several developed countries, human exposure continues to be of concern because of possible health hazards associated with the indoor application of wood preservatives or paints containing PCP. This book cites over 600 studies in an effort to determine the risks to man and the environment posed by the production and use of PCP.

A Textbook of Modern Toxicology. By Ernest Hodgson and Patricia E. Levy. Published by Elsevier Science Publishing Co., Inc., PO Box 1663, Grand Central Station, New York, NY 10163, 1987. 386 pp. Price: United States and Canada $39.50; outside North America Dfl. 150.00. ISBN 0-444-01131-5.

Emphasis in this textbook is placed on mechanisms of toxic actions and the rationale of testing procedures. It covers the following areas: uptake, distribution, metabolism and excretion of toxicants, mechanisms of toxic action, testing for toxicity, analysis of toxicants, treatment of toxicity, and regulation of toxic chemicals.

AUTHORS IN THIS ISSUE

Aboul-Enein, Hassan Y., 31
Abounassif, Mohammad A., 31
Agarwal, Vipin K., 19
Bourgeois, Claude Fernand, 12
Carman, Allen S., 41
Ciba, Nadine, 12
Cole, Eugene C., 9
Cole, Richard J., 43
Dorner, Joe W., 43
Francis, Octave J., Jr, 41
Gad-Kariem, El-Rasheed A., 31
Gallicano, Keith D., 48
Handa, Swadesh K., 51
Holak, Walter, 1
Ianniello, Robert M., 29
Ibrahim, Mahmoud E., 31
Johnson, Frank J., 7
Kirschenheuter, Gary P., 41
Klein, Clifford, 33
Kuan, Shia S., 41
Lane, J. Perry, 38
Laneville, Ann, 53
Learson, Robert J., 38
Lupan, Stephen, 26
Neale, Michael E., 53
Norris, Kathleen M., 16
Park, Hyun, 48
Potts, Donald, 16
Reyes, Erlinda S. P., 16
Rutala, William A., 9
Saschenbrecker, Peter W., 48
Shostak, Donald, 33
Slahck, Stephen C., 23
Taylor, Cheryl, 16
Trivedi, Ramesh J., 36
Wahbi, Abdel-Aziz M., 31
Ware, George M., 41
Weaver, Kenneth M., 53
Yee, John, 48
Young, Larry M., 48

Improving and Developing Analytical Methods . . . A Continuing Challenge

WALTER HOLAK
Food and Drug Administration, New York Regional Laboratory, 850 Third Ave, Brooklyn, NY 11232-1593

In recognition of his skill at conceiving rugged, elegant analytical methods that have helped further the consumer protection mission of the U.S. Food and Drug Administration (FDA), the 1987 AOAC Harvey W. Wiley Award was presented to Walter Holak at the 101st AOAC Annual International Meeting and Exposition in San Francisco, CA, Sept. 14, 1987. Holak began working for FDA on Dec. 10, 1962, the same year he became a member of AOAC. In the ensuing 25 years, he has developed many methods, perhaps the most significant being those to measure methyl mercury and iodine and that to determine "free" and "total" sulfites in foods. FDA recognized his achievements with a Commendable Service Award in 1979, and in the same year AOAC gave him the double honor of naming him Associate Referee of the Year and electing him a Fellow.

It is indeed a great honor for me to have been chosen by the Association of Official Analytical Chemists to be the 1987 recipient of the Harvey W. Wiley Award. Coincidentally, this prestigious award comes on the 25th anniversary of my career as a chemist with the Food and Drug Administration, New York Regional Laboratory, and my membership in AOAC. I believe that this year's award is especially notable due to the fact that it is the first Wiley Award given to an individual employed at an FDA field laboratory. According to the Awards Committee, this year's award is given for "sustained contribution to the methodology necessary for the FDA to protect consumers, especially in the area of toxic substances."

The primary mission of an FDA field laboratory is to analyze samples. Traditionally, the responsibility for research and method development lies mainly with headquarters and research centers; however, alloted time for research and method development has always been considered an important tool for a field laboratory. As we are well aware, oftentimes the most creative ideas for research projects have developed from sample analysis. Winning this award was possible because management personnel at the New York Regional Laboratory believe in, and are committed to, research as an integral part of an efficiently functioning laboratory. This commitment allowed me sufficient time to pursue my research projects. In reality, this award is for the entire New York Regional Laboratory, for its teamwork. The staff through their input and cooperation contributed greatly toward the success of these projects.

Presented at the 101st Annual International Meeting and Exposition of the AOAC, Sept. 14–17, 1987, San Francisco, CA.

The importance of consumer protection today becomes obvious if we consider the changes that have occurred in our society. Formerly, consumers had control over their incoming food supply. Over the past 100 years or so, they have lost their personal control, and the result is that trust must now be placed in others to maintain food safety. This applies to every level of production, including packaging and transportation of food.

For 101 years, AOAC has provided the scientist with accurate and practical analytical techniques for testing all sorts of consumer products. However, the status of method development is a dynamic one. As we move forward technologically, methods must be developed and improved as accuracy, dependability, and sensitivity requirements change. The result of this advance in technology is that regulatory agencies are forced to deal with new challenges concerning regulation and changes in the food, drug, and cosmetic areas. The analyst has the important function of keeping abreast of changes in methods to meet or exceed these needs.

First, I would like to illustrate my point by going back in history, to the very beginning of AOAC, and follow the evolution of analytical methods in the area of 3 topics for which I am currently serving as an Associate Referee, sulfites, mercury, and iodine in foods. I will then briefly describe our contributions to the field.

Sulfites

Sulfites, in particular the sulfites and bisulfites of sodium and potassium, are important in the processing of many foods such as corn syrup and dried fruits, in preventing "black spot" in shrimp, and in keeping fruits and vegetables fresh. However, the addition of sulfites to foods has been under

reexamination recently because questions have been raised concerning their safety.

Sulfites have been used as preservatives in foods for many centuries. It is not surprising, therefore, that a method for the determination of sulfites appears in the Bureau of Chemistry Bulletin (1) covering the period of 1887–1892, 100 years ago. Those bulletins, edited by Harvey W. Wiley, contained the proceedings and reports of AOAC up to the year 1912. In the early days of AOAC, there was much interest in determining sulfites in wines and beers. The first AOAC method for sulfites, their determination in malt liquors, consisted of distilling SO_2 from phosphoric acid solution into an iodine trapping solution, which oxidized sulfite to sulfate. In the determinative step, the quantitation was performed gravimetrically as barium sulfate. In the Bulletin of 1894–1899 (2), sulfite in wine was determined by distilling with a current of CO_2 and trapping in standard iodine solution. Excess iodine was then titrated with sodium thiosulfate. The first method for "free" sulfite in wine appeared in the Bulletin of 1900–1901 (3) and consisted of titrating sulfurous acid directly with standard iodine solution. The "total" sulfite was similarly determined after treatment with potassium hydroxide. In the Bulletin of 1907–1908 (4), a report pointed out the interferences in the latter method. These were hydrogen sulfide and organic substances that also volatilize and reduce iodine. Methods proposed to eliminate these interferences included cadmium sulfide traps followed by a gravimetric determination as barium sulfate instead of titrimetry. At that time, Dr. Wiley pointed out the importance of differentiating sulfite from hydrogen sulfide, and a mere trace of substance which may be present naturally ought not be considered as satisfactory evidence that it has been added.

In the Bulletin of 1907–1908 (5), a definitive study presented evidence that sulfurous acid and sulfites have no value to health or nutrition but are only deleterious to health. A paper presented in 1909 (6) reported a method for sulfites in foods using steam, rather than direct, distillation. At the 24th annual meeting of AOAC (7), a report on sulfite in molasses indicated that more SO_2 is released by distillation if the sample is greatly diluted. In the Bulletins of 1911–1912 (8), bromine solution was substituted for iodine, and the determination was performed gravimetrically in an attempt to eliminate interferences.

A 1929 report (9) presented a comparison of the Monier-Williams method and the accepted AOAC method for sulfites in foods. In the Monier-Williams method, the acidified sample was refluxed, and SO_2 was carried into a hydrogen peroxide trap with a current of carbon dioxide, whereas the AOAC method used steam distillation into an iodine solution. The sulfuric acid produced by the oxidation of SO_2 was determined either volumetrically by titration with sodium hydroxide or gravimetrically as barium sulfate. In the following decades, numerous studies and modifications were made of the Monier-Williams method. In 1932 (10), it was reported that hydrochloric acid was more effective in releasing SO_2 than the previously used phosphoric acid. In 1933 (11), the Monier-Williams method was recommended for dried fruits but required a distillation time of 1½ h. Four methods for SO_2 in alcoholic beverages were studied in 1937 (12): Monier-Williams, direct distillation, AOAC steam distillation, and the Ripper method, which uses direct titration with iodine solution. The Monier-Williams method was found to be the only one universally reliable. A number of collaborative studies for beers and wines (13–17) demonstrated the reliability of the Monier-Williams method. However, several collaborative studies were unsuccessful due to sample instability. In 1961 (18), a colorimetric method for sulfites was presented based on the purple color formed when *p*-rosaniline hydrochloride, formaldehyde, and sulfur dioxide react to form *p*-rosaniline methylsulfonic acid. Also, in the same year, a collaborative study was successfully conducted using modifications to the Monier-Williams method for SO_2 in dried apricots (19). These modifications were adopted as official in the following year (20). The *p*-rosaniline method was collaboratively studied in the years 1963–1965 (21–23) for beer and dried fruits and gave results comparable to the Monier-Williams method.

Over the years, the Monier-Williams method became the standard to which other methods have been compared. In 1966 (24), however, it was shown that it is not applicable to certain foods, such as onions, leeks, and cabbage because of distillation of interfering sulfur-containing compounds.

A rapid screening procedure was proposed in 1976 (25) for preservatives in ground beef, including sulfites, based on colorimetry with *p*-rosaniline, and a successful collaborative study was conducted in 1980 (26). In the same year, a collaborative study (27) of the Ripper method for sulfites in wine revealed poor precision and large systematic error.

In 1983, a symposium on Sulfite Analysis Methods for Food Products was held at the 99th AOAC Annual International Meeting to consider the technological aspects of the use of sulfiting agents in food (28) and to reevaluate existing methods (29–32). An ion chromatographic method was also presented (33).

Recently, the addition of sulfites to foods has become an important safety issue because of a number of documented adverse reactions in hypersensitive individuals. This prompted government agencies to review the use of sulfites as preservatives in foods and to issue new regulations. The addition of sulfites to fresh fruits and certain vegetables is now prohibited by FDA, and a label declaration requirement has been issued for SO_2 in foods at levels as low as 10 ppm.

There has been concern that existing methods would be inadequate to meet the new requirements. An AOAC Sulfite Methods Task Force was organized in 1985 to address the problems. A number of methods have been proposed including the optimized Monier-Williams (34), flow injection analysis (35), differential pulse polarography (36), and others.

The differential pulse polarographic method, which we proposed, is outlined in Figure 1. The rationale for this approach has been to adapt the time-tested Monier-Williams procedure for isolating SO_2 from the sample, i.e., by purging with nitrogen, but using a simplified apparatus with a sensitive and specific mode of detection. The sample is blended with 5% alcohol to minimize the oxidation of sulfite by atmospheric oxygen. "Free" sulfite is released from the sample at pH 1.5 by purging with nitrogen at room temperature. "Total" sulfite is similarly released from a more strongly acid solution with an application of heat. Both are trapped in a pH 5.2 acetate buffer, containing alcohol, in which sulfite is then polarographed. The apparatus in which the above operations are performed is shown in Figure 2. The apparatus is quite simple and can be easily constructed by any laboratory. The same apparatus can be used under a variety of conditions. For example, the sample can be treated with an alkali to release aldehyde-bound sulfite, acidified, and then purged with nitrogen at room temperature. This procedure is advantageous for foods containing high levels of volatile sulfur-containing compounds, such as garlic. A collaborative study of the method has been conducted and was adopted

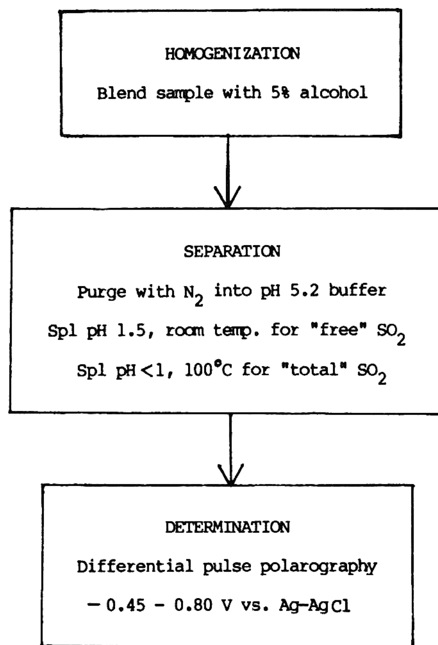

Figure 1. Outline of DPP method for sulfites in foods.

official first action for total sulfite at the last year's meeting of AOAC.

We are currently conducting a mini-collaborative study of the method using sulfite-stabilized food samples to determine the precision more effectively.

Mercury

The interest in determining mercury lies in the fact that it is a toxic element. It is widely distributed in the environment, from both natural geological activity and industrial pollution. Microbial action in waters converts inorganic forms of mercury to organic forms. A large portion of mercury found in fish, therefore, occurs in the form of methyl mercury.

The first AOAC method for mercury appeared in 1923 (37) for antiseptic tablets, and it utilized iodometric titration. Seven years later, in 1930 (38), a method for organic mercurial seed disinfectants was published that was based on volatilization and collection of mercury on gold, precipitation as mercuric sulfide, and titration of excess cyanide with silver nitrate. From 1935 to 1963, mercury methods were generally based on titration or spectrophotometry with dithizone (39–46). The difficulties to be overcome included interference from copper, instability of the dithizonate to light, and volatility losses of mercury during the digestion. Sample digestions were, therefore, performed under reflux or closed systems, such as oxygen combustion flasks.

In 1965 (47), a direct microdetermination of mercury in color additives by photometric-mercury vapor procedure was presented. During the following year, an apparatus for cold vapor/atomic absorption (AA) of mercury was published (48). The cold vapor/AA technique became the most popular technique for mercury determination and is still widely used today. Numerous reports have been presented dealing with applications of the technique to various types of samples, such as wheat (49), fish and eggs (50), rice (51), and biological materials (52).

In the early 1970s, we experienced in the United States the so-called "mercury crisis." It started with the revelation that some tuna samples contained levels of mercury in excess of FDA's tolerance level of 0.5 ppm at that time. To assess

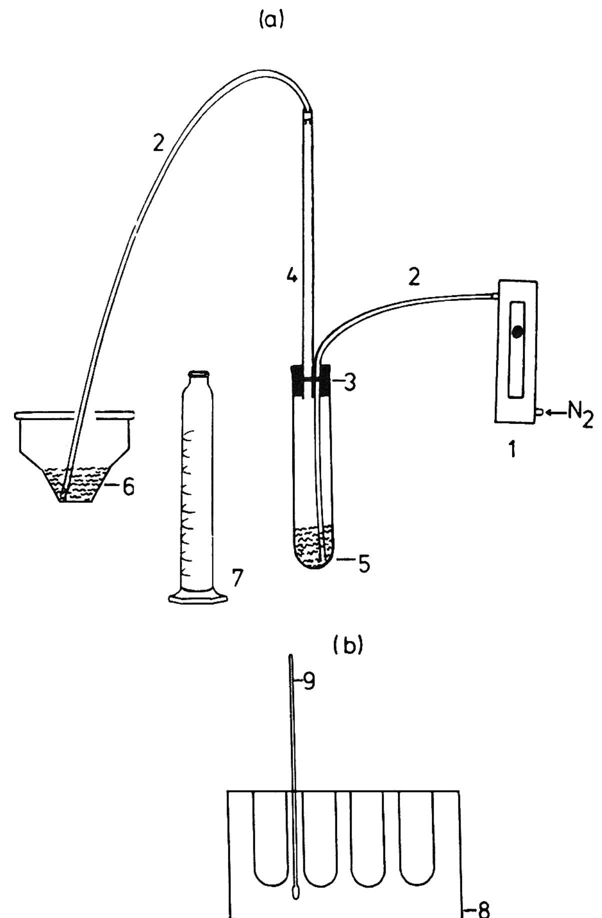

Figure 2. Purge-trap apparatus for SO_2 determination: (a) 1, flow meter; 2, Teflon tubing, 2 mm id; 3, rubber stopper; 4, glass tubing, 1.0 cm id × 40 cm; 5, sample test tube, 25 × 200 mm; 6, polarographic cell, connected to polarograph in usual way, containing 10.0 mL electrolyte-trapping solution; 7, graduated cylinder, containing 10.0 mL electrolyte-trapping solution. (b) 8, block heater; 9, thermometer.

the health hazard, hundreds of samples had to be analyzed. The AOAC official colorimetric method was not sufficiently sensitive. A new method (53) based on flameless atomic absorption was quickly collaborated and adopted official first action. Because the most troublesome part of the mercury determination is sample digestion, subsequent methods (54–59) dealt with this problem.

An atomic absorption method for inorganic and organic mercury in blood based on selective volatilization (60) and a procedure for the determination of methyl mercury in fish by gas chromatography (61) appeared in 1972. The methods dealing with the determination of total mercury (62–73) by flameless atomic absorption, however, still dominated the field. In the 1980s, the importance of speciation of mercury compounds present in seafood was realized, since organic forms of mercury are more toxic than inorganic forms. This realization is exemplified by the publication of methods that determined methyl mercury by gas chromatography (74–77). Currently, FDA's tolerance level for mercury in food, derived from methyl mercury, is 1.0 ppm. Existing GC methods use organic solvent extraction for methyl mercury from the food matrix and electron capture for detection. We have proposed a method for methyl mercury that couples 2 popular instruments found in almost every laboratory: liquid chromatography for separating organo-mercury compounds and flameless AA for sensitive and specific detection. The outline of

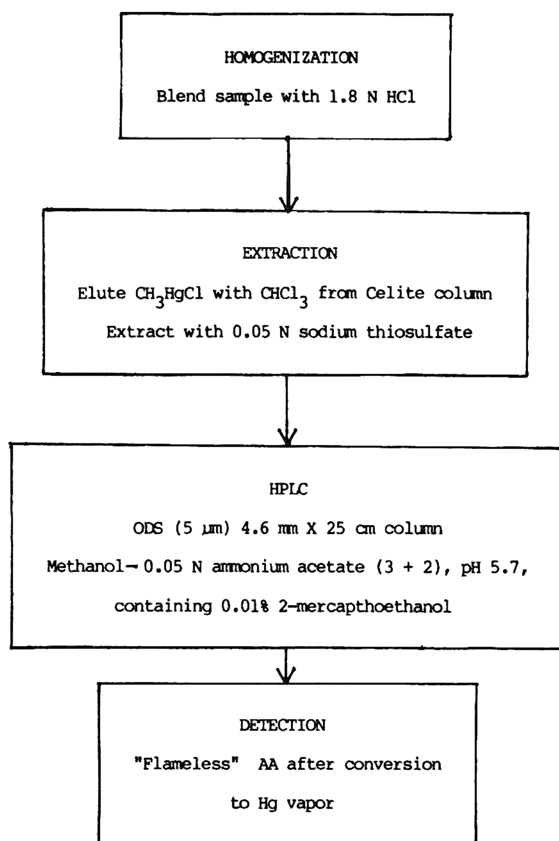

Figure 3. Outline of methyl mercury detection by liquid chromatography/atomic absorption.

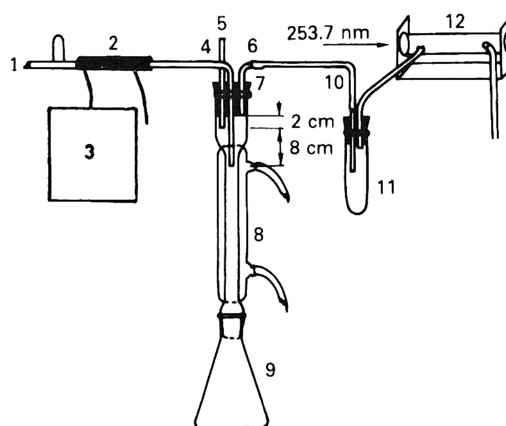

Figure 4. Liquid chromatography/atomic absorption interface. 1, LC eluate; 2, heater; 3, thermocouple; 4, 5, and 6, stainless steel tubes (5 for nitrogen inlet); 7, rubber stopper; 8, water-cooled condenser; 9, standard taper Erlenmeyer flask; 10, narrow PTFE tubing; 11, test tube solvent trap; 12, 10 cm cell with quartz windows.

the method is shown in Figure 3. The sample is blended with hydrochloric acid to form methyl mercuric chloride. The mixture is then packed into a column with Celite, and methyl mercuric chloride is eluted with chloroform and then extracted into aqueous phase containing sodium thiosulfate. The latter solution is suitable for analysis by reverse-phase liquid chromatography. The mobile phase is a methanol–acetate solution containing a small amount of 2-mercapto-ethanol to form a neutral methyl mercury complex. Detection by flameless atomic absorption requires the conversion of methyl mercury to the atomic vapor. This is accomplished through an interface as shown in Figure 4. The effluent from the liquid chromatograph is heated to produce mercury vapor from organo-mercury compounds. The mercury vapor, together with vaporized mobile phase, is directed into a water-cooled condenser where the mobile phase is liquefied. Subsequently, the mercury vapor is swept with nitrogen into the absorption cell placed in the light path of an atomic absorption spectrophotometer. Although there is some unavoidable peak broadening with such a system, it is, nevertheless, analytically useful. We have used the method routinely for approximately one year for the determination of methyl mercury in seafood. The method is currently undergoing a collaborative study.

Iodine

Iodine, an essential micronutrient, is used by the thyroid gland for the production of the hormones triiodothyronine and thyroxine, both known to regulate the basal metabolic rate. Diet is a source of iodine and is therefore important in the production of thyroid hormones. In the United States, the recommended daily allowance (RDA) of iodine for adults is 150 μg/day. It has been estimated that the average iodine intake in the United States greatly exceeds the RDA.

The first AOAC iodine method, a qualitative test in mineral water, was published in 1904 (78). A sample made alkaline was evaporated to dryness and acidified, and iodide was oxidized to iodine with potassium nitrite and extracted with carbon disulfide. The pink color in the organic phase indicated the presence of iodine. There was not much activity in this area until 1931 when a method for iodine in limestone rocks, rock phosphate, and soils was published (79). The sample was combusted at over 1000°C in a quartz tube, and iodine vapor was aspirated through a potassium carbonate solution, oxidized with potassium nitrite, and extracted with carbon disulfide. The sample treatment procedures generally used for iodine for the next several years (80–82), were combustion or fusion with an alkali followed by oxidation and absorption in carbon disulfide. In 1943, a volumetric method (83) was published for iodine in iodized salt. Bromine water was used to oxidize iodine to iodate, and, after addition of excess potassium nitrate to liberate free iodine, the determination was performed by titration with standard sodium thiosulfate solution. In 1957 (84), a Carius combustion method was presented. The following year (85), a report on the spectrophotometric determination of iodine in foods described a method in which the sample was ashed and oxidized with potassium permanganate, and the iodine was extracted into *o*-xylene and determined spectrophotometrically at 494 nm. In 1965 (86), a microchemical method used an oxygen flask combustion for the sample treatment. Later (87), iodide was determined in feeds and plants by ion selective electrode. A collaborative study (88) of the microchemical method with the oxygen flask combination was conducted in 1973. In 1977 (89), a chromatographic method for total iodine in milk was published. Iodine was converted to iodobutanone in this method. In 1978 (90), iodine was determined in nutritional and beverage products by ion selective electrode. A collaborative study conducted in 1979 (91), which used neutron activation analysis and the Ce-As-I catalytic method, demonstrated wide variability in the results, thereby pointing out difficulties in reliably determining iodine in foods. In 1981 (92), iodine was determined in foods also by iodide-catalyzed reduction of cerium by arsenite after sample digestion with nitric, sulfuric, and perchloric acids. In the same year, iodine in total diet composites was determined by neutron activa-

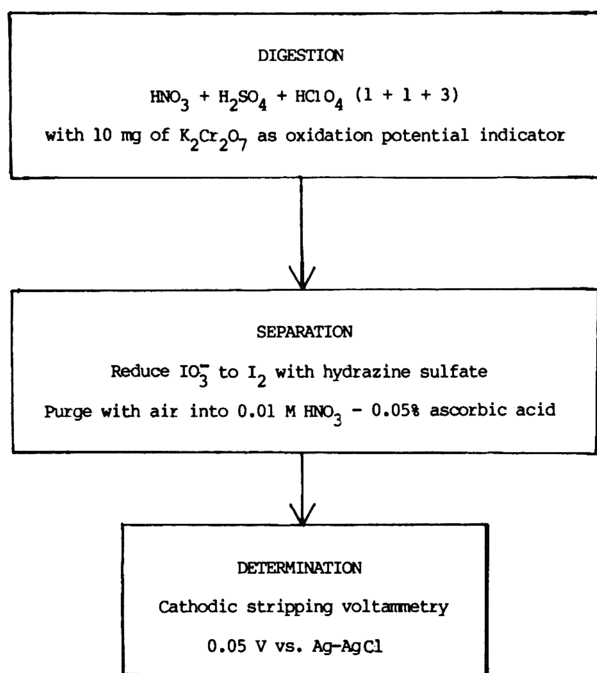

Figure 5. Outline of CSV method for iodine in foods.

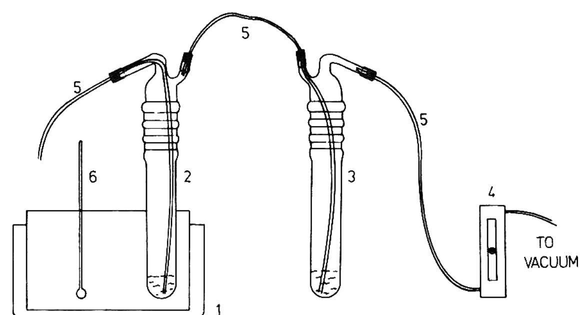

Figure 6. Distillation/trapping apparatus for iodine. 1, heating block; 2, digestion tube, 20 × 160 mm, S 24/40 with trapping solution; 4, flow meter; 5, Teflon tubing, 0.04 in. id; 6, thermometer.

tion analysis (93). A differential pulse polarographic (DPP) method for the determination of iodine in thyroid tablets (94) was collaboratively studied and adopted official first action in 1982. Two additional DPP methods for the iodine in foods were published, one in 1982 (95) and the other in 1983 (96).

The importance of iodine determination in foods lies in the fact that although it is an essential micronutrient, high levels may lead to thyroid-related problems. Its history shows that a reliable determination of iodine in foods is very difficult because of the low levels at which it may be present (0.1 ppm) and because losses of this element occur during sample digestion, isolation, and trapping. We believe that we have overcome most of these problems by the development of a method shown in Figure 5. The sample is digested with nitric, sulfuric, and perchloric acids with a little potassium dichromate which acts as an oxidation potential indicator. The mixture is maintained under reflux when the color is green, indicating reducing conditions. The digestion is carried to completion when the acid mixture remains orange throughout, indicating oxidizing conditions and that iodine is present in the nonvolatile iodic acid state. Iodine is isolated from the sulfuric acid digest by reducing with hydrazine sulfate and aspirating the iodine vapor into an electrolyte-containing ascorbic acid which reduces it to the iodide state. The determination is performed by cathodic stripping voltammetry, a very sensitive technique capable of measuring iodine at parts per billion levels. Figure 6 shows the apparatus for distillation and trapping of the iodine. The tube on the left contains the sample digest, and the one on the right contains the trapping solution. The system is connected to a vacuum that draws air through the inlet tube (No. 5) where the reagent is introduced. A collaborative study of the method is being planned.

Conclusion

The historic perspective of the AOAC methods for the determination of sulfites, mercury, and iodine clearly demonstrates the role the analyst plays in the scheme of consumer protection. As the need arises for improved, more sensitive, and more specific analytical methods, numerous publications appear in the literature to meet the demand. This has been true in the case of mercury since the early 1970s and in the case of methyl mercury since the 1980s. The same situation applies to sulfites and iodine today. In the future, the importance of the analyst's contribution can be expected to increase as the demand for better analytical methods increases. I would like to cite product tampering as one example. Tampering by use of common poisons in foods and drugs has increased over the last several years. Analysts can be expected to develop new and efficient analytical methods that will expedite investigations into incidents of tampering and ultimately increase the safety of products for the consumer.

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President's Address

"Association"—"Official"—"Analytical Chemist"

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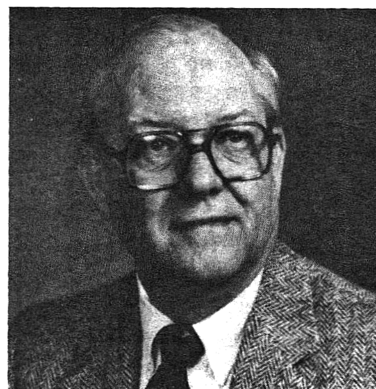
One of the many privileges associated with serving as president of this or any scientific organization is the opportunity to write and deliver the presidential address. As each of us has pursued his or her career in the world of science, we have found ourselves at a speaker's podium many times. We are assigned 15 minutes to report on research or a collaborative study with 5 minutes allowed for questions. It is a difficult task to condense everything we have to say into the time frame, and we must stay strictly to the subject at hand. However, when you have the privilege of a presidential address, there is no time limitation—within reason, there is no selected subject, and you are not required to accept questions from the audience! You are free to mix science with a little history, perhaps some personal philosophy, and some suggestions for the future.

I have chosen 3 terms from our organization's name to dissect and discuss. The dissection is merely a vehicle to be able to share some thoughts and ideas that I have developed throughout this past year.



Association is a term that has been part of our name since our formation 103 years ago. At that formative meeting, only about 2 dozen were in attendance, and they elected as their first president Samuel Johnson of Connecticut, the only other Johnson to serve as president until the current year. Through the years, our Association grew under the guidance of Professor Harvey W. Wiley and under the benevolent wing of, first, the U.S. Department of Agriculture and, later, the Food and Drug Administration, and, finally, as an independent scientific organization. We grew from a regional, one-dimensional organization concerned only about fertilizer to one that encompassed all of agriculture and finally beyond. Our mission, however, has never changed. We have always pursued uniform, validated, analytical methodology in whatever matrix or commodity we choose to study.

Our Association today has grown to well over 3000 members, and they are citizens of almost 70 countries. We address hundreds of topics with dedication and commitment from the army of volunteer collaborators, associate referees, general referees, and methods committee members with the support of elected officers and staff.

We have about 20 standing committees that address topics ranging from Awards to Ways and Means. These committees are made up of volunteers who are appointed by the president, and they pursue their assignments with the same vigor as the previously mentioned group.

One of these standing committees, the Long-Range Planning Committee, has developed a long-range plan for the Association that will shape our future. During the past year, the staff developed the strategy to carry out the plan, all committees worked to refine the strategies, and the Board of Directors adopted the Strategic Plan. At the business meeting on September 17, you will be asked to vote on the first facets of the implementation of the Strategic Plan—changes to our bylaws. I urge you to consider these changes very carefully. It is the considered opinion of the Board that the issues of membership, voting, and reshaping of the Board of Directors and the Official Methods Board are primary requirements for new, vigorous growth. If you cannot attend the meeting, vote by proxy so your voice can be heard.

Webster defines "Association" as an organization of persons with a common interest. Most of my remarks to this point have addressed that definition and how we collectively have been able to grow and accomplish many objectives. Before I leave the term "Association," allow me to reflect on one other slightly different aspect of the word. Many of us have spent years working on AOAC projects and attending meetings such as this one, and we have developed a special relationship with many individuals. These personal associations have strengthened us professionally and made our lives fuller by having experienced them. Whether we think about the effect of corporate Association or individual association, I believe we are all better for having experienced it through AOAC.

Official is another term that has been in our name since 1884. During the organizational meeting, a decision was reached that only official chemists could be members and vote, but fertilizer industry representatives were welcome to attend and enter into the discussions. Voting on acceptance of methods, therefore, was solely in the hands of the regulators. This fact immediately set AOAC apart from other societies and has held true throughout our history. Lawmakers from all levels of government have recognized this difference and have consistently designated AOAC methods in drafting legislation to equip regulators in the United States and Canada. In more recent years, this practice has spread to many other countries of the world.

Presented at the 101st Annual International Meeting of the AOAC, Sept. 14-17, 1987, San Francisco, CA.

Earlier, I alluded to the changes to the bylaws that will be placed before the membership at the business meeting. One of these changes grants full membership to regulated industry scientists. This is a major divergence from our heritage and was considered at great length by the Board of Directors and others before offering it for vote. Two things have occurred in recent years that convinced me this was the proper course to take. In his remarks following the banquet at the 1985 annual meeting, Joseph Hile, Associate Commissioner for Regulatory Affairs, FDA, stated, "I urge the Association to consider broadening industry's role in AOAC by doing away with associate membership and extending full membership privileges to all industry scientists."

The second thing which further confirmed my position was a letter presented to the Board in July 1986 from FDA's Conflict of Interest Policy Officer that stated, "Full membership of industry [scientists] in AOAC does not, on the surface, create a problem with FDA nor do we believe that there would be more potential for conflict of interest." The letter proceeded to say this could only change if industry gained control of AOAC.

With the safeguards written into the bylaw changes to maintain a majority of regulators as members of the Board of Directors and the Official Methods Board, I feel we have no concerns about jeopardizing our position as an official organization. What we have to gain is full participation and support of industry members who already make up over half of our collaborators! Industry representatives who currently hold refereeships are some of the most productive and committed, but to date they have been relegated to associate membership. In my opinion, the time has arrived when we can no longer afford to accord our fellow scientists from the industrial sector anything but full membership in the Association.

The third and final term I will explore with you today is *Analytical Chemists.* This term has been a part of our name only since 1966, but those previous "Agricultural Chemists" practiced analytical chemistry exclusively. We have always concerned ourselves with analytical methods as developed and tested by "Analytical Chemists."

Analytical chemistry has been practiced for centuries, but only in recent years has it been recognized as a scientific discipline in its own right. This relatively new discipline has drawn from the fields of physics, biology, electronics, and all other subgroups of chemistry to become a highly sophisticated branch of chemistry. Those of us who practice this profession must become proficient in our chosen occupation by extended education and experience.

A world-renowned analytical chemist has written that to conduct successful scientific research, one must acquire intelligence, imagination, and intuition; and these three will act together in the development of new ideas. He also stated the three basic concepts of analytical chemistry—quality, quantity, and structure—are all interdependent on the sample to be analyzed. Only an exact knowledge of the history of the sample ensures the obtaining of reliable analytical information. In keeping with this philosophy, 2 things have occurred within the past year that require mentioning at this time. The Official Methods Board has begun the process of establishing a task force to study the science of sampling. Last year at the annual meeting, sampling of many commodities was emphasized, and with the creation of this new task force, we should begin to see and understand the importance of sampling and of maintaining sample integrity during custody.

The other event of the past year that addresses our subject is the suggestion of one of our Board members to initiate an Analyst Certification program. It would recognize and certify certain AOAC analytical chemists who had participated in or conducted a collaborative study. This program is under study, and you should be hearing more about it in years to come.

To summarize my thoughts on analytical chemists, let me say they are, in general, highly trained individuals who respond to the needs of their clientele with resourcefulness and wisdom. They are proud problem solvers who display honesty and integrity as they go about their jobs in regulatory positions, research institutions, production facilities, and as teachers in academia.

In reviewing past presidents' addresses, I found they fell into 2 categories—either "State of the Association" speeches or scholarly scientific presentations. Obviously, to this point, I have done neither. It is appropriate to highlight some of the activities and changes that are currently underway in AOAC. This is the second annual meeting held outside Washington, and 3 more are planned for Palm Beach, St. Louis, and New Orleans. I feel moving the meeting each year has proven to be a wise choice, and I hope we continue the practice. This year marks the last year for the Spring Training Workshop, which, incidentally, was highly successful—the organizers in Ottawa deserve a special vote of gratitude. The reasons for cancelling the meeting after this spring were twofold—the annual meeting is now more accessible, and regional sections are becoming more active. We have chartered 7 regional sections to date, and more are in the formative stage. This appears to be the direction that AOAC needs to pursue. Large geographic areas are not presently served by a regional section on this continent, and in the future, we may want to extend this concept to other areas of the world.

In most of the addresses of the last 10 years, the president has included some comments on our financial condition. Our condition can best be described as *fragile* in spite of several new sources of funding. We have increased sustaining and individual members' dues, solicited contributions for the annual meeting, and instituted page charges for the *Journal of the AOAC* to help defray ever-increasing costs of doing business. *Official Methods of Analysis* is still the major source of revenue, and if we cannot find significant new sources, it may be necessary to curtail services offered.

In closing, allow me to express my appreciation to my fellow Board members who have served faithfully and pursued their assignments diligently; to the AOAC staff who continually provide outstanding services; and last, but most important, to the hundreds of volunteers who make AOAC go. Without volunteers to serve on committees, as Referees, as collaborators, as reviewers, as statisticians, etc., etc., there would be no AOAC and the world would be a lesser place to live.

DISINFECTANTS

Bacterial Numbers on Penicylinders Used in Disinfectant Testing: Use of 24 Hour Adjusted Broth Cultures

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The current AOAC use-dilution methods of disinfectant efficacy testing require the use of 48–54 h unadjusted broth cultures of *Salmonella choleraesuis, Staphylococcus aureus,* and *Pseudomonas aeruginosa* for the inoculation of stainless steel penicylinders. The use of unadjusted broth cultures contributes to noncomparable numbers of organisms on penicylinders among the test strains due to relative efficacy of bacterial attachment to penicylinders and to bacterial numbers in broth. To achieve comparable numbers of cells on the penicylinders among the 3 test strains. the cell densities of *S. aureus* and *P. aeruginosa* in broth culture were visually adjusted. Growth studies were conducted using *S. choleraesuis* and *P. aeruginosa* to determine the numbers of cells in broth at timed intervals and the corresponding numbers of cells attaching to the penicylinders. Results showed that the use of the 24 h broth cultures for all 3 test strains, with adjustment of *S. aureus* and *P. aeruginosa* broths, contributes to more comparable numbers of organisms attached to the penicylinders used in disinfectant testing.

The AOAC use-dilution methods (1) require the use of 48–54 h bacterial broth cultures for the inoculation of stainless steel penicylinders used in disinfectant testing. Previous investigations have demonstrated significant differences in the numbers of bacteria dried onto the surfaces of these stainless steel penicylinders (2). The numbers of the 3 AOAC test bacteria adhering to the penicylinders varied significantly, with more than 10^7 cells/penicylinder for *Pseudomonas aeruginosa,* approximately 5×10^6 for *Staphylococcus aureus,* and 10^6 or less for *Salmonella choleraesuis.* Presumably, this wide range is due to morphological and physiological differences among the 3 test organisms that affect their adherence and affect the bacterial numbers in broth that are reached at stationary phase. A comparable microbial load is preferred for disinfectant testing because the time required for complete killing of the test bacteria is directly related to the starting dosage. Thus, unless the microbial loads are similar, the disinfectants are not similarly challenged with the AOAC test bacteria.

Disinfectant test methodologies used in several other countries utilize 24 h broth cultures (3, 4), as do 5 of the other AOAC disinfectant test methods (1). In general, 18–24 h broth cultures have been found to be the most suitable for disinfectant testing (3). Presumably, such cultures represent the maximum cell density.

In an attempt to ensure more equitable bacterial numbers on the penicylinders, the turbidity of *S. aureus* and *P. aeruginosa* in 24 h nutrient and synthetic broth was adjusted. Using *S. choleraesuis* and *P. aeruginosa,* growth studies were performed, and the numbers of cells in broth at various time intervals and the numbers of cells that had attached to the penicylinders were counted. The results of these studies are presented here.

METHOD

Media and Reagents

(a) *Nutrient broth.*—See sec. **4.001(a)** (1), 10 mL in 20 × 150 mm tubes using Anatone (American Laboratories, Inc., Omaha, NE 68127), and Beef Extract (Difco Laboratories, Detroit, MI 48232) as specified. Prepare according to directions using glass-distilled water. Adjust pH to 6.8 before sterilization.

(b) *Synthetic broth.*—Bacto Synthetic Broth AOAC (Difco Laboratories), formulated to comply with sec. **4.001(b)** (1).

(c) *Pour plate agar.*—Bacto Plate Count Agar (Difco Laboratories).

(d) *Distilled water.*—Sterile Water for Irrigation, USP (Travenol Laboratories, Inc., Deerfield, IL 60015).

(e) *Asparagine solution, 0.1%.*—See sec. **4.007(e)** (1).

(f) *Phosphate buffer dilution water (PBDW).* See sec. **4.020(f)** (1).

(g) *Barium chloride.*—Crystals, reagent grade.

(h) *Sulfuric acid.*—ACS reagent grade.

(i) *McFarland standards.*—Prepare 1% (w/v) aqueous sulfuric acid solution using distilled water. For 1.0 standard, add 0.1 mL 1% barium chloride solution to 9.9 mL 1% sulfuric acid. This will approximate bacterial density of 3.0×10^8 cells/mL. For 0.5 standard, add 0.05 mL 1% barium chloride solution to 9.95 mL 1% sulfuric acid. This will approximate bacterial density of 1.5×10^8 cells/mL. Prepare fresh standards each week and protect from light.

Organisms

Staphylococcus aureus 6538, *Salmonella choleraesuis* 10708, and *Pseudomonas aeruginosa* 15442 (American Type Culture Collection, Rockville, MD 20852).

Apparatus

(a) *Vortex mixer.*—Vortex-Genie (Fisher Scientific, Pittsburgh, PA 15219).

(b) *Colony counter.*—New Brunswick Scientific, Edison, NJ 08817, or equivalent.

(c) *Penicylinders.*—S&L Metal Products Corp., Maspeth, NY 11378.

(d) *Sonic cleaner.*—E/MC Model 450 (RAI Research Corp., Hauppauge, NY 11787), or equivalent.

(e) *Inoculating loop.*—4 mm id platinum/rhodium loop (Scientific Products, McGaw Park, IL 60085).

Culture Preparation

From stock culture slant, make ≥4 consecutive broth transfers (using 4 mm id loop) of test organisms in 10 mL portions of broth. Keep caps loose and incubate tubes at 37°C. For penicylinder inoculation, broth must be in 25 × 150 mm tubes. Incubate tubes 24 h at 37°C.

Penicylinder Preparation

Decontaminate previously used penicylinders by steam sterilization; then sonicate ≥5 min, rinse with distilled water, and process as in sec. **4.009** (1). Place clean penicylinders in groups of 10 into 20 × 150 mm screw-cap tubes and cover with 0.1% asparagine. Sterilize tubes at 121°C 15 min and let cool.

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Table 1. Mean numbers of *S. choleraesuis* ATCC 10708 and *P. aeruginosa* ATCC 15442 in nutrient broth (per mL) and on penicylinders

Time, h	Broth[a]	Penicylinders[b]	% Attached
	S. choleraesuis		
6	1.6×10^8		
12	3.8×10^8		
18	7.7×10^8	3.1×10^6	0.37
24	5.9×10^8	2.2×10^6	0.37
36	6.3×10^8	2.4×10^6	0.38
48	5.9×10^8	1.9×10^6	0.32
54	3.9×10^8	1.8×10^6	0.46
	P. aeruginosa		
6	5.1×10^8		
12	5.5×10^8		
18	8.4×10^8	3.2×10^7	3.8
24	1.1×10^9	3.3×10^7	3.0
36	1.2×10^9	2.7×10^7	2.3
48	1.1×10^8	2.8×10^7	2.6
54	5.2×10^8	9.3×10^6	1.8

[a] Mean values of 2 replicates.
[b] Mean values of 3 replicates.

Adjustment of Culture Broths

For inoculation of penicylinders in which 24 h broths are used, use *S. choleraesuis* broth undiluted; adjust turbidity of *S. aureus* to that visually comparable with No. 1 McFarland standard; and adjust turbidity of *P. aeruginosa* to that visually comparable with 0.5 McFarland standard. For *P. aeruginosa* remove aliquot of broth to be adjusted by tilting tube and guiding pipet 5–10 mm under pellicle. Alternatively, sterile Pasteur pipet with vacuum source may be used to remove pellicle. Adjust turbidity of broth cultures with PBDW to obtain turbidity visually comparable to that of turbidity standard. Agitate turbidity standard and broth tube on vortex mixer immediately before comparing. For turbidity adjustment, simultaneously view white background and contrasting black line with adequate light source through both tubes.

Inoculation of Penicylinders

Transfer 10 mL portions of test broths to separate 25 × 150 mm sterile tubes and briefly vortex (1.0 s) tube. Pour off asparagine from prepared carriers, using sterile pipet to withdraw any remaining solution in bottom of tube. Using flamed wire hook, aseptically transfer 6 penicylinders to test broth. Tube may be shaken to rearrange cylinders so all are submerged. Let tube sit undisturbed at room temperature 15 min. Aseptically remove carriers from inoculation tube and place on end in sterile glass petri dish matted with 2 layers of 9 cm Whatman No. 2 filter paper. Incubate covered dish at 37°C 40 min at 25–40% relative humidity.

Removal and Quantitation of Organisms

Place 6 inoculated penicylinders in separate 20 × 150 mm screw-cap tubes each containing 10 mL sterile PBDW. Spin tubes on vortex mixer, setting 4, for 1 minute. (The efficiency of this removal method has been demonstrated [2].) Using 1.0 mL transfer aliquots, prepare serial dilutions in PBDW to 10^{-4}. Using pour plate method, plate 10^{-3} and 10^{-4} dilutions in duplicate with 1.0 mL samples and 15–17 mL plate count agar at 42–45°C. Incubate plates 48 h at 37°C and count those with colonies numbering between 30 and 300.

Cell Attachment According to Growth Curves

Since preliminary data indicated that the greatest difference in numbers of bacteria attached to penicylinders was between *S. choleraesuis* and *P. aeruginosa*, growth studies of both organisms were conducted. Seven 10 mL tubes of nutrient broth for each organism were inoculated and transferred as described above (*Culture Preparation*). At specific intervals (6, 12, 18 h, etc.), two 1.0 mL aliquots from each broth tube were removed and serially diluted in PBDW, and duplicate pour plates were prepared. Plates were incubated and quantitated as described above. At 18, 24, 36, 48, and 54 h after removal of aliquots, 5.0 mL broth culture of each organism was placed in sterile 25 × 150 mm tubes for inoculation of penicylinders and subsequent cell removal as described above.

Table 2. Mean numbers of bacteria on penicylinders (n = 6) after inoculation with adjusted[a] 24 h broths

Organism	Nutrient broth	Synthetic broth
S. aureus	3.4×10^6	2.4×10^6
S. choleraesuis	3.8×10^6[b]	3.8×10^6[c]
P. aeruginosa[d,e]	1.4×10^7	8.4×10^6

[a] *S. aureus* broth adjusted to turbidity of 1.0 McFarland standard; *P. aeruginosa* broth adjusted to turbidity of 0.5 McFarland standard; *S. choleraesuis* broth undiluted.
[b] Mean value of 12 replicates.
[c] Mean value of 5 replicates.
[d] Difference in cell numbers between broths is significant ($P < 0.001$).
[e] Difference in cell numbers when compared with *S. aureus* and *S. choleraesuis* is significant for both broths ($P < 0.001$).

Statistical Analysis

Statistical comparisons between numbers of organisms at different times were made by log transforming the data and then applying either t-tests (2 groups) or an analysis of variance (>2 groups).

Results and Discussion

Growth studies of *S. choleraesuis* showed maximum cell numbers in nutrient broth and attachment to penicylinders at 18 h, with a mean of 7.7×10^8 cells/mL and 3.1×10^6 cells/penicylinders, respectively (Table 1). The numbers of *S. choleraesuis* in broth and on penicylinders at 18 h, however, were not significantly different ($P > 0.05$) from those at 24, 36, and 48 h. The mean percentage of *S. choleraesuis* cells in broth culture which attached to the penicylinders was only 0.38. *P. aeruginosa* also demonstrated a maximum number of cells in nutrient broth at 18–48 h with a mean of 1.1×10^9 cells/mL (Table 1). Optimal attachment of *P. aeruginosa* to penicylinders occurred from 18 to 48 h with a mean of 3.0×10^7 cells/penicylinder or approximately 3% of the cells in broth.

Results of all 3 test bacteria attaching to penicylinders in both nutrient and synthetic broths are presented in Table 2. *S. aureus*, with turbidity visually adjusted to that of a 1.0 McFarland standard, demonstrated mean numbers of organisms on penicylinders comparable to those of *S. choleraesuis* for both broths ($P > 0.05$). Numbers of *P. aeruginosa* attaching to penicylinders, after broth culture dilution comparable to a 0.5 McFarland standard, remained significantly higher than the other 2 test strains ($P < 0.001$) for both broths.

These results demonstrate that the use of 24 h broth cultures can consistently increase the numbers of *S. choleraesuis* above 10^6, thus producing a bacterial challenge to germicide testing that is comparable to that of adjusted *S. aureus*. *P. aeruginosa* broth cultures would require further dilution beyond that employed in this study to achieve comparability with the other 2 test bacteria. This is largely attributable to

P. aeruginosa attaching to penicylinders 10 times more efficiently than *S. choleraesuis*.

It is suggested that unadjusted 24 h broth cultures for *S. choleraesuis* and adjusted 24 h broth cultures for *S. aureus* and *P. aeruginosa* be considered in future revision of the AOAC use-dilution methods. This should result in a more comparable bacterial challenge among the 3 test bacteria used in disinfectant testing.

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VITAMINS AND OTHER NUTRIENTS

Disposable Cartridge Extraction of Retinol and Alpha-Tocopherol from Fatty Samples

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A new approach is proposed for liquid/solid extraction of retinol and α-tocopherol from samples, using a disposable kieselguhr cartridge. The substitution of the mixture methanol-ethanol-*n*-butanol (4 + 3 + 1) for methanol in the alkaline hydrolysis solution makes it now possible to process fatty samples. Methanol is necessary to solubilize the antioxidant ascorbic acid, and a linear chain alcohol such as *n*-butanol is necessary to reduce the size of soap micelles so that they can penetrate into the kieselguhr pores. In comparisons of the proposed method with conventional methods on mineral premixes and fatty feedstuffs, recovery and accuracy are at least as good by the proposed method. Advantages are increased rate of determinations and the ability to hydrolyze and extract retinol and α-tocopherol together from the same sample.

Methods for processing samples prior to analytical determination of retinol and α-tocopherol have not progressed to a great extent in previous decades. Automated determination of fat-soluble vitamins by modern methods is hampered by the initial processing of the sample and, particularly, by the extraction step. Recently, Bourgeois and coworkers (1) reviewed extraction techniques for retinol and α-tocopherol and discussed this problem. They proposed a new approach to extraction of fat-soluble vitamins: liquid/solid extraction of the unsaponifiables with small kieselguhr disposable cartridges. They used the new technique on several thousand samples of feed, food, and mineral premixes. Yet, for fatty samples they found it necessary to use the traditional liquid/liquid extraction with an organic solvent.

The present work is based on the assumption that in the case of fatty samples, large soap micelles are formed which obstruct the kieselguhr pores. Consequently, the hydrolysis solution traverses the column without being adsorbed. Soap micelles are formed in presence of water and methanol. They are destroyed by heavier alcohols such as ethanol. Methanol is used in the hydrolysis solution for the following reason:

Retinol and α-tocopherol are easily oxidized in the presence of air, so that it is necessary to use a rather high concentration of a strong antioxidant—ascorbic acid—to protect them during adsorption on kieselguhr. In the absence of the antioxidant, retinol and α-tocopherol recoveries are 20 to 30% lower. Methanol is a better solvent than ethanol for ascorbic acid, but methanol forms micelles. Use of fat-soluble antioxidants in place of ascorbic acid would create new problems: Such antioxidants are oxidized to quinones, which are very unstable in basic media and form many degradation products. These products could increase the background blanks to a prejudicial degree, and fat-soluble antioxidants may not be as efficient as ascorbic acid. Use of methanol–ethanol (1 + 1) brought little improvement, so it was necessary to address the problem on a more theoretical basis.

In studies of the effect of alcohols on the critical micelle concentration (CMC) of surfactants (2–8), researchers have shown that alcohols decrease CMC. Harkins et al. (4) and Schulmann et al. (5) showed that alcohols penetrate the micelle and the hydroxyl group remains bound to the surface in the palisade layer. Shinoda (6), Harva (7), and Vikingstad (8) studied the action of alcohols on soap micelles. Shinoda demonstrated that the longer the alcohol, the more effective its action on CMC. Herzfeld and coworkers (9) showed that CMC decrease is greater with 1-propanol than with 2-propanol. More recently, Guveli et al. (10, 11) showed that 1-alkanols reduce the viscosity of emulsions and decrease the size of micelles. Zana and coworkers (12) showed that linear alcohols dissolve in the micelle and decrease CMC and the micelle molecular weight.

From the literature survey, we determined that linear alcohols better penetrate soap micelles. They reduce emulsion viscosity and micelle size. The longer the alkyl chain, the more effective the alcohol and the smaller the amount necessary to obtain a given effect.

METHOD

Reagents and Materials

(a) *Pyrogallol solution.*—Dissolve 1 g pyrogallol in 40 mL water. Add 120 mL 1-butanol, 360 mL ethanol, and 480 mL methanol and shake. Prepare daily.

(b) *Potassium hydroxide aqueous solution.*—Dissolve 700 g KOH (86% w/w) in 600 mL water and adjust to 1 L.

(c) *Ascorbic acid aqueous solution.*—10% (w/v) in water. Prepare daily.

(d) *Disposable cartridges.*—Extrelut cartridges 11737 and fillings 11738 (E. Merck, Darmstadt, FRG): small plastic columns, 3 cm in diameter, and weighing ca 10 g. Operate columns under normal pressure. Columns can be reloaded 20 times or more; series of 8 or even 16 columns can be manipulated in parallel.

Hydrolysis

Weigh 50 or 100 g feed sample (for α-tocopherol or for retinol and retinol plus α-tocopherol) or 10–20 g premix in 1 L low actinic long-neck boiling flask. Add 400 mL pyrogallol solution and 80 mL KOH solution. Weigh flask, attach reflux condenser, and heat 30 min under nitrogen. Shake flask from time to time or, preferably, use magnetic stirrer. Rinse condenser with 20 mL water, cool to room temperature, and weigh flask again. Bring weight to its initial value plus 20 g with ethanol if necessary.

Preparation of Cartridges

Already-filled columns can be used, but we usually fill empty columns with kieselguhr powder sold in small bags. Fill 10 g powder in columns and homogenize with vibrating agitator. (*Note:* We previously described a small loading-press designed to facilitate loading [1]. This press is useful but not absolutely necessary for filling columns.)

Extraction

Manipulation should be done under a hood. Let hydrolysis solution cool to room temperature and pipet 40 mL aliquot

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Table 1. Composition of the alkaline hydrolysis solution[a] and maximum amounts of fats that can be assayed

Solution components	% Component in solutions			
	A	B	C	D
Water	4	4	4	4
Methanol	76	48	48	—
Ethanol	20	48	36	96
n-Butanol	—	—	12	—
Fat processed, g:				
Lard	2.4	2.9	4.8	30
Soybean oil	3.6	5.1	7.0	>30

[a] Hydrolysis was carried out with 400 mL of this solution, plus 0.4 g pyrogallol, and 80 mL KOH.

into 50 mL low-actinic volumetric flask. Adjust to mark with ascorbic acid solution. Shake and transfer 20 mL of this solution onto cartridge. Hydrolysis solution should be completely retained in the column. If it is not, start again with 15 mL. Wait 15 min, set 50 mL volumetric flask under column, and elute with 60 mL isooctane. Elution lasts ca 25 min, and ca 40 mL isooctane is collected. Keep flask in freezer (−18°C) and adjust volume with isooctane just before determination.

Just after use, discard adsorbent, and dip column in aqueous solution of Mucapur® (Merz Co., Frankfurt, FRG) for 1 h. Then column may be washed with rest of glassware. Column can be reloaded at least 20 times.

Results and Discussion

In all cases, a 20 mL aliquot of the hydrolysis solution was layered onto the column. Table 1 shows the influence of the composition of the hydrolysis solution on the maximum amounts of fats that can be processed. Lard and soybean oil were chosen as models for saturated and unsaturated fats. The given amounts are contained in the whole hydrolysis solution, i.e., 480 mL without assayed sample. Solution A corresponds to the one that was proposed previously (1) and routinely used in our laboratory. Solution B gave little improvement. Solution C gave satisfactory results: the maximum amounts of fats are twice as great as for solution A. This solution was chosen for the proposed method. All feed samples tested with it gave good results, as will be shown. Solution D gave the best results as far as the maximum amounts of fats are concerned, and it confirmed our initial assumption that micelles block kieselguhr pores. Because micelles do not form in ethanol, elevated amounts of fats can be assayed. Yet, the recoveries of retinol and α-tocopherol from 30 g lard were not satisfactory with solution D (see Table 2). Retinol was not completely recovered and elution of α-tocopherol was sluggish. This is due to the retentive action exerted by too large an amount of lard. In other experiments, elution of both vitamins with solution D was satisfactory when the amount of fat was smaller. Yet it is not possible to use ascorbic acid as an antioxidant with solution D, since it is not soluble enough in ethanol. Use of a strong antioxidant to stabilize retinol and α-tocopherol is imperative. This is the reason solution C was finally chosen.

Table 3. Effect of different geometrical isomers of butanol on the maximum amount of fat that can be assayed[a]

Alcohol	Amount of fat assayed, g	
	Lard	Soybean oil
1-Butanol	4.8	7.0
2-Butanol	4.1	6.1
Isobutanol	4.2	5.5
Tertiobutanol	3.8	5.3

[a] Compositions of hydrolysis solutions are identical to solution C of Table 1 except for the geometry of the isomer.

Before testing *n*-butanol, we first tried isobutanol. Assays were performed with mixtures containing 50% methanol and various proportions (%) of ethanol and isobutanol: 30 + 20, 25 + 25, 37.5 + 12.5, 12.5 + 37.5, and 43.75 + 6.25. The second and third mixtures gave best results; we chose the third one (50% methanol, 37.5% ethanol, and 12.5% isobutanol), because it contains less isobutanol which is slightly more expensive.

Maintaining these proportions of the 3 alcohols, we tested different geometrical isomers of butanol. Table 3 shows that the linear isomer *n*-butanol gives best results, in accordance with the literature survey and with our initial assumption on the prejudicial effect of soap micelles. *n*-Butanol penetrates the micelles and reduces their size. The smaller micelles can then penetrate the kieselguhr pores rather than blocking them. The experiment described below also confirms the assumption about micelles.

In four long-neck boiling flasks, 5 g samples of lard were saponified in parallel with solutions A, B, C, and D (see Table 1). After completion, the flasks were taken off the heating bath and allowed to cool to room temperature. At the beginning all solutions were yellow and clear. After 15 min, flask A showed a slight precipitate which grew; after 50 min the precipitate occupied half the flask. At the same time, a slight precipitate appeared in flask B. Flasks C and D remained clear. After 2 h, a gel formed in flask A and a flocculate formed in flask B. After 4 h, the gel in flask A was opaque; a white crystalline precipitate had formed in B; and C and D remained clear. After 1 day, C and D were still clear. The probable explanation is that solutions A and B contain large soap micelles. On cooling, the soap molecules crystallize inside the micelles at given temperatures corresponding to the Krafft Points of the various fatty acids (13, 14). Soap micelles and soap crystals block the kieselguhr pores, and the hydrolysis solution traverses the column unretained. In solution D, there are probably no micelles; in

Table 2. Isooctane elution of retinol and α-tocopherol from the cartridge in 10 mL fractions after hydrolysis of 30 g lard with solution D

Fraction	Recovery, %	
	Retinol[a]	α-Tocopherol[b]
1	27.0	12.0
2	19.7	11.5
3	18.0	20.4
4	13.3	56.1
Total	78.0	100

[a] 20 IU layered onto cartridge.
[b] 0.18 mg layered onto cartridge.

Table 4. Recovery of retinol and α-tocopherol from cartridge when maximum amounts of fats are hydrolyzed with solution C[a]

	Retinol, IU	α-Tocopherol, mg
Without fat	34.6	0.084
	35.3	0.086
Lard, 4.8 g	34.2	0.083
	35.6	0.087
Soybean oil, 7.0 g	34.6	0.087
	34.9	0.085

[a] Theoretical amounts of fats hydrolyzed and layered onto cartridges were 36 IU retinol and 0.088 mg α-tocopherol.

Table 5. Isooctane elution of retinol and α-tocopherol from cartridge in 10 mL fractions after hydrolysis with solution C[a]

Fraction	Recovery, %	
	Retinol	α-Tocopherol
1	61.6–64.0	64.1–67.9
2	29.4–26.5	30.3–25.5
3	6.8–7.5	5.6–6.6
4	2.2–2.0	0–0
Total	100	100

[a] 39 IU retinol and 0.086 mg α-tocopherol were layered onto the cartridges.

solution C, there are small micelles stabilized by *n*-butanol. The latter may be a thermodynamically stable microemulsion. The concentration of aqueous ascorbic acid used to stabilize solution C after hydrolysis is 10% (w/v) instead of 20% as for solution A in our previous study (1), because solution C contains less methanol than A, and therefore there is a risk of precipitation of ascorbate. However, the antioxidant is still in large excess.

Table 4 shows that recoveries of retinol and α-tocopherol are good with solution C, even when maximum amounts of lard and soybean oil are hydrolyzed. Vitamins were layered onto the columns in amounts corresponding to average values in feedstuffs.

The eluates were separated in four 10 mL fractions, and retinol or α-tocopherol was determined in each fraction. Elution patterns of retinol and α-tocopherol with solution C are presented in Table 5. Elution patterns of these vitamins with solution A were presented in Tables 6 and 7 of reference 1. Comparison shows that elution patterns with solutions A and C are much the same.

Table 8. Recovery of retinol and α-tocopherol from feedstuffs and premixes[a]

Sample	Retinol concn, IU/kg	Retinol rec., %	α-Tocopherol concn, ppm	α-Tocopherol rec., %
Broiler growth 1	8 660	103.5	20.52	107.5
Broiler growth 2	7 700	90.8	20.35	105.1
Piglet	9 970	102.8	18.25	91.4
Dairy cow	5 540	100.3	17.80	97.1
Turkey-cock and guinea fowl	6 054	95.0	22.80	99.8
Chick MVP	1 507 000	103.4	934	107.3
Cattle MVP	2 201 000	103.5	557	99.6
Sow MVP	276 600	102.1	1276	102.6
Piglet MVP 1	475 000	101.6	709	111.2
Piglet MVP 2	2 440 000	103.9	2308	101.2
Average, %		100.7		102.3

[a] Results are averages of duplicate determinations.

Comparison of Liquid/Liquid and Liquid/Solid Extraction Techniques

The method proposed here has been compared with our former liquid/liquid extraction methods (1) for retinol and α-tocopherol (called here "conventional methods"). Retinol was determined by liquid chromatography and α-tocopherol by our continuous-flow method (15). Mineral and vitamin premixes (MVP) contained calcium and organoelements such as manganese, magnesium, iron, and copper. High amounts of minerals were chosen so that the stabilization effect of the ascorbic acid could be tested. For example the sow MVP contained 2000 ppm copper and 10 000 ppm iron. The piglet MVPs 1 and 2 contained, respectively, 12 000 and 25 000

Table 6. Parallel extractions of retinol with cartridges and conventional liquid/liquid partition

Sample	Fat, %	Conventional technique, IU/kg	Cartridge technique, IU/kg	Cart./Conv. × 100, %
Broiler growth 1	7	8060, 8606[a]	8065, 9255	103.9
Broiler growth 2	9	5574, 6672	7691, 7715	125.8
Piglet	7.5	9822, 10922	9940, 9996	96.0
Dairy cow	1.5	4600, 5217	4980, 6100	112.8
Turkey-cock and guinea fowl	2.0	6308, 6899	5884, 6224	91.7
Chick MVP	—	1473.10^{+3}, 1554.10^{+3}	1437.10^{+3}, 1578.10^{+3}	99.6
Cattle MVP	—	1950.10^{+3}, 2210.10^{+3}	2140.10^{+3}, 2262.10^{3}	105.8
Sow MVP	—	3009.10^{+3}, 3145.10^{+3}	2730.10^{+3}, 2801.10^{+3}	89.9
Piglet MVP 1	—	3410.10^{+3}, 4250.10^{+3}	4720.10^{+3}, 4787.10^{+3}	124.0
Piglet MVP 2	—	2386.10^{+3}, 2390.10^{+3}	2420.10^{+3}, 2460.10^{+3}	102.1
Average, %				105.2

[a] Duplicate determinations.

Table 7. Parallel extractions of α-tocopherol with cartridges and conventional liquid/liquid partition

Sample	Fat, %	Conventional technique, ppm	Cartridge technique, ppm	Cart./Conv. × 100, %
Broiler growth 1	7	20.02, 20.37[a]	20.47, 20.57	101.6
Broiler growth 2	9	17.80, 19.24	20.20, 20.50	110.0
Piglet	7.5	17.17, 17.55	18.1, 18.4	105.1
Dairy cow	1.5	17.10, 18.0	17.65, 18.0	101.4
Turkey-cock and guinea fowl	2.0	21.70, 22.86	22.60, 22.99	102.2
Chick MVP	—	940, 990	926, 939	96.8
Cattle MVP	—	533, 534	552, 562	104.5
Sow MVP	—	1189, 1224	1256, 1300	105.8
Piglet MVP 1	—	637, 643	689, 730	110.8
Piglet MVP 2	—	1950, 2144	2306, 2310	112.7
Turkey	9	29.85, 31.93	31.10, 35.20	107.4
Average, %				105.3

[a] Duplicate determinations.

ppm copper and 10 000 and 15 000 ppm iron. High fat contents of most feed samples were also chosen.

As can be seen in Tables 6 and 7, the results obtained with the proposed and conventional techniques are in good agreement. Only for the last sample of Table 7, a feedstuff for turkey, was it necessary to diminish the volume of solution layered onto the cartridge from 20 mL to 15 mL. The proposed method shows a slight tendency to higher values, as found before (1). This may be due either to incomplete extraction or to a slight degradation of the vitamins during liquid/liquid partition. The difference was significant only in the case of α-tocopherol in feedstuffs.

Statistical Evaluation

The paired t-test was applied to results in Tables 6 and 7 for feedstuffs and for mineral premixes. At the 5% level, only the results obtained for α-tocopherol in feed were significantly higher by the proposed method (calculated t-value = 2.912 vs theoretical Student t-value = 2.571). The other t-values found were not significant (1.598 for α-tocopherol in concentrates, 1.566 and 0.826 for retinol in concentrates and feedstuffs).

Recovery

Samples were first assayed to determine actual levels of retinol and α-tocopherol. Then aliquots of standard solutions were added after the alkaline hydrolysis step. The known amounts of added vitamins were approximately equal to the amounts originally present in the samples. The recovery factor was calculated by subtracting the result obtained for the unsupplemented sample from the one obtained for the supplemented sample and dividing by the known added amount. Table 8 shows the results obtained from duplicate determinations. The average recovery factors were 100.7% for retinol and 102.3% for α-tocopherol.

Reproducibility

The coefficients of variation (CV) for the extractions from hydrolyzed standard solutions containing 832 IU retinol acetate and 2.3 mg DL α-tocopherol were 2.8 and 1.2%, respectively. Ten assays were carried out in each case. Suppression of one retinol result would decrease the CV from 2.8 to 1.3%. These CV values correspond to the whole assay: hydrolysis, liquid/solid extraction, and automated determination of the vitamins.

Maximum Capacity of Cartridges

The amounts of retinol and α-tocopherol pipetted onto the columns are usually about 30 IU and 0.1 mg, respectively, for feedstuffs. We have tried to layer amounts of 3000 IU retinol and 10 mg α-tocopherol (2 assays each). The obtained recovery was 100.3% for retinol and 98.5% for α-tocopherol.

Conclusions

It has been shown that use of an alcohol mixture of methanol–ethanol–n-butanol (4 + 3 + 1) in the alkaline hydrolysis solution allows the processing of fatty samples of feedstuffs. The proposed technique has been shown to be at least as quantitative and as accurate as traditional liquid/liquid partition techniques; it allows appreciably increased rates; and it offers the possibility of hydrolizing and extracting retinol and α-tocopherol together in the same sample.

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Comparison of Paired-Ion Liquid Chromatographic Method with AOAC Fluorometric and Microbiological Methods for Riboflavin Determination in Selected Foods

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A paired-ion liquid chromatographic (LC) technique coupled with fluorometric detection to determine riboflavin in various food matrices is described. Chromatograms of many foods showed 2 peaks of interest due to presence of riboflavin and flavin mononucleotide (FMN). Relatively high levels of FMN were found in raw beef, corned beef, chicken liver, and canned mushrooms. When riboflavin and FMN contents were summed, LC values were comparable to those obtained by the AOAC standard procedures. The LC technique was sensitive, rapid, and simple, yielding a mean standard deviation of 3.1% which was comparable to the AOAC fluorometric method (3.0%) and better than the AOAC microbiological assay (9.6%). Mean spike recoveries were 91.8% for LC compared to 90.5% and 89.6% for the AOAC fluorometric and microbiological methods, respectively.

Riboflavin can be analyzed by various microbiological, biological, colorimetric, polarographic, and fluorometric methods (1). It is routinely determined in foods, using the AOAC wet chemical fluorometric method (2).

Liquid chromatography (LC) has been used to separate and differentiate closely related nonvolatile compounds such as the B vitamins. Liquid chromatography with fluorescence detection has been reported to offer the advantages of speed, accuracy, and sensitivity and has been used in the assay of riboflavin in cereal and cereal products (3, 4), meat and meat products (5), and various other foods (6–8). LC results have been found to agree well with those obtained by microbiological assay (6, 9). However, lower values for the LC method compared to the AOAC method in some foods have also been reported (1, 7).

We have developed a more rapid and sensitive LC method for analysis of riboflavin. In our preliminary work with LC, we encountered a fluorescing peak aside from the riboflavin peak. The reported agreement between LC and AOAC methods (1, 7) and the result of our preliminary experiment led us to pursue further studies to compare the fluorometric LC and wet chemical AOAC methods, to characterize the fluorescing compound as to its similarity to riboflavin, and to quantify riboflavin in some foods by microbiological assay.

Experimental

Apparatus

The liquid chromatographic system consisted of:

(a) *Pump.*—Beckman Model 110 A pump (Beckman Instruments Inc., Fullerton, CA 92634).

(b) *Autosampler.*—WISP 710B (Waters Associates Inc., Milford, MA 01757).

(c) *Detector.*—Varian spectrofluorometer, SF-330 with filters to provide 440 nm excitation and 565 nm emission wavelengths (Varian Associates Inc., Palo Alto, CA 94303). Sensitivity 100×; selector, 10×.

(d) *Column.*—10 μm Hibar Lichrosorb, RP-8, 4.0 × 250 mm (E. Merck, Darmstadt, FRG), or Brownlee Labs 10 μm, MPLC RP-8, 4.0 × 250 mm (Brownlee Labs, Santa Clara, CA 95050). Operating conditions: column temperature, ambient; mobile phase, 1.0 mL/min.

(e) *Recorder.*—Fisher Recordall Series 500 (Houston Instruments, Austin, TX 78753); 1 V; chart speed, 0.25 or 0.5 cm/min.

Reagents

(a) *LC elution solvent.*—0.005M 1-hexane sulfonic acid and methanol (60 + 40). A vial of Pic B-6 (Waters Associates) and 400 mL glass-distilled methanol (Caledon Laboratories, Georgetown, Ontario, Canada) were diluted to 1 L with water, filtered through 0.45-μm filter, and degassed by sonification prior to use.

(b) *Riboflavin stock solution.*—100 μg/mL in 0.02M acetic acid, prepared from dry USP grade standard (Hoffmann-La Roche Ltd, Vaudreuil, Quebec), stored under toluene at refrigerator temperature (4°C). Dilute standards were prepared fresh daily from stock solution. Stock and dilute standards were protected from light.

(c) *Flavin mononucleotide stock solution.*—100 μg/mL in methanol, prepared from grade 1 sodium salt standard (Sigma Chemical Co., St. Louis, MO 63178), stored at refrigerator temperature (4°C). Dilute standards were prepared fresh daily from stock solution. Stock and dilute standards were protected from light.

(d) *Lactobacillus casei.*—ATCC 7469 (DIFCO Laboratories, Detroit, MI 48232).

Samples

The samples used represent raw and processed foods. They were flour, bread, raw ground beef, corned beef, raw chicken liver, cooked chicken liver, raw mushrooms, canned mushrooms (including liquid), instant skim milk, raw whole milk, UHT 2% milk, dry whole milk, chocolate milk, cheese, and AACC cereal check sample. Bread and cooked liver were prepared from the flour and raw liver used in this study. Raw whole milk was provided by Donlands Dairy, Toronto, Ontario. The rest of the samples were purchased from commercial stores.

Sample Preparation

Samples were prepared according to the AOAC official method, sec. **43.041** (2).

To an accurately weighed sample containing about 10 μg riboflavin, ca 75 mL 0.1M HCl was added and autoclaved 30 min at 121°C. Solution was cooled and adjusted to pH 6 with constant stirring using 1M NaOH. Immediately, 1M HCl was added to bring pH to 4.5. Solution was diluted to 100 mL with water and filtered through Whatman No. 42 ashless filter paper. For the LC assay, the sample extracts were clarified by passing them through 0.45 μm Millipore filter. Extracts from high-fat samples were extracted with hexane prior to clarification of the aqueous layer. Each sample was analyzed 3 or 4 times. Spike recovery studies were done singly on 14 samples.

All operations involving sample preparation and subsequent LC, AOAC fluorometric, and microbiological assays were performed under subdued light.

LC Determination

Three 50 μL portions of clarified extract were injected per sample, and standard was injected after every 5 samples.

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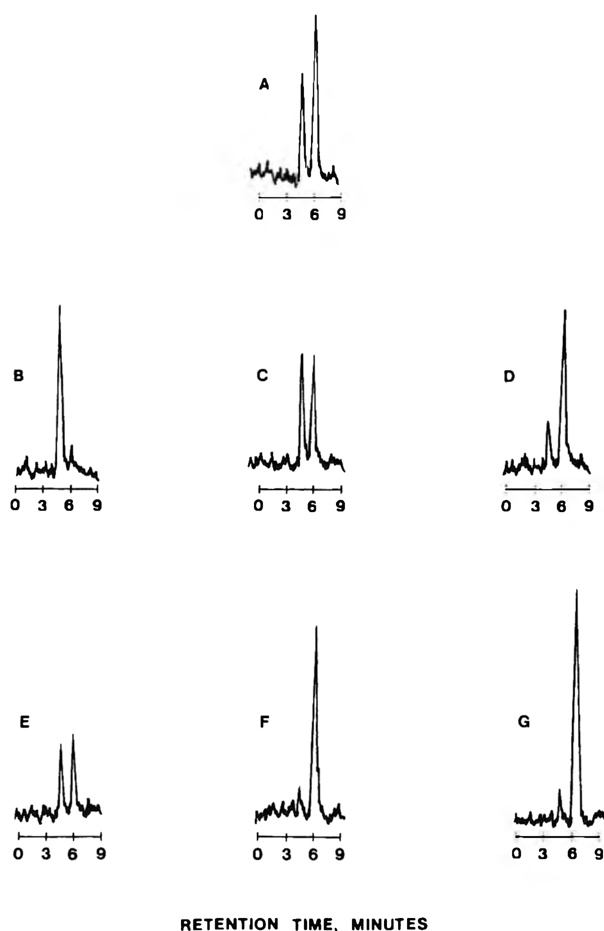

Figure 1. Chromatograms of standards (A), raw chicken liver (B), cooked chicken liver (C), fresh mushrooms (D), canned mushrooms (E), dry whole milk (F), and cheese (G).

Quantitation was performed by manual measurement of peak height.

AOAC Fluorometric Method

The filtrate was assayed as described in AOAC official method, secs **43.039-43.042** (2). Fluorescence was measured using a Varian spectrofluorometer at 440 nm excitation and 565 nm emission.

Microbiological Assay

Biological activity was estimated according to the *Methods of Vitamin Assay* (10) and the AOAC secs **43.168-43.176** (2) using the microorganism *Lactobacillus casei* (ATCC no. 7469). The following sample filtrates were chosen for microbiological assay: flour, raw and cooked chicken livers, corned beef, fresh and canned mushrooms, chocolate milk, and AACC cereal check sample.

Results and Discussion

The 2 types of RP-8 analytical columns used in this study gave equally good separation. Long-term use decreased column efficiency, but resolution was improved to some degree by cleaning the column with successive washings of water, methanol, chloroform, and methanol.

Detector response for riboflavin standard concentrations of 2.5–15 ng was linear and passed through the origin. Slope (m), 1.361, was obtained by correlation $y = mx + b$, and calculated correlation value (R) was 0.99999. Detection limit for riboflavin was 1.0 ng, similar to that found by Fellman et al. (8) using 360 nm excitation and 425 nm emission, and

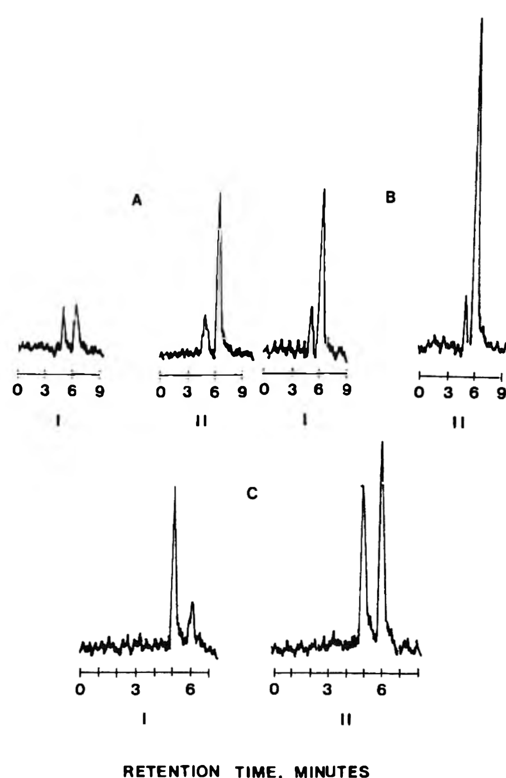

Figure 2. Chromatograms of canned mushrooms (A), chocolate milk (B), and corned beef (C) before (I) and after (II) spiking with riboflavin.

levels as low as 0.02 mg/100 g sample can be measured accurately. This sensitivity confirmed the suitability of the detection method for analysis of foods containing microgram quantities of riboflavin.

Figure 1 shows typical chromatograms of riboflavin in various foods obtained using the described LC conditions. A component whose amount varied from one sample type to another eluted just before riboflavin. Riboflavin had a retention time of 6 min and was well separated from the first peak (4.8 min retention time). The first peak and the riboflavin peak will be referred to as peaks A and B, respectively. Riboflavin values found by LC analysis were lower than those obtained by the manual fluorometric assay for most products.

Table 1. Recoveries of riboflavin standard added to samples before autoclaving

Sample	Added riboflavin, µg	Recovery, %: HPLC[a]	Standard procedures: Fluorometric	Standard procedures: Microbiological
Flour	5.24	93.3	90.1	70.4
Bread	5.24	96.6	89.9	
Ground raw beef	10.48	94.6	90.3	
Corned beef	10.48	96.7	86.4	91.1
Raw chicken liver	5.24	98.5	88.0	98.1
Cooked chicken liver	5.24	94.3	83.2	74.2
Fresh mushrooms	10.48	89.0	93.2	86.6
Canned mushrooms	7.86	87.5	90.4	89.4
Raw whole milk	10.48	89.9	84.2	
UHT 2% milk	10.48	90.4	98.0	
Dry whole milk	7.86	91.1	89.3	
Chocolate milk	10.48	86.2	98.7	99.2
Cheese	2.62	88.9	114.9	
AACC cereal check	10.48	87.5	70.0	107.7
Mean ± SD, %		91.8 ± 7	90.5 ± 25	89.6 ± 13

[a] Calculation based on the riboflavin peak only.

Table 2. Comparison of LC and AOAC standard methods

Sample	mg Riboflavin/100 g sample[a]			
	LC		AOAC	
	Riboflavin	FMN + riboflavin	Fluorometric	Microbiological
Flour	0.21 ± 0.00	0.26 ± 0.00	0.24 ± 0.01	0.23 ± 0.02
Bread	0.19 ± 0.02	0.27 ± 0.02	0.25 ± 0.01	
Ground raw beef	0.03 ± 0.00	0.13 ± 0.01	0.12 ± 0.00	
Corned beef	0.04 ± 0.00	0.17 ± 0.02	0.17 ± 0.01	0.20 ± 0.02
Raw chicken liver	0.27 ± 0.02	2.17 ± 0.03	2.06 ± 0.04	1.95 ± 0.19
Cooked chicken liver	0.92 ± 0.12	2.38 ± 0.04	2.23 ± 0.07	2.31 ± 0.18
Fresh mushrooms	0.28 ± 0.01	0.36 ± 0.01	0.40 ± 0.01	0.36 ± 0.04
Canned mushrooms	0.07 ± 0.00	0.16 ± 0.01	0.15 ± 0.01	0.15 ± 0.02
Instant skim milk	1.38 ± 0.01	1.50 ± 0.02	1.55 ± 0.06	
Raw whole milk	0.13 ± 0.00	0.16 ± 0.01	0.16 ± 0.00	
UHT 2% milk	0.12 ± 0.00	0.14 ± 0.00	0.14 ± 0.00	
Dry whole milk	1.14 ± 0.00	1.30 ± 0.01	1.29 ± 0.02	
Chocolate milk	1.09 ± 0.02	1.36 ± 0.04	1.40 ± 0.08	1.32 ± 0.08
Cheese	0.30 ± 0.00	0.33 ± 0.00	0.30 ± 0.01	
AACC cereal check[b]	1.61 ± 0.06	1.71 ± 0.05	1.93 ± 0.05	1.79 ± 0.18
Mean CV, %	2.7	3.5	3.0	9.6

[a] Mean ± standard deviation, $n = 4$ or 3.
[b] Theoretical value is 1.79 ± 0.20.

These findings are consistent with those in fish and bread samples (7) and in eggs and dairy products (1) using fluorometric and ultraviolet (UV) detection, respectively.

The position of riboflavin in the chromatograms was confirmed through spiked sample recovery (Figure 2). The LC recoveries of added spikes ranged from 86.2 to 98.5% (Table 1) with a mean of 91.8%, which was slightly higher than the conventional method, 90.5%.

In the AOAC official method, the sample filtrate is treated with $KMnO_4$ to remove fluorescing interferences, then H_2O_2 is added to reduce the excess permanganate prior to fluorescence measurement. A blank is carried out by adding sodium dithionite, which destroys riboflavin.

The canned mushroom and raw liver samples which contained significant amounts of peak A, gave low fluorometric blank readings. Successive treatment with permanganate and peroxide had no effect on peaks A and B in cooked liver extract. On the basis of retention time, peak A is neither lumichrome nor lumiflavin, 2 known degradation products of riboflavin. Photodegradation and conditions used in sample preparation such as autoclaving and pH adjustment failed to produce peak A from the standard. UV irradiation of alkaline cooked liver extract produced lumiflavin from both compounds A and B. Excitation spectra of peaks A and B were determined at wavelengths at 420–520 nm, with emission wavelength fixed at 565 nm. Both peaks had maximum excitation wavelengths at 475–480 nm. Further tests showed that peak A has the same retention time as flavin mononucleotide (FMN). FMN, a phosphorylated riboflavin, occurs in biological organs and tissues (11). When cooked liver extract was spiked with FMN, peak A and FMN produced a single peak (Figure 3).

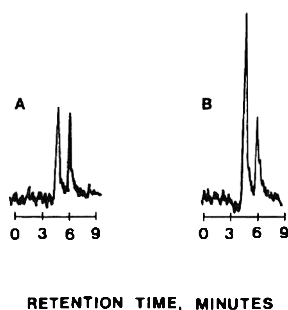

Figure 3. Cooked chicken liver before (A) and after (B) spiking with FMN.

Interestingly, the ratio of the 2 peaks depended on how the sample was processed. When the high-FMN-containing chicken liver sample was cooked at low heat, its riboflavin content increased while its FMN content decreased. Heating might have activated some phosphatase type of enzymes in raw liver, cleaving the phosphate group from FMN, thus forming more riboflavin.

When the data resulting from microbiological assays of selected samples (Table 2) were statistically analyzed using *t*-test, they were not significantly different from those obtained by conventional methods or by LC (sum of riboflavin and FMN). This finding verifies a previous report showing positive agreement between the microbiological and wet chemical methods (8). More importantly, this suggests that the secondary fluorescing compound has biological activity similar to that of riboflavin. A higher relative standard deviation (9.6%) was obtained with the microbiological method than with the 2 other methods, with a mean spike recovery of 89.6%. Also, the microbiological assay was time-consuming, requiring at least 24 h after sample preparation before any result could be obtained.

For samples containing minor amounts of FMN (<25% of the sum of FMN and riboflavin), FMN and riboflavin peak heights were summed and calculated as total riboflavin without significant error. However, for samples with significant amounts of FMN—such as raw beef, corned beef, fresh liver, cooked liver, and canned mushrooms—FMN and riboflavin contents were calculated separately using their corresponding KF factors, then were summed and reported as total riboflavin. The KF factor is the ratio of concentration of standard to peak height. FMN had a higher KF factor than riboflavin.

Conclusion

Our findings confirm that riboflavin values measured by LC can be lower in samples containing a high amount of FMN. Summation of riboflavin and FMN gives a good correlation with standard procedures. The LC analytical procedure is sensitive, rapid, simple, and accurate enough that we recommend undertaking a collaborative study.

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Liquid Chromatographic Determination of Vitamin D in Fortified Milk

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A method for the determination of vitamins D_2 + D_3 in fortified milk is described. Vitamins D_2 and D_3 are extracted from the saponified sample and converted to isotachysterols with antimony trichloride. The isotachysterols are quantitated using liquid chromatography with ultraviolet detection at 301 nm, which is the absorption maximum. At this wavelength other materials present in the sample do not interfere with the analysis of isotachysterols and therefore a cleanup step is avoided. Recoveries of vitamin D added to skim milk were 98.1% (SD 5.3), 96.7% (SD 3.3), and 96.0% (SD 5.1) for samples fortified with 200, 400, and 600 IU/quart, respectively. For whole milk, recoveries were 102.0% (SD 6.5) and 97.1% (SD 3.5) in samples fortified with vitamin D equivalent to 200 and 400 IU/quart, respectively. The detection limit for vitamin D is 40 IU/quart.

Vitamin D_2 (ergocalciferol) or vitamin D_3 (cholecalciferol) is added to fortify milk at 400 IU/quart (10 μg/quart) (1). The main problem encountered in quantitation of vitamin D in milk is the presence of excessive amounts of fats, proteins, and sterols including cholesterol. Some of these compounds have similar chemical and physical properties and interfere with the analysis (2). In addition, vitamin D is sensitive to heat and light and is subject to oxidation, thus lengthy extraction and cleanup procedures may result in lower recoveries (3).

Liquid chromatography (LC) has been successfully applied for the determination of vitamin D in fortified milk (3–11). All LC methods require saponification of the milk to hydrolyze fats, followed by extraction to collect all fat-soluble material. A further cleanup step is needed to separate other interfering materials from vitamin D before it is quantitated by LC.

This report describes an LC method for the determination of vitamin D in milk that does not require a cleanup step.

METHOD

Reagents and Standards

(a) *Solvents.*—LC grade hexane, methanol, and ethyl acetate and analytical grade chloroform (Fisher Scientific Co., Springfield, NJ 07081).

(b) *Aqueous KOH solution.*—Dissolve 400 g reagent grade KOH in water, cool, and dilute to 500 mL with water.

(c) *Alcoholic KOH solution.*—Dissolve 15 g reagent grade KOH in 400 mL water and dilute to 500 mL with ethanol.

(d) *Ethanolic pyrogallol solution.*—Dissolve 1.0 g pyrogallol in 100 mL ethanol.

(e) *Antimony trichloride solution.*—Dissolve 2.0 g antimony trichloride (Aldrich Chemical Co., Milwaukee, WI 53201) in 10 mL chloroform, or use prepared antimony trichloride in chloroform (Carr-Price reagent from Sigma Chemical Co., St. Louis, MO 63178).

(f) *Tartaric acid solution.*—Dissolve 4.0 g tartaric acid (Sigma Chemical Co.) in 10 mL water.

(g) *Mobile phase for LC.*—Hexane–ethyl acetate–methanol (97 + 2.5 + 0.05).

(h) *Vitamin D standard solutions.*—*(1) Stock solutions:* Separately dissolve 100 mg each of crystalline D_2 (ergocalciferol) and D_3 (cholecalciferol) (both from Sigma Chemical Co.) and dilute to 200 mL with methanol. *(2) Intermediate standard solutions:* Separately dilute 5 mL of each stock solution to 100 mL with methanol. *(3) Working standard solutions:* Dilute 1 mL of each intermediate standard to 50 mL with methanol.

Apparatus

(a) *Centrifuge tubes.*—25 mL conical centrifuge tubes with glass stoppers, used for isomerization.

(b) *Vortex mixer.*—Bronwill vortex mixer, or equivalent.

(c) *Liquid chromatography apparatus.*—Laboratory Data Control Constametric III pump equipped with Rheodyne 7105 syringe injection valve with 20 μL sample loop, Spectromonitor III variable wavelength detector (LDC, Riviera Beach, FL 33404), and Hewlett-Packard 3390A integrator (Hewlett-Packard, Palo Alto, CA 94304).

(d) *Chromatographic column.*—Spherisorb normal phase, 3 μm, in stainless steel column, 25 cm × 2 mm id (Phase Separation Inc., Norwalk, CT 06854).

Saponification and Extraction

The procedure described by Wickroski and McLean (10) was followed: Place 25 mL milk sample in 250 mL low-actinic glass Erlenmeyer flask. Add 50 mL ethanolic pyro-

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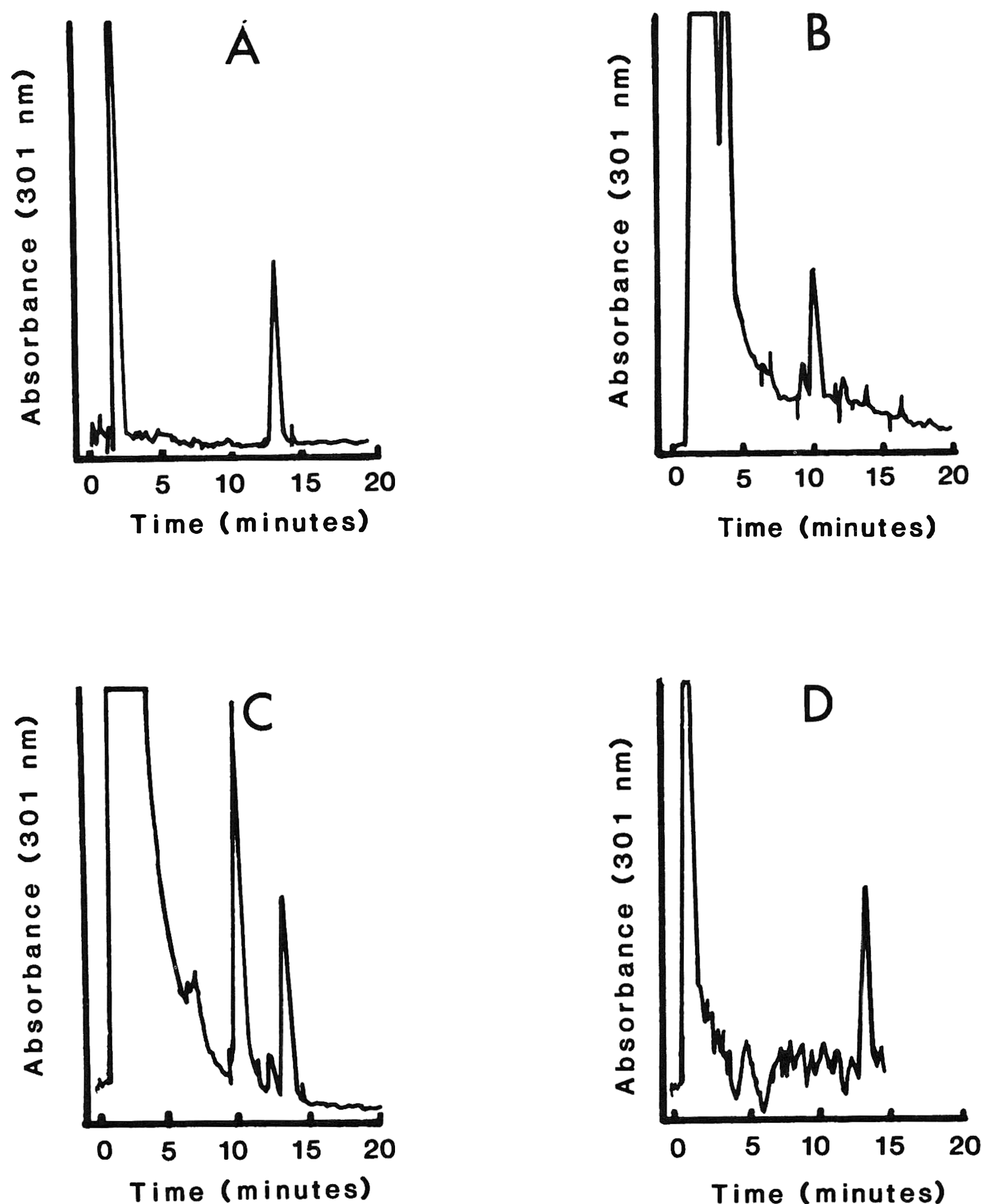

Figure 1. Chromatograms of A, standard isotachysterol D_3; B, unfortified reconstituted partly skim milk; C, reconstituted partly skim milk fortified with 400 IU/quart of vitamin D_3; D, standard isotachysterol D_3 equivalent to 0.03 IU.

gallol solution and 15 mL aqueous KOH solution. Place small magnetic stirring bar in flask and saponify mixture overnight at room temperature with slow constant stirring. Transfer saponified mixture to 500 mL separatory funnel. Rinse flask with two 40 mL portions of water, 20 mL ethanol, and 50 mL hexane, separately. Add all these washings to separatory funnel. Shake funnel vigorously to extract vitamins into hexane layer. Remove aqueous layer and extract 3 times with 50 mL of hexane. Combine hexane extracts and wash 3 times with 60 mL alcoholic KOH solution. Wash combined hexane extract with 50 mL portions of water until there is no color to phenolphthalein in aqueous layer. Remove water from hexane extract with anhydrous sodium sulfate. Concentrate extract to ca 5 mL on rotatory evaporator, keeping bath temperature below 40°C. Transfer concentrated extract to 25 mL centrifuge tube and evaporate to dryness by using warm water bath and slow stream of nitrogen. Dissolve extract in 0.5 mL chloroform.

Isomerization

The method followed by DeLeenheer and Cruyl was adopted (12) for both standards and samples. For standards, isomerization was carried out using levels of vitamin D_2 and D_3 ranging from 5 to 50 IU (0.125 to 1.25 μg) dissolved in 0.5 mL chloroform. Add 2 mL antimony trichloride solution in chloroform to centrifuge tube containing standards or samples, shake well on vortex-type mixer for 15–20 s, and leave at room temperature 30 s, shaking 2 or 3 times during interval. Immediately add 3 mL tartaric acid solution and shake well 15–20 s on vortex mixer. Add 2 mL water and shake again on vortex mixer 15–20 s. Remove aqueous phase by using pipet and filter chloroform layer through small column filled with anhydrous sodium sulfate. (Disposable pipet can be used for this purpose.) Evaporate chloroform extract under slow stream of nitrogen and dissolve the sample in 500 μL hexane.

Table 1. Recoveries of vitamin D_3 added to reconstituted, partly skimmed milk at 200, 400, and 600 IU/quart

	200 IU/quart		400 IU/quart		600 IU/quart	
Test	IU found	Rec., %	IU found	Rec., %	IU found	Rec., %
1	195	97.5	363	90.7	568	94.7
2	186	93.0	400	100.0	576	96.0
3	181	90.5	407	101.7	551	91.8
4	205	102.5	393	98.2	592	98.6
5	197	98.5	378	94.5	632	105.3
6	211	105.5	393	98.2	608	101.3
7	186	93.0	385	96.2	538	89.7
8	209	104.5	378	94.5	545	90.8
Av.	196.2	98.1	387.1	96.7	576.2	96.0
SD	10.6	5.3	13.1	3.3	30.6	5.1

Liquid Chromatography

Use the following operating conditions: ambient temperature; mobile phase, hexane–ethyl acetate–methanol (97 + 2.5 + 0.05); flow rate, 0.7 mL/min; detector, 301 nm; sensitivity, 0.01 AUFS. Quantitate by measuring peak heights. Retention time of isotachysterol should be between 12 and 16 min.

Results and Discussion

Vitamin D in milk was quantitated by estimation of isotachysterol D_2 or D_3 which is obtained by isomerization of vitamin D_2 and D_3. Isotachysterol(s) formed from D_2 and D_3 had the same retention time on LC analysis. This was verified by carrying out the isomerization reaction on vitamin D_2 and D_3 separately as well as on a mixture of equal amounts of D_2 and D_3. Therefore, recovery studies were carried out using only vitamin D_3.

Figure 1A shows a chromatogram of standard isotachysterol D_3 obtained after isomerization. Linearity of the isomerization step was confirmed by carrying out the reaction on the standard vitamin D_3 in concentrations ranging from 5 to 50 IU (0.125–1.25 μg). The yields for isomerization were above 90%. Linearity for the isomerization step in the range of 0 to 250 μg also has been established by DeLeenheer and Cruyl (12), who reported an efficiency of 93%. In our experiments, it was necessary to carry out isomerization at concentrations ranging from 5 to 20 IU, because the sample size used was 25 mL milk which should contain 10 IU vitamin D if fortified with 400 IU/quart.

Figure 1B is a chromatogram of an unfortified sample of reconstituted partly skimmed milk showing the absence of interfering peaks. A 25 mL portion of reconstituted unfortified partly skimmed milk (fat content 0.8%) was fortified before saponification with varying amounts of vitamin D_3, ranging from 200 to 600 IU/quart. Figure 1C shows a chromatogram of partly skimmed milk fortified with 400 IU/quart of vitamin D_3 and shows a well-resolved isotachysterol D_3 peak. In samples fortified with 200 IU/quart, recoveries ranged from 91 to 106% (average 98%, standard deviation 5.3) (Table 1). Recoveries ranged from 91 to 102% (average 97%, standard deviation 3.3) in samples fortified with 400 IU/quart and 90 to 105% (average 96%, standard deviation 5.1) in samples fortified with 600 IU/quart.

Table 2. Recoveries of vitamin D_3 added to unfortified homogenized milk at 200 and 400 IU/quart[a]

	200 IU/quart		400 IU/quart	
Test	IU found	Rec., %	IU found	Rec., %
1	262	104.8	428	95.1
2	226	90.4	450	100.0
3	258	103.2	466	103.5
4	269	107.6	452	100.4
5	232	92.8	423	94.0
6	253	101.2	431	95.8
7	274	109.6	426	94.7
8	266	106.4	419	93.1
Av.	255	102.0	436.9	97.1
SD	16.2	6.5	15.8	3.5

[a] 50 IU/quart which was found in the unfortified sample was taken into account.

Table 3. Recovery of vitamin D_3 from commercially fortified whole milk

Test	Label claim, IU/quart	Found, IU/quart
1	400	484
2	400	469
3	400	443
4	400	483
5	400	484
6	400	498
Av.		477
SD		17.3

A like experiment was carried out with unfortified homogenized milk. This milk, however, showed the presence of a small amount of vitamin D which accounted for about 50 IU/quart. This value was taken into account for calculation of recoveries after fortification with standard vitamin D_3. This milk was fortified with 200 and 400 IU/quart of vitamin D_3 and recoveries are shown in Table 2. The chromatogram of the whole milk sample was similar to the chromatogram of skim milk (Figure 1C). Recoveries ranged from 90 to 110% (average 102%, standard deviation 6.5) in samples fortified with 200 IU/quart and 93 to 104% (average 97%, standard deviation 3.5) in samples fortified with 400 IU/quart.

A commercially available fortified whole milk guaranteed to contain 400 IU vitamin D per quart was analyzed using the proposed method. The results are given in Table 3. Recovery ranged from 443 to 498 IU/quart with an average of 477 IU/quart and standard deviation of 17.3.

The present procedure completely eliminates the cleanup step required in all LC methods (3–11). Figure 1D is a chromatogram of standard isotachysterol D_3 equivalent to 0.03 IU which is the lower detection limit for pure standard. In milk samples, as little as 40 IU/quart can be detected and quantitated.

The aim of this work was the development of a method suitable for the quantitative determination of vitamin D in fortified milk and similar products, without a cleanup step. A major problem in using the present official method (9) is the conversion of vitamin D to previtamin D during saponification. Since quantitation of previtamin D is not possible due to other interferences, a correction factor of 1.25 is applied to quantitate vitamin D (9). In the proposed method, saponification is carried out at room temperature which avoids formation of previtamin D.

All LC methods reported to date require a cleanup step after extraction. In the AOAC official method, two LC systems are required, one for sample cleanup and a second for analysis.

It has been reported that vitamin D is also sensitive to evaporation (13). In our method, only one evaporation step is needed before vitamin D is converted to isotachysterol D, and isotachysterol is stable to heat, light, and evaporation (12) and can be handled easily.

Isomerization of vitamin D_2 and D_3 by treatment with antimony trichloride has several advantages. The extinction of isotachysterol is more than double compared to vitamin D, and therefore sensitivity is increased. The wavelength maximums for isotachysterol(s) are 278, 288, and 301 nm which allows the detection of isotachysterol at 301 nm (13). At this wavelength, interferences from compounds present in the sample matrix are completely eliminated. The only disadvantage of the proposed method is that vitamins D_2 and D_3 cannot be determined separately.

Acknowledgment

The author thanks L. McLean for technical assistance.

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Letters in Support of AOAC Fellows Awards February 15, 1988

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PESTICIDE FORMULATIONS

Liquid Chromatographic Method for Determination of Anilazine in Formulated Products: Collaborative Study

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A liquid chromatographic method for the determination of anilazine (DYRENE®) in formulated products has been developed and subjected to a collaborative study involving 15 participating laboratories. Each collaborator was furnished with reference standard, internal standard, and blind duplicate samples of DYRENE 80% concentrate, 75% wettable powder (75 WP), 50 WP, and 4 flowable formulation. Samples are dissolved in acetonitrile and analyzed by reverse phase chromatography using octanophenone as an internal standard. Collaborators were instructed to evaluate the method by either peak area or peak height measurements. Seven laboratories performed the analyses using peak areas; 8 laboratories used peak heights. Peak area measurements for the wettable powder samples resulted in statistically high bias vs peak height measurements. This was apparently due to inconsistent integration parameters among collaborators. Difficulties in establishing the correct integration parameters are illustrated along with t-test values for the 2 measurement techniques. Coefficients of variation of the peak height values obtained on the 80 concentrate, 75 WP, 50 WP, and 4 flowable were 1.68, 1.29, 1.74, 1.87%, respectively. The method has been adopted official first action.

Anilazine, 4,6-dichloro-*N*-(2-chlorophenyl)-1,3,5-triazin-2-amine, is commercially available as DYRENE® in several formulations including an 80% concentrate, 75% wettable powder (75 WP), 50 WP, and a 4 flowable formulation. Anilazine is a widely used fungicide which is highly effective against a variety of plant diseases that attack agricultural crops, ornamental plants, and turfgrasses. Anilazine has been evaluated by potentiometric titration (1), colorimetry (2, 3), gas chromatography (4–7), and liquid chromatography (LC) (8, 9).

Titrimetric results are subject to significant interferences including the major impurity 6-chloro-*N,N'*-bis(2-chlorophenyl)-1,3,5-triazine-2,4-diamine (bis-compound). The structure of this compound and anilazine are shown in Figure 1. As expected, colorimetric determinations are subject to a variety of interferences. Gas chromatographic (GC) methods have not been entirely satisfactory; quantitation difficulties in GC methods have been attributed to adsorptions on packing materials (5, 6). This premise appears quite reasonable considering the low volatility of anilazine and several of its impurities, for example, the bis-compound is considerably less volatile than is anilazine. Methanol was used in one LC method for anilazine (8), but anilazine is known to react with methanol quite readily to form the dimethoxy analog (10).

The present report describes a collaborative study of a reverse phase LC method with UV detection at 250 nm and octanophenone as internal standard. The method separates all known impurities in the product.

Collaborative Study

The LC method was submitted to study by 15 participating laboratories. Collaborators were furnished blind duplicate subsamples of the formulations, a reference standard, the internal standard, and the method. Collaborators were instructed to refrigerate all materials until sufficient time was available to perform the study. The anilazine standard provided included about 1% of the bis-compound as a reference. Collaborators were instructed that this compound must be resolved from the anilazine and internal standard peaks (Figure 2). Presence of the bis-compound allowed the analyst to determine whether resolution was adequate and provided an indication of the overall quality of each laboratory's LC system. The study was designed according to suggestions given by Youden and Steiner (11).

Anilazine in Pesticide Formulations
Liquid Chromatographic Method
First Action
AOAC-CIPAC Method

(Method is suitable for formulations with anilazine as only active ingredient.)

Principle

Anilazine is detd by liq. chromatgy using octanophenone as internal std. Adequate resolution is controlled by monitoring sepn of bis-compound (major impurity of anilazine) from anilazine and internal std peaks.

Apparatus

(a) *Liquid chromatograph.*—Able to generate >7 MPa (>1000 psi) and measure *A* at 250 nm. Operating conditions: column temp. ambient; mobile phase flow rate ca 1.7 mL/min (ca 800 psi); chart speed 0.5 cm/min; injection vol. 20 μL; *A* range 0.32 AUFS. Retention times: anilazine ca 2.5 min, bis-compound ca 4.0 min, octanophenone ca 6.6 min. Pump LC mobile phase thru column until system is equilibrated (flat baseline). Allow 1 min after elution of internal std before next injection.

(b) *Chromatographic column.*—250 × 4.6 mm id packed with ≤10 μm C_{18} bonded silica gel capable of resolving bis-compound from anilazine and internal std peaks (Du Pont ODS, or equiv.).

(c) *Chart recorder.*—Min. 250 mm span, 10 mV range, 30 cm/h speed.

(d) *Bath.*—Ultrasonic.

(e) *Filters.*—0.45 μm porosity (Gelman Acrodisc-CR, or equiv.).

Reagents

(a) *Acetonitrile.*—LC grade or distd in glass (Burdick & Jackson Laboratories, Inc., or equiv.).

(b) *Octanophenone internal std soln.*—Dil. 4 mL octanophenone (Aldrich Chemical Co., Inc., or equiv.) to 250 mL with CH_3CN.

Submitted for publication June 2, 1987.
This report of the Associate Referee was presented at the 100th AOAC Annual International Meeting, Sept. 15–18, 1986, at Scottsdale, AZ.
The recommendation of the Associate Referee was approved interim official first action by the General Referee, the Committee on Pesticide Formulations and Disinfectants, and the Chairman of the Official Methods Board. The method was adopted official first action at the 101st AOAC Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. See the General Referee and Committee reports, *J. Assoc. Off. Anal. Chem.* (1988) **71**, January/February issue.

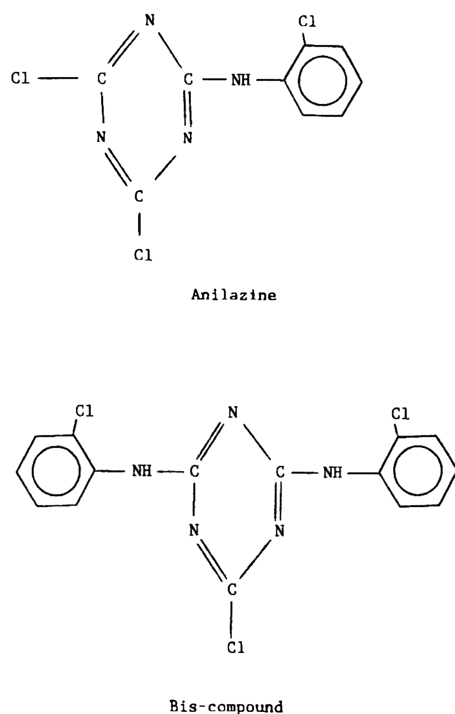

Figure 1. Structure of anilazine and its major impurity, 6-chloro-*N,N'*-bis(2-chlorophenyl)-1,3,5-triazine-2,4-diamine (bis-compound).

(c) *Water.*—LC grade or distd in glass (Burdick & Jackson Laboratories, Inc., or equiv.).

(d) *LC mobile phase.*—CH_3CN–H_2O (80 + 20).

(e) *Anilazine std soln.*—Accurately weigh ca 230 mg anilazine ref. std (Mobay Corp., Agricultural Chemicals Div., PO Box 4913, Hawthorne Rd., Kansas City, MO 64120-0013) into 100 mL vol. flask. Pipet 10 mL internal std soln into flask, dil. to vol. with CH_3CN, and mix well. Pipet 5 mL of this soln into 100 mL vol. flask, dil. to vol. with CH_3CN, and mix well. Filter portion of final soln for LC analysis.

Preparation of Sample

(a) *Formulations excluding flowable.*—Accurately weigh sample contg ca 230 mg anilazine into 100 mL vol. flask. Pipet 10 mL internal std soln into flask, dil. to vol. with CH_3CN, and sonicate 1 min. Mix well. Pipet 5 mL of this soln into 100 mL vol. flask, dil. to vol. with CH_3CN, and mix well. Filter portion of final soln for LC analysis.

(b) *Flowable.*—Accurately weigh sample contg ca 230 mg anilazine into 100 mL vol. flask. Add 5 mL LC grade or distd in glass H_2O and swirl until sample is thoroughly dispersed. Pipet 10 mL internal std soln into flask, dil. to vol. with CH_3CN, and sonicate 1 min. Mix well. Pipet 5 mL of this soln into 100 mL vol. flask, dil. to vol. with CH_3CN, and mix well. Filter portion of final soln for LC analysis.

Determination

Inject anilazine std soln and adjust operating parameters so that anilazine elutes in 2.5–3.0 min. Adjust injection vol. and attenuation to give largest possible on-scale peaks. Bis-compound must be resolved from anilazine and octanophenone peaks. If not, change or repack column.

Using same injection vol. for all sample and std injections, make repetitive injections of ref. std soln and calc. response ratios by dividing anilazine peak ht by internal std peak ht. Response ratios must agree ±1%. Average duplicate response ratios obtained with std solns.

Inject duplicate aliquots of each sample soln. Average duplicate response ratios for each sample soln. Response ratios must agree ±1%. If not, repeat detn, starting with std injections.

Re-inject ref. std soln twice. Average response ratios of stds immediately preceding and following sample injection. These must agree within ±1%. If not, repeat detn.

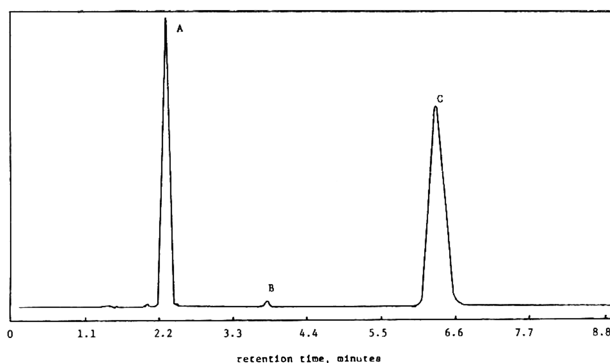

Figure 2. Typical LC chromatogram showing (A) anilazine, (B) bis-compound, and (C) octanophenone internal standard.

Calculation

$$\text{Anilazine, wt \%} = (R/R') \times (W'/W) \times P$$

where R and R' = av. response ratios for sample and std solns, resp., W' and W = wt (mg) of anilazine in std and sample solns, resp., and P = % purity of anilazine std.

CAS-101-05-3 (anilazine)

Results and Discussion

A complete set of results was received from each of the 15 collaborators. The collaborators used a variety of equipment to perform the analyses: 7 brands of pumps, 9 brands of detectors, 7 brands of columns, and 7 brands of injectors. Sample volumes injected varied from 5 to 30 μL. Pressures obtained covered a range of 3.5–19 MPa for flow rates of 1.0–1.9 mL/min.

Seven collaborators determined response ratios by using peak area measurements obtained from laboratory data systems; 8 collaborators used manually obtained peak height measurements (Tables 1 and 2).

The results on 4 flowable from collaborator 10 are outliers on the basis of the Dixon test of sums. Collaborator 15 is clearly an outlier by the Dixon outlier test. This collaborator's

Table 1. Collaborative results for determination of anilazine (%) in blind duplicate samples: measurements by peak area

Coll.	80 conc.		75 WP		50 WP		4 flowable	
1	78.1	78.9	71.5	71.6	49.9	49.6	38.2	38.3
2	79.1	80.9	73.6	73.9	50.4	50.9	39.4	39.0
3	78.0	78.9	70.4	70.7	49.3	49.4	37.0	37.7
4	79.4	76.8	71.0	70.7	51.3	49.3	38.1	38.6
5	78.5	78.2	69.7	70.5	49.5	48.6	38.6	36.8
6	78.9	78.8	71.7	71.7	49.8	49.8	38.8	38.9
7	79.4	80.0	72.0	71.4	49.8	50.0	38.4	38.3

Table 2. Collaborative results for determination of anilazine (%) in blind duplicate samples: measurements by peak height

Coll.	80 conc.		75 WP		50 WP		4 flowable	
8	76.7	76.2	69.6	69.6	48.1	48.2	37.3	37.0
9	79.1	79.1	71.6	71.8	49.8	49.7	38.3	38.2
10	81.1	80.4	70.2	72.5	50.3	50.4	41.3[a]	40.7[a]
11	78.7	77.8	71.1	70.9	50.0	50.4	39.3	39.0
12	79.0	78.5	70.7	70.7	48.7	48.3	37.9	37.8
13	78.2	77.9	70.6	69.6	49.2	48.8	38.6	38.6
14	78.3	78.2	69.6	70.6	49.2	48.8	38.6	38.6
15[b]	71.5	72.6	64.0	64.4	43.0	42.8	30.5	32.1

[a] Outlier by Dixon test on laboratory sums.
[b] All results for this collaborator are outliers on basis of Dixon outlier test.

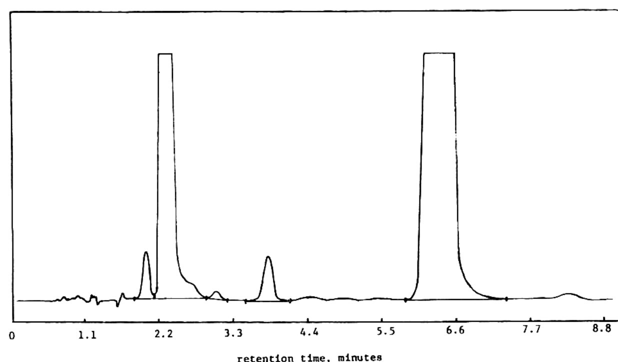

Figure 3. LC chromatogram of wettable powder formulation showing ideal baseline interpretations (0.4 mV/min slope sensitivity setting).

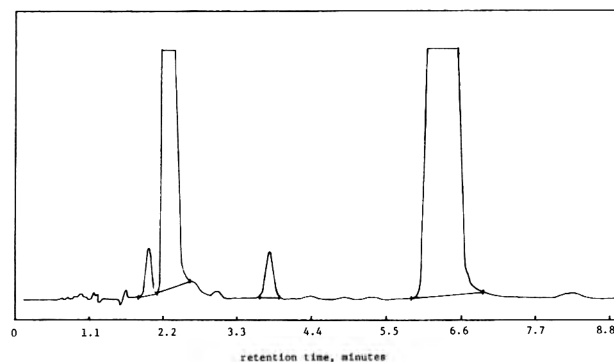

Figure 4. LC chromatogram of wettable powder formulation showing undesirable baselines obtained from implementing a 2.0 mV/min slope sensitivity setting.

results might be explained by a significant reference standard weighing error because all of the values for each formulation deviate from the average by approximately the same factor.

An examination of the statistics regarding peak area vs peak height measurements is shown in Table 3. Each laboratory performed either peak height or area percent calculations, so the *t*-test for independent samples must be used. Examination of the calculated *t*-test results relative to the standard table values shows that the analyses of the 80 concentrate and the 4 flowable are not significantly different whether analyzed by peak height or peak area. However, the 2 sets of data for the wettable powders are so significantly different (for a type 1 error of 0.10) as to not allow averaging of results.

Although this LC method is known to resolve all anilazine impurities, excipients in the wettable powder formulation produce extremely small absorbances that are resolved, but which reside on the tail of the anilazine peak and can easily create significant errors in area measurements if integration parameters are not carefully controlled. Visually, these absorbances are almost imperceptible; however, analog-to-digital processing prior to attenuation of the detector signal provides ample sensitivity to detect these components.

Table 3. Statistical treatment of collaborative data[a]

	Peak area	Peak height
80 conc.:	Mean = 78.85	Mean = 78.51
	S_x = 0.97	S_x = 1.32
	S_O = 0.92	S_O = 0.37
	S_L = 0.31	S_L = 1.27
	CV_x, % = 1.23	CV_x, % = 1.68
	t calcd = 0.79	
75 WP:	Mean = 71.46	Mean = 70.65
	S_x = 1.20	S_x = 0.91
	S_O = 0.30	S_O = 0.73
	S_L = 1.16	S_L = 0.55
	CV_x, % = 1.68	CV_x, % = 1.29
	t calcd = 2.01	
50 WP:	Mean = 49.83	Mean = 49.28
	S_x = 0.69	S_x = 0.86
	S_O = 0.61	S_O = 0.22
	S_L = 0.32	S_L = 0.83
	CV_x, % = 1.39	CV_x, % = 1.74
	t calcd = 1.87	
4 flow:	Mean = 38.29	Mean = 38.26
	S_x = 0.74	S_x = 0.72
	S_O = 0.55	S_O = 0.11
	S_L = 0.68	S_L = 0.71
	CV_x, % = 1.93	CV_x, % = 1.87
	t calcd = 0.11	

[a] t-table value for a Type I error of 0.10 with 26 degrees of freedom is 1.71.

Figure 3 shows a typical baseline of an anilazine wettable powder injection when integration parameters are carefully controlled. Although a component is evident at ca 2.8 min, it is resolved from the anilazine for peak height determinations, and its area is negligible. Figures 4 and 5 show erroneous interpretations of the baselines which can readily occur if integration parameters are not carefully controlled. In Figure 4, the slope sensitivity was set to a less sensitive setting. This condition will produce a low bias due to incomplete integration of the anilazine peak at about 2.5 min. In Figure 5, the slope sensitivity was set to a more sensitive setting. This results in a misinterpretation of baseline leading to a high bias from a very large error in integration of the anilazine peak. The high bias of the peak area measurements of the wettable powders in the collaborative study is apparently due to such an error. Nearly all peak height measurements were performed manually without the aid of a data system. This technique effectively eliminates the peak area measurement problems shown in Figures 4 and 5.

Unfortunately, each collaborator's area integration parameters are variables of the study which are not readily controlled. The inability to control these parameters (e.g., slope sensitivity, tangent skimming, baseline interpretations) and predict their effect on processing the digital raw data file probably is the largest error in most collaborative studies.

The difficulties involved in eliminating this situation are varied. Obviously, all collaborators will not have the same laboratory data systems available. This generates a problem in comprehending the operation of a wide selection of systems. In addition, there is the general lack of graphics display terminals and software among laboratories to display exactly

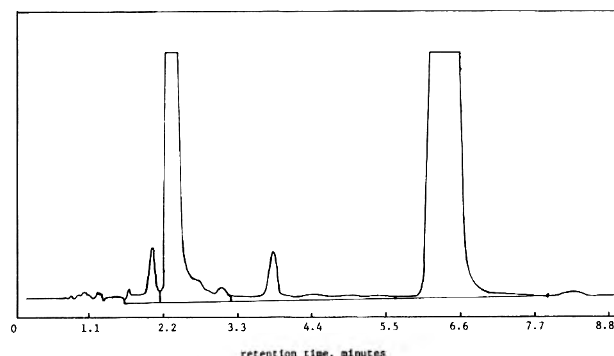

Figure 5. LC chromatogram of wettable powder formulation showing undesirable baselines obtained from implementing a 0.04 mV/min slope sensitivity setting.

how the data system interpreted and established baselines. These resources are crucial to establishment of similar integration parameters for all collaborators.

Recommendation

It is recommended that the LC method be adopted official first action as an AOAC-CIPAC method.

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Liquid Chromatographic Method for Determination of Cyhexatin in Technical and Formulated Products: Collaborative Study

STEPHEN LUPAN
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Collaborators: J. Basters; M. Buys; I. Dainis; D. Glawitsch; J. Hiemstra; P. Hitos; J. Kaiser; P. Kool; S. Lupan; A. Martijn; T. van der Molen; P. Pasma; P. Roque; A. de Reijke; C. Self; T. Stevens; R. Suter; W. Timmer

Cyhexatin technical (95%), 25 and 50% wettable powders, and a 600 g/L suspension concentrate were analyzed by liquid chromatography in a collaborative study of the method. Paired samples of cyhexatin technical and the 3 formulations were extracted with a mixture of methanol-water-acetic acid (950 + 49 + 1) containing *n*-decylbenzene as internal standard. Components were separated on a Lichrosorb RP-18 column using methanol-water-HCl-NaCl (93 + 7 + 0.001M + 0.005M) at 2.0 mL/min, and then detected at 214 nm. Results were received from 18 of the 20 laboratories who agreed to participate in the study; 14 sets of data were suitable for statistical evaluation. Repeatability coefficients of variation (CV_0 ranged from 0.46% for the 50% wettable powder to 1.14% for the suspension concentrate. Reproducibility coefficients of variation (CV_x) ranged from 0.70 to 1.85% for the 4 types of samples. The method has been adopted official first action as a CIPAC-AOAC method.

Cyhexatin (tricyclohexatin hydroxide) is an acaricide that is effective by contact against the motile stages of a wide range of phytophagus mites on deciduous fruits, vines, vegetables, and ornamentals. Cyhexatin is formulated as either a wettable powder (50 or 25% cyhexatin) or a 600 g/L suspension concentrate, and is marketed under the trade name Plictran (trademark of the Dow Chemical Co.).

The current method of analysis, nonaqueous titration, has given a number of problems particularly during the analysis of formulated product ("Analytical Methods of Dow Chemical Co." Plictran Tech., Ref. 87698; Plictran 25 W, Ref. 61912a). This together with the nonspecificity of the method propagated development of an alternative method.

Initial work using gas chromatography showed that derivatization to the chloride was possible but reproducibility was poor. Liquid chromatographic (LC) analysis also proved unsuccessful until ion-pairing reagents, e.g., methanesulfonic acid, were introduced together with reverse phase columns. Work finally culminated when it was found that cyhexatin

Submitted for publication June 10, 1987.
This report was presented by S. Lupan at the CIPAC Symposium, Sept. 3, 1985, at Copenhagen, Denmark.
The recommendation of S. Lupan and the General Referee, J. Launer, was approved interim official first action by the Committee on Pesticide Formulations and Disinfectants and the Chairman of the Official Methods Board. The method was adopted official first action at the 101st AOAC Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. See the General Referee and Committee reports, *J. Assoc. Off. Anal. Chem.* (1988) **71**, January/February issue.
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could be suitably chromatographed in the presence of chloride ions (HCl + NaCl). A collaborative study of the LC method is reported here.

Collaborative Study

Four pairs of samples (as closely matched pairs) containing cyhexatin (technical, 25 and 50% wettable powders, and a 600 g/L suspension concentrate) were sent to 20 laboratories. Collaborators were supplied with an internal standard (*n*-decylbenzene) and a procedure on how to perform the study.

The samples were to be analyzed singly (by duplicate injection) on 2 different days in a predetermined pattern. Each sequence of 2 sample injections was to be preceded and followed by a standard injection. The average calibration factor, determined from these 2 standards, was to be used to calculate the interdispersed sample solution. The calibration factor was to be recorded to 3 decimal places and the sample results to 2 decimals. Identity was taken to be confirmed if the retention times for cyhexatin samples relative to that for the internal standard agreed with the relative retention time for cyhexatin standard.

Cyhexatin Technical and in Pesticide Formulations

Liquid Chromatographic Method

First Action

CIPAC-AOAC Method

Principle

Sample is extd with *n*-decylbenzene internal std soln contg HOAc, MeOH, and H_2O. Cyhexatin is detd by liq. chromatgy using peak ht for quantitation.

Apparatus

(**a**) *Liquid chromatograph.*—With peak ht integrator or recorder, 10 μL sample loop, and detector at 214 nm. Operating conditions: column ambient; flow rate 2.0 mL/min; *A* range 1.0 AUFS; injection vol. 10 μL; retention times, cyhexatin ca 7 min and internal std ca 10 min. Adjust parameters to give peak ht for cyhexatin ca 75% full scale.

(**b**) *Chromatographic column.*—ODS bonded silica, 10 μm particle size, stainless steel, 25 cm × 4.6 mm id (E. Merck, available from Curtin Matheson Scientific, Inc., or VWR Scientific), or equiv.

Reagents

(**a**) *Methanol.*—LC grade.

(**b**) *Acetic acid.*—Glacial.

(**c**) *HCl soln.*—1M.

(**d**) *Sodium chloride.*—Analytical reagent grade.

(**e**) *Mobile phase.*—MeOH–H_2O–HCl–NaCl (93 + 7 + 0.001M + 0.005M). In 1 L g-s flask, dissolve 1 mL 1M HCl, 69 mL H_2O, and 0.29 g NaCl. Add 930 mL MeOH, mix, and degas.

(**f**) *n-Decylbenzene.*—Eastman Laboratory Chemicals No. 9195 (Eastman Kodak Co.), or equiv.

(**g**) *Cyhexatin reference std.*—Available from Dow Chemical Co.

Preparation of Standards

(**a**) *n-Decylbenzene internal std soln.*—Weigh 1.0 g *n*-decylbenzene into 1 L vol. flask. Add 49 mL H_2O and 1 mL HOAc. Dil. to vol. with MeOH and sonicate until dissolved.

(**b**) *Cyhexatin std soln.*—Accurately weigh ca 110 mg pure cyhexatin ref. std into 150 mL g-s flask. Add by pipet 100 mL internal std soln, shake well, sonicate for 10 min, and cool to ambient temp.

Preparation of Sample

(**a**) *Technical material.*—Accurately weigh ca 120 mg sample into 150 mL g-s flask. Add by pipet 100 mL internal std soln, shake well, and sonicate for 10 min. Cool to ambient temp. and centrifuge at 2000 rpm.

(**b**) *Wettable powder.*—Accurately weigh sample contg ca 110 mg cyhexatin into 150 mL g-s flask. Proceed as for *Technical material* beginning, "Add by pipet . . . ."

(**c**) *Suspension concentrate.*—Accurately weigh sample contg ca 120 mg cyhexatin into 150 mL g-s flask. Add 10 mL H_2O and swirl until completely homogeneous. Proceed as for *Technical material* beginning, "Add by pipet . . . ."

Determination

Inject 10 μL std solns until response ratio (cyhexatin peak ht/internal std peak ht) varies <2%. Make 2 sample injections followed by 1 std injection. Average peak ht ratios of stds immediately preceding and following sample injections, and average peak ht ratios of the 2 samples. Calc. cyhexatin as follows:

$$\text{Cyhexatin, \%} = (R/R') \times (W'/W) \times P$$

where R and R' = av. peak ht ratios for sample and std, resp.; W' = g cyhexatin in std soln; W = g sample extd for analysis; and P = % purity of std.

CAS-13121-70-5 (cyhexatin)

Results and Recommendation

Of the 20 laboratories who originally requested to participate in the study, 18 replied with data in the time allowed. Of these 18 responses, 4 laboratories had failed to follow the protocol for the study: collaborator 3 used peak area instead of peak height for quantitation. Collaborator 18, who also used peak area for quantitation, made only single injections to obtain the second set of values. Collaborator 5 deviated from the calibration procedure by making insufficient injections of standard. Collaborator 8 could not achieve adequate separation and submitted no data.

A number of calculation errors were detected when the data were studied. In these cases, the results were recalculated. The raw data, which includes recalculated values where necessary, are shown in Table 1 as the mean values for duplicate injections. Table 1 includes results of analyses by collaborators 3, 5, and 18, but those data were not included in the statistical evaluation. Visual examination of the remaining 14 sets of data did not show outliers that deviated so much from other entries that they were considered suitable for Dixon's test.

Results of the statistical evaluation, also shown in Table 1, include repeatability and reproducibility standard deviations, S_O and S_X, respectively, and the corresponding coefficients of variation, CV_O and CV_X. Values for CV_O ranged from 0.46 to 1.14% and for CV_X from 0.70 to 1.85% for the 8 samples.

In the evaluation of the method using ISO (International Organization for Standardization) protocols, repeatability and reproducibility were calculated. These values are related to CV_O and CV_X, respectively, as shown in the following equations:

$$r = (2 \times 2^{1/2} \times m) \times (CV_O/100)$$
$$R = (2 \times 2^{1/2} \times m) \times (CV_X/100)$$

where m = mean value.

Recommendations

The statistical evaluation of the collaborative results (Table 1) shows that method performance was satisfactory. It is recommended that the LC method for cyhexatin in technical and formulated products be adopted official first action as a CIPAC-AOAC method.

Acknowledgments

The author thanks all the collaborators for their cooperation in the completion of this study. Thanks are especially

Table 1. Results (%) for collaborative study of LC method for cyhexatin in technical and formulated products[a]

	Tech. 95%		Wettable powd. 25%		Wettable powd. 50%		Susp. conc. 60%	
Coll.[b]	1	1A	2	2A	3	3A	4	4A
1	94.09	95.51	25.21	25.03	51.04	51.24	50.51	54.10
	95.38	95.33	25.35	25.09	51.05	51.15	50.30	53.39
2	94.94	95.40	25.29	25.28	51.53	51.25	50.35	52.93
	94.96	95.09	25.36	25.26	51.21	51.59	49.75	53.12
3[c]	97.02	96.73	25.51	25.59	53.27	51.54	51.67	53.08
	97.23	97.33	25.59	25.59	52.70	52.24	52.07	53.41
4	94.90	94.86	25.19	25.06	51.34	51.33	51.02	53.88
	94.32	95.33	25.20	25.32	51.04	51.86	50.36	53.75
5[d]	92.27	94.55	24.54	25.85	49.84	50.82	53.77	55.39
	91.39	94.07	22.73	25.64	49.60	47.09	54.44	55.52
6	95.35	95.97	25.47	25.47	51.77	51.86	51.37	53.60
	93.77	95.76	25.38	25.56	51.78	51.70	51.01	53.50
7	95.38	95.36	24.27	24.18	51.62	51.55	50.28	54.30
	94.94	96.73	25.35	25.42	51.35	51.80	50.74	53.91
9	94.45	95.34	25.37	25.37	51.57	51.55	51.19	53.79
	95.58	96.33	25.61	25.53	52.06	52.00	51.42	54.26
10	95.75	96.62	25.37	25.42	51.72	51.35	50.86	54.00
	94.71	96.64	25.11	25.16	51.08	51.17	50.12	53.50
11	94.24	94.54	24.92	24.97	50.69	50.81	50.00	52.48
	94.66	94.76	24.78	25.04	51.14	50.89	49.91	51.84
12	93.38	93.30	24.78	24.61	50.90	51.02	50.06	53.04
	94.73	94.62	25.04	25.10	51.46	50.67	49.77	52.81
13	96.87	96.97	25.61	25.33	51.87	51.91	52.14	55.64
	94.44	96.01	25.51	25.67	51.83	52.49	54.25	55.84
14	94.60	95.27	24.82	25.30	50.69	50.67	50.93	53.18
	94.78	94.98	25.07	25.25	50.98	50.94	50.52	53.27
15	94.92	95.82	25.41	25.20	51.58	51.54	50.82	53.00
	94.31	94.74	25.36	25.13	51.37	51.40	50.36	53.15
16	95.67	95.61	25.58	25.58	51.57	51.18	51.53	54.23
	95.78	95.93	25.55	25.39	51.45	52.72	50.01	56.57
17	96.87	96.62	25.41	25.28	51.45	51.57	50.70	53.20
	94.36	95.14	25.21	25.14	51.55	51.29	50.16	53.65
18[e]	95.47	95.73	25.13	24.68	50.35	50.35	54.69	57.02
Mean, %	94.94	95.52	25.24	25.22	51.38	51.45	50.73	53.73
S_O	0.88	0.58	0.23	0.28	0.24	0.37	0.58	0.51
S_X	0.88 (0.80)[f]	0.82	0.31	0.30	0.36	0.50	0.91	1.01
CV_O, %	0.93	0.61	0.92	1.09	0.46	0.71	1.14	0.96
CV_X, %	0.93 (0.84)	0.84	1.22	1.19	0.70	0.97	1.79	1.85

[a] Samples were distributed as closely matched pairs of each product, which were analyzed separately on 2 different days. Each value is the average of duplicate injections.

[b] Collaborator 8 could not achieve adequate separation; no data were submitted.

[c] Protocol not followed: used peak area for quantitation. Data not included in statistical evaluation.

[d] Protocol not followed: made insufficient standard injections. Data not included in statistical evaluation.

[e] Protocol not followed: used peak area for quantitation. Made only single injections to obtain second data set, which was not reported. Data for first set not included in statistical evaluation.

[f] Results in parentheses are actual values.

due to all the members of the Dutch Pesticide Panel (NEGAG) for their assistance in evaluating the methodology and the protocol.

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DRUGS

Determination of Ammonium Ion in Povidone by Derivatization and Square Wave Voltammetry

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A rapid and precise method is described for the determination of residual ammonium ion in povidone (polyvinylpyrrolidone). Formaldehyde reacts with ammonium ion at pH 4 to yield hexamethylenetetramine, which is reducible at ca −0.9 V vs Ag/AgCl. The derivative is detected using square wave voltammetry and is quantitated by the method of standard additions.

Povidone (polyvinylpyrrolidone), used in a variety of pharmaceutical products as an excipient, is produced by the free radical-initiated polymerization of vinylpyrrolidone. Ammonia/hydrogen peroxide can be used as an initiator system. Excessive residual ammonia can impart an objectionable odor to the product. It is therefore necessary to monitor ammonia levels to ensure product quality.

Attempts to measure ammonia in the presence of povidone by direct methods in our laboratory have not been successful because of interference by the matrix. These attempts have included ion chromatography, ammonia gas sensing electrode, and potentiometric titration methods. Usually, a separation step is needed to isolate the analyte from the matrix. Digestionless Kjeldahl analysis was reliable but required the use of large sample sizes (for adequate sensitivity) and specialized glassware. In addition, the analysis was time consuming and not readily adaptable to automation.

A rapid and accurate method has been developed for the determination of residual ammonium ion in povidone. Ammonium ion is reacted with an excess of formaldehyde at pH 4 to form hexamethylenetetramine, which is electrochemically active at the hanging mercury drop electrode. Using square wave voltammetry and the standard additions method, ammonium ion can be measured in the presence of povidone at levels ≥ 15 ppm.

METHOD

Apparatus

(a) *Polarograph.*—BAS-100 electrochemical analyzer (Bioanalytical Systems, West Lafayette, IN 47906), or equivalent, equipped with Model 303A static mercury dropping electrode and Model 305 stirrer (Princeton Applied Research, Princeton, NJ).

(b) *X-Y recorder.*—DMP-40 digital plotter (Houston Instruments, Austin, TX 78753), or equivalent.

(c) *Sample vials.*—30 mL glass with plastic screw caps and Teflon cap liners (ACE Scientific, E. Brunswick, NJ 08816), or equivalent.

Reagents

(a) *Distilled, deionized water (DDW).*—18 Mohm/cm (Barnstead Co., Boston, MA 02131), or equivalent.

(b) *Formaldehyde.*—37% aqueous solution, ACS reagent grade (GFS Chemicals, Columbus, OH).

(c) *Buffer/electrolyte.*—Dissolve 10.4 g sodium acetate and 32 mL glacial acetic acid in 100 mL DDW and dilute to 200 mL with DDW. Add 200 mL 37% formaldehyde solution and mix thoroughly. Store in tightly stoppered glass bottle which has been previously cleaned with alcoholic KOH, DDW, 6M HNO_3, and DDW (in sequence).

(d) *Ammonium ion standard solution.*—700 ppm ammonium ion. Weigh 0.2080 g (to nearest 0.1 mg) ammonium chloride (Baker Analyzed Reagent, J. T. Baker Co., Phillipsburg, NJ 08865) into clean, dry 100 mL volumetric flask. Dissolve and dilute to volume with DDW.

Determination

Clean all glassware before use with alcoholic KOH, DDW, 6M HNO_3, and DDW. Accurately weigh 0.80–1.20 g duplicate samples into glass vials. Add 25 g buffer/electrolyte solution to each sample and manually cap tightly. Dissolve solid completely by shaking or vortex-mixing. Transfer 10.0 mL sample solution to glass cell containing Teflon stirring bar (12.7 × 3 mm). Clamp cell in place over stirring motor and initiate stirring/purge with electrolyte-saturated argon for 5 min. Set initial potential −0.70 V, final potential −1.05 V; square wave amplitude 25 mV, square wave frequency 15 Hz, step size 4 mV, current sensitivity commensurate with concentration; large drop size; stirring rate 400 rpm. All measurements should be done at 23.0 ± 0.5°C using Ag/AgCl reference electrode.

Quantitation

Use method of standard additions of ammonium chloride standard to sample cell. Measure peak current from tangent fitted to peak base. Use linear regression to determine amount of analyte (μg) from absolute value of x-intercept of peak current vs amount analyte added plot. Amount of analyte in sample is calculated from equation:

$$\text{Analyte concentration, ppm} = (\mu g \text{ sample} \times \text{dilution factor})/\text{sample weight, g}$$

Dilution factor is original sample solution weight divided by weight of aliquot added to voltammetry cell.

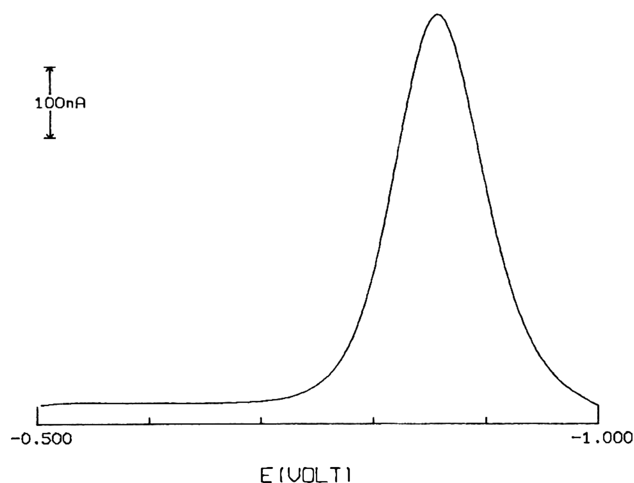

Figure 1. Ammonium ion standard (10 ppm) in presence of supporting electrolyte. Conditions given in text.

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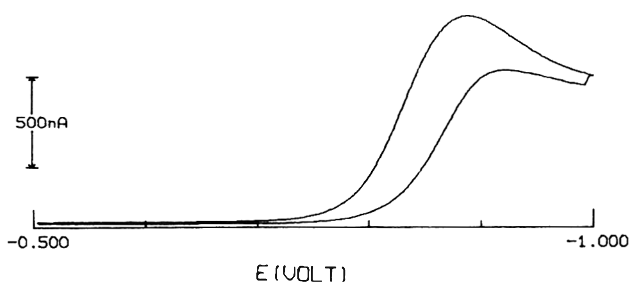

Figure 2. Forward and reverse square wave voltammetric currents of 10 ppm ammonium ion standard in supporting electrolyte.

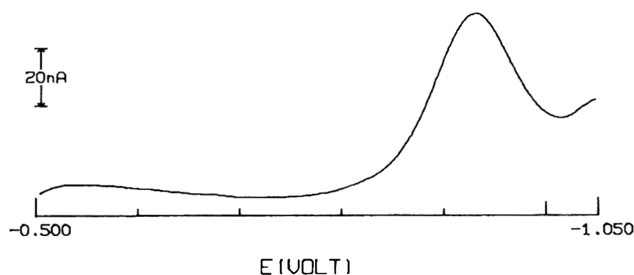

Figure 3. 4% (w/w) Sample 3 dissolved in supporting electrolyte.

Results and Discussion

Aliphatic aldehydes readily react with ammonia and primary amines to form imines (Schiff base). Formaldehyde, however, will form an unstable imine with ammonia at low pH which undergoes further condensation to yield hexamethylenetetramine (1,3,5,7-tetraazatricyclo[3.3.1.1^{3,7}] decane) (1). While a variety of methods exist for the determination of hexamethylenetetramine (2), most use decomposition and measurement of the products and are, therefore, not direct procedures. However, hexamethylenetetramine is reducible at the mercury drop electrode (3–5), which allows for its direct determination. A typical square wave voltammogram of the NH_4^+/HCHO adduct in the presence of the supporting electrolyte is shown in Figure 1. In this case, irreversible reduction (Figure 2) occurs at −0.85 V vs Ag/AgCl, which confirms the identity of the adduct.

Figure 3 shows the reduction of the adduct in the presence of povidone. A shift of peak potential to −0.94 V is observed. The cathodic shift of peak potentials for irreversible reductions in the presence of povidone has been observed previously (6). This behavior necessitates the use of the standard additions procedure for quantitative analysis. Low levels (equivalent to 50 ppm) of ammonium ion were observed in the reagent blank when the apparatus and samples were not carefully prepared. If acid-washed glassware and high purity reagents are used, the reagent blank can be reduced to the equivalent of 0.1 ppm NH_4^+.

The time needed to obtain the maximum current response under the experimental conditions was evaluated by examining the peak current after addition of NH_4^+ to the supporting electrolyte at specified time intervals from 0 to 11 min. The peak current of a 4.73 ppm ammonium ion standard reached a maximum value at about 5 min and was stable for up to 11 min. Similar behavior was observed for povidone samples containing NH_4^+. Thus, a 5 min reaction time was used for all standard and sample analyses.

Response linearity was determined for ammonium ion standard solutions in the 0.5–30 ppm range. The equation of linear regression is:

$$\text{Peak current (nA)} = (23.40 \pm 0.19 \text{ nA/ppm})C + 2.02 \pm 1.72 \text{ nA}$$

(where C = solution concentration [ppm]) with a correlation coefficient of 0.9996 and a standard error of 3.10. The detection limit, defined as the ammonium ion level producing a peak amplitude twice that of the noise level, was 0.5 ppm. This corresponds to 15 ppm analyte in the sample.

The precision of the proposed method was examined for 3 different molecular weight grades of povidone from 2 manufacturers. Table 1 shows the results compared with those obtained by the digestionless Kjeldahl procedure. Agreement between the 2 methods is excellent. The detection limit for the electrochemical method (15 ppm) is superior to the Kjeldahl procedure (about 30 ppm), with the added advantages of speed, small sample size, and ease of sample preparation. The overall coefficient of variation for ammonium ion levels detected in the samples is 3.4%.

Known amounts of ammonium ion standard were added to povidone samples to evaluate recovery. Sample solutions were fortified with ammonium ion in the 4.0–40 μg range. The mean recovery of ammonium ion over the entire range of povidone molecular weights was 100.3 ± 6% at the 95% confidence level (Table 2).

Specificity for ammonium ion was evaluated by spiking

Table 1. Precision of proposed method vs digestionless Kjeldahl method

Sample	Manuf.	NH_4^+, ppm	
		Proposed method	Kjeldahl method
1	A	1042	1050
		1039	1020
		1011	1190
		1047	1030
2	B	698	670
		685	670
		695	680
		613	640
3	A	356	370
		370	374
		374	360
		374	380
4	B	220	200
		222	290
		219	250
		213	240
5	A	ND[a]	30
		ND	30
		ND	60
		ND	30
6	B	119	139
		128	149
		107	150
		112	120

[a] ND = not detected; detection limit = 15 ppm.

Table 2. Recovery of ammonium ion in povidone

Sample	Added, μg	Found, μg	Rec., %
1	39.79	39.16	98
	34.71	36.96	105
	35.54	36.12	101
3	25.49	26.66	104
	27.24	27.81	102
	26.85	26.43	98
5	4.99	4.68	94
	4.01	4.14	103
	4.01	3.92	98

sample solutions with all known process impurities at high levels (0.1% w/w) and examining the current response. Hydrazine gave rise to an intense peak at about −0.70 V vs Ag/AgCl, which partially overlapped the analyte peak. However, because hydrazine is typically present at levels much lower than the USP limit of 3 ppm (7), no interference would be observed. Other compounds that form reducible derivations with formaldehyde will cause interference if present. However, these compounds, which include primary amines, hydroxylamine, and semicarbazide, are not normal process impurities.

Conclusions

Derivatization of ammonium ion and detection by square wave voltammetry allow for its fast, accurate, and sensitive determination in povidone. The procedure has the added advantage of simplicity of sample preparation and a lower detection limit compared to the digestionless Kjeldahl procedure.

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Determination of Aztreonam and L-Arginine Combination in Parenteral Formulations

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A rapid, sensitive and precise liquid chromatographic method is presented for the determination of aztreonam alone, in the presence of its degradation product, and in a parenteral formulation containing L-arginine. A reverse phase column and 0.2M phosphate buffer (pH 6)-methanol (95 + 5) with mobile phase at a flow rate of 2 mL/min is used. The method is sensitive for the range of 10-50 μg/mL with a relative standard deviation of less than 2%. The method has been applied to a parenteral formulation containing aztreonam and L-arginine. L-Arginine is also determined by a nonaqueous titrimetric method.

Aztreonam (Figure 1) is a synthetic monocyclic β-lactam antibiotic highly active against a broad range of gram-negative organisms (1, 2). Aztreonam has been determined in sera and urine by a reverse-phase liquid chromatographic (LC) method (3). The results were in good correlation with those from the microbiological assay. The stability of intravenous admixtures of aztreonam and clindamycin phosphate has been studied using liquid chromatography (4).

Aztreonam is prescribed as a parenteral formulation with L-arginine (Azactam®, a blend of aztreonam and L-arginine, contains approximately 780 mg L-arginine per gram aztreonam). The present paper describes a rapid, sensitive, and precise LC method for the determination of aztreonam and a nonaqueous titration method for the determination of L-arginine in the formulation.

METHOD

Apparatus and Reagents

(a) *Liquid chromatograph.*—Varian Model 5000 equipped with variable wavelength Varian UV 50 detector and Varian Model 9176 recorder (Varian Instrument Group, Sunnyvale, CA 94089) and Rheodyne Model 7125 injector (Rheodyne, Inc., Cotati, CA 94928). Chromatographic parameters were controlled by Varian data system (DCS 111 L). Optimum parameters established were as follows: mobile phase, 0.2M phosphate buffer (pH 6)–methanol (95 + 5); stationary phase, reverse-phase MicroPak MCH-10 (30 cm × 4 mm) column (Varian); flow rate, 2 mL/min; chromatography time, 4.15 min; detection at 254 nm.

(b) *Solvents and reagents.*—Chromatographic grade methanol (Merck, Darmstadt, FRG). Phosphate buffer (pH 6): 50 mL 0.2M KH_2PO_4 + 5.70 mL 0.2M NaOH, diluted with water to 200 mL. All reagents were AR grade.

(c) *Standard solutions.*—Prepare aztreonam standard (Squibb & Sons, New York, NY) solution in water (0.100% w/v). Dilute 10 mL aliquot to 100 mL with mobile phase to obtain 0.010% w/v solution. Prepare 5 dilutions to cover the concentration range 10–50 μg/mL, using mobile phase. Inject 10 μL onto column, and average 3 readings for each concentration. Prepare calibration curve for peak area and concentration, and compute linear equation by regression analysis.

Sample Preparation for Liquid Chromatography

Accurately weigh quantity of Azactam powder (ca 89 mg) equivalent to ca 50 mg aztreonam and dissolve in water in 100 mL volumetric flask. Dilute 10.0 mL to 50 mL with mobile phase. Dilute 5 mL of this solution to 20 mL with mobile phase. Inject 10 μL onto column and take average of 3 readings. Interpolate concentration from calibration curve.

Nonaqueous Titration of L-Arginine

Dry sample of Azactam 2 h at 35°C under vacuum. Accurately weigh quantity equivalent to ca 44 mg L-arginine

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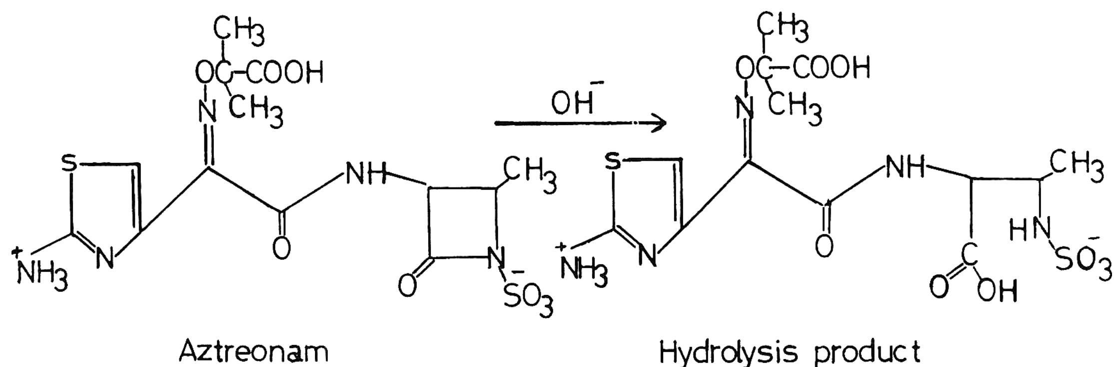

Figure 1. Aztreonam hydrolysis product.

and add 1 mL formic acid (99–100%) to dissolve powder. To the clear solution, add 50 mL glacial acetic acid and 2 drops of crystal violet solution. Titrate with 0.1M $HClO_4$ until color changes to blue, which corresponds to maximum value of $\Delta E/dV$ (where E is electromotive force and V is volume of titrant) in a potentiometric titration. Determine quantity of L-arginine, taking into consideration that 1 mL 0.1N $HClO_4$ is equivalent to 0.00871 g L-arginine.

Preparation of Alkali-Induced Degradation Product

Add 5 mL of 1M NaOH to 10 mL of 0.1% w/v aztreonam standard solution. After 30 min, add 5 mL of 1M HCl to neutralize solution. Quantitatively transfer solution to 100 mL volumetric flask and dilute to volume with mobile phase. Dilute 2 mL of this solution to 20.0 mL with mobile phase in volumetric flask. Inject 10 μL onto column under conditions previously described.

Results and Discussion

Cleavage of the β-lactam ring in aztreonam takes place after treatment with NaOH. The hydrolysis product is the corresponding acid (Figure 1). Under the conditions described, the retention times for the hydrolytic product and aztreonam were 1.35 and 4.15 min, respectively (Figure 2). The hydrolytic product, being more polar, appeared earlier than aztreonam.

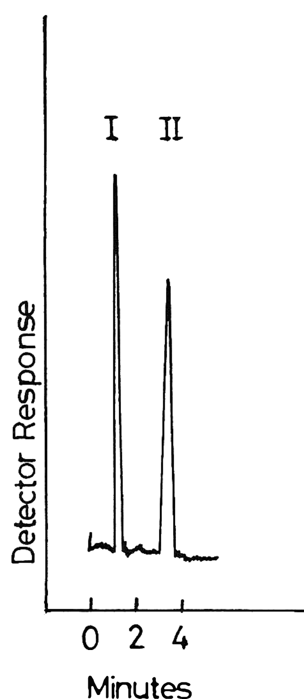

Figure 2. LC analysis of aztreonam II in presence of hydrolysis product I.

The calibration curve (peak area vs concentration) was constructed with 5 points, using a series of concentrations of aztreonam (10–50 μg/mL). Each point was the average of 3 injections. Regression analysis indicated a linear relationship between peak area, y, and concentration (μg/mL), c, with the following equation:

$$y = -6688.6 + 23955.2c$$

The correlation coefficient (r) was 0.9999. The value of the intercept was shown to be not significantly different from zero according to the t-test.

The mean percentage recoveries of aztreonam (authentic sample) and aztreonam in parenteral formulations were 100.5 and 100.7, respectively (Table 1). Excellent precision of the method for the determination of Aztreonam alone and in parenteral formulations is indicated by the relative standard deviations of the mean percent recovery which were 1.1% and 1.2%, respectively.

Three mixtures of aztreonam–hydrolytic product (2:1) were prepared and assayed for aztreonam. The mean percentage recovery (±SD) of aztreonam was 100.4 ± 1.0. Recovery experiments for added aztreonam to azactam injection formulation in the ratio 2:1 (5 experiments) and 1:2 (5 experiments) gave mean values of 100.4 ± 1.4 and 101.1 ± 1.1, respectively. All these experiments indicate the efficiency, selectivity, and reproducibility of the method. Furthermore, the minimum detectable concentrations of aztreonam and its hydrolytic product were 5 and 0.25 μg/mL, respectively.

L-Arginine in the parenteral formulation, dried under vac-

Table 1. Precision and recovery for LC determination of aztreonam

Sample	*n*	Mean % rec.	Precision (RSD,%)
Aztreonam (authentic)	5	100.5	1.08
Azactam injection[a]	6	100.7	1.18
Mixtures (ratios)		Mean % rec. of added aztreonam (±SD)	
Aztreonam + hydrolytic product (2:1)	3	100.4 ± 0.98	
Aztreonam + Azactam[a] (2:1)	5	100.4 ± 1.36	
Aztreonam + Azactam[a] (1:2)	5	101.1 ± 1.06	

[a] Azactam injection powder labeled to contain 1 g aztreonam + 0.780 g L-arginine.

uum, was determined by the nonaqueous titration method using 0.1M $HClO_4$ as a titrant. The sample gave a turbid solution in glacial acetic acid. Formic acid (99–100%, 1 mL) was used to effect a clear solution suitable for titration. The end point was detected using the potentiometric method and indicator method (crystal violet). The change of the indicator color at the maximum of the differential potentiometric titration curve was from violet to blue.

The mean percentage found of L-arginine in the parenteral formulation was 99.1 ± 0.5%, based on 5 separate determinations for quantities ranging from 70 to 100 mg dried sample. Recovery experiments of added L-arginine to the parenteral formulation were performed. Three different quantities of L-arginine (25, 40, and 50 mg) were separately added to three 50 mg quantities of Azactam. The mean percentage recovery of the added L-arginine was 99.1 ± 0.7%. The authentic sample of L-arginine used in this work was assayed by the same method and was 99.9% (2 determinations). Aztreonam did not interfere with the nonaqueous determination of L-arginine by the present method.

Acknowledgment

The authors thank Squibb & Sons, New York, NY, for supplying an authentic sample of aztreonam.

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Liquid Chromatographic Determination of Flucytosine in Capsules: Collaborative Study

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A liquid chromatographic method for the determination of flucytosine in capsules was collaboratively studied by 7 laboratories. The method uses a C_{18} reverse phase column, water-methanol-acetic acid mobile phase containing 1-octanesulfonic acid sodium salt, *p*-aminobenzoic acid as internal standard, and photometric detection at 285 nm. The mean recovery value (±SD) of flucytosine from a synthetic formulation representing capsules was 99.2 ± 1.72% (CV = 1.73%). Composited samples of 250 and 500 mg commercial capsules gave assay values of (mean ± SD) 103.17 ± 2.21 and 99.29 ± 1.29% of declared, respectively. CV values were 2.15 and 1.30%. Reproducibility and repeatability CVs were 2.19 and 1.50%, respectively, for the 250 mg capsules, and 1.34 and 0.63%, respectively, for the 500 mg capsules. The method has been adopted official first action.

Flucytosine (5-fluorocytosine) is a fluorinated pyrimidine with antifungal properties, which is used medicinally against *Candida* and *Cryptococcus* infections. A liquid chromatographic (LC) procedure was developed for the identification and assay of flucytosine in capsules (1). This method also detects fluorouracil, which can be present as a synthesis contaminant or degradation product (2). The USP assay is a nonspecific spectrophotometric procedure (3). Although several LC methods for flucytosine have been reported in the literature (4–6), none has dealt with pharmaceutical samples. Because of its simplicity and specificity, the LC procedure was collaboratively studied.

Collaborative Study

Collaborators from 7 laboratories received blind duplicate powdered samples of 2 commercial capsule formulations and one synthetic capsule formulation. Each collaborator was also furnished with an instruction sheet and a copy of the method. No restriction was placed on the source of the chromatographic column to be used in the study.

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This report of the Associate Referee, D. Shostak, was presented at the 99th AOAC Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.
The recommendation of the Associate Referee was approved interim official first action by the General Referee, the Committee on Drugs and Related Topics, and the Chairman of the Official Methods Board. The method was adopted official first action at the 101st AOAC Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. See the General Referee and Committee reports, *J. Assoc. Off. Anal. Chem.* (1988) **71**, January/February issue.

Flucytosine in Drug Capsules
Liquid Chromatographic Method
First Action

Principle

Flucytosine content of capsules is detd by liq. chromatgy on C_{18} reverse phase column, using H_2O–MeOH–HOAc mobile phase contg 1-octanesulfonic acid Na salt, *p*-aminobenzoic acid as internal std, and UV detection at 285 nm.

Apparatus

(a) *Liquid chromatograph.*—Model 8800 solv. pump with variable wavelength detector capable of monitoring elution at 285 nm (Du Pont Instruments Div.), injection valve with 20 μL sample loop (Valco Instruments Co., Inc., PO Box 55603, Houston, TX 77055), and suitable strip chart recorder. Equiv. LC system may be used. Operating conditions: flow rate 1.5 mL/min; detector 285 nm, 0.32 AUFS; chart speed 0.5 cm/min; temp. ambient; injection vol. 20 μL. To detect fluorouracil, main degradation/precursor product of flucytosine, monitor sepn at 266 nm to maximize sensitivity.

(b) *Chromatographic column.*—Stainless steel, 300 mm × 3.9 mm id, packed with 10 μm μBondapak C_{18} (Waters Associates, Inc.), or equiv. column that meets LC system suitability requirements.

Reagents

(a) *1-Octanesulfonic acid Na salt.*—(Eastman Kodak Co.).

(b) *p-Aminobenzoic acid.*—Certified ACS grade (Fisher Scientific Co.), or equiv.

(c) *Mobile phase.*—H_2O–MeOHc–HOAc (785 + 200 + 15 v/v/v) contg 2 g/L of 1-octanesulfonic acid Na salt.

(d) *Internal std soln.*—Accurately weigh ca 160 mg *p*-aminobenzoic acid and transfer to 200 mL vol. flask. Add 100 mL mobile phase, sonicate 5 min, shake mech. 25 min, dil. to vol. with mobile phase, and mix well.

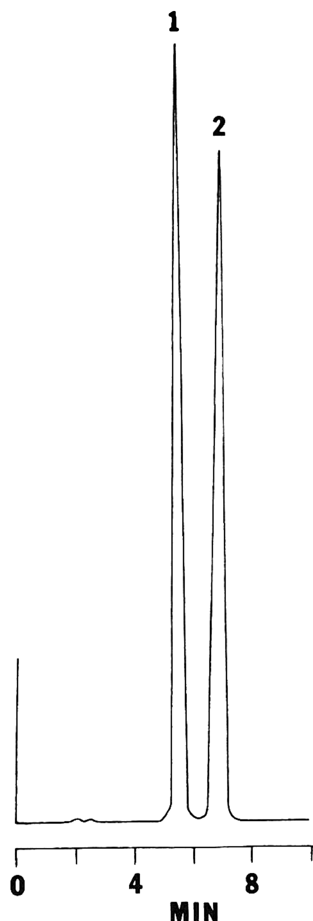

Figure 1. LC separation of (1) flucytosine and (2) *p*-aminobenzoic acid internal standard. Conditions as described in method.

Figure 2. LC chromatogram of (1) fluorouracil and (2) flucytosine. Chromatographic conditions: column, mobile phase, flow rate, and temperature as described in method. Wavelength 266 nm; detector attenuation 0.08 AUFS; chart speed 0.5 cm/min.

(e) *Flucytosine std soln.*—Accurately weigh ca 30 mg USP Flucytosine Ref. Std and transfer to 50 mL vol. flask. Add 25 mL mobile phase, sonicate 5 min with gentle swirling, shake mech. 25 min, dil. to vol. with mobile phase, and mix. Transfer 10.0 mL of this soln to 100 mL vol. flask, add 5.0 mL internal std soln, dil. to vol with mobile phase, and mix. Prep. this soln fresh daily.

Sample Preparation

Accurately weigh contents of ≥20 flucytosine capsules and det. av. wt/capsule. Accurately weigh portion of powder equiv. to ca 100 mg flucytosine and transfer to 100 mL vol. flask. Add 50 mL mobile phase, sonicate 5 min with gentle swirling, shake mech. 25 min, dil. to vol. with mobile phase, and mix well. Filter portion of soln thru suitable paper or 0.45 μm membrane filter, discarding first 10 mL filtrate. Transfer 6.0 mL filtrate to 100 mL vol. flask, add 5.0 mL internal std soln, dil. to vol. with mobile phase, and mix well. From this point, complete detn on same day.

Suitability Test and Determination

Inject 20 μL each of sample and std solns into LC system by using sampling valve or high pressure microsyringe. Operate system as described in *Apparatus*. Adjust detector sensitivity so peak response for flucytosine is between 40 and 75% full scale. In suitable chromatogram, CV of peak ht (or area) ratios for 6 replicate injections of std soln should be ≤2.0%, and resolution factor, R, for flucytosine peak and internal std peak should be ≥2.

Calc. resolution factor as follows:

$$R = [2(t_2 - t_1)]/(W_2 + W_1)$$

where t_2 and t_1 = retention times of 2 components, and W_2 and W_1 = corresponding widths of peaks, measured by extrapolating sides of peaks to baseline.

Calculation

Calc. amt flucytosine in dosage form, using response ratios based on either peak hts or peak areas, according to following equation:

$$\text{Flucytosine, mg/capsule} = 1.667C \times (R/R') \times (T/W)$$

where C = concn, μg/mL, of flucytosine in std soln; R and R' = response ratios for flucytosine peak to internal std peak for sample and std, resp.; T = av. capsule wt, mg; W = wt sample taken for assay, mg.

CAS-2022-85-7 (flucytosine)

Results and Discussion

When the liquid chromatograph was operated isocratically with a mobile phase flow rate of 1.5 mL/min, flucytosine and *p*-aminobenzoic acid were eluted with approximate retention times of 5.5 and 7 min, respectively. As shown in Figure 1, baseline resolution between the 2 compounds was achieved under the described experimental conditions. The system was linear for a concentration of flucytosine from 30 to 90 μg/mL. During development of the method we also found that it was possible to resolve flucytosine from fluorouracil, its main degradation/precursor product, which may be present in pharmaceutical dosage forms. Fluorouracil typically eluted at about 2.5 min and was detectable to a minimum level of about 0.1%. To maximize sensitivity for the detection of fluorouracil, it is recommended to monitor the separation at 266 nm (Figure 2). None of the samples examined in this study contained fluorouracil.

The collaborative results together with their statistical evaluation are presented in Table 1. All assays were conducted in duplicate. During the study the collaborators used 4 different types of LC columns representing 3 different man-

Table 1. Collaborative results for LC determination of flucytosine in commercial capsules and synthetic capsule formulation as blind duplicate samples

Coll.	Found, % of declared				Rec., %[c]	
	250 mg/cap.[a]		500 mg/cap.[b]			
1	102.8	102.4	99.5	99.7	99.0	99.6
2	99.2	99.1	99.6	97.3	97.8	99.0
3	104.1	103.0	99.2	99.4	99.6	100.5
4	102.3	104.5	97.8	97.9	98.0	97.1
5	103.3	106.9	98.1	98.5	96.7	96.8
6	101.6	105.4	100.0	100.0	101.8	99.5
7	104.8	105.8	101.6	101.5	101.3	101.5
Mean	103.17		99.29		99.16	
SD	2.21		1.29		1.72	
CV, %	2.15		1.30		1.73	
Reproducibility SD	2.26		1.33		1.78	
Reproducibility CV, %	2.19		1.34		1.79	
Repeatability SD	1.55		0.63		0.79	
Repeatability CV, %	1.50		0.63		0.80	

[a] Av. capsule weight = 389.1 mg.
[b] Av. capsule weight = 616.3 mg.
[c] Prepared to contain 64.8 mg flucytosine/100 mg formulation.

ufacturers. All of the columns used produced statistically valid results. None of the 7 participating laboratories reported any difficulties with the analytical procedure.

Recommendation

It is recommended that the liquid chromatographic method for the determination of flucytosine in capsules be adopted official first action.

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B. W. Williams, FDA, Winchester, MA

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COSMETICS

Stability-Indicating Liquid Chromatographic Determination of Alpha-Ionone in Toothpaste

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A simple, sensitive, and rapid liquid chromatographic method for quantitating α-ionone in toothpaste at levels of 20 ppm in the presence of large amounts of flavor has been developed. The method is accurate, precise, cost-effective, and specific for α-ionone. Average recovery of a laboratory-prepared sample was 99.0% with the relative standard deviation was 1.29% (n = 6).

Toothpaste contains fluoride to prevent cavities, α-ionone for breath protection, and thymol for plaque reduction. It also contains additional abrasives and flavor to mask the α-ionone/thymol tastes. Parabens are added as preservatives. α-Ionone is usually incorporated into toothpaste because of its olfactory properties at low concentration. At levels below 20 ppm, it is a challenge to isolate α-ionone from a complex sample matrix for subsequent quantitation.

A library search indicated that there are no liquid chromatographic (LC) or thin-layer chromatographic (TLC) methods in the literature, but several gas chromatographic (GC) methods have been published (1, 2). The LC method reported here is preferred over the GC method because it is specific, simple, has no interferences from flavor components, and is cost-effective since it does not require elaborate sample preparation. The method affords complete baseline separation of α-ionone from the sample matrix and offers excellent precision, recovery, and linearity over a wide range.

METHOD

Apparatus and Reagents

(**a**) *LC apparatus.*—Perkin-Elmer Series 3B pump, LC-75 variable wavelength spectrophotometric detector with LC auto controller, LC-420B auto sampler, and Sigma 15 chromatography data station, or equivalent. Operating conditions: flow rate, 2.0 mL/min; ultraviolet detector at variable wavelength programmed as follows (according to product manual), A = 265 nm, 1 = 230 nm, 2 = 0 nm, T_1 = 9.0 min, T_2 = 15.0 min; injection volume, 100 μL loop; chart speed, 5 mm/min.

(**b**) *LC column.*—300 mm × 4.5 mm id, packed with 10 μm C_{18} packing (Analytical Sciences, Inc., Santa Clara, CA), or equivalent.

(**c**) *Mobile phase.*—Methanol–acetonitrile–0.01M monobasic potassium phosphate (35 + 20 + 45). Filter separately through appropriate filter paper. Degas under vacuum while stirring for ca 30 min.

(**d**) *Standard solutions.*—*Stock solution 1.*—Accurately weigh ca 0.050 g reference standard α-ionone into 100 mL volumetric flask. Dissolve in and dilute to volume with methanol. Mix well. *Stock solution 2.*—Pipet 10 mL stock solution 1 into 500 mL volumetric flask. Dilute to volume with methanol. Mix well. *Working standard solution.*—Pipet 10 mL stock solution 2 into 100 mL volumetric flask and then add 50 mL (graduated cylinder) deionized water. Mix well and let solution equilibrate to room temperature. Add methanol to volume and mix well. Solution is stable for 3 days.

Sample Preparation

Accurately weigh ca 5.0 g sample into a 100 mL volumetric flask. Make sure sample is delivered to bottom of flask (a syringe with long plastic tubing is recommended). Add 50 mL deionized water (graduated cylinder) into flask and shake for ca 30 min using mechanical shaker. Add 40 mL methanol to volume and mix well. Let flask sit while residue settles to bottom. Filter supernatant liquid into a sample vial using 0.45 μm disposable filter unit (Millipore Co., or equivalent).

Procedure

Equilibrate column to a steady baseline by pumping a mobile phase at 2 mL/min. When column reaches equilibrium at the chromatographic conditions, inject 100 μL aliquots of working standard solution until constant response is obtained.

Inject sample solutions into liquid chromatograph. Compute sample value from data obtained from standard solutions.

Calculations

% α-ionone (as is) =

$$\frac{\text{PA sample}}{\text{PA standard}} \times \frac{\text{Concn (g working std./100 mL)}}{\text{wt of sample (g)}} \times 100$$

where PA = area counts.

Results and Discussion

The toothpaste contains methyl- and propylparabens and α-ionone at concentrations of 0.05, 0.10, and 0.0020%, respectively. Our objective was to quantitate these analytes using a single sample preparation. The detector was initially programmed to 265 nm to quantitate parabens and then was changed after 9 min to the maximum absorption of 230 nm for α-ionone. Thus, by using a variable-wavelength detector, we were able to quantitate 3 analytes with a single sample preparation. The method was not validated for methyl- and propylparabens since we were generating paraben numbers for information only.

The precision and recovery data (0.00196–0.00203 for 6 samples, mean 0.00198, standard deviation 0.00003, percent

Table 1. Linearity of detector response in LC determination of α-ionone in toothpaste

Detn no.	Concn, mg/mL	Area counts
1	0.00058	1.4804
2	0.000928	2.3852
3	0.00116	2.9488
4	0.00174	4.4224
5	0.00232	5.8867
6	0.0029	7.3337

Correlation coefficient = 0.9999
= slope·x + intercept
Area = 2.5213E + 03·amount + 3.0390E-02
Amount = 3.9662E-04·area + −1.2053E-05

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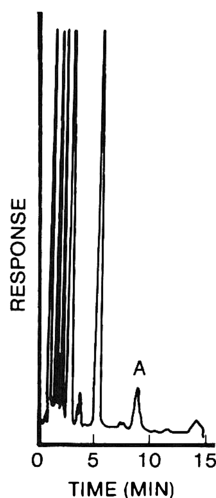

Figure 1. Chromatogram of α-ionone (A) in toothpaste sample.

relative standard deviation 1.29, and percent recovery 99.0) from the laboratory spiked samples (placebos spiked with α-ionone at label claim of 0.0020%) validate the method for the determination of α-ionone in toothpaste without interference from other ingredients (parabens) and excipients. The linearity of response of the detector (correlation coefficient 0.999) was established in each case for the concentration range 0.00058 to 0.0029 mg/mL (Table 1). Figures 1 and 2 show chromatograms of α-ionone in a toothpaste sample and of the standard containing methyl- and propylparabens, respectively.

Table 2. Stability of α-ionone[a] in toothpaste over 3-month period

Sample	Room temperature	Storage temperature	
		37°C	45°C
Formulation A			
Initial	0.0021	—	—
1 month	—	—	0.0017
2 month	—	0.0017	0.0018
3 month	0.0020	0.0018	0.0016
Formulation B			
Initial	0.0019	—	—
2 month	0.0021	0.0020	—
3 month	0.0019	0.0020	0.0017
Formulation C			
Initial	0.0020	—	—
1 month	—	0.0020	0.0018
2 month	—	0.0021	0.0019
3 month	0.0020	0.0018	0.0019

[a] Theoretical α-ionone, 0.0020%.

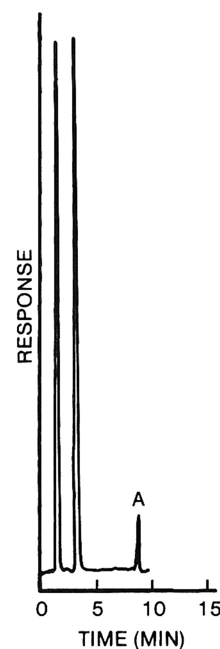

Figure 2. Chromatogram of standard containing methyl- and propylparabens. A = α-ionone.

The data of formulation C (in Table 2) verify that the method is stability indicating, since the active concentration remained close to the target amount after 3 months of study. Whereas, in the case of formulations A and B, we lost α-ionone concentration at 45°C after 3 months.

It will be very difficult to study the decomposition products of α-ionone at the 20 ppm level. Hence, we did not develop a chromatographic procedure for assaying α-ionone in presence of its decomposition products. We assumed that α-ionone decomposition will result in changing its concentration and, consequently, the detector response, as we observed in formulations A and B.

In conclusion, on the basis of the data, the method is precise, accurate, simple, cost-effective, and stability indicating.

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FISH AND OTHER MARINE PRODUCTS

Manual Determination of Minced Fish Flesh in Mixed Fillet-Minced Cod Blocks: Collaborative Study

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A simple, rapid method for determining the amount of minced fish in mixed fillet-minced cod blocks was tested by 9 collaborators. Each collaborator first examined 2 practice blocks containing 20% mince, and then examined 6 blind duplicate samples of 5 lb cod blocks from each of 3 test lots containing, respectively, 26.25, 18.75, and 12.5% mince. The data from one of the 9 collaborators was not usable because of a malfunctioning scale. Standard deviations (SD) for the 3 lots (all 8 collaborators combined) were 1.77, 1.51, and 1.65, and coefficients of variation (CV) were 6.8, 8.9, and 16.2%, respectively. For comparison, the results of an informal collaborative study by 10 participants on 16.5 lb mixed cod blocks containing 20.1% mince were SD 2.72 and CV 0.136%. Collaborators reported no problems with the method, and statistical analysis shows the method to be sufficiently precise for this type of determination. The method has been adopted official first action for cod.

Material recovered by use of a meat/bone separating machine from fish frames or small headed and gutted fish is called minced fish. In some countries other than the United States, producers add minced fish to fish blocks made from fillets. They claim that this reduces breakage when the blocks are processed into fish sticks and fish portions.

U.S. producers of fish sticks and portions have expressed an interest in having the Department of Commerce develop U.S. Standards for Grades of Mixed Fillet-Minced Fish Blocks. One prerequisite for developing such standards is the availability of a method to determine the amount of minced fish in a mixed block. An industry-government committee identified several methods that had been used to determine minced fish in mixed fillet-minced fish blocks. These methods were evaluated for practicality of use in a production situation (1).

One method was selected as practical in terms of time, equipment, and materials required to perform the test. The selected method was evaluated by 3 members of the government-industry committee. Their results on blocks at 3 levels of minced fish, 15, 20, and 25% (non-U.S. fish block manufacturers generally use 20 ± 5% minced fish) looked promising (2), so a collaborative study was proposed. This paper reports the results of the collaborative study.

Collaborative Study

A collaborative study was designed to evaluate the proposed separation method. Nine government and industry laboratories agreed to participate. A total of twenty 5 lb cod (*Gadus morhua*) mixed blocks were prepared in the Associate Referee's laboratory for each of the collaborators. Each collaborator received 2 blocks with 20% mince, which were identified and used as practice blocks. The collaborators also received 6 blocks with 26.25% mince, 6 with 18.75% mince, and 6 with 12.5% mince. The blocks were randomly numbered and distributed among the collaborators along with instructions and the method. Only the practice blocks were identified as to mince content. The collaborators were directed to analyze each sample and to submit their results to the Associate Referee.

Minced Fish Flesh in Mixed Fillet-Minced Cod Blocks
Physical Separation Method
First Action

Principle

Mixed fillet-minced cod block is air-thawed, drained, weighed, and immersed in cold H_2O bath. Fillets are sepd from mince by hand and placed on perforated tray to drain. H_2O from bath is poured thru sieve, and any fillet pieces on sieve are removed and added to drain tray. Fillets are drained 15 min and weighed. Amt of minced fish is calcd from drained wt of block and drained wt of fillets.

Apparatus

(**a**) *Trays*.—Al or galvanized. (*1*) Shallow; large enough to hold 1 fish block for thawing. (*2*) Perforated, with holes 3/8 in. diam. drilled to cover entire bottom of tray, or made from U.S. Standard No. 8 metal wire cloth.
(**b**) *Container*.—Tub of sufficient size to hold fish block plus H_2O.
(**c**) *Scale*.—Sensitive to 0.25 oz (7 g).
(**d**) *Sieve*.—U.S. No. 8.

Determination

Thaw sample (entire fish block) on preweighed tray at ambient (room) temp. (usually takes overnight). Perform detn within 8 h after block is completely thawed.

Drain any exuded fluid (thaw-drip) and det. wt of drained fish block (wt = W_B).

Immerse drained fish flesh in tub of cold, 10–21° (50–70°F), H_2O. Use ratio of 2–3 parts H_2O to 1 part fish by wt. Sep. fillets by hand and wash minced fish flesh from fillets in tub. Place washed fillets on upper section of preweighed perforated tray. Incline tray at angle of 20–40° to facilitate draining.

Pour H_2O contg minced fish and any small pieces of fillet from tub thru sieve. Remove any fillet pieces from sieve, place them on drain tray with fillets, and let fillets drain 15 min. Remove any excess H_2O from lower section of tray and weigh drained fillets (wt = W_F).

Calculation

$$\text{minced fish, \%} = [(W_B - W_F)/W_B] \times 100$$

where W_B = wt of thawed, drained fish block, and W_F = wt of drained fillets.

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This report of the Associate Referee, J. P. Lane, was presented at the 101st AOAC Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA.
The recommendation of the Associate Referee was approved interim official first action by the General Referee, the Committee on Foods I, and the Chairman of the Official Methods Board. The method was adopted official first action at the 101st AOAC Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. See the General Referee and Committee reports, *J. Assoc. Off. Anal. Chem.* (1988) **71**, January/February issue.
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Table 1. Collaborative results for determination of % minced fish flesh in 5 lb randomly numbered blocks

	Collaborator[a]							
Sample	2	3	4	5	6	7	8	9
				26.25% mince				
1	26.5	26.6	25.0	27.0	25.1	25.9	25.6	27.4
2	26.3	24.4	23.7	26.5	25.1	21.8	26.3	29.3
3	27.1	26.2	24.9	25.8	22.9	24.9	24.7	28.4
4	23.7	29.5	24.5	26.2	24.6	26.0	27.4	28.3
5	27.3	25.0	25.1	26.7	25.1	23.5	26.5	28.7
6	27.4	26.2	25.5	28.0	25.1	22.4	28.3	29.3
Average	26.4	26.3	24.8	26.7	24.7	24.1	26.5	28.6
Range	23.7–27.4	24.4–29.5	23.7–25.5	25.8–28.0	22.9–25.1	21.8–26.0	24.7–28.3	27.4–29.3
SD	1.39	1.77	0.62	0.76	0.88	1.79	1.28	0.71
				18.75% mince				
1	17.9	14.1	15.9	15.1	16.5	15.1	17.9	17.8
2	17.7	15.5	15.9	16.6	14.8	16.6	16.7	18.8
3	18.3	17.8	16.5	19.1	18.3	15.0	17.2	18.6
4	19.2	15.3	16.1	17.4	17.4	14.9	17.9	19.5
5	17.8	15.5	17.0	19.1	17.5	16.9	18.3	18.3
6	18.7	13.9	16.1	16.1	16.5	14.2	18.8	19.0
Average	18.3	15.4	16.3	17.2	16.8	15.5	17.8	18.7
Range	17.7–19.2	13.9–17.8	15.9–17.0	15.1–19.1	14.8–18.3	14.2–16.9	16.7–18.8	17.8–19.5
SD	0.59	1.39	0.43	1.63	1.21	1.06	0.75	0.59
				12.5% mince				
1	10.8	9.3	8.8	10.9	9.5	8.6	10.9	11.7
2	10.0	9.4	9.2	10.5	9.3	10.7	10.6	12.2
3	10.7	8.1	7.9	11.7	11.0	9.5	9.8	13.4
4	6.5	9.4	9.4	16.8	9.0	9.1	11.7	12.2
5	11.1	10.5	8.4	10.3	9.5	10.1	10.9	12.2
6	9.2	9.5	7.4	11.4	9.5	9.9	10.5	10.9
Average	9.7	9.4	8.5	11.9	9.6	9.7	10.7	12.1
Range	6.5–11.1	8.1–10.5	7.4–9.4	10.3–16.8	9.0–11.0	8.6–10.7	9.8–11.7	10.9–13.4
SD	1.72	0.76	0.77	2.44	0.70	0.75	0.62	0.81

[a] Data for collaborator 1 not included; used incorrectly calibrated balance.

Results and Discussion

A complete set of data was received from each of the 9 collaborators. However, after collaborator 1 had completed analyses of the samples, it was found that incorrectly calibrated scales had been used; therefore, those data were discarded. The data from the other 8 collaborators are shown in Table 1; the statistical summary is given in Table 2.

Five lb blocks were used in this study because of the expense involved in preparing sufficient samples at 3 levels of mince for all collaborators. However, the common block sizes used in industry are 16.5 and 18.5 lb. We also ran an informal collaborative study with 10 collaborators who each examined 16.5 lb fish block samples containing 20.1% mince. The results of this informal study are given in Table 3 for comparison. Although these blocks were over 3 times the weight, the average SD was only about 1% larger, indicating that the 5 lb samples were representative of the 16.5 lb commercial fish block.

To determine where potential errors may occur in the determination, samples of blocks containing only fillets were examined using exactly the same procedure. The results of this test showed that during the washing procedure, the fillets absorbed some water, indicating a larger error with a higher percentage of fillets. The results of the collaborative study support this; the average error increased with the increased percentage of fillets in the test samples.

The collaborators in the formal study found that the method was simple and relatively rapid, and required only one specialized piece of equipment, i.e., the drain tray. The accuracy is well within the reported degree (±5%) used by those non-U.S. processors who prepare mixed blocks. However, only cod was used in this study; therefore, it is recommended that this method be adopted official first action for mixed blocks prepared from cod. Cod and other gadoid species such as pollack, haddock, and whiting represent approximately 70% of the current fish block production. The species in this family are discussed in another report (3).

Recommendation

The Associate Referee recommends that this method be adopted official first action for cod.

Table 2. Statistical summary of collaborative data for % mince in fish blocks

	Mince, %		
Statistic	26.25	18.75	12.5
N	48	48	48
Mean	26.0	17.0	10.2
Range	21.8–29.5	13.9–19.5	6.5–16.8
SD	1.77	1.51	1.65
CV, %	6.8	8.9	16.2
Rel. std dev., %			
Repeatability	5.9	8.2	13.8 (8.5)[a]
Reproducibility	6.9	8.9	15.5 (12.5)[a]

[a] Numbers in parentheses reflect removal of 1 Cochran outlier (laboratory 2, sample 4).

Table 3. Data for % minced fish flesh—informal collaborative study on 16.5 lb mixed fillet-minced cod blocks containing 20.1% minced cod

	Collaborator									
Sample	1	2	3	4	5	6	7	8	9	10
1	18.3	25.2	20.9	18.5	19.2	17.9	18.6	21.8	21.0	11.9
2	18.7	23.5	20.4	18.4	23.0	17.7	18.9	21.7	21.4	20.0
3	19.6	23.4	21.2	21.3	18.7	19.8	18.7	24.0	21.5	17.9
4	18.4	23.7	21.4	19.9	21.8	16.5	19.0	21.3	21.3	19.1
5	20.6	21.9	19.6	18.0	22.3	18.0	19.0	26.1	21.1	17.3
6	17.9	22.7	20.1	20.7	22.3	19.6	14.6	21.8	22.2	18.5
7	19.2	23.3	21.6	17.8	20.6	18.9	16.1	21.7	20.8	18.6
8	19.2	22.6	23.1	19.6	22.2	18.9	18.5	22.2	22.4	12.2
9	18.6	22.2	17.8	20.7	21.4	18.5	10.9	24.7	20.5	13.7
10	19.1	20.4	21.0	21.5	21.5	18.9	13.6	22.5	22.0	17.1
Average	19.0	22.9	20.7	19.6	21.3	18.5	16.8	22.8	21.4	16.6
Range	17.9–20.6	20.4–25.2	17.8–23.1	17.8–21.5	18.7–23.0	16.5–19.8	10.9–19.0	21.3–26.1	20.5–22.4	11.9–20.0
SD	0.77	1.27	1.40	1.39	1.40	0.98	2.87	1.60	0.62	2.94
N	10	10	10	10	10	10	10	10	10	10

Overall values:
N = 100
Mean = 20.0
SD = 2.72
CV = 13.6%

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MYCOTOXINS

Use of Ten Gram Samples of Corn for Determination of Mycotoxins

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Data were gathered, during a study on the development of an automated system for the extraction, cleanup, and quantitation of mycotoxins in corn, to determine if it was scientifically sound to reduce the analytical sample size. Five, 10, and 25 g test portions were analyzed and statistically compared with 50 g test portions of the same composites for aflatoxin concentration variance. Statistical tests used to determine whether the 10 and 50 g sample sizes differed significantly showed a satisfactory observed variance ratio (F_{obs}) of 2.03 for computations of pooled standard deviations; paired *t*-test values of 0.952, 1.43, and 0.224 were computed for each of the 3 study samples. The results meet acceptable limits, since each sample's *t*-test result is less than the published value of the $|t|$, which is 1.6909 for the test conditions. The null hypothesis is retained since the sample sizes do not give significantly different values for the mean analyte concentration. The percent coefficients of variation (CVs) for all samples tested were within the expected range. In addition, the variance due to sample mixing was evaluated using radioisotope-labeled materials, yielding an acceptable CV of 22.2%. The variance due to the assay procedure was also evaluated and showed an aflatoxin B_1 recovery of 78.9% and a CV of 11.4%. Results support the original premise that a sufficiently ground and blended sample would produce an analyte variance for a 10 g sample that was statistically comparable with that for a 50 g sample.

Recently, published reports (1–3) have presented results ranging from 5 to 25 g of mycotoxin determinations conducted on analytical samples of grains. In addition, automated equipment (4) reportedly capable of grinding and extracting small quantities of solids, semisolids, and powders has become available. Experimental evaluation of the use of these smaller analytical samples for mycotoxin determination was needed because erroneous results may be obtained due to the nonuniform nature of this type of contamination and due to the inherent difficulties associated with preparing analytical samples representative of the parent lots. The heterogeneity of this type of contamination becomes apparent when the random nature of aflatoxin occurrence and contamination, along with the varied particle sizes associated with grains, is taken into account. Under such circumstances, it is reasonable to advocate the use of larger analytical samples or multiple analysis of the subject material, the conclusion being that the larger sample sizes or multiple aliquots are statistically more representative of the parent lots. For analyte distribution in solids, homogeneity increases as the size of the particles that constitute these solids is reduced. Therefore, it is reasonable to conclude that when grains are sufficiently ground and mixed, the analyte variance obtained from the analysis of a small sample aliquot should not differ significantly from results obtained from a larger portion of the same sample. The present research was designed to determine experimentally the analyte dispersion or variance between the larger (50 g) and smaller (principally 10 g) samples.

Experimental data are presented showing the distribution and variation of aflatoxin contamination when 5–50 g samples of ground corn are analyzed. The variability associated with testing corn for aflatoxin has been shown by Whitaker and Dickens (5). Their data were obtained from analysis of 50 and 500 g samples along with statistical functional relationships developed to predict the aflatoxin variance for any size sample. That report expanded on results that had been presented earlier by Stoloff et al. (6), Cuculla et al. (7), and Shotwell and Stubblefield (8), who studied the variability of aflatoxin contamination in peanuts, peanut kernels, and corn, respectively. The Shotwell and Stubblefield report is the basis for the AOAC official method (9) for the sample preparation of corn and soybeans for the determination of aflatoxins. It stipulates that the entire sample be coarse-ground to pass a No. 14 sieve and that a 1–2 kg subsample of the coarse-ground material be reground to pass a No. 20 sieve (0.850 mm). For sample preparation, this laboratory and others, such as that of the North Carolina Department of Agriculture, improved on the recommended coarse-fine grinding procedure. This laboratory comminutes the entire sample to pass a sieve opening of 0.889; the North Carolina laboratory comminutes the entire sample to pass a sieve opening of 1.00 mm (10). This procedure increases the precision of the aflatoxin determination because the entire sample is finely reduced and mixed.

Experimental

Bulk Sample Preparation

Three 15 lb (6.8 kg) samples of aflatoxin-contaminated corn were ground to pass a 0.889 mm sieve opening (No. 19 mesh), using a Model 3600 Comitrol Mill (custom modified) (Urschel Laboratories, Inc., Plano, TX 75023). The ground samples were individually blended in a planetary mixer for 20 min.

Analytical Samples

From sample A, thirty 10 g portions, six 50 g portions, six 25 g portions, and six 5 g portions were removed for aflatoxin determination. From sample B, thirty 10 g and six 50 g portions were removed for aflatoxin determination. From sample C, twenty-four 10 g and twelve 50 g portions were removed for aflatoxin determination; 15 additional 10 g portions were removed from sample C for the radioisotope distribution study.

Sample Mixing Efficiency and Analyte Distribution Study

Thirty g sample C was spiked with tritium-labeled aflatoxin B_1 (^{3}H-B_1) as follows: 10 μL ^{3}H-B_1, specific activity 60 Ci/mmol (Moravek Biochemicals, Inc., Brea, CA 92621) was dissolved in 20 mL methylene chloride (CH_2Cl_2) and added to the 30 g sample. The mixture was tumbled in a 500 mL indented round-bottom powder mixing flask (RE-35, Brinkmann Instruments, Inc., Westbury, NY 11590) for 10 min in a rotary evaporator without vacuum. Using vacuum and a 40°C water bath, the CH_2Cl_2 was completely evaporated

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Table 1. Precision measurements to determine aflatoxin distribution and sample mixing efficiency in corn samples[a]

Sample	Sample size, g	Aflatoxin found: Mean, µg/kg	SD	CV, %
A	50[b]	34.3	3.18	9.3
	25[b]	36.1	3.02	8.4
	5[b]	36.1	2.10	5.8
	10[c]	30.2	7.32	24.2
B	50[b]	26.3	9.35	35.5
	10[c]	32.6	9.90	30.3
C	50[d]	30.3	5.77	19.1
	10[e]	31.0	9.98	31.9
B, column spike	—[f]	78.9[g]	9.01	11.4

[a] Application of the Dixon test (12) for measurement with a 1 in 20 probability of a wrong decision (5% risk of unjust value rejection) shows no outliers.
[b] $n = 6$.
[c] $n = 30$.
[d] $n = 12$.
[e] $n = 24$.
[f] $n = 8$.
[g] Percentage of added aflatoxin B_1 recovered.

by tumbling and mixing the sample overnight. The spiked corn was combined with an additional 270 g ^3H-free sample C corn in a 1 L powder mixing flask and then was blended on the rotary evaporator 30 min without vacuum. Fifteen 10 g portions of this ^3H-spiked composite were removed for assay. Portions of ^3H-free samples were also used for instrument calibrations and to determine the variances of individually spiked samples when the sample sizes were held constant while the radioactivity was varied and vice versa.

All samples were extracted with 10 mL reagent grade toluene by shaking for 30 min in glass-stopper flasks. Extracts were filtered through plastic disposable columns with 20 µm frits (Quik-Sep, Isolab Inc., Akron, OH 44321).

Aliquots (3.0 mL) of filtrate from each sample were prepared by diluting to 6.0 mL with Beckman's Ready Solvent N.A. scintillation fluid. Samples were counted 1 min each on a Beckman LS 1800 instrument, and data were computed in disintegrations per minute (dpm).

Aflatoxin Determination

All samples were analyzed using the CB procedure (11). Extracts were spotted on thin-layer chromatographic plates and quantitated by densitometry (Shimadzu Scientific Instruments, Inc., Columbia, MD 21046).

Results and Discussion

Investigators have hypothesized the degree of random occurrence of aflatoxin contamination in solid commodities with sound statistical reasoning to cover extreme situations (6). Aflatoxin methods have an inherent imprecision, as is validated by collaborative study (8) and as is also determined in the present study (Table 1). The total analytical variance associated with laboratory samples of this type is attributed to the analyte distribution associated with grinding and mixing and to the variance of the analytical method. The study reported here was primarily concerned with the variance related to the former. Due to the physical nature of the materials involved, at best we can only decrease the variance by reducing the sample particles to a very fine grind. The use of 3 different naturally contaminated samples provided a good measure of the grinding procedure and analyte distribution after mixing. Using 3 different samples rather than one large sample also eliminated the possibility of later concluding that results were biased, since one sample could be abnormally homogeneous or heterogeneous in its analyte distribution.

The results of the analyte distribution study (Table 1) show good agreement for all weights of samples analyzed. The percent coefficients of variation (CVs) obtained for sample A, as well as those obtained for samples B and C, at both the 10 and 50 g sample sizes, are all well within the expected, acceptable range. The results obtained for the 10 g samples are in close agreement with results previously obtained for 50 g samples (8). A two-tailed F-test was conducted on the experimental data of the 10 and 50 g samples to determine whether the standard deviations (SDs) of the 2 sample sizes differed significantly. Computation of pooled SDs (Table 2) for samples A, B, and C for the 10 and 50 g sizes shows an observed variance ratio (F_{obs}) of 2.03, which is satisfactory since that value is less than the published value (13) of 2.16 at the 5% confidence level for $F_{81,21}$. Paired t-tests, used for comparing the means of the 2 sample sizes, had computed values of 0.952, 1.43, and 0.224 for samples A, B, and C, respectively. These results are also within satisfactory limits because $|t|$ for each sample is less than the published value (13), where $|t|$ is 1.6909 for the degree of freedom (df = 34), for each sample. The null hypothesis is retained because the sample sizes do not give significantly different values for the mean analyte concentrations. The data from this study support the original premise, which was that a corn sample that was ground to pass a No. 20 or finer sieve and that was well mixed would produce analytical results for sample weights of <50 g whose analyte level and variance would be statistically comparable to results obtained for 50 g samples.

The design of this study provided an opportunity to evaluate the variance due to sample mixing by using a radioactive tracer, which also provided a corroborative means for evaluation of the aforementioned experimental data. This experiment was designed to simulate a (1 + 9) contaminated–noncontaminated sample ratio, using tritium-spiked corn (sample C) to represent the contaminated sample portion.

Table 2. Variation of 2 sample sizes (10 and 50 g) for determination of aflatoxins

	Two-tailed F-test at 5% confidence level						Computation of pooled SDs minus sums of squares			
	10 g			50 g			10 g		50 g	
Sample	$\bar{x}$	s	n	x	s	n	df	df × s^2	df	df × s^2
A	30.3	7.32	30	34.3	3.18	6	29	1553.89	5	50.56
B	32.6	9.90	30	26.3	9.35	6	29	2842.29	5	437.11
C	31.0	9.98	24	30.3	5.77	12	23	2290.81	11	366.22
Sum							81	6686.99	21	853.89
S^2p								82.56		40.66
Sp								9.09		6.38

The dpm were 18.8 × 10^4 (4.18 × 10^3 SD) for n = 15 determinations. These results show that the distribution of the isotope-labeled material was acceptable, yielding a 22.2% CV. These data indicate that acceptable sample mixing can be achieved even when analyte distribution is very heterogeneous.

Another experiment that was part of this study evaluated the variance of the assay procedure. A series of eight 10 g silica gel columns prepared and processed in accordance with the CB procedure (11) were each directly spiked with 250 ng aflatoxin B_1. The results (Table 1) reveal an average B_1 recovery of 78.9% and a CV of 11.4%. These data were not needed to complete the study but, as in the previous case, served as a reference to establish that study procedures were satisfactory. The combined CVs for this work and the isotope-labeled material study totaled 33.6%, which agrees with published data (8).

The use of smaller analytical sample sizes offers the advantages of reduced cost per sample analysis, faster analysis time, amenability to automation, and reduction in the amounts of toxic and obnoxious solvents used in current methods, consequently affording the user the option of increasing sample surveillance. Therefore, we suggest that laboratories involved in mycotoxin surveys consider using 10 g samples of grains. However, we suggest that positive or regulatory borderline samples be reanalyzed by the official method, using larger analytical samples.

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Rapid Determination of Aflatoxins in Raw Peanuts by Liquid Chromatography with Postcolumn Iodination and Modified Minicolumn Cleanup

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A method is described for rapid cleanup followed by reverse-phase liquid chromatographic (LC) quantitation of aflatoxins in raw peanuts. A modified minicolumn cleanup is used for sample preparation, and a preliminary estimation of aflatoxin content by minicolumn can be made so that highly contaminated samples can be diluted before LC analysis. The use of the simple, quick minicolumn cleanup eliminates the need for further column or cartridge cleanup, thus greatly reducing sample preparation time. Sensitive quantitation is achieved using a phenyl column, a mobile phase of water-tetrahydrofuran (80 + 20, v/v), and postcolumn derivatization with water-saturated iodine followed by fluorescence detection. The recoveries of aflatoxins B_1, B_2, G_1, and G_2 from peanut meal spiked at 3 levels ranged from 71.7 to 88.3% (average 80%) with coefficients of variation from 2.7 to 10.4%.

Aflatoxin contamination of peanuts is the most significant economic problem facing the peanut industry. Contamination can occur in the field before harvest in response to drought stress, and it can occur during improper storage (1). Thus, all peanuts and peanut products destined for human and animal consumption must be monitored for the presence of aflatoxins throughout all phases of production. Numerous procedures have been published on the analysis of agricultural commodities for aflatoxins using various methods of extraction, cleanup, and detection. The AOAC has adopted 2 official thin-layer chromatographic (TLC) methods (CB method, secs **26.026–26.031**, and BF method, secs **26.032–26.036**) for the determination of aflatoxins in peanuts and peanut products with visual analysis of TLC plates as the method of detection and quantitation (2). Hutchins and Hagler (3) noted that while the CB method is reliable and is widely used, it is very time-consuming and expensive. This is probably one of the reasons that many "rapid" methods have been developed and published, many of which use liquid chromatography (LC) for separation, detection, and quantitation of the aflatoxins.

In most of the LC methods published recently using reverse-phase chromatography with a C_{18} column and fluorescence detection of aflatoxins, trifluoroacetic acid (TFA) is used for derivatization. The derivatization procedure occurs following sample cleanup but before injection into the LC system, and converts the weakly fluorescent aflatoxins B_1 and G_1 to their respective highly fluorescent hemiacetals, B_{2a} and

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G_{2a}. Aflatoxins B_2 and G_2 do not react but are already highly fluorescent in aqueous mobile phases.

More recently, a technique has been developed using postcolumn derivatization with aqueous iodine to enhance the fluorescence of B_1 and G_1. The procedure was first reported by Thorpe et al. (4) and was based on observations by Davis and Diener (5) that B_1 and G_1 could be reacted with iodine-saturated water to yield derivatives as fluorescent as B_2 in aqueous mobile phases. Subsequent modifications and usage have been reported by Tuinstra and Haasnoot (6), Thiel et al. (7), and Gilbert and Shepherd (8). Shepherd and Gilbert (9) investigated various combinations of reaction coil tubing dimensions, coil temperature, eluant and reagent flow rates, and reagent concentration and reported the optimum for each. This postcolumn derivatization procedure has 2 distinct advantages over precolumn derivatization with TFA. First, it is faster because the derivatization is accomplished automatically without human manipulation. Second, confirmation of the presence of B_1 and G_1, if desired, is done simply by reinjecting the same sample with the postcolumn reagent pump turned off. This results in a drastic reduction in the height of peaks corresponding to B_1 and G_1 and provides a simple, dependable confirmation.

One of the problems encountered in our laboratory when we analyze peanuts for aflatoxin by liquid chromatography is the wide range in the levels of contamination. Aflatoxin content may range from <1 to $>10\,000$ ng/g. When diversely contaminated samples are analyzed using the same LC system parameters, many samples will not fall within the linear range established for such a system. Therefore, a method was needed in which a preliminary estimation of the aflatoxin concentration could be made quickly before LC analysis. Highly contaminated extracts could then be diluted to fall within the linear range of the LC system. This report describes a method developed to achieve these goals using minicolumn chromatography and liquid chromatography with postcolumn iodination.

METHOD

Apparatus

Equipment specified is not restrictive; other suitable equipment can be substituted.

(a) *Liquid chromatograph.*—Two M6000A pumps, Model 712 WISP automatic injector, Model 730 data module, with Intelink (Waters Chromatography Division, Millipore Corp., Milford, MA 01757).

(b) *LC guard column.*—30 × 4.6 mm 5 μm Spheri-5 MPLC phenyl cartridge (Brownlee Labs, Inc., Santa Clara, CA 95050).

(c) *LC column.*—10 cm × 8 mm Nova-Pak phenyl Radial-Pak cartridge used with Model RCM-100 radial compression module (Waters-Millipore).

(d) *Postcolumn reactor.*—Model PCRS 510 heater system for LC postcolumn reactions containing 0.5 mL reaction coil (8.2 m × 0.48 mm; Kratos Analytical, Ramsey, NJ 07446).

(e) *LC fluorescence detector.*—Model LS-1 LC, with excitation wavelength of 365 nm and emission cutoff at 418 nm (Perkin-Elmer, Norwalk, CT 06856).

(f) *Separatory funnel.*—30 mL, graduated to 0.5 mL with tapered, reduced diameter (5 mm od) tip and 18 mm top id to accept disposable plastic tube closure (Tudor Glass Co., Belvedere, SC 29841).

(g) *Sample vials.*—4 mL, with open top caps, PTFE septa, self-sealing septa, and limited volume inserts with springs for use with autoinjector (Waters-Millipore).

(h) *Blenders.*—Waring Mini-Jar, 37 mL, 110 mL, 250 mL; Waring glass jar, 1250 mL; Waring 2 qt; and Waring 1 gal. (Thomas Scientific).

(i) *Vortex mixer.*—Fisherbrand Touch (Fisher Scientific).

(j) *Filter pulp.*—Schleicher and Schuell, ash-free analytical.

Reagents

(a) *Aflatoxin standards.*—Commercial mixed standards of aflatoxins B_1 and G_1 (5.0 μg/mL) and B_2 and G_2 (1.5 μg/mL) in benzene–acetonitrile (98 + 2) (Applied Science Laboratories, State College, PA 16901). In silanized test tube, evaporate to dryness, under stream of nitrogen, 50 μL commercial standard solution and redissolve in 10 mL injection solvent. Transfer to 4 mL vials for placement in autoinjector.

(b) *LC mobile phase.*—LC grade water (80%) and LC grade tetrahydrofuran (THF) (20%).

(c) *Reaction solvent.*—Saturated aqueous iodine solution prepared by stirring ca 100 mg iodine in 1 L LC grade water overnight in closed container protected from light. Vacuum-filter saturated solution through 0.45 μm filter just before use and replace daily.

(d) *Injection solvent.*—Mix 80 mL LC grade water and 20 mL THF and 0.1 mL LC grade acetic acid.

(e) *Solvents.*—ACS grade methanol, chloroform, methylene chloride, acetone, and distilled water.

Sample Preparation, Extraction, and Preliminary Estimation of Aflatoxin Content

Proceed as in Holaday-Velasco minicolumn method for corn, secs **26.020-26.025** (2), with following exceptions:

(a) *Preparation of Sample.*—Samples can be prepared as in sec. **26.020** (2). However, to eliminate sampling error, blend entire sample at high speed for 2 min with volume of methanol–water equal to twice sample weight in suitable size blender. For example, Mini-Jars are ideal for <75 g samples because small samples are not ground and blended well in larger blenders. Choose blender capacity that produces fine, homogeneous blend. Maximum sample size is ca 1700 g in 1 gal. blender.

(b) *Reagents.*—Substitute chloroform for benzene and methylene chloride–acetone (9 + 1, v/v) for chloroform–acetone (9 + 1, v/v) in sec. **26.022** (2).

(c) *Preparation of column.*—Use Holaday-Lansden minicolumn (10), which is prepared by plugging bottom of column with filter pulp followed by addition of ca 15 mm Florisil, 15 mm activity V neutral alumina, and top plug of filter pulp.

(d) *Extraction.*—After adding salt solution to initial filtrate and shaking, filter 15 mL contents into separatory funnel, add 3 mL chloroform, shake, and let layers separate.

(e) *Chromatography.*—Collect 2.5 mL chloroform layer in 4 mL LC vial. Withdraw 1 mL and apply to top of minicolumn and let extract drain into column. Wash with 2 mL methylene chloride–acetone under vacuum and examine column for blue fluorescent band at top of Florisil layer as in sec. **26.019** (2). Compare with standard minicolumn to which has been added 1 mL chloroform containing 62.5 ng total aflatoxin. This corresponds to 50 ng/g concentration in original sample and is near maximum concentration allowable for accurate LC quantitation without dilution. If fluorescence of sample minicolumn is less than or equal to that of standard minicolumn, evaporate remainder of sample (1.5 mL) in LC vial to dryness under stream of nitrogen and redissolve in 1.5 mL injection solvent. Mix ca 10 s with vortex mixer and load into autosampler. If fluorescence of sample minicolumn is greater than that of standard minicolumn, dilute remaining

sample as necessary (1:10, 1:100, 1:1000, etc.) so that minicolumn analysis of diluted sample yields fluorescence less than or equal to that of standard minicolumn. Volume of diluted sample should be exactly 2.5 mL if it is to be checked by minicolumn. If additional minicolumn analysis of diluted sample is deemed unnecessary, sample should be diluted to 1.5 mL. After proper dilution has been made, proceed as with undiluted sample.

LC Quantitation

Set mobile phase flow rate at 1.5 mL/min and reagent flow rate at 0.4 mL/min. Obtain 40°C column temperature and 75°C postcolumn reactor temperature. Let system equilibrate until steady baseline is achieved and then repeat injections (20 μL) of aflatoxin standard while adjusting detector settings to give 50% full scale deflection for B_1 (equivalent to 0.5 ng B_1 or 20 ng/g peanuts). Optimize peak integration parameters as described in data module manual and calibrate data module for external standard quantitation with following values: B_1 = 20; G_1 = 20; B_2 = 6; G_2 = 6. These values (in ng/g) correspond to 100% recovery of aflatoxins from peanuts with 20 μL injection for standard and samples.

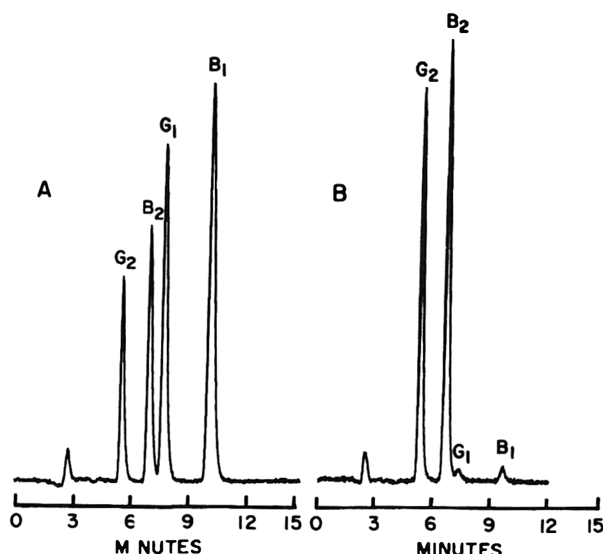

Figure 1. LC separation of aflatoxins with postcolumn iodination and fluorescence detection. Conditions described in *Method*. A, chromatogram of aflatoxin standard solution; B, chromatogram of aflatoxin standard solution with iodine reagent pump turned off and mobile phase flow rate increased to 1.6 mL/min.

Results and Discussion

The LC conditions reported here are similar to those in earlier reports (4, 6–9) with 3 major exceptions: use of a phenyl column, use of a water-THF mobile phase, and use of a postcolumn reactor. The latter makes control of the reaction simpler and more reliable; however, coiled Teflon tubing in a temperature-controlled water bath can be substituted (9). Figure 1 illustrates a typical separation of the aflatoxin standard solution with and without the iodination reaction. The elution order of G_1 and B_2 is reversed when compared with most reverse-phase separations. This observation has also been made by Shepherd (11). Figure 1B shows how the presence of aflatoxins B and G_1 can be confirmed by shutting off the iodine reagent pump and reinjecting the sample. In that case, the B_1 and G_1 peak heights are drastically reduced. On the other hand, other peaks that are present, such as B_2 and G_2, usually increase in height in the absence of the iodine reagent. Also, a slight shift in retention times occurs when the iodine pump (0.4 mL/min) is turned off. Exact retention times can be maintained during a confirmation run by substituting mobile phase for aqueous iodine in the reagent pump.

The detection limit using the system as described was 0.1 ng B_1 and G_1/g, which corresponds to an injection of 2.5 pg, and 0.06 ng B_2 and G_2/g, which corresponds to an injection of 1.5 pg. Repeated injections of the standard solution showed the response to be linear in the range of 0.025–1.25 ng B_1 and G_1, which corresponds to 1–50 ng/g, and 0.0075–0.375 ng B_2 and G_2, corresponding to 0.3–15 ng/g. If required, the detector sensitivity can be increased to achieve a lower detection limit and a reduced linear range.

A recovery study using this method was carried out with aflatoxin-free peanut meal spiked at 3 concentrations each of B_1, B_2, G_1, and G_2. Twelve 50 g samples were spiked with the aflatoxin standard mixture—4 each at 5, 20, and 50 ng B_1 or G_1/g and 1.5, 6.0, and 15.0 ng B_2 or G_2/g. Data presented in Table 1 show that recoveries of the 4 aflatoxins were reasonably uniform (range 71.7–88.3%) with acceptable coefficients of variation (CVs). Chloroform was substituted for benzene in the partitioning phase of the Holaday-Velasco method (sec. **26.024**) (2) because our data (unpublished) showed poor recovery of aflatoxins from the aqueous phase by benzene (about 12% for G_2 to about 40% for B_1). Recoveries (Table 1) using the current method were much more uniform, and the procedure was more efficient when chloroform was substituted for benzene. Use of chloroform in the Holaday-Velasco minicolumn method, particularly when that method is used quantitatively, requires certain concessions. With the chloroform layer on the bottom, pipetting 1 mL extract for transfer to the minicolumn is not feasible without also removing an unacceptable portion of the aqueous phase. However, the use of separatory funnels eliminated this problem because the chloroform is drained from the bottom directly into the vials. The chloroform also may extract impurities that make quantitation by minicolumn difficult. This usually occurs in samples which have been extensively damaged. However, the use of chloroform as described here was acceptable since the minicolumn was not used for accurate quantitation, but only as a screen to identify highly contaminated samples that required dilution.

Table 1. Recovery of aflatoxins from 50 g spiked peanut meal

Statistic	Aflatoxins											
	B_1			G_1			B_2			G_2		
Added ng/g	5.0	20.0	50.0	5.0	20.0	50.0	1.5	6.0	15.0	1.5	6.0	15.0
Recd, ng/g[a]	3.85	16.33	40.44	3.74	15.56	35.83	1.23	5.30	11.71	1.25	5.20	11.28
Std dev., ng/g	0.16	1.10	1.08	0.14	1.31	1.14	0.07	0.19	0.33	0.13	0.23	0.46
CV, %	4.2	6.7	2.7	3.7	8.4	3.2	5.7	3.6	2.8	10.4	4.4	4.1
Rec., %	77.0	81.7	80.9	74.8	77.8	71.7	82.0	88.3	78.1	83.3	86.7	75.2

[a] Mean of 4 determinations. Aflatoxin was not detected in the unspiked meal.

Table 2. Precision study of proposed method using extracts of 3 samples of field-contaminated peanuts

	Aflatoxin, ng/g				
Statistic	B_1	G_1	B_2	G_2	Total
Sample 1					
Range	10.0–10.9		0.6–0.9		10.8–11.7
Mean[a]	10.4		0.8		11.2
Std dev.	0.3		0.1		0.3
CV, %	2.9		12.5		2.7
Sample 2					
Range	41.7–46.4	23.0–26.3	5.4–6.1	3.2–4.3	73.4–83.1
Mean	44.3	24.8	5.8	3.8	78.6
Std dev.	1.5	1.0	0.2	0.4	2.9
CV, %	3.4	4.0	3.4	10.5	3.7
Sample 3					
Range	143.7–168.5	267.1–312.3	7.0–10.4	17.9–26.3	450.3–515.5
Mean	159.2	298.2	8.8	22.9	489.1
Std dev.	7.5	13.5	1.1	2.8	20.4
CV, %	4.7	4.5	12.5	12.2	4.2

[a] Mean of 10 determinations of an extract of 150 g peanuts. Ten 15 ml portions of the extract were processed by the method as described to determine method precision.

The precision of this procedure was evaluated in peanuts naturally contaminated with low, medium, and high levels of aflatoxin. For each sample, 150 g was extracted and filtered. The filtrate was then divided into ten 15 mL portions, and each was prepared according to the procedure outlined in *Method*. This study was done in this manner so that any variations would result from the procedure itself and not from variable contamination within the peanut samples. Data from this study are presented in Table 2 and show that a high degree of confidence can be placed in the method regardless of whether a sample is slightly or heavily contaminated. The CV for total aflatoxins ranged from 2.7 to 4.2% for the 3 samples. The CVs for B_1 and G_1 were <5% for all samples. The CVs for B_2 and G_2 (range 3.4–12.5%) were generally higher than for B_1 and G_1, and this will usually be true because the contamination level for B_2 and G_2 is generally about 10% that of B_1 and G_1. Because B_1 and G_1 are the more important toxins, the system was designed to optimize their quantitation with some resulting loss in precision for B_2 and G_2. These results illustrate the degree of precision that can be expected with this method when analyzing samples that have variable levels of contamination, including some extremely high levels.

Figures 2A and B compare the chromatograms obtained with an extract of aflatoxin-free peanuts and peanuts spiked with the aflatoxin standard mixture at 20, 20, 6, and 6 ng B_1, G_1, B_2, and G_2/g, respectively. This illustrates that raw peanuts did not require extensive, time-consuming cleanup to yield extracts suitable for accurate LC quantitation. The option of running confirmatory chromatograms on samples suspected of containing a compound(s) interfering with B_1 or G_1 ensures confidence in using the simple, rapid extraction-cleanup procedure. Figure 2C shows the chromatogram of a naturally contaminated peanut sample used in the precision study (Table 2, sample 3), and illustrates the advantage of using the minicolumn for preliminary estimation of the level of aflatoxins in the sample. The extract was diluted 1:10 before LC injection based on 1 minicolumn analysis. In this case, accurate quantitation of the undiluted sample would have been impossible because it was well beyond the linear range of the system. However, it took <2 min to analyze the sample by minicolumn and to make the appropriate dilution needed for accurate LC quantitation. In most cases, a slightly experienced analyst can determine the degree of dilution needed to bring the sample into the linear range for LC analysis (usually 1:10, 1:100, or 1:1000 is adequate) with 1 mini-

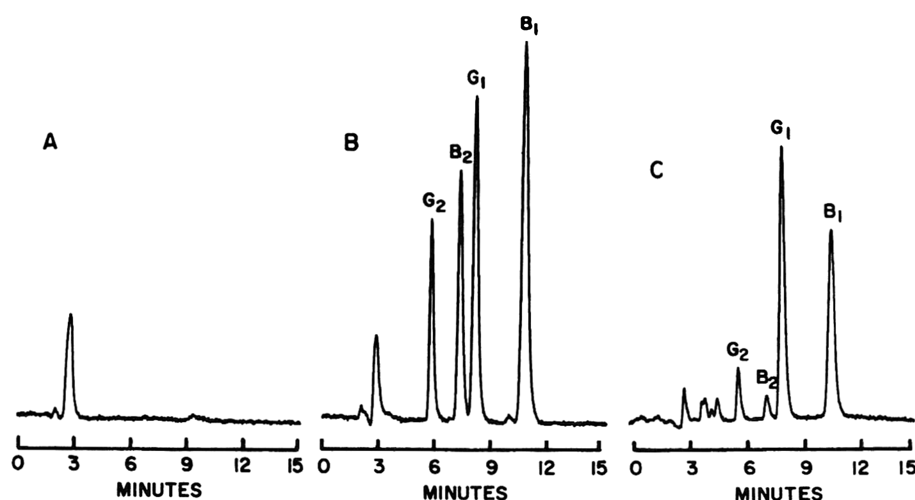

Figure 2. A, chromatogram of aflatoxin-free peanut sample prepared as described in *Method*; B, chromatogram of aflatoxin-free peanut sample that was spiked with standard solution to give 20 ng B_1 and G_1/g and 6 ng B_2 and G_2/g before extraction and cleanup; C, chromatogram of naturally contaminated peanut sample diluted 1:10 before LC injection based on minicolumn analysis.

column analysis based on the intensity of the fluorescent band in the minicolumn. However, if necessary, a second minicolumn analysis can be made with the diluted sample to ensure that a proper dilution has been made. The remainder of the diluted sample (1.5 mL) is then evaporated and processed in the normal fashion.

The method described here was found to be sensitive, efficient, and extremely rapid. The greatest time-saving came from using the simple sample preparation procedure, which, when used in conjunction with the LC conditions described, produced a chromatogram that was free of interferences. This, therefore, eliminated the need for further column or cartridge cleanup before LC injection. Certain aspects of this method can be streamlined to increase speed and efficiency. First, use of the graduated separatory funnel allows simultaneous filtration of the initial filtered extract plus salt solution and collection of 15 mL of this filtrate. Second, the 2.5 mL portion of the chloroform layer from the separatory funnel can be collected in the LC vial in 1 step with a vial holder prepared in the following manner. Using a 9/32 in. drill bit, bore several holes in a 9–12 in. length of wood (2 × 4) to a precise depth so that when a vial (containing 2.5 mL chloroform) is placed in the hole, the meniscus is exactly in line with the top level of the wooden block. With an empty vial placed in such a vial holder, lower the tip of the separatory funnel into the vial (this is the reason for the special, tapered 5 mm od tip on the separatory funnel) and drain the chloroform into the vial until the meniscus is in line with the top level of the vial holder. In this way 2.5 mL chloroform can be quickly delivered directly from a set of separatory funnels to a corresponding set of vials without pipet transfer.

In conclusion, this method provides the following combined advantages over most existing LC methods: First, the derivatization step with iodine is automatic and does not require human manipulation as is the case for precolumn derivatization. Second, it provides the option for a simple but effective confirmation of aflatoxins B_1 and G_1 by reinjecting the same sample with the iodine pump turned off. Third, the analysis is extremely rapid, enabling 2 experienced analysts to perform up to 60 analyses in a normal work day. Fourth, the method does not significantly compromise precision for this reduced analysis time. Finally, the method provides a high degree of flexibility in that highly contaminated samples can be quickly identified and diluted so that they can be chromatographed along with samples of very low contamination.

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DRUG RESIDUES IN ANIMAL TISSUE

Simultaneous Liquid Chromatographic Screening of Five Coccidiostats in Chicken Liver

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A reverse-phase liquid chromatographic (LC) method is described for simultaneously determining 5 coccidiostats—aklomide, dinsed, ethopabate, nitromide, and zoalene in chicken liver. The method entails blender extraction of 10 g liver with ethyl acetate, column chromatography through Sephadex LH-20 and neutral alumina, and LC analysis on a C_{18} column with UV detection at 260 nm. The drugs were eluted from Sephadex with methanol-benzene (10 + 90), from alumina with methanol-dichloromethane (10 + 90), and from C_{18} with acetonitrile-water (linear gradient: 25% acetonitrile for 10 min, increasing to 55% over 15 min; flow rate 1 mL/min). Liquid chromatography was completed in 40 min and calculations were based on peak height measurements. Average recoveries of the coccidiostats from fortified liver ranged from 72 to 97%, except for dinsed, which showed a relatively constant average recovery of 57%. The detection limit for the standards was 2.5 ng on column. Levels as low as 50 ng/g were detected in fortified liver samples.

The addition of chemotherapeutics to animal feeds as an aid to increase feed efficiency and prevent disease has caused considerable debate regarding potential danger to human health. Regulatory tolerances and withdrawal periods have been established to minimize human exposure (1, 2). Analytical methods to detect and quantitate low levels of such drugs in edible tissues are therefore important.

Several analytical methods involving gas chromatography (3, 4), liquid chromatography (LC) with ultraviolet (UV) (5–7), fluorescence (8), or electrochemical (9) detection, thin-layer chromatography (10, 11), and colorimetry (12–14) are available for detecting individual coccidiostats or chemically and structurally related groups of coccidiostats in animal feeds and tissues. However, few procedures are capable of simple multiresidue screening for quantitating chemically different feed additives. This paper describes an LC procedure for simultaneous identification in chicken liver of 5 coccidiostats that belong to 3 distinctly different chemical classes, namely nitrobenzamides (aklomide, nitromide, and zoalene), nitrobenzene sulfonamides (dinsed), and *N*-phenylacetamides (ethopabate). Aklomide (2-chloro-4-nitrobenzamide) was used in Canada until March 1985 at a level of 250 mg/kg (0.025%) in combination with sulfanitran at a level of 200 mg/kg (0.020%) to prevent coccidiosis in chicken. This entry has now been deleted from the Medicating Feed Ingredients Brochures (1). Nitromide (3,5-dinitrobenzamide) is used at a level of 250 mg/kg (0.025%) in combination with sulfanitran and roxarsone as an aid in preventing deaths from coccidiosis in chicken and to improve feed efficiency and pigmentation in broilers and replacement chickens. A 5 day withdrawal period is prescribed. Zoalene (3,5-dinitro-*o*-toluamide) is used at a level of 125 mg/kg (0.0125%) to prevent deaths from coccidiosis in broilers and replacement chickens and as a prophylactic against intestinal coccidiosis in turkeys. A withdrawal period has not been prescribed, but there are tolerances of 2 ppm in chicken fat, 3 ppm in turkey fat and chicken and turkey muscle, and 6 ppm in liver and kidney of chickens and turkeys (1).

Dinsed (dinitrodiphenylsulfonylethylenediamine) is used at a level of 200 mg/kg (0.02%) in combination with sulfanitran, dibutyltin dilaurate, and roxarsone in a multi-ingredient medication to prevent coccidiosis and as an anthelmintic in chicken and turkeys. A 5 day withdrawal period is in effect.

Ethopabate is used at levels of 4 mg/kg (0.0004%) and 40 mg/kg (0.004%) in combination with amprolium at 125 mg/kg (0.0125%) to prevent coccidiosis in replacement chickens intended as caged layers and to prevent deaths from coccidiosis in broilers. Withdrawal is to commence 3 days prior to slaughter.

The aim of the LC method described here is to monitor any significant residual levels (>50 ng/g) of the 5 coccidiostats that may be found in chicken liver.

METHOD

Reagents and Materials

(**a**) *Solvents.*—Distilled-in-glass ethyl acetate, benzene, hexane, dichloromethane and methanol; LC grade acetonitrile, (Caledon Laboratories, Georgetown, Ontario).

(**b**) *Pipet tip.*—Pasteur disposable pipet, glass, 5 mm id × 145 mm long (Maple Leaf brand, Cat. No. 14672-029).

(**c**) *Filter paper.*—Grade 934-AH, glass microfiber, 0.304 mm thick, 7 cm diam (Whatman, Canlab Cat. No. F2835-7).

(**d**) *Neutral alumina.*—Brockmann Activity I, 80–200 mesh (Fisher Scientific Co.). Plug pipet with silanized glass wool and fill with alumina to height of 5 cm, tapping gently to pack adsorbent. Add 0.5 cm anhydrous sodium sulfate. Activate column at 180°C overnight, cool to room temperature in desiccator, and wash with 5 mL hexane–dichloromethane (10 + 90) before use. (The column has been activated at 180°C for 3 days with no detrimental effects.)

(**e**) *Sephadex LH-20.*—Bead size 25–100 μm (Sigma Chemical Co., St. Louis, MO 63178). Add 2.5 g Sephadex to 30 mL methanol inside 31 cm × 11 mm id glass column equipped with Teflon stopcock. Equilibrate Sephadex 1 h. Drain methanol to top of column packing and wash column sequentially with 30 mL each methanol, benzene, and hexane–benzene (30 + 70), letting it equilibrate 1 h in each solvent before draining. Prior to chromatography, let column equilibrate an additional 1 h in hexane–benzene mixture. After chromatography, regenerate column by adding 30 mL methanol and repeating equilibration sequence as described above. Do not let column run dry at any time.

(**f**) *Coccidiostats.*—Aklomide, nitromide, dinsed, and zoalene (Salsbury Laboratories, Charles City, IA 50616); ethopabate (Merck Frosst Canada, Pointe-Claire, Dorval, Quebec).

(**g**) *Standard solutions.*—*Individual stock solutions.*—1.0 mg analytical standard of each individual coccidiostat/mL acetonitrile. *Individual intermediate stock solutions.*—100.00

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Table 1. Recovery of coccidiostats added to 10 g chicken liver

	Recovery, %[a]				
Added, μg/g	Aklomide	Nitromide	Zoalene	Ethopabate	Dinsed
0.2	93.9 ± 9.0	100.0 ± 11.7	93.4 ± 11.0	71.1 ± 6.0	53.5 ± 11.7
0.5	92.9 ± 7.1	98.3 ± 7.4	92.4 ± 5.5	68.9 ± 6.9	58.4 ± 12.5
1.0	93.7 ± 5.9	94.6 ± 7.5	93.3 ± 8.1	72.3 ± 7.8	61.3 ± 11.2
2.0	92.6 ± 9.3	93.2 ± 11.3	93.0 ± 10.9	73.3 ± 9.8	55.5 ± 11.1

[a] Mean and standard deviation of 12 determinations at each concentration, based on LC peak height responses of drug recovered relative to quantity of drug added.

μg of each of the 5 coccidiostats/mL. Dilute 1.0 mL stock solution to 10 mL with acetonitrile. *Mixed working solutions.*—Prepare a set of 12 working solutions, i.e., 0.10, 0.50, 0.75, 1.0, 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5, 20.0 μg/mL acetonitrile, containing all 5 coccidiostats at the same concentration from individual intermediate stock solutions (100 μg/mL). *Individual working solutions.*—1.0 μg/mL of each of the 5 coccidiostats/mL. Dilute 1.0 mL of individual intermediate stock solutions to 100 mL with acetonitrile. *Standard curve solutions.*—0.10–20 μg of each of the 5 coccidiostats/mL. Dilute aliquots of individual intermediate stock solutions with acetonitrile to yield desired concentrations. Refrigerate all standard solutions.

Apparatus

(a) *Tissue grinder.*—Brinkmann Polytron homogenizer (Brinkmann Instruments Inc., Westbury, NY 11590).

(b) *Evaporator.*—Rotavapor Model RE 121 equipped with a Model 461 water bath (Büchi, Flavil, Switzerland).

(c) *Liquid chromatograph.*—Waters Associates LC system consisting of 2 M45 pumps, WISP 710B autosampler, Model 480 variable-wavelength UV detector, 5 μm Radial-Pak C_{18} column enclosed in a radial compression unit (RCM-100), M730 data module, and system controller (Waters Associates, Milford, MA 01757). Operating conditions: column temperature, ambient; gradient, mobile phase of acetonitrile–water starting at (25 + 75) for 10 min, increasing linearly to (55 + 45) over 15 min, and maintaining this composition for a further 15 min; total run time, 40 min; flow rate, 1 mL/min; sensitivity, 0.01 AUFS; wavelength, 260 nm; chart speed, 0.5 cm/min.

Analytical Procedure

Weigh 10 g macerated, partially thawed chicken liver into 250 mL Erlenmeyer flask. Add 100 mL ethyl acetate and blend at medium speed 30 s with Polytron homogenizer. Filter mixture with suction through Whatman glass-fiber filter paper placed on Buchner funnel. Collect filtrate in 500 mL evaporating flask, concentrate filtrate to ca 10 mL on evaporator (40°C water bath), and transfer extract to 50 mL screw-cap test tube. Rinse flask twice with two 5 mL portions of ethyl acetate. Wash combined extract with 20 mL of 7% sodium chloride solution, back-extracting aqueous layer twice with two 20 mL portions of ethyl acetate. Dry combined ethyl acetate extracts over anhydrous sodium sulfate in 500 mL evaporating flask. Filter dried extract and remove solvent on evaporator (40°C water bath). Reconstitute residue in 2 mL hexane–benzene (30 + 70) 3 times and transfer each aliquot to Sephadex LH-20 column, equilibrated in solvent mixture. Discard first 8 mL eluate and elute coccidiostats with methanol–benzene (10 + 90), collecting next 20 mL eluate in 30 mL beaker. Evaporate solvent at 45°C under stream of nitrogen. Transfer residue with four 2 mL portions hexane–dichloromethane (10 + 90) to an alumina column prewashed with 5 mL solvent mixture. Discard first 10 mL eluate and elute coccidiostats with methanol–dichloromethane (10 + 90) and then collect the next 13 mL eluate in 15 mL test tube. Evaporate solvent at 45°C under stream of nitrogen. Reconstitute residue in 2 mL acetonitrile–water (30 + 70) and inject one 25 μL aliquot into LC system.

Linearity, Precision, and Recovery

Linearity of the UV detector response for each coccidiostat was determined from a set of 12 working standards of concentrations ranging from 0.1 to 20 μg/mL. Each working standard contained all 5 coccidiostats at the same concentration and was prepared by evaporating the appropriate volume of mixed stock solution (100 μg/mL in acetonitrile) to dryness and reconstituting the residue in 2 mL acetonitrile–water (30 + 70). A single calibration curve was constructed for each coccidiostat by plotting peak height response vs amount expected on column from a 25 μL injection. To monitor instrumental precision, 25 μL of 1.0 μg/mL standard was injected into the LC system once per day for 12 days.

For recovery studies, 10 g samples of frozen chicken liver, obtained locally, were fortified at 5 different concentrations, 0.0, 0.2, 0.5, 1.0, and 2.0 μg/g, for each coccidiostat. Twelve livers were spiked with each concentration by using the appropriate volume of acetonitrile-mixed stock solution (0, 20, 50, 100, and 200 μL, respectively) and were held frozen 1 h before extraction and LC analysis as described in *Analytical Procedure.* All recoveries were determined by comparing the amount of drug recovered from liver to the amount of drug spiked onto liver, calculated from the linearity curve, $PH = mx + b$, where PH = peak height of drug recovered, m = slope of calibration curve, x = amount of coccidiostat expected on column from a 25 μL injection, and b = y-intercept of calibration curve.

$$\% \text{ Coccidiostat recovery} = [2000\ \mu L(PH - b) \times 100] / [C_L \times 10\ g \times 25\ \mu L \times m]$$

where C_L = concentration of spiked drug in liver (μg/g).

Results and Discussion

The UV detector response was linear over the range studied, 2.5–500 ng coccidiostat on column. Linear regression analyses of the data (n = 12) produced the following linear equations and correlation coefficients:

$$\text{aklomide PH} = 9.23x - 3.54, 0.9996$$
$$\text{ethopabate PH} = 16.29x - 174.5, 0.9997$$
$$\text{nitromide PH} = 12.53x + 11.85, 0.9998$$
$$\text{zoalene PH} = 14.20x + 28.02, 0.9999$$
$$\text{dinsed PH} = 8.35x - 18.97, 0.9999$$

Instrumental precision was monitored for 25 ng coccidiostat on column over 12 days. The coefficient of variation (CV) of peak height measurements indicated good reproducibility for all 5 drugs. The CVs for dinsed, aklomide, zoalene, ethopabate, and nitromide were 0.9, 6.1, 6.7, 6.9,

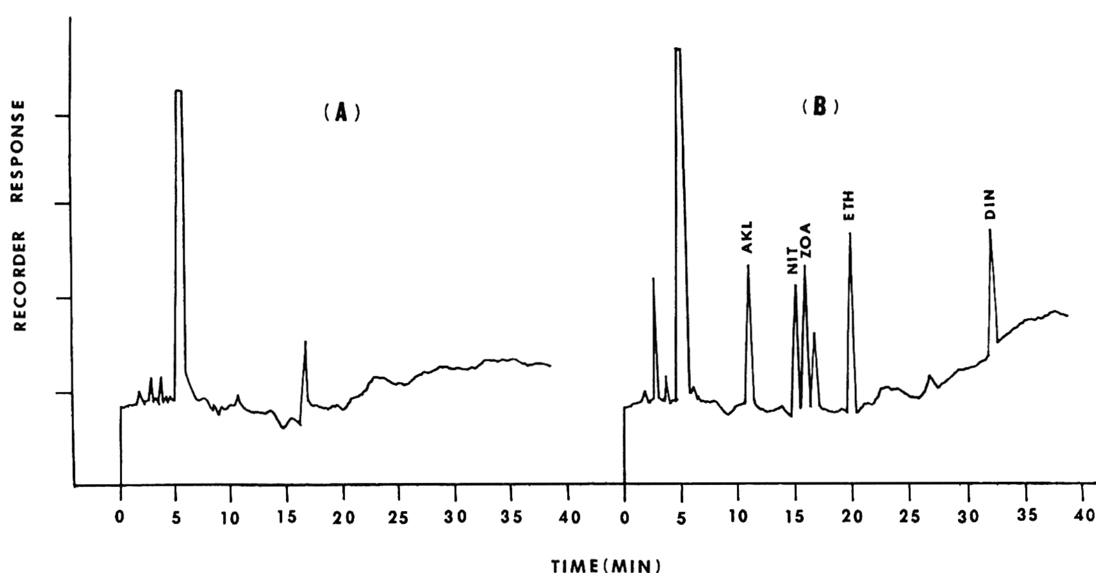

Figure 1. Liquid chromatograms of organic residue remaining after extraction and Sephadex and alumina cleanup of 10 g of (A) blank liver and (B) blank liver fortified with 1000 ng each of aklomide (AKL), nitromide (NIT), zoalene (ZOA), ethopabate (ETH), and dinsed (DIN). Retention times, min: AKL 11.36; NIT 15.53; ZOA 16.44; ETH 20.58; DIN 33.16. Amount on column was 12.5 ng each.

and 7.1%, respectively. The excellent reproducibility of dinsed stands in sharp contrast to its lower average recovery.

The percent recoveries of the spiked coccidiostats taken through the entire analytical procedure are summarized in Table 1. Recoveries were similar at each concentration and were highest for the nitrobenzamides and lowest for dinsed. Most of the dinsed was lost on the alumina column, which was expected because of the acidic nature of the drug. To obtain high recoveries of the nitrobenzamides it is necessary to spike the drugs into frozen liver because of in vitro metabolism at room temperature (10).

The optimal LC operating conditions were studied by varying the gradient composition, column particle size, and UV detector wavelength. For equal concentrations, the UV detector response was greatest for ethopabate at the selected wavelength of 260 nm. Nitromide and zoalene were not separated on a 10 μm C_{18} column using the gradient scheme outlined in *Apparatus* (c). However, full separation was achieved on a 5 μm C_{18} column. All 5 coccidiostats were separated within 40 min. Figure 1A shows the LC chromatogram of a blank liver sample; Figure 1B shows the same sample fortified with 100 ng/g coccidiostats. Both alumina and Sephadex column cleanup of the samples was necessary to lower the UV background absorbance and to eliminate interfering peaks on the chromatograms.

The method was field tested in a survey of 211 chicken liver samples, randomly selected from registered meat packing establishments across Canada; it demonstrated that residue levels for all 5 coccidiostats were below the threshold of quantitation. The detection limit for standards was 2.5 ng on column. Levels as low as 50 ng/g were easily discernible by this method for fortified samples, although concentrations of 0.5 ng/g have been achieved for ethopabate by LC with fluorescence detection (8). Work is currently in progress to expand this technique to the detection of known metabolites of these drugs and to examine various feed additive combinations to substantiate further the absence of interferences, however unlikely such an occurrence may be.

Acknowledgments

The authors thank Linda Jentz and Katheryn Kadota for their skillful assistance in the development of this method and in performing tissue analyses.

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TECHNICAL COMMUNICATIONS

Spectrophotometric Method for the Microdetermination of Bendiocarb Standard Residues in Water

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A spectrophotometric method has been developed for the microdetermination of bendiocarb in water. The method is based on the reaction of bendiocarb phenol resulting from the hydrolysis of bendiocarb with nitric acid to form a yellow complex with an adsorption maximum at 420 nm. The method is applicable for estimation of residues of bendiocarb in the range of 10-100 μg/5 mL solution.

Bendiocarb (2,3-isopropylidenedioxyphenyl *N*-methyl carbamate), formulated as 80% wettable powder Ficam W, has been evaluated as a control agent against several agricultural pests in corn, sugar beet, maize, and other crops (1–4). Acute oral and dermal LD_{50} values for the male rat are 45–48 and 566 mg/kg, respectively. After bendiocarb is applied, its residues may be present in lakes, streams, and ponds because of runoff from treated areas. A rapid, sensitive, and reliable method is required to study its persistence in aquatic systems. A survey of literature revealed that only gas (5) and liquid (6) chromatographic methods have been reported for the analysis of residues of bendiocarb; so far, no spectrophotometric method has been reported. There was, therefore, a need to develop a simple spectrophotometric method for the determination of bendiocarb residues in water. A quantitative color reaction of dilute nitric acid and bendiocarb phenol (2,2-dimethyl-1,3-benzodioxol-4-ol) is the basis of the present method for bendiocarb determination.

METHOD

Apparatus

(**a**) *Spectrophotometer.*—Perkin-Elmer Model 402 with 1 cm silica cells.

(**b**) *Colorimeter.*—Bausch and Lomb Spectronic 20 with 12 × 100 mm tube.

(**c**) *Glass columns.*—10 mm × 200 mm length.

(**d**) *Test tubes.*—30 mL capacity with B_{19} sockets and stopper.

Reagents

All are laboratory reagent grade unless otherwise specified.

(**a**) *Methylene chloride.*

(**b**) *Sodium sulfate.*

(**c**) *Nitric acid.*—50%. Analytical grade (sp. gr. 1.42).

(**d**) *Ethyl acetate.*

(**e**) *Silica gel.*—60–80 mesh.

(**f**) *Bendiocarb.*—Analytical grade, 99% purity, obtained from Rallis India, Bangalore, India.

Preparation of Standard Solution

Dissolve 100 mg bendiocarb in acetone and dilute to 100 mL with acetone in calibrated flask. Transfer 2 mL of this solution to 100 mL calibrated flask and dilute to volume with acetone, thus obtaining a solution containing 20 μg/mL of bendiocarb.

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Preparation of Standard Curve

Transfer 0, 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 mL portions of bendiocarb standard solution into a series of clean, dry test tubes. Dilute solution in each test tube to 5.0 mL with acetone and then evaporate solvent under current of dry air. Into each tube, pipet 1.0 mL 1N methanolic potassium hydroxide solution. Rotate tubes 5 min to wet sides with solution and then evaporate methanol from each test tube. Next pipet 5 mL nitric acid into each tube. Place tubes in water bath 25 min at 50°C, remove them from bath, and cool in beaker of cold water. Read absorbance of each solution against blank in spectrophotometer at 420 nm.

Extraction of Water Samples

Filter 100 mL water samples, saturate with sodium chloride, and extract with methylene chloride (3 × 50 mL). Wash methylene chloride extract with 0.1M K_2CO_3 solution to remove any free phenols present in samples, including any bendiocarb phenol that may be present as a residue. Dry methylene chloride extract by passing it through sodium sulfate in filter funnel and then collect in 500 mL flask. Concentrate methylene chloride to about 10 mL in Kuderna-Danish evaporator.

Silica Gel Cleanup

Pack glass column with silica gel to height of 100 mm and place layer of sodium sulfate on top of silica gel. Wash column with methylene chloride (50 mL) and add methylene chloride extract containing bendiocarb to column. Elute column with ethyl acetate–methylene chloride (60 mL, 1 + 9, v/v) solution, concentrate solution, and dilute with methylene chloride to 25 mL in calibrated flask.

Determination

Place suitable portion of methylene chloride extract (expected to contain 10–100 μg of bendiocarb) in test tube, evaporate solution, and proceed exactly as described under *Preparation of Standard Curve.*

Results and Discussion

Recovery

Recovery of bendiocarb was checked by adding a definite amount of insecticide to samples of tap and pond water and

Table 1. Recovery of bendiocarb from water samples[a]

No.	Fortification level, ppm	Tap water: Amt recovered, ppm	Tap water: Rec., %, ±SD	Pond water: Amt recovered, ppm	Pond water: Rec., %, ±SD
1	0.1	0.085	85.0 ± 0.95	0.087	87.0 ± 0.45
2	1.0	0.92	92.0 ± 1.0	0.90	90.0 ± 0.50
3	1.5	1.30	86.6 ± 1.1	1.35	90.0 ± 0.80
4	2.0	1.82	91.0 ± 0.86	1.85	92.50 ± 1.0

[a] Each value is the mean of 4 separate determinations.

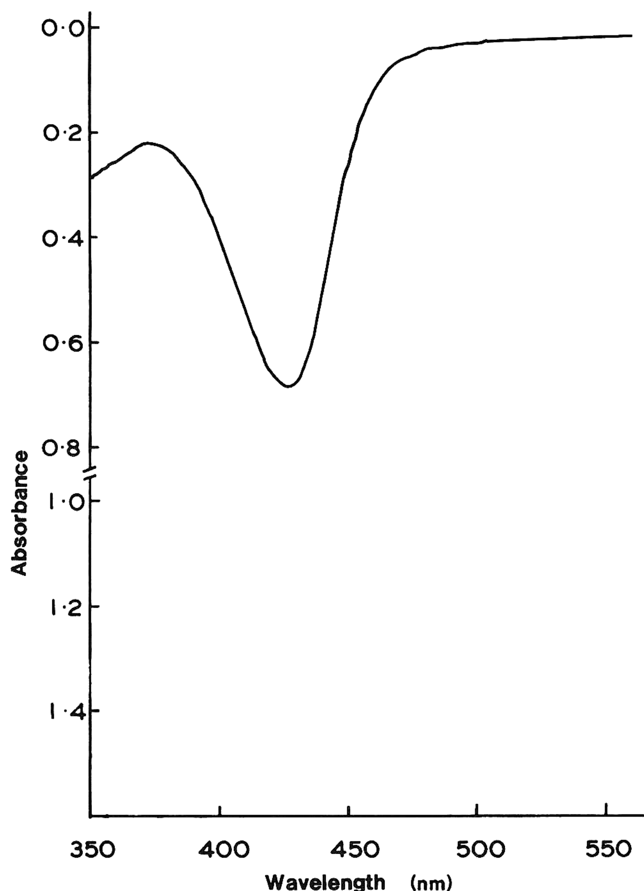

Figure 1. Absorption spectrum of colored compound.

then extracting as described in the method of analysis. The results given in Table 1 indicate that acceptable recoveries (85–92%) of bendiocarb were obtained with the proposed procedure.

Color Reaction

Under normal conditions, phenols undergo nitration with an electrophillic reagent such as nitric acid by substitution in the ring (7). These nitration reactions were performed on bendiocarb phenol from bendiocarb, using an appropriate concentration of nitric acid.

Characteristics.—The absorption spectrum of the yellow complex obtained from bendiocarb phenol and dilute nitric acid was determined on the Perkin-Elmer spectrophotometer in the wavelength range of 350–650 nm. Figure 1 shows the spectrum obtained when 40 μg of bendiocarb was present in 5 mL of solution. Maximum adsorption occurred at 420 nm.

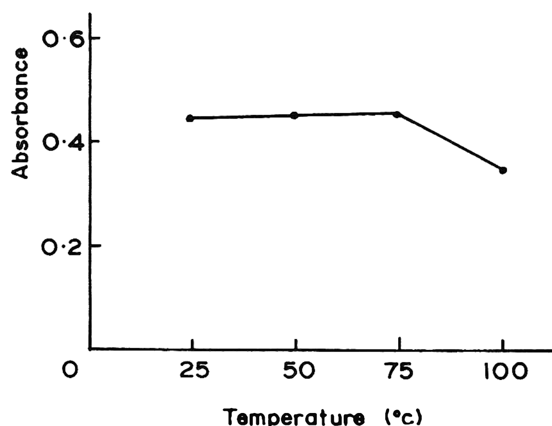

Figure 2. Effect of temperature on colored compound.

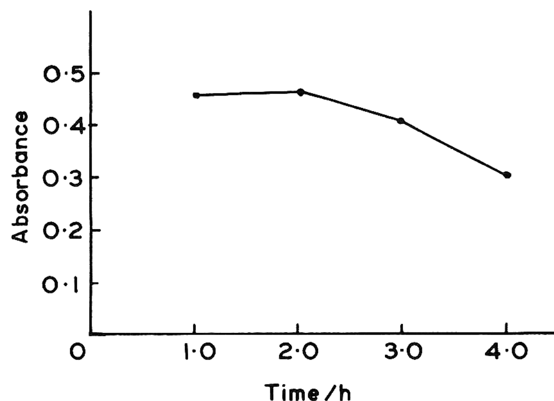

Figure 3. Stability of colored compound.

The relationship between the concentration and the color intensity obeyed Beer's Law in the range 1 to 20 μg/mL of solution.

Effect of heat.—The absorbances of the color developed with 40 μg of bendiocarb at various temperatures between 20 and 100°C were studied. The relationship between the temperature of reaction and absorbance at 420 nm is shown in Figure 2. The color complex was stable up to a temperature of 70°C, after which it decomposed steadily with increase in temperature.

Stability.—The stability of the color complex developed with 40 μg bendiocarb was studied by reading absorbances at 420 nm at an interval of 1 h. The color was stable for 2 h, after which it gradually deteriorated (Figure 3).

Effect of nitric acid concentration.—Different concentrations of nitric acid ranging from 10 to 60% were used for formation of the color complex. At a concentration of 50% nitric acid, the absorbance reached a maximum and remained constant up to a 60% concentration.

Conclusions

A sensitive and precise method for the determination of bendiocarb in field water samples is presented. It is based on the reaction of bendiocarb phenol resulting from hydrolysis of bendiocarb with dilute nitric acid. The method is applicable for residues of bendiocarb in the range 10–100 μg/5 mL solution. The method can be used for the determination of bendiocarb in lake, stream, and pond water samples.

Acknowledgment

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Liquid Chromatographic Method for Determination of Piperine in *Piper nigrum* (Black and White Pepper)

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A method was developed for the detection and quantitation of piperine in *Piper nigrum*. A reverse-phase liquid chromatographic system equipped with a C_{18} column with detection at 340 nm was used. A rapid 1 h acetone extraction followed by solvent dilution was used to avoid sample cleanup. The detection limit is 3 ng injected piperine, with 97.5-100.5% recovery of added piperine.

Piperine has been determined by ultraviolet (UV) spectrophotometric (1-3), colorimetric (4), and Kjeldahl nitrogen methods (5). The UV and colorimetric methods were compared (6), and an early liquid chromatographic (LC) method was compared with both the UV and Kjeldahl methods (7). These researchers (6, 7) noted that the non-LC methods were nonspecific because they determined other components along with piperine. Galetto et al. (7) reported that LC analysis, with detection at 254 nm, tended to minimize the UV-interfering effect of piperettine (λmax 364 nm), the second most abundant alkaloid in *Piper nigrum*. A more recently developed LC method (8) involves the isolation of a nonvolatile ether extract before analysis; the analyte is eluted in 3 min.

These authors discussed above presumed that the resolution of piperine from other alkaloids was incomplete and that piperine was not specifically determined. We have developed a method that gives results equivalent to those obtained with the currently accepted UV method (1). The method uses an isocratic reverse-phase LC system similar to that reported previously for capsaicin determination (9). UV detection is performed at 340 nm, which is almost optimal for piperine (λmax 343 nm). This system was selected to make the method suitable for laboratories using fixed wavelength detectors and single pump LC systems.

METHOD

Reagents

(a) *Acetone.*—ACS grade (Anachemia Chemicals, Mississauga, Ontario L5C 1T9).

(b) *Methanol.*—LC grade (Caledon Labs, Georgetown, Ontario L7G 4R9).

(c) *Water.*—LC grade (Caledon Labs).

(d) *Sodium pentane sulfonate.*—Regis Chemical Co., Morton Grove, IL 60053.

(e) *Acetic acid.*—Distilled-in-glass (Caledon Labs).

(f) *Methanol–water solution.*—(70 + 30).

(g) *Eluting solvent.*—Dissolve 0.95 g sodium pentane sulfonate in 160 mL water; add 335 mL methanol and 5 mL acetic acid. Filter and degas through 0.5 μm filter.

(h) *Piperine.*—Aldrich Chemical Co., Milwaukee, WI 53233.

(i) *Other pepper alkaloids.*—Piperlonguminine, piperylin, piperanine, piperettyline, and piperettine (Figure 1) were synthesized and supplied as powders by McCormick & Co., Hunt Valley, MD 21031. Each was dissolved in methanol to give a concentration of ca 9.15 mg/mL. The solutions were stored at −20°C in amber containers. Before use, a 1 mL aliquot of each was diluted to 10 mL with the methanol–water solution in separate volumetric flasks.

Apparatus

(a) *Liquid chromatograph.*—Beckman Model 332 with 2 Model 110A pumps, Model 420 controller, and Model 210 injection valve (Altex, Berkeley, CA 94719).

(b) *Detector.*—Hewlett-Packard Model 1040A diode array, HP85B computer, 2225A printer, and 7470A plotter (Hewlett-Packard, Palo Alto, CA 94304).

(c) *UV spectrophotometer.*—Pye Unicam SP1700 (Pye Unicam, Cambridge, UK).

(d) *Column.*—C18 (ion pair), 5 μm, 4.6 × 250 mm (Altex).

(e) *Amber glassware.*—250 mL boiling flasks, 10 and 100 mL volumetric flasks.

Samples

A 1 kg sample of peppercorns from each of 8 cultivars was obtained from McCormick & Co., and a 5 kg sample of Malabar black pepper was obtained from Stange Canada, Inc.

Preparation.—Samples were ground to pass a U.S. 20 mesh screen.

Moisture determination.—The moisture content of each sample was determined using ASTA Method 2.0 (10).

Spectrophotometry

The UV spectrophotometric analysis was carried out in duplicate on each sample according to ASTA Method 12.1 (1).

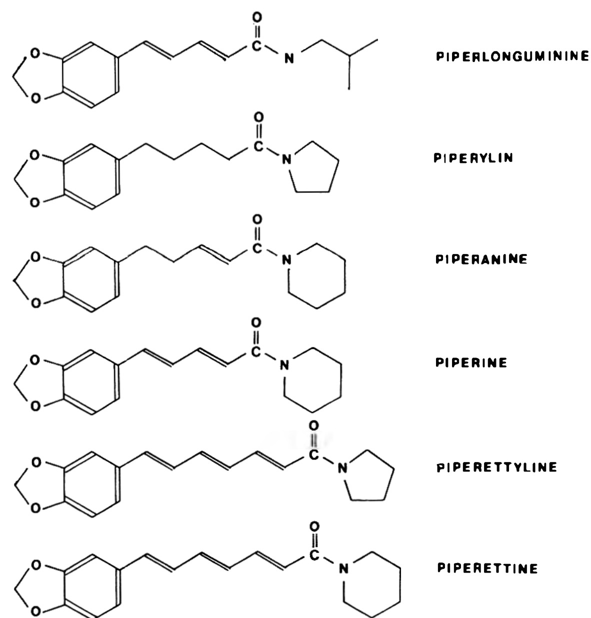

Figure 1. Structures of the pepper alkaloids.

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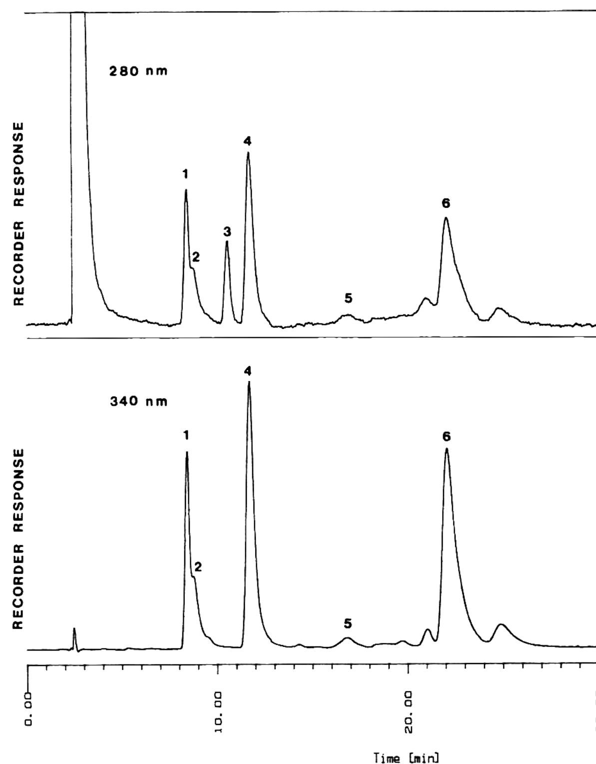

Figure 2. LC chromatograms of pepper alkaloids at 280 and 340 nm. Peaks: 1, piperlonguminine; 2, piperylin; 3, piperanine; 4, piperine; 5, piperettyline; 6, piperettine.

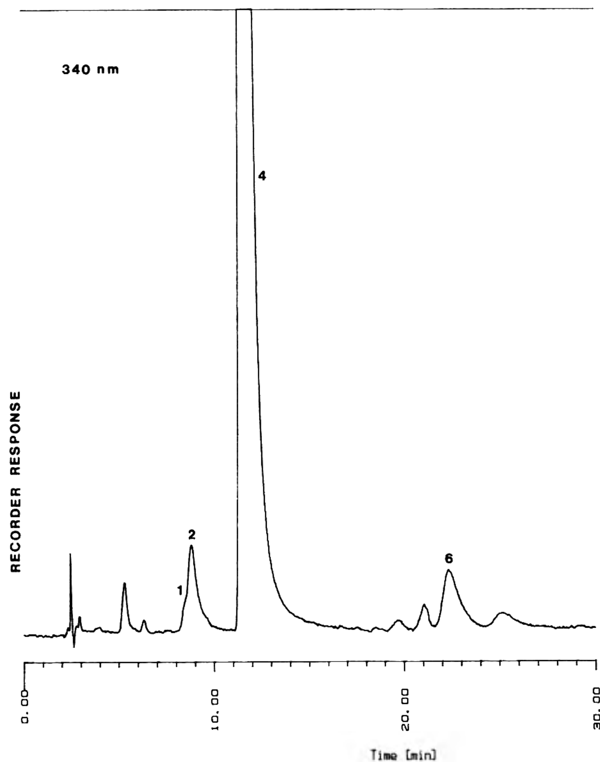

Figure 3. Typical LC chromatogram of black pepper at 340 nm. See Figure 2 for peak numbers.

Determination

Set flow rate of liquid chromatograph at 1.0 mL/min and use attenuation of 0.08 AUFS with detector set at 340 and 280 nm. Prepare calibration curves by making triplicate 20 μL injections from standard solutions of each alkaloid and then plotting μg injected vs peak area count. Prepare standards to give concentrations between 0.1 and 1.0 μg injected.

Prepare ground pepper samples for LC analysis by weighing 2 g (±0.001 g) into 250 mL amber boiling flask connected to 300 mm water-cooled condenser and reflux 1 h using heating mantle. After cooling, dilute 0.4 mL aliquot to 10.0 mL using methanol–water solution. Inject 20 μL onto column.

Calculation

Calculate content of each alkaloid from its calibration curve and convert to dry basis (db) using following equation:

Alkaloid, % (db) = (alkaloid, μg [from calibration curve] × 100)/(sample size [μg injected] × 100)/(100 − % moisture)

Results and Discussion

Under the conditions of the method, piperine was well separated from the other 5 common alkaloids. The elution order was piperlonguminine, piperylin, piperanine, piperine, piperettyline, and piperettine. The chromatograms (Figure 2) show that piperanine was not detected at 340 nm and therefore does not contribute to the UV determination of piperine.

Table 1. Analysis of Malabar black pepper using LC and UV methods

	LC results, % dry basis			UV results, % dry basis
Detn	Piperine	Piperettine	Piperine + piperettine	All alkaloids as piperine
1	3.72	0.63	4.35	4.38
2	3.75	0.49	4.24	4.40
3	3.81	0.50	4.31	4.77
4	3.81	0.51	4.32	4.33
5	3.93	0.54	4.47	4.32
6	3.91	0.52	4.43	4.32
7	3.74	0.52	4.26	4.33
8	3.73	0.60	4.33	4.54
9	3.88	0.52	4.40	4.53
10	3.91	0.52	4.43	4.49
11	3.94	0.57	4.51	4.41
12	3.84	0.52	4.36	4.27
13	3.79	0.49	4.28	4.37
14	3.78	0.50	4.28	4.34
15	3.84	0.52	4.36	4.33
16	3.91	0.54	4.45	4.49
Mean	3.83	0.53	4.36	4.41
SD	0.076	0.039	0.080	0.125
CV, %	1.97	7.33	1.84	2.84

Table 2. Analysis of pepper varieties by LC and UV methods

	Results, % dry basis			UV results, % dry basis
Variety	Piperine	Piperettine	Piperine + piperettine	All alkaloids as piperine
Lampong	4.69	0.21	4.90	5.06
Tellicherry	3.05	0.56	3.61	3.40
Muntok (black)	5.52	0.38	5.90	5.92
Lampong/Pegama	4.55	0.24	4.79	4.50
Sarawak (white)	3.03	0.39	3.42	3.37
Brazilian	3.30	0.50	3.80	3.93
Muntok (white)	4.20	0.51	4.71	4.73
Sarawak (black)	5.00	0.64	5.64	5.68
Malabar	3.83	0.53	4.36	4.41

Table 3. Response factors of 4 alkaloids relative to piperine

Alkaloid	340 nm	280 nm
Piperlonguminine	0.93	1.06
Piperylin	1.04	1.01
Piperanine	0	0.27
Piperine	1.00	1.00
Piperettine	0.90	0.65

Table 4. Recovery of piperine added to black pepper

Piperine in sample, mg	Added, mg	Total, mg	Found, mg	Rec., %
76.6	50.6	127.2	127.8	100.5
77.0	40.1	117.1	115.5	98.6
76.2	25.4	101.6	99.1	97.5

Table 1 shows the results of 16 replicate analyses performed on a Malabar black pepper sample using the LC and UV methods. A *t*-test of these data shows no significant difference ($P < 0.005$) in the results. The alkaloid contents were calculated on a dry basis to prevent possible variations caused by moisture. This would not be required for routine analysis, however, because piperine is normally reported on an "as is" basis.

The UV method measures all UV-absorbing material at 343 nm as piperine. The sum of piperine and piperettine as determined by LC is presented since they represent approximately 98% of the total alkaloids. In all cases, the levels of piperlonguminine and piperylin were <2% of the total alkaloid content; piperanine does not adsorb at 340 nm, and piperettyline levels were insignificant.

Table 2 shows the results of analysis of each of the 9 pepper varieties using LC and UV analysis. The data were analyzed by linear regression analysis, and the correlation coefficient between piperine (UV) and piperine + piperettine (LC) was 0.987 ($P < 0.01$).

Table 3 shows the response factors of the pepper alkaloids in relation to pure piperine. These are shown because the alkaloids are not commercially available. Piperettine was not included since it was not found in any of the samples.

Recovery experiments were conducted by adding a known amount of pure piperine to the flask containing a ground sample just before extraction. Recoveries ranged from 97.5 to 100.5% (Table 4). The piperine used was checked for purity by measuring its extinction coefficient, which was 99.4%.

The minimum detectable level of piperine was calculated to be 170 ppm in the sample, based on the sample weight, dilutions, and instrument settings using a minimum area count of 15 on the integrator. Lower levels (<10 ppm) can be detected by concentrating the first extract. Over the range 0.1–1.0 μg/20 μL, the relationship between detector response and μg injected was linear ($r = 0.985$).

An acceptable correlation has been shown between the currently accepted method for piperine determination (1) and the LC method with detection at 340 nm and using the sum of piperine and piperettine. Furthermore, since the ratio of piperine to piperettine can also be determined, the method may be used to indicate sample origin or to detect adulteration (6). This aspect was not pursued, however, due to the small number of pepper samples. It is suggested that the LC method be subjected to a collaborative study by organizations interested in pepper analysis.

Acknowledgments

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GENERAL REFEREE REPORTS: COMMITTEE ON PESTICIDE FORMULATIONS AND DISINFECTANTS

Disinfectants

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Use-Dilution Test.—In the past year, a second collaborative test incorporating 31 standardized items was carried out with 12 laboratories. In the first study, 18 laboratories took part. The same disinfectants were used in both collaborative studies. In the second study, all laboratories used the same culture provided by the Associate Referee. The results show no significant decrease in variability of pass/fail results when the 31 standardized items were incorporated into the test method. The AR conducted tests to determine the concentration of disinfectants that would show complete kill in the test and found that some showed increased kill with increasing concentration of disinfectant, while others did not (3 quaternary ammonium chlorides and 3 substituted phenols were tested).

A third collaborative study using higher concentrations of disinfectants that show complete kill in pre-trial tests against *Pseudomonas aeruginosa* was requested.

Tests were also done using sodium hypochlorite, at the request of the General Referee, with the intent of specifying a known chemical with known bacterial killing ability. By using such a chemical, the variability of the test could be defined and new pass/fail criteria could be developed.

The AR felt that this would pose a new problem. By using a chemical that could be classified as a "Class I" disinfectant (able to kill viruses, bacterial spores, vegetative bacteria, and tuberculosis bacteria), it would be inconsistent to compare such test results with generally used disinfectants applied in noncritical medical environments where "Class I" disinfection is not needed.

In addition to these largely philosophical problems, the second collaborative test was not a true test of the 31 standardized portions of the method because the laboratories involved were reluctant to buy equipment, materials, or chemicals for a test still in development. Thus, both the disinfectants used and the equipment requirements of the test vitiated against successful completion of a method modification.

The Office of Pesticide Programs, U.S. Environmental Protection Agency, as the regulatory agency with purview over disinfectants, strongly supports retention of the use-dilution test as the test of choice in assessing the performance of disinfectants. The AR has conclusively shown that the major causes of variation in the test are due to wide variability in the numbers of bacteria on the steel cylinders, together with intrinsic variations in and on the surfaces of the cylinder itself, plus the inherent cellular characteristics of the test bacterium.

Parallel studies, using washed test bacterial cells, of a suspension test also showed variability, but not to the same extent as the use-dilution test. The AR believes that a new suspension test should be developed and used in lieu of the present test because of (1) greater control over bacterial test numbers; (2) a 7- to 8-log reduction in bacterial numbers in 10^{10} to minimum (10^{10} cells/mL, start) which is sufficient for control of bacteria in noncritical medical environments; and (3) a greater ability to statistically define pass/fail in the collaborating laboratories.

The General Referee believes that every effort should be made to complete the update of the current method. To this end, a new steel carrier will be recommended and much more attention will be paid to specific details before the next collaborative test is run. Continued study is recommended.

Tuberculocidal Test.—The tuberculocidal test method has been submitted to the Committee on Pesticide Formulations and Disinfectants for determining whether the test is sufficiently precise to warrant a collaborative test. The Associate Referee has experienced some difficulty with repeatability of test results. Comments have been received from the Committee statistician on the number of replicates required in the collaborative study. These comments are being evaluated by the Associate Referee before the final proposal is submitted to the General Referee for testing. Continued study is recommended.

Sporicidal Test.—It is recommended that this topic be deleted until an Associate Referee can be appointed.

Recommendations

(1) Delete the topic Sporicidal Tests.

(2) Continue study on all other topics.

This report of the General Referee was presented at the 101st AOAC Annual International Meeting, Sept. 14-17, 1987, at San Francisco, CA. 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

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No studies have been done in this area during the past year. The following is a status report of selected topics in this section:

Anilazine.—This method was granted interim official first action and was presented in a poster session in September by Associate Referee Stephen Slahck.

Benomyl.—Associate Referee Mikio Chiba reports no progress and has no plans for the next year to collaborate on his method because of funding problems.

Oxythioquinox.—The method of analysis was recommended for official final action during the year.

Triadimefon (Bayleton).—The method of analysis was recommended for official final action during the year.

Triphenyltin.—Associate Referee P. Pasma reports no progress during the past year.

Recommendations

(1) Delete Dioxins in Pentachlorophenol as an active topic.

(2) Adopt as official first action the LC method for anilazine in formulated products.

(3) Adopt as official final action the methods for oxythioquinox (**6.B34-6.B39**) and triadimefon (**6.A31-6.A36**).

(4) Continue study in all other areas.

This report of the General Referee was presented at the 101st AOAC Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. The recommendations were reviewed by the Committee on Pesticide Formulations and Disinfectants. See the report of the commmittee, this issue.

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

Pesticide Formulations: Herbicides I; Other Organophosphorus Insecticides: Rodenticides and Miscellaneous Pesticides

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Two outstanding Associate Referees, A. Aner Carlstrom and Robert B. Grorud, retired this past year. Many thanks for all the work performed over the years by these 2 dedicated individuals.

The following is a summation of activities this past year.

Herbicides I

Chlorphenoxy Herbicides.—Richard D. Larson has been appointed to continue the work Robert Grorud was doing.

Dicamba.—Benjamin Belkind is continuing to study a method.

Other Organophosphorus Insecticides

Crotoxyphos.—Wendy King is continuing to study a method.

Fenamiphos (Nemacur).—Carl Gregg has a method written and is ready for a collaborative study.

Naled.—Robert Iwamoto has replaced A. A. Carlstrom.

Rodenticides and Miscellaneous Pesticides

Brodifacoum.—Jess Parker will be appointed to replace Richard Freedlander. He should have the method for wax bait formulations ready for collaborative study during the next year.

Recommendation

Continue study on all topics.

This report of the General Referee was presented at the 101st AOAC Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. 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. Reregistrations, data callins from the EPA and several states, as well as reorganizations in several companies have all prevented the use of analytical resources for collaborative studies. There has, however, been considerable progress in improving and validating in-house methodology. Most significant is the increased use of capillary GC and the routine use of wide-bore GC for formulation analyses. LC analysis is now quite routine in almost all laboratories, as are computerized integration systems and the use of automated injectors.

Associate Referees are needed for substituted urea herbicides, oryzalin, dinoseb, metasulfuron-methyl, sulfometuron-methyl, and chlorsulfuron. The status of current assignments is as follows:

Bensulide (Betasan).—W. Ja has developed an improved in-house LC method. It is possible that a collaborative study could be initiated in 1988.

Bromacil.—P. Tseng is ready for collaborative study of an LC method as soon as time permits.

Fluometuron (Cotoran®).—A. Hofberg has continued to test an LC method in-house and is ready to initiate a collaborative study which will be completed in 1988.

Methazole (Probe).—B. Belkind has completed development and testing of an LC normal-phase method. He is ready for a collaborative study.

Naptalam (Alanap).—P. Parkins has been unable to complete the needed in-house method testing. He hopes to complete this in 1988 and possibly be ready for a collaborative study.

Thiocarbamates.—W. Ja will continue to study the use of capillary GC analysis for these compounds as time allows.

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 101st AOAC Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. The recommendations were reviewed by the Committee on Pesticide Formulations and Disinfectants. See the report of the committee, this issue.

Pesticide Formulations: Herbicides III

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No collaborative studies were carried out during the year, although one had been planned. A conflict was highlighted between the rules of acceptance of CIPAC and AOAC methods which needs to be resolved. Two new Associate Referees were appointed—one of them in a new study area on the chemical fomesafen.

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

Alachlor, Butachlor, and Propachlor.—Associate Referee David Tomkins reports considerable interest worldwide in the propachlor/butachlor methods. No negative comments have been received. The method for alachlor in microencapsulated formulations was granted interim official first action. He plans to carry out a collaborative study on a method of analysis for atrazine and alachlor in mixed formulations.

Bentazon.—Associate Referee Thomas Schmidt has delayed consideration of a further collaborative study until the IUPAC general assembly meeting in Boston in September 1987 to see if new harmonized protocols for CIPAC–AOAC studies are developed.

Bromoxynil.—Associate Referee Laurence Helfant reports that he has put on hold a collaborative study of a method for the analysis of MCPA esters and alkanoic esters of bromoxynil, following the takeover of his company by another chemical company and the potential rationalization of product ranges.

Cyanazine.—James Launer kindly redrafted the CIPAC

method into AOAC format and recalculated the statistics. These have been submitted to the statistician for review.

Dalapon.—Associate Referee Timothy Stevens recommends that dalapon be dropped as a subject area now that the method (**6.296-6.301**) is official final action.

Fluazifop-Butyl.—Associate Referee Steve Eitelman recommends that the subject area be dropped now that the method (**6.353-6.357**) is official final action.

Pesticides in Fertilizers.—Associate Referee Paul Korger has identified a problem with the application of AOAC methods (**6.321** and **6.387** for fertilizer samples impregnated with 2,4-D IOE and MCPP IOE. The extraction procedure has been modified to improve recovery, and a mini-collaborative study is planned this year. If the data from this study are acceptable, then it is proposed that an AOAC collaborative study be initiated in 1988.

Fomesafen.—A new Associate Referee has been appointed, Steven Eitelman. He has an LC method of analysis for fomesafen and plans a collaborative study in the next year.

Propanil.—A new Associate Referee has been appointed, Steve Gazaway. He plans to collaboratively study an LC method of analysis for propanil in the next year.

Recommendations

(1) Adopt as official first action the GC method for alachlor microencapsulated formulations.

(2) Adopt as official final action the GC methods for butachlor (**6.B12-6.B16**) and propachlor (**6.B17-6.B21**).

(3) Remove the subject areas of dalapon, fluazifop-butyl, metribuzin, and glyphosate from further consideration.

(4) Continue study in all other areas.

This report of the General Referee was presented at the 101st AOAC Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. 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 edition, and to "Changes in Official Methods," *J. Assoc. Off. Anal. Chem.* (1986) **69**, 349–390 (B methods).

Pesticide Formulations: Organohalogen Insecticides; Other Insecticides, Synergists, and Insect Repellents

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The General Referee submitted a CIPAC provisional GC method for the determination of methyl, ethyl, propyl, butyl, Three Associate Referees were appointed on 3 topics this year: N. S. Birdie on resmethrin, C. F. Harper on benzene hexachloride (and lindane), and J. F. Muniz on methoxychlor. Associate Referees are needed for aluminum phosphide, cyhexatin, and fenvalerate. The General Referee rewrote the full CIPAC method for cyanazine into AOAC style.

The following is the present status of selected topics assigned to Associate Referees:

Organohalogen Insecticides

Ethylan (Perthane).—D. Bradway is investigating GC and LC methods.

Fenvalerate.—The General Referee recommends adoption of the provisional CIPAC gas chromatographic method as an official first action CIPAC–AOAC method.

Methoxychlor.—J. Muniz is investigating GC and LC methods.

Trichlorfon.—D. Bradway is investigating GC and LC methods.

Other Insecticides, Synergists, and Insect Repellents

Cyhexatin (Plictran).—The full CIPAC liquid chromatographic method received AOAC interim official first action approval.

Nicotine.—R. Bushway is investigating LC methods.

Permethrin.—The General Referee recommends adoption of the official CIPAC–AOAC gas chromatographic method (**6.B06-6.B11**) as official final action.

Rotenone.—R. Bushway plans a collaborative study of modifications to the official first action AOAC liquid chromatographic method (**6.182-6.186**).

Recommendations

(1) Continue first action status of the following methods: (a) titrimetric method for technical allethrin (**6.165-6.170**); (b) CIPAC–AOAC gas chromatographic method for cypermethrin (**6.B01-6.B05**); (c) hydrolyzable chloride method for dicofol (**6.332-6.337**); (d) liquid chromatographic method for rotenone (**6.182-6.186**); and the infrared method for rotenone (**6.179-6.180**).

(2) Adopt as official first action the CIPAC method for cyhexatin.

(3) Consider for adoption the following methods: (a) full CIPAC method for deltamethrin; (b) provisional CIPAC GC method for fenvalerate.

(4) Adopt as official final action the CIPAC–AOAC GC method for permethrin (**6.B06-6.B11**).

(5) Continue study on all other topics.

This report of the General Referee was presented at the 101st AOAC Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. 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 edition, and to "Changes in Official Methods," *J. Assoc. Off. Anal. Chem.* (1986) **69**, 349–390 (B methods).

GENERAL REFEREE REPORTS: COMMITTEE ON DRUGS AND RELATED TOPICS

Cosmetics

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Aloe.—This topic was initiated to develop analytical methods for the estimation of aloe in cosmetic raw materials and finished products to detect adulteration. The method for aloe assay would probably involve the quantitative determination of one or more compounds that occur in aloe, and could involve the determination of aloe-emodin, an anthraquinone derivative, or aloin, the glycoside of aloe-emodin. The problem with this approach is that the chemical composition of aloe will vary depending on factors such as climate, soil, rainfall, and species. One other factor complicating any assay method is the deliberate adulteration with compounds that are used as purity indicators. Aloin, for example, is commercially available at a cost of ten cents per gram. Depending on the levels normally present in the plant extract, it may be economically feasible to use it as an adulterant.

Essential Oils and Fragrance Materials, Composition.—The Associate Referee, Harris H. Wisneski, has completed work on the development and evaluation of a gas chromatographic method for the determination of musk ambrette (2,4-dinitro-3-methyl-6-tert.-butylanisole) in fragrance composition. The method will be submitted for collaborative study. He is presently drafting directions for collaborators and preparing the kits.

Preservatives.—Associate Referee Ann R. Stack has completed work on the development of a liquid chromatographic method for the determination of methyl, ethyl, propyl, butyl, and isobutyl parabens in cosmetic creams and lotions. The method has been evaluated within the Division. Directions for collaborators are being drafted.

Water and Ethyl Alcohol.—This topic is being revived to develop a method to replace **35.001-35.006**.

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 101st AOAC Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. 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 edition.

Diagnostics and Test Kits

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The recent establishment of the Diagnostics and Test Kits General Refereeship recognizes the rapid expansion of this area. These diagnostics and kits offer relatively simple and quick analyses of a variety of specimens ranging from foods and drugs to clinical samples. Analytical techniques that are employed also vary considerably. They include biochemical automated microbial identification, ELISA procedures for pregnancy detection, enzyme-based test strips to determine blood glucose levels, either by the strip alone or with a portable reflectometer, and so forth. Many products are marketed for home use. Present efforts are focused on recruiting Associate Referees and establishing mechanisms for developing analytical procedures, protocols, test mechanisms, reference standards, and other means of characterizing product performance. As these issues are addressed by Associate Referees, we will proceed toward collaborative studies. Thus far, 7 individuals have indicated their willingness to serve as Associate Referees.

Five Associate Referees have actually been appointed. The areas covered include: gene probes for TB and enteric infections; immunological and diagnostic assay of pesticides, hormones, and enzymes; automated microbial identification systems; and multicomponent analysis of clinical specimens.

Recommendations

(1) Discontinue study on the topic Oxalates.

(2) Continue study on all other topics.

This report of the General Referee was presented at the 101st AOAC Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. The recommendations were reviewed by the Committee on Drugs and Related Topics. See the report of the committee, this issue.

Drug Residues in Animal Tissues

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Two U.S. federal agencies, the Food and Drug Administration (FDA) of the Department of Health and Human Services, and the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture, have leading roles in the animal drug residue area. FSIS leads in monitoring residues in animal tissues that are offered for human consumption. FDA (*1*) approves drugs that can be safely used in animals, (*2*) establishes tolerances for drug residues in food-producing animals, and (*3*) leads in the approval of analytical methods for monitoring drug residues in edible animal tissues.

In last year's report, I mentioned the formation of a task force on animal drug methodology needs with representatives from FDA and FSIS. This task force has evaluated the available determinative and confirmatory procedures and has established a list of principal needs for improved analytical procedures. FDA has developed a prioritized list of drugs that need improved analytical methods. Some sponsors of animal drugs have already been directed by FDA to improve their analytical procedures.

Both federal agencies have increased their staffing and contract funding targeted for the development of analytical methods for drug residues in edible animal tissues. Attempts are being made to exploit new technologies, for example, supercritical fluid technology. Efforts to utilize competitive binding techniques, especially as a foundation for rapid

screening procedures, and sample cleanup by solid phase extractions continue.

Although analytical methodology needs are numerous and considerable work is being done by both the drug sponsors and the agencies, it is difficult to recruit scientists to serve as AOAC Associate Referees. Perhaps interest would be stimulated if AOAC could establish a means of recognition for animal drug residue methods that have been approved by FDA and FSIS.

No Associate Referee reports were received on Dimetridazole, Levamisole, or Benzimidazoles. Work on the latter was done under an FDA-sponsored contract. The draft final contract report, including a collaborative study of the multiresidue method for thiabendazole, 5-hydroxythiabendazole, fenbendazole, oxfendazole, and mebendazole, has been received and reviewed by the Project Advisory Group. Additional information was requested in April 1987. The final contract report and the additional information were due August 15, 1987.

Recommendations

(1) Discontinue the topics Dimetridazole; Estrogenic Compounds; Levamisole; and Tiamulin (Screening Method).

(2) Continue study on all other topics.

This report of the General Referee was presented at the 101st AOAC Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. The recommendations were reviewed by the Committee on Drugs and Related Topics. See the report of the committee, this issue.

Drugs I

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p-Aminobenzoic Acid and Salicylic Acid Salts in Pharmaceuticals.—The Associate Referee, Richard D. Thompson, reported that all collaborative study results were received and that he was in the process of compiling the data and preparing his manuscript in AOAC format. The LC method on which the collaborative study was based was recently published by M. Carlson and the Associate Referee (*J. Liq. Chromatogr.* [1987] **10**, 997–1009). Mr. Thompson plans to present the collaborative study results at the 102nd Annual Meeting of AOAC.

Diethylpropion Hydrochloride.—The Associate Referee, Walter Dunbar, will present the results of his collaborative study at the 101st Annual Meeting of AOAC in San Francisco, CA. The interim official method is currently scheduled for publication in the January/February 1988 issue of the *Journal of the AOAC.*

Three topics (Aspirin and Caffeine with Other Drugs; Phenothiazine and Tricyclic Antidepressants in Formulations by LC; and Sulfamethoxazole and Trimethoprim) were discontinued.

Recommendations

(1) Continue official first action status of the liquid chromatographic method for determination of acetaminophen in tablets, **37.C01-37.C06**. As previously reported (*J. Assoc. Off. Anal. Chem.* [1987] **70**, 212–214), this method is applicable to both single-component tablets and multi-component tablets containing aspirin and caffeine. Continue study.

(2) Adopt as official first action the interim official method for LC quantitation of diethylpropion hydrochloride for bulk drug substance and tablets. Continue study.

(3) Adopt as official final action the official first action LC method for primidone in tablets, **37.A17-37.A22**.

(4) Discontinue study on the topics Aspirin and Caffeine with Other Drugs; Phenothiazines and Tricyclic Antidepressants in Formulations by LC; Primidone; and Sulfamethoxazole and Trimethoprim.

(5) Continue study on the topics Acetaminophen with Codeine Phosphate; *p*-Aminobenzoic Acid and Salicylic Acid Salts in Pharmaceuticals; and Phenothiazine-Type Drugs by TLC.

This report of the General Referee was presented at the 101st AOAC Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. The recommendations were reviewed by the Committee on Drugs and Related Topics. 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) and *J. Assoc. Off. Anal. Chem.* (1987) **70**, 385–403 (C methods).

Drugs II

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Aminacrine.—The Associate Referee, Elaine A. Bunch, reported that she is investigating LC methods for determination of aminacrine HCl in drug preparations in the presence of degradation products. The results of her recent collaborative study were published (1).

Atropine in Morphine and Atropine Tablets and Injections.—The Associate Referee, I. J. Holcomb, reported that no further work has been accomplished and recommends that the topic be discontinued because these products are no longer widely available.

Belladonna Alkaloids.—A replacement for this vacant Associate Refereeship is being sought. LC methodology for determination of the D- and L-hyoscyamine content is available (2–4). The scope of this topic would also include determination of individual alkaloids as well as ensuring the suitability of the procedure to detect any decomposition product that may be present.

Colchicine in Tablets.—The Associate Referee, Richard D. Thompson, reported that he is planning a collaborative study of an LC method for determination of colchicine in tablets in combination with probenecid. More work is being planned for methodology for determination of the decomposition product colchiceine.

Curare Alkaloids.—The Associate Referee, John R. Hohmann, reported that he investigated a proposed LC procedure for metocurine iodide (5) or tubocurarine chloride (6) content in bulk drug and injections. Initial results look promising; previous problems of interferences from decomposition products of the preservatives appear to be overcome. He recommends that it be considered for a collaborative study. Other peaks detected should also be identified.

Dicyclomine Capsules.—The Associate Referee, Henry S. I. Tan, reported the results of an intralaboratory study of his proposed GC method for dicyclomine in capsules. On the basis of these results, he is recommending that the proposed method be subjected to a collaborative study.

Epinephrine–Lidocaine Combinations.—This topic is still vacant.

Epinephrine and Related Compounds by LC—Electrochemical Detectors.—The Associate Referee, John Newton, reported that no further work was accomplished.

Ergot Alkaloids.—The Associate Referee, Thomas C. Knott, reported no further work was accomplished on this topic.

Homatropine Methyl Bromide in Tablets.—This topic is still vacant.

Morphine Sulfate in Morphine Sulfate Injection.—The Associate Referee, Ada C. Bello, reported the results of a collaborative study at the AOAC meeting (7). The proposed LC method was demonstrated to be suitable for determination of the morphine content of these products even where the samples contained decomposition products and some known contaminants. On the basis of these results, the Associate Referee recommends that the proposed LC method be adopted official first action for determination of morphine sulfate and certain contaminants in bulk drug and injections.

Neostigmine.—The Associate Referee, Rita Kling Jhangiani, reported that no further work was done and she recommends that the topic be discontinued.

Phenethylamine Drugs, Semiautomated Individual Unit Analysis.—The Associate Referee, Percy A. McCullen, reported no further work was accomplished on this topic.

Pheniramine with Pyrilamine, Phenylpropanolamine, and Phenylephrine.—The Associate Referee, Henry S. Scroggins, reported no further work was accomplished.

Physostigmine and Its Salts.—The Associate Referee, Norlin W. Tymes, reported no further work on this topic.

Pilocarpine.—The Associate Referee, Irving W. Wainer, reported no further work on this topic.

Rauwolfia Alkaloids (Reserpine and Rescinnamine).—A new Associate Referee is needed for this topic.

Rauwolfia serpentina.—The Associate Referee, Ugo R. Cieri, reported no further work on this topic. More validation work is needed before any collaborative study is undertaken.

Recommendations

(1) Adopt as official first action the LC method for determination of morphine sulfate and certain contaminants in injections and bulk drug material.

(2) Discontinue the following topics: Atropine in Morphine and Atropine Tablets and Injections; Epinephrine–Lidocaine Combinations; Homatropine Methyl Bromide in Tablets; Neostigmine; Phenethylamine Drugs, Semiautomated Individual Unit Analysis.

(3) Declare open any topic that has been inactive for an extended period (more than 2 years).

(4) Appoint new Associate Referees for the following topics: Belladonna Alkaloids; Pheniramine with Pyrilamine, Phenylpropanolamine, and Phenylephrine; Rauwolfia Alkaloids.

(5) Continue study on all other topics.

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This report of the General Referee was presented at the 101st AOAC Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. The recommendations were reviewed by the Committee on Drugs and Related Topics. See the report of the committee, this issue.

Drugs III

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Ampicillin and Amoxicillin.—No report was received this year. No further collaborative studies are anticipated. It is recommended that the topic be dropped.

Coumarin Anticoagulants.—The method described in the manuscript, "Liquid Chromatographic Determination of Coumarin Anticoagulants in Dosage Forms," by Associate Referee Ella Moore (FDA, New York Regional Laboratory) has been granted interim official first action status.

Flucytosine.—The revised method in the manuscript, "LC Determination of Flucytosine in Capsules," by Donald Shostak and Clifford Klein (FDA, New York Regional Laboratory) has been granted interim official first action status.

Halogenated Hydroxyquinoline Drugs.—No further work has been done on this topic since the reporting of the collaborative study results and the submission of a manuscript for interim first action consideration.

Hydralazine.—The revised version of the paper titled "UV Spectrophotometric Determination of Hydralazine Hydrochloride in Tablets" by Associate Referee Barry Mopper (FDA, New York Regional Laboratory) has been submitted for consideration as interim official first action.

Insulin by Liquid Chromatography.—No report has been received. This project has been inactive for over 2 years. It is recommended that the topic be dropped.

Levodopa.—The method described in the manuscript, "LC Determination of Levodopa and Levodopa-Carbidopa in Tablets," by Associate Referee Susan Ting (FDA, New York Regional Laboratory) has been granted interim official first action status.

Medicinal Gases.—This topic continues to be of considerable interest to the U.S. Food and Drug Administration. It is hoped that collaborators can be found for this study. Interested persons should contact the Associate Referee, Martin Woodhouse, at FDA, New York Regional Laboratory.

Meprobamate.—No report was received. The topic should be dropped.

Mercury-Containing Drugs.—This topic should be dropped.

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) is currently being prepared for resubmission 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.

Trimethobenzamide.—This topic should be dropped.

Recommendations

(1) Discontinue the topics Ampicillin and Amoxicillin; Insulin by Liquid Chromatography; Fluoride; Meprobamate; Mercury-Containing Drugs.

(2) Adopt as official first action the LC method for flu-

cytosine, the LC method for coumarin anticoagulants, and the LC method for levodopa and levodopa-carbidopa.

(3) Adopt as interim first action the UV spectrophotometric method for hydralazine HCl.

(4) Adopt as official final action the ion-pair column chromatographic procedure for trimethobenzamide, **36.A01-36.A06**; discontinue the topic.

(5) Adopt as official final action the microchemical identification tests for promethazine, triflupromazine, perphenazine, and triethylperazine as indicated in **36.A07-36.A10**; discontinue the topic.

(6) Adopt as interim first action the LC method for halogenated hydroxyquinoline drugs.

(7) Continue study on all other topics.

This report of the General Referee was presented at the 101st AOAC Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. The recommendations were reviewed by the Committee on Drugs and Related Topics. 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).

Drugs IV

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D- and L-Amphetamines.—Associate Referee Irving Wainer reported that a liquid chromatographic method has been collaboratively studied. The method has been adopted interim first action:

Benzodiazepines.—Associate Referee Eileen Bargo is planning a collaborative study on flurazepam. For oxazepam in tablets and capsules, **40.014**, she plans to evaluate one more manufacturer 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 set up a collaborative study for heroin HCl. Of the 12 volunteers, only 3 submitted results. He is planning a new study, using different collaborators.

Recommendations

(1) Adopt as official final action the official first action liquid chromatographic method for determination of diazepam in drug tablets, **40.A01-40.A06**. Continue the topic.

(2) Adopt as official first action the interim official first action liquid chromatographic method for D- and L-amphetamines.

(3) Continue study on all other topics.

This report of the General Referee was presented at the 101st AOAC Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. 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 edition, and to "Changes in Official Methods," *J. Assoc. Off. Anal. Chem.* (1985) **68**, 369–411 (A methods).

Drugs V

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Conjugated Estrogens in Drugs.—The elaborate colorimetric method (**39.001-39.005**) is tedious and nonspecific. The substances which are indicated (equilin, estrone, and estrone sodium sulfate) currently have LC methods in the *U.S. Pharmacopeia* (USP). The Referee recommends surplusing this method.

Conjugated Estrogens by LC.—The Associate Referee's developmental work was published (*J. Chromatogr. Sci.* [1985] **74**, 201–204). However, this derivative-fluorescence approach now appears to be too cumbersome. Continued study is recommended, but using a more direct approach such as ultraviolet absorption measurement.

Dexamethasone Phosphate in Drugs.—The Referee recommends continuation of the first action status of this nonspecific, spectrophotometric method (**39.056-39.060**). In addition, it is recommended that this method be surplused. The USP has an LC method for these preparations.

Digitoxin, Automated Individual Tablet Analysis.—The Associate Referee has been unable to work on this topic because of more pressing assignments. Furthermore, it now appears that LC analysis is a better approach. Discontinuation of the topic is recommended.

Hydrocortisone.—The method (**39.047-39.055**) has been official first action for over 2 years. It is being used regularly and no problems have been reported. The Referee recommends adoption as official final action and discontinuation of the topic.

Methocarbamol.—The method, (**36.212-36.218**) has been official first action for over 2 years. It has been used successfully on numerous occasions and there have been no adverse reports. Adoption of the method as official final action and discontinuation of the topic is recommended.

Miconazole.—The Associate Referee has changed positions and is no longer able to work on this topic. A gas chromatographic method is specified in USP. Discontinuation of the topic is recommended.

Progestins in Tablets.—The Associate Referee has not been able to work on this topic due to other priorities and he does not foresee any future opportunities. A collaborative study was conducted using an automated scheme, but with fewer than the required participants. The results indicated that the method works well and it is now being used successfully. In view of the difficulties of obtaining the proper number of participants for this type of a study, it is recommended that the information obtained be published and that the topic be discontinued.

Steroid Acetates.—Adoption as official first action of the method now undergoing interim review is recommended. The results were most satisfactory and the method indeed shows great promise. Continued study is recommended.

Steroid Phosphates.—Adoption of the interim method as official first action is recommended. There are 4 parts to this study: liquid chromatographic determination of dexamethasone in bulks and elixers, infrared identification of dexamethasone, thin layer chromatographic identification of dexamethasone in elixers, and gas chromatographic determination of alcohol in elixirs. All 4 parts had excellent collaborative results. Continued study is recommended.

Recommendations

(1) Surplus the following methods: conjugated estrogens in drugs, **39.001-39.005**; dexamethasone phosphate in drugs, **39.056-39.060**.

(2) Adopt as official first action the following interim official first action methods: LC method for determination of dexamethasone acetate in suspensions; LC determination of dexamethasone, TLC identification of dexamethasone in bulk and elixirs, IR identification of dexamethasone, and GC determination of alcohol in dexamethasone elixirs. Continue study.

(3) Adopt as official final action the following official first action methods: LC determination and IR identification of hydrocortisone in bulk and tablets, **39.047-39.055**; LC determination of methocarbamol in injections and tablets, **36.212-36.218**.

(4) Discontinue the topics Digitoxin, Automated Individual Tablet Analysis; Hydrocortisone; Miconazole; Progestins in Tablets—Automated Methods.

(5) Continue study on all other topics.

This report of the General Referee was presented at the 101st AOAC Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. 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 edition.

Immunochemistry

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Development of Quality Standards Criteria for Monoclonal Antibodies Used in Immunoassays

In the development of any immunoassay, i.e., ELISA, RIA, etc., criteria for selection of the antibody is defined with regard to "affinity" and "specificity." There are various levels of effort in antibody selection, and successful immunoassays are usually associated with more rigorous determinations of the antibody quality. To enable evaluation of a "kit" or immunoassay product, a user should be informed of the antibody quality and hence the quality of the assay itself. For these reasons as well as others, e.g., insurance of "long term quality" and reproducibility, the first project in this area will be to develop criteria for evaluation of monoclonal antibody quality and later to apply the standards developed in collaborative studies of various immunoassay applications.

Background and Approach: With support of the California Department of Agriculture, projects have been initiated and are underway for development of monoclonal antibodies to various environmental compounds in the laboratories of Bruce Hammock, University of California at Davis, in collaboration with Alex Karu, University of California at Berkeley. Target compounds that have been defined include: (a) triazines; (b) carborfurans; (c) thiocarbamates; and (d) certain bacterial toxins used as biorational pesticides. Once the monoclones have been selected and antibodies produced, collaborative evaluation studies will be undertaken in various laboratories.

Timetable: In 1988, at least one monoclonal antibody evaluation will be planned and initiated with at least 4 other participating laboratories. A draft document will be prepared by the Associate and General Referees, outlining the study protocol. A position paper will also be drafted outlining the proposed quality evaluation criteria and standards.

Development of Study Design and Protocol for Collaborative Study on Immunoassay of Pyridostigmine

Pyridostigmine (3-hydroxy-1-methyl pyridinium bromide dimethylcarbamate) is a drug used in the treatment of organophosphate poisoning and in the treatment of the autoimmune disease, myasthenia gravis. Plasma and metabolite assays are important for assessing and planning of treatment and assessment of toxicity. An immunoassay for pyridostigmine, utilizing polyclonal antibody preparation, has been developed and is now being evaluated.

Background and Development of Collaborative Study Design: Antibodies to pyridostigmine have been developed in laboratories of the U.S. Army Medical Research and Development Command. A number of studies have been carried out with regard to the quality and potential of the immunoassay for quantitation of pyridostigmine. The data support consideration of an interlaboratory study on the immunoassay method. In 1988, a protocol will be developed and submitted for approval. Participating laboratories and an Associate Referee will be chosen and the study will be initiated.

This report of the General Referee was presented at the 101st AOAC Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. 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, was to have reported on his study on ash. However, because of other work he still has not finished a written report although the collaborative work has been completed. Dr. Farrell has also completed a study under ISO auspices which he intends to write in AOAC format for submission as an AOAC method.

John W. Newton, Associate Referee on Methyl Xanthines, lacked the time to work on his planned work on this topic.

During the year, Daniel J. Zuccarello was appointed Associate Referee to study a liquid chromatographic method for caffeine in coffee. The appointment came so late that he was unable to get his study started this year.

B. Denis Page, Associate Referee on Solvent Residues in Decaffeinated Coffee and Tea, did not make a report.

Although not appointed as an AOAC Associate Referee on Water Extract in Tea, Francis Farrell has been actively leading an ISO study of a modification of the AOAC method. He will submit his report when he has converted it to AOAC format.

Recommendations

(1) Appoint an Associate Referee on the topic Water Extract in Tea.

(2) Continue study on all topics.

This report of the General Referee was presented at the 101st Annual International Meeting, Sept. 14-17, 1987, at San Francisco, CA. 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.—Associate Referee Graham MacEachern has been asked to consider the International Dairy Federation (IDF) studies on fatty acid analysis and their relevance to adulteration monitoring.

Automated Methods for Fat, Turbidimetric Methods.—Associate Referee Frank Shipe suggests that these studies be discontinued since the infrared methods have superseded turbidimetry except with in-line milk plant control instruments. He also suggests discontinuation of the **16.074-16.082** method. Aldo Conetta, Technicon, agrees, and Associate Referee Ray King has not forwarded comments.

Babcock, Mojonnier, and Kjeldahl Tests.—David M. Barbano was appointed Associated Referee, has submitted a collaborative study, and is submitting a poster this year to justify modifications of the Babcock and Mojonnier methods for fat in milk.

Babcock Test and Babcock Glassware.—Associate Referee Robert L. Bradley requested changes in *Official Methods of Analysis* to standardize the test for cream to allow 8 min centrifuge time. He is conducting a collaborative study on improperly marked Babcock test bottles, and he is obtaining bottles with different markings that may be easier to read.

Calcium, Phosphorus, and Magnesium in Cheese.—Associate Referee Roger Pollman conducted a collaborative study, but the overall variance for Ca/P was too large. He is requesting an additional study with modifications to improve these statistics.

Casein and Caseinates.—Associate Referee Charles Pyne reported no activity in this area.

Fat in Butter, Direct Method.—Associate Referee Doug Engebretsen suggested repeal of the method **16.233** in favor of the Kohman method (SMEDP-15, 18.9C) because the latter has significantly lower estimates for fat.

Fat Test in Chocolate Milk.—Associate Referee James Marshall reported no work being done in this area and requested discontinuation of the study.

Gerber Test for Fat in Milk.—Associate Referee Dick Kleyn reports that publication is pending in the *J. Assoc. Off. Anal. Chem.*

Ice Cream and Frozen Desserts.—Margreet Lauwaars requested returning **16.309** from surplus status because of Codex requirements.

Infrared Milk Analyses (IRMA).—Associate Referee D. L. Biggs and the General Referee have sought considerable assistance in writing the calibration procedures for infrared instrumentation. These should be completed this year.

Iodine.—Associate Referee Dave Sertl is organizing a collaborative study on the method reported last year.

Laboratory Quality Control.—Associate Referee Ray Matulis has no plans to publish the poster paper guidelines for control sampling.

Lactose by Chromatographic Determination.—Leslie West is publishing the method in the *J. Assoc. Off. Anal. Chem.* but requests replacement as Associate Referee.

Lactose by Enzymatic Determination.—Associate Referee Dick Kleyn reported that no studies are underway in this area.

Moisture in Cheese (Microwave).—Associate Referee Judy Arey requests official status for the microwave oven method for moisture in Cheddar and cottage cheese and requests that the current write-up be changed to reflect the data generated in recent studies.

Moisture in Cheese.—Associate Referee Ron Case is cooperating with the National Cheese Institute to supervise extensive comparative studies at the University of Wisconsin, Madison. The studies will examine Cheddar, Mozzarella, and processed cheeses, and cheese spread using several drying and preparation techniques. The in-depth comparative study is considered adequate to refine the techniques for this assay.

Mojonnier Robot.—The General Referee requested that Robert L. Bradley serve as Associate Referee for the Zymate II method assembled by Forcoven Products, Humble, TX. Three units may be operational by 1988 so that a collaborative study can be initiated.

Protein Reducing Substances Test.—Associate Referee Joe Cardwell suggests continuation of studies to rapidly identify milk powder and other proteins added to milk.

Protein in Milk (Dye Binding).—Associate Referee Doyle Udy has been asked to assemble the data to allow the acid

orange-12 dye binding method to be more usable in calibration of mid-infrared instrumentation.

Reactivated Phosphatase.—Associate Referee G. K. Murthy is conducting a collaborative study on the American Public Health Association rapid colorimetric method and AOAC official method **16.304-16.306** for phosphatase in selected cheeses. Associate Referee Dick Kleyn is conducting reactivation studies on alkaline phosphatase.

Tyramine.—Thea Reuvers accepted the opportunity to serve as Associate Referee and is preparing a collaborative study and seeking collaborators.

Whey Proteins in Nonfat Dry Milk.—Method was again recommended for official first action.

The General Referee recommended appointment of Dick Kleyn, Rutgers University, as Associate Referee for Fat in Milk, Gerber Method, and Robert L. Bradley as Associate Referee for Mojonnier Robot.

No reports were received on the topics Chloride Meters, Whey Proteins in Nonfat Dry Milk, Milk Fat Test—AutoAnalyzer, Nitrates in Cheese, Phosphorus, Protein Constituents in Processed Dairy Products, Protein in Milk—Rapid Methods, and Nonfat Solids.

Invitations to serve as Associate Referees for gas chromatographic determination and titrimetric determination of free fatty acids, pH measurements in cheese, detection of heat treatment of milk, methods for whey protein in nonfat dry milk, fat in milk (turbidimetric), and fat analysis in butter have not been accepted. We need an Associate Referee to accept the challenge to work on vitamins A and D fortification problems in response to a challenge from the National Association of State Departments of Agriculture (Policy No. DFD-23, 1986).

Recommendations

(1) Approve the changes suggested in the Babcock and Mojonnier tests for fat.

(2) Appoint Robert L. Bradley Associate Referee for robotic Mojonnier method.

(3) Include in "Changes in Methods," 4th supplement, the following change in paragraph **16.178(a)**: "... centrf. 5 min. Fill bottle to neck with hot H_2O and centrf. 2 min. Add hot ..."

(4) Approve as official first action the Olieman method for measurement of whey proteins in nonfat dry milk.

(5) Approve as official first action the microwave methods for moisture in Cheddar and cottage cheese.

(6) Include precision statistics by Drudgington for fat in dry milk in *Official Methods of Analysis,* 15th Ed.

(7) Approve as official first action the mid-infrared spectroscopic method for fat, lactose, protein, and total solids in milk (**16.083-16.092**) and approve the use of "A," "B," and "A + B" filters for measurement of fat in milk.

(8) Return **16.309** (**16.220-16.221**, 11th Ed.) from surplus status.

(9) Initiate repeal of method **16.233**, the direct method for fat in butter.

(10) Approve "Determination of Phosphorus in Processed Cheese," IDF Bulletin 207, pp. 41–93.

(11) Approve "Enzymatic Determination of the Lactic Acid and Lactates Content of Dried Milk," IDF Bulletin 207, pp. 94–121.

(12) Approve "Flame Photometric Determination of Sodium, Potassium, and Calcium Contents of Dried Milk," IDF Bulletin 207, pp. 122–132.

(13) Add precision values to Thermistor Method **16.096** from "Determination of the Freezing Point of Milk by Means of the Thermistor Cryoscope," IDF Bulletin 207, pp. 198–207.

(14) Request that Tim Peeler be invited to provide precision parameters for inclusion in *Official Methods of Analysis,* 15th Ed.

(15) Establish a procedure whereby methods previously approved by the American Public Health Association Technical Committee working on *Standard Methods for the Examination of Dairy Products,* 15th Ed., may be included in *Official Methods of Analysis.*

(16) Discontinue Fat Test in Chocolate Milk.

(17) Replace Ray Matulis with Ron Case (same address) as Associate Referee for Moisture in Cheese.

(18) Discontinue studies on Automated Methods for Fat Analysis, Turbidimetric Methods, as suggested by Frank Shipe. These include methods **16.074-16.082** and **16.068-16.073**. Initiate repeal of method **16.074-16.082**.

(19) Appoint Doyle Udy to be Associate Referee for Fat in Dairy Products, Udy Turbidity Test.

This report of the General Referee was presented at the 101st Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. 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 edition.

Decomposition and Filth in Foods (Chemical Methods)

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Ammonia in Dogfish.—Associate Referee Beverly Hunter reported that the second collaborative study on the enzymatic procedure for the determination of ammonia in dogfish is ready to begin. The General Referee concurs with the recommendation of the Associate Referee that the study be continued.

Gas and Liquid Chromatography.—Samples of canned tuna are in preparation for a collaborative study of the GC procedure for the determination of cadaverine and putrescine (*J. Assoc. Off. Anal. Chem.* [1981] **64,** 584–591). The levels of the compounds to be included in the study cover the range 0.5–50 μg/g. The Referee recommends continued study.

Although there were no reports on the topics Ethanol in Seafoods, Coprostanol, Crabmeat, GC Determination of Volatile Amines-TMA and DMA, and TLC Determination of Amines in Fishery Products, the General Referee recommends that study be continued.

Recommendations

(1) Appoint an Associate Referee on the topic Shellfish Decomposition and continue study.

(2) Continue study on all other topics.

This report of the General Referee was presented at the 101st Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. The recommendations were reviewed by the Committee on Foods I. See the report of the committee, this issue.

Fish and Other Marine Products

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Determination of Fish Content in Coated Products (Breaded or in Batter).—Associate Referees Frederick J. King and H. Houwing did not submit a report this year. In a personal communication with King, he has advised that the West European Fish Technologists' Association (WEFTA) has not had an opportunity to extend the study of the 3 procedures because of other priorities. WEFTA is currently concentrating its effort to develop improved methodology for microbiological analyses of fish products. It is, therefore, recommended that the AOAC European representative contact Associate Referee H. Houwing to determine whether WEFTA will have the time and resources to continue with the subject study. The General Referee recommends continued study.

Drained Weight of Block Frozen, Raw, Peeled Shrimp.—The Associate Referee initiated the collaborative study, but completion of the study has been delayed because of the seasonal nature of the product. The study consists of examining 3 groups of 12 blocks of frozen, raw peeled shrimp by the procedure developed by the Associate Referee. The first group of blocks has been examined, and the results have been submitted. The second group of shrimp blocks has now been prepared and shipped to the collaborators. The Associate Referee is confident that the final group of blocks of frozen shrimp can be prepared and shipped to the collaborators within the next several months. The General Referee recommends continued study.

Minced Fish in Fish Fillet Blocks.—Associate Referee J. Perry Lane submitted a report last year describing the results he obtained in a collaborative study that he conducted using only cod fillets. Since then, Lane retired and Frederick King was appointed Associate Referee to continue the study. After a title change in the Associate Referee's report, the method was accepted and a recommendation was made to adopt it as an interim first action method. The subject was changed to Minced Fish Flesh in Mixed Fillets–Minced Cod Blocks. The newly appointed Associate Referee has recommended that other Gadoid species, such as Alaska pollock, be studied. The General Referee concurs with this recommendation.

Nitrites in Smoked Fish.—The General Referee recommends that an Associate Referee be appointed to conduct a collaborative study using the method developed.

Organometallics in Fish.—By mutual agreement with the General Referee for Metals and Other Elements and with the concurrence of the Association, this subject was recommended to be reassigned to the General Referee for Metals and Other Elements. The general Referee concurs with this recommendation.

Other Topics.—The current official final action method for "Total Solids for All Marine Products except Raw Oysters" is method **18.023**. This method uses asbestos fibers in the determination. However, because of current safety concerns regarding the use of asbestos fibers, the General Referee is recommending that sand be substituted for asbestos in the cited method. Ten to 15 g washed sand would be a suitable substitute for the 2 g asbestos fibers. In the original "Report on Total Solids in Fish and Other Marine Products," *J. Assoc. Off. Anal. Chem.* (1952) **35,** 216, the Associate Referee mixed the product with sand, pumice, or asbestos to facilitate the removal of moisture. All 3 gave satisfactory results although the Associate Referee judged that the results obtained with asbestos fibers were slightly superior.

Recommendations

(1) Adopt as official first action the interim official action method for minced fish flesh in mixed fillets–mixed cod blocks.

(2) Continue study on all other topics.

This report of the General Referee was presented at the 101st Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. 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 edition.

Food Additives

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Antioxidants.—A paper was presented at the Spring Workshop by the Associate Referee on the "Confirmation of Antioxidants by HPLC-Diode Array Detection." The diode array detector was most useful for the confirmation analysis of the more interference-prone dried foods, e.g., snack foods and soup mixes as well as salad dressings.

In the FDA laboratory, work was undertaken to study the reactions of BHA in deep-fat frying with a model fat system consisting of 90% methyl stearate and 10% methyl linoleate. The results closely paralleled those obtained with studies utilizing animal fat to prepare french fried potatoes.

In summary, the BHA level dropped to less than 15% of its initial level, whereas >85% of the decomposition products of BHA were retained in the fat. Chromatographic analysis of the reaction products revealed the presence of a wide variety of substances directly derived from BHA. Two prominent peaks did, however, rise above a broad spectrum of products that coalesced into a single elongated mound. All of these products on reverse phase chromatography were less polar than BHA; however, thin-layer chromatography (TLC) on silica gel revealed that the decomposition products are much more polar than BHA. This phenomenon may be explained by assuming that BHA-derived products are stable in deep-fat frying medium, but that they react readily with oxygen when deposited on a TLC plate. This is exactly what one would expect for substances that provide the carry-through antioxidant activity found in french fries prepared in BHA-protected fat. Work is presently underway to identify these substances.

Brominated Vegetable Oils (BVOs).—Little work was done during this past year due to higher priority projects. Capillary gas chromatography (GC) after acid-catalyzed transesterification appears to be the gel technique for characterizing BVOs. Although both the liquid chromatographic (LC) and the capillary GC systems completely resolve the di-, tetra-, and hexabrominated methyl stearates, gas chromatography is better than liquid chromatography for analyzing citrus based soft drinks. Plans to collaboratively study the capillary GC method next year are under consideration.

The LC method has been published (*J. Liq. Chromatogr.* [1987] **10,** 205).

Indirect Additives from Food Packaging.—The following is a summary report of the activities worked on during the past year.

(1) PVC: Extensive studies of the migration characteristics

of rigid and flexible PVC have been conducted with one exception—the verification that 95% ethanol is a suitable food-stimulating solvent for plasticized polyvinyl chloride. Initial experiments indicate this to be true.

(2) PET: A rapid screening procedure has been used to obtain qualitative analysis of apparent residuals present in polyethylene terephthalate (PET) soft drink bottles obtained from 4 different manufacturers and resin obtained from one commercial source. The identifications were made based on LC retention volumes and have not been confirmed by mass spectroscopy. Extractions of PET were performed in dimethyl acetamide at 60°C. Assuming somewhat similar UV absorption coefficients, the relative amounts of residuals in the bottles followed this order: MET = E1/2AE > DMT > BHET > TA, where MET = methyl ethyl terephthalate, E1/2AE = half ethyl ester of TA, TA = terephthalic acid, DMT = dimethyl terephthalate, BHET = Bis hydroxy ethyl terephthalate. All had TA present, some having more than others.

(3) CPET: Crystallized PET (CPET), a primary polymer used for dual ovenable trays, has been examined. Trays from commercial manufacturers have been characterized. The LC elution profile of the CPET tray extracts appears similar to that of the PET bottle, except MET is more abundant.

Work is underway to find a suitable fatty food-simulating solvent for studying migration of residues from PET food packaging materials. Such investigations require that the initial concentration of the adjuvant in the polymer be known before migration experiments are undertaken. Two methods have been compared. One is based on dissolving and reprecipitating the polymer and analyzing the resulting solution. The other is based on using size exclusion chromatography to separate solution fractions for separate analysis by reverse-phase liquid chromatography.

The solution/reprecipitation method is based on dissolving the polymer (PET) in trifluoroacetic acid and slowly precipitating the polymer by adding chloroform, acetone, water, and ammonium hydroxide. The precipitated polymer and solution are separated, and the solution is analyzed by liquid chromatography.

Size exclusion chromatography is being studied as the technique of choice to separate and quantitate DMT and TA in PET articles. This method involves dissolving the polymer in hexafluoroisopropyl alcohol and collecting the low molecular weight fraction to be quantitatively analyzed by liquid chromatography.

(4) Benzene in Polypropylene: A new catalyst is being used in the production of polypropylene (PP). In addition to increasing polymer yield, it also increases the level of benzene residue in the polymer.

Work was initiated to (a) develop a screening method for determining low levels (ppb) of benzene in PP articles and (b) survey a limited sample of food packaging articles for residual benzene. The screening method employs a photoionization detector permitting detection limits of 10 to 20 ppb. Analysis is done by dissolving 10 g polypropylene in 150°C hexadecane in a septum-sealed vial, sparging with helium, and then trapping the volatiles in a 0°C methanol trap. An equal volume of water is added to the methanol, and the solution is analyzed by headspace gas chromatography on an OV-1 megabore column.

In screening for benzene in polypropylene food contact materials, relatively low levels of apparent benzene ($\leq$0.5 ppm) have been detected in the 19 samples examined. Somewhat higher levels (low ppm) of benzene were previously found in other food contact materials such as polystyrene cups and tubs. Samples examined included cookie trays, syrup bottles, salad containers, general use cups, and yogurt cups. Apparent benzene findings ranged from about 3 to 530 ppb. Samples containing the higher levels of apparent benzene are being examined by mass spectrometry for confirmation.

Nitrosamines in Foods.—A considerable effort was applied to methods development, instrument modification, and detection systems for the determination of nonvolatile *N*-nitroso compounds in foods and food-contact materials.

The Associate Referee compared 3 methods for the determination of *N*-nitrosamino acids in cured meats: (*1*) Sen et al. (1980) *IARC Sci. Publ.* **31,** 457–463; (*2*) Tricker et al. (1984) *Food Addit. Contam.* **1,** 245–252; (*3*) Sen et al. (1982) *IARC Sci. Publ.* **41,** 185–197.

Although all 3 methods gave comparable results for NPRO and NTCA, (*1*) and (*2*) produced cleaner extracts and resulted in cleaner chromatograms and would thus be suitable for AOAC collaborative studies. The last method (*3*) is better suited for rapid screening purposes.

In addition, 2 reports must be written and submitted for publication by N. P. Sen and coworkers: "Further Studies on the Formation of Nitrosamines in Cured Pork Products Packaged in Elastic Rubber Nettings" reports that traces of *N*-nitrosodibutylamine (NDBA) and *N*-nitrosodiethylamine (NDEA) can form due to the interaction of nitrite in meat and amines in rubber, in cured pork products packaged in elastic rubber netting. Studies were conducted to determine if taking the netting off immediately after processing or if placing a layer of collagen as a barrier between nettings and meat could prevent or minimize formation of the aforementioned nitrosamines. None were effective. Cured pork products packaged in a reformulated rubber netting, made with different amine additives, contained either none or only negligible levels of NDBA or NDEA, but, they did contain 15–104 ppb levels of a new nitrosamine, *N*-nitrosodibenzylamine, which is reported to be noncarcinogenic.

The second study, "An Investigation on the Possible Presence of Morpholine and *N*-Nitrosomorpholine in Apples Coated With Waxes" reports that fairly high levels (1.1–3.85%) of morpholine (MOR) and traces (140–670 ppb) of *N*-nitrosomorpholine (NMOR) were detected in 8 commercial samples of liquid waxes used for coating apples. Also, low levels (0.25–7.7 ppm) of MOR, but no NMOR, were detected in 17 batches of apples coated with these waxes. Uncoated apples were negative for both MOR and NMOR. Although MOR is easily nitrosated, incubation of wax-coated apples with 10 ppm nitrite under simulated gastric conditions did not form any detectable levels of NMOR (detection limit, 0.5 ppb). Therefore, the possibility of formation of NMOR in the human stomach, after ingestion of such wax-coated apples, is highly unlikely.

Polydimethylsiloxane in Foods.—Two methods have been developed: (*1*) "Polydimethylsiloxane in Pineapple Juice By Atomic Absorption Spectroscopy" and (*2*) "Polydimethylsiloxane in Vegetable Oils By Atomic Absorption Spectroscopy." The methods and collaborative study protocols have been reviewed and approved. Participants for the studies have been obtained, primarily from the European scientific community. Preliminary ruggedness and familiarization studies are currently underway.

Polycyclic Aromatic Hydrocarbons in Foods.—Work in this area involved the completion of a survey of fast-food (hamburger) establishments. Hamburgers from 10 different restaurants, all using an open flame to charbroil the meat, were examined. Eight PAHs were found and confirmed by liquid

chromatography-scanning UV. The compounds were fluoranthene, pyrene, benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, benzo(ghi)perylene, and indeno(cd)pyrene. The levels found ranged from undetected to over 20 ppb and varied from establishment to establishment. Apparently, the position of the flame relative to the meat being cooked was a significant factor in the deposition of PAHs on the hamburgers. A manuscript is in preparation for publication.

Future studies will involve determination of PAHs in foods and food contact materials (packaging, etc.).

Sulfiting Agents in Foods.—A collaborative study jointly sponsored by the National Food Processors Association and FDA was conducted of FDA's optimized Monier-Williams method for determining sulfites in foods (*Fed. Regist.* [July 9, 1986] **51** (131), 25012–25024). Twenty-one industry and government laboratories participated in the study. The study design involved 3 food matrixes (hominy, juice, and seafood), each prepared at 4 sulfite levels—the regulatory level, half the regulatory level, twice the regulatory level, and a blank. All test portions were analyzed as blind duplicates, giving each collaborator a total of 24 samples. Samples were monitored throughout the course of the study.

Results indicated a reproducibility (among laboratories) coefficient of variation, for those 3 foods at 10 ppm, ranging from 15.5 to 26.6%.

It is recommended that the method be adopted official first action for the quantitation of sulfites in foods.

The Associate Referee, Charles R. Warner, has developed an LC method and has submitted it for publication. The manuscript is entitled "Sulfite Stabilization and High Performance Liquid Chromatographic Determination: A Reference Method for Free and Reversibly Bound Sulfite in Food." Briefly, a liquid chromatographic (LC) postcolumn reactor combination was developed for the determination of sulfite (free and reversibly bound) in 15 different foods. The foods were treated with a pH 5.1 buffered aqueous 2% formaldehyde solution to convert the labile sulfite to the relatively stable hydroxymethylsulfonate. Reverse-phase, ion-pairing liquid chromatography with postcolumn detection consisting of an initial reaction with KOH followed by colorimetric reaction with Ellman's reagent buffered at pH 6.3 was used for separation and quantitation.

The photometric detector at 412 nm quantitated the strongly absorbing 3-carboxy-4-nitrothiophenolate anion, the product of the sulfite reaction with Ellman's reagent. The recovery at levels of 5–100 ppm as sulfur dioxide was >90% by reverse isotope dilution assay. Repetitive analysis of a single grape juice extract containing 20 ppm SO_2 showed a relative standard deviation of 2.2%.

Recommendations

(1) Adopt as official first action 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.

This report of the General Referee was presented at the 101st Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. The recommendations were reviewed by the Committee on Foods I. See the report of the committee, this issue.

Meat, Poultry, and Meat and Poultry Products

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During the past year, collaborative studies were conducted in 5 topic areas: Minimum Processing Temperature, Nitrosamines in Bacon, Nonmeat Proteins in Meat, Protein in Meat and Meat Products—Mercury vs Copper Catalysts, and Sample Preparation Techniques for Meat Analysis. Four Associate Refereeships remain vacant.

A mini collaborative study has been conducted by CEM Corp. for the use of Freon TF in lieu of methylene chloride in the CEM microwave instrument for total fat analysis in meats. The results of that study will be evaluated for possible inclusion of Freon TF as an alternative solvent in method **24.A04**.

The U.S. Department of Agriculture, Food Safety and Inspection Service (FSIS), conducted two 96-sample studies on new methodologies. The first was done with CEM Corp. on their microwave digestion instrumentation for determination of total protein in meat. Analytical values compared favorably with those obtained from the same samples analyzed by official method **24.027**. When more commercial instruments are available, CEM would like to do a full collaborative study. To that end, a new Associate Refereeship is recommended. The second 96-sample study was conducted with Udy Corp. for the dye binding technique for total protein in meat. This test was found not to be useful for regulatory purposes due to excessive variability. No collaborative study is anticipated.

The Codex Alimentarius Commission Joint FAO/WHO Food Standards Program met in Rome, Italy, in June 1987. The Codex Committee on Residues of Veterinary Drugs in Foods issued a priority list of veterinary drugs requiring evaluation: chloramphenicol, anabolic agents (estradiol, progesterone, testosterone, trenbolone acetate, zeranol), sulfonamides, nitrofurons, nitroimidazoles, quinoxaline-di-*N*-oxides, and trypanocides. AOAC should take an active role in studying analytical methodology for these compounds in meat.

Automated Methods.—Vacant.

Bioassay Methods for Meat and Poultry Products.—Vacant.

Chemical Antibiotic Methods.—The Associate Referee, William Moats, is presenting a paper at the 1987 Annual International Meeting on a newly developed method for novobiocin residues and hopes to conduct a collaborative study during the coming year.

Crude Protein Analysis of Meat (Peroxymonosulfuric Acid Digestion and Improved Nesslerization).—The Associate Referee, David Christians, continues study on this topic. The concomitant total phosphorus method has been successfully collaboratively studied and reviewed by the Committee on Foods I. It will be considered for adoption when the authors complete recommended manuscript changes.

Fat in Meat Products.—The Associate Referee, Max Foster, presented a paper at the 1987 Annual International Meeting comparing total fat analysis in various meat products by the Soxhlet and the Tecator Soxtec Systems (Randall application of the Soxhlet procedure). He has designed a collaborative study for the Soxtec apparatus.

Immunochemical Identification of Additives in Meat Products.—The Associate Referee, Ronald Berger, has developed

an immunochemical method for the identification of species in cooked products and hopes to conduct a collaborative study during the coming year, as soon as reagents become commercially available.

Histological Identification Methods.—Vacant.

LC Methods for Meat Products.—The Associate Referee, M. Sher Ali, is preparing a manuscript for publication of the LC method developed for the simultaneous determination of 10 different carbamate pesticide residues in meat.

3-Methyl Histidine.—The Associate Referee, Roger Wood, reports that no further progress was made on this topic during the past year.

Minimum Processing Temperature.—Jim Eye has resigned as Associate Referee for this topic. The new Associate Referee is Grover Pickle, Special Projects Chemist, USDA-FSIS, Science, Eastern Laboratory, Athens, GA.

During the past year, a collaborative study on the use of the APIZYM and Boehringer Mannheim enzyme test kits to detect undercooked canned hams was conducted. USDA requires all pork products from countries not free from exotic animal diseases be cooked to 156°F prior to exportation to the United States. If test kits could be used at ports of entry to detect undercooked product, USDA would save a considerable amount of resources. The Boehringer kit for acid phosphatase enzyme activity appears to be insensitive for the amount of enzyme present in the product tested between 150°–156°F and, therefore, could not be used for this screening determination. Of the 19 enzymes tested by the APIZYM system, 2, valine and leucine amino peptidases, might be useful for a "go/no go" screen at 156°F. A manuscript describing this study and its evaluation is being prepared for submission to *J. Assoc. Off. Anal. Chem.*

Nitrates and Nitrites.—Vacant. Interest continues in this topic, especially on the question of reagent and sample blanks.

Nitrosamines in Bacon.—The Associate Referee, Walter Fiddler, presented a paper at the Institute of Food Technologists' annual meeting, June 1987, in Las Vegas, NV, on a collaborative survey for nitrosopyrrolidine (NPYR) in dry cured bacon after frying. Conclusions included that NPYR is not of major concern for this product. During the coming year a collaborative study for dimethylnitrosamine in minced fish/meat formulations will be conducted under the General Referee for Food Additives, Thomas Fazio.

NMR Systems for Meat Analysis.—The Associate Referee, Michael A. Wilson, reports no activity on this topic due to the lack of funding. It is recommended that this topic be discontinued.

Nonmeat Proteins in Meat.—The Associate Referee, Christopher Hitchcock, reports that the official first action semiquantitative ELISA test for soy in meat has been converted to a commercially available kit form, and a collaborative study for the kit has been completed. A manuscript entitled "Determination of Soya Protein in Meat Products by a Commercial Enzyme Immunoassay Procedure: Collaborative Trial" has been submitted to *J. Assoc. Publ. Anal.* for publication. In addition, a poster was presented at the 1987 AOAC Annual International Meeting.

Protein in Meat and Meat Products—Mercury vs Copper Catalysts.—The Associate Referee, Carolyn Henry, reports that the U.S. Department of Agriculture, FSIS, has completed a collaborative study on this topic under contract with Webb Foodlab, Inc., Raleigh, NC. Preliminary evaluation of the data indicates that copper catalysts have about a 0.1% negative bias for protein in meat when compared to mercury. However, due to environmental concerns and local ordinances against the use of mercury, many laboratories have switched to copper catalysts. For most regulatory and quality control work, the low bias may not be of concern. Further study on optimization of the copper catalyst formulation may eliminate the bias. A manuscript of this study is being prepared for publication and recommendation to AOAC.

Sample Preparation Techniques for Meat Analysis.—This is a new topic this year. The Associate Referee, Sylvan Eisenberg, reports on a collaborative study underway for the use of commercial food processors to prepare meat samples for analysis in lieu of the conventional meat chopper (grinder). He conducted a poster presentation at the 1987 AOAC Annual International meeting.

Serological Identification of Animal and Poultry Species.—The Associate Referee, Arthur Marin, reports that the "ORBIT" and "PROFIT" tests are now official first action. He recommends that the U.S. Department of Agriculture, FSIS, collaboratively study its newly developed method for the determination of species in cooked product. The developers of the procedure, Richard Mageau and Ronald Berger, have prepared a manuscript describing the procedure and are considering an interlaboratory study.

Species Identification by "Orbit" and "Profit".—The Associate Referee, Mark Cutrufelli, has successfully studied these procedures and they have become official methods. The General Referee and the Association recognize Mark's exemplary efforts. This topic may now be discontinued.

Specific Ion Electrode Applications.—The Associate Referee, Randy Simpson, reports no further work on this topic during the past year. Additional statistical evaluation is being prepared on the collaborative study for sodium, potassium, and chloride in processed meat products using ion selective electrode analysis.

Recommendations

(1) Seek new Associate Referees for the topics Automated Methods, and Nitrates and Nitrites.

(2) Discontinue the topics Bioassay Methods for Meat and Poultry Products, Histological Identification Methods, NMR Systems for Meat Analysis, and Species Identification by "ORBIT" and "PROFIT."

(3) Initiate a new Associate Refereeship for Microwave Digestion of Meat and Poultry Samples for Total Protein Determination.

(4) Associate Referee Ronald Berger, Immunochemical Identification of Additives in Meat Products, should conduct a collaborative study on the U.S. Department of Agriculture's method for species identification in cooked product.

(5) Evaluate manuscripts (when submitted) for possible official action recommendations on the completed collaborative studies for Protein in Meat and Meat Products—Mercury vs Copper Catalysts and the Use of Food Processors for Meat Sample Preparation in Lieu of the Conventional Meat Chopper.

(6) Continue study on all other topics.

This report of the General Referee was presented at the 101st Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. 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).

Mycotoxins

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Recent developments in mycotoxin methodology include the first published applications of some new instrumental techniques to the estimation of mycotoxins in foodstuffs. Capillary column supercritical fluid chromatography-mass spectrometry (MS) of T-2 toxin and diacetoxyscirpenol in wheat extracted with the same supercritical fluid (CO_2) has been demonstrated (1). Also liquid chromatography (LC) with diode-array UV detection and the more sensitive thermospray LC/MS technique have been used to determine patulin, ochratoxin A, zearalenone, and 4 trichothecenes in wheat (2). Going from complex to the simple, the development of rapid immunochemical test kits for aflatoxins and other mycotoxins continues at a rapid pace and further collaborative testing is planned. A new Associate Refereeship on Immunochemical Methods for Mycotoxins is proposed. Any person interested in developing this topic, working where necessary with the Associate Referee concerned with a particular mycotoxin, is invited to contact the General Referee.

A new book that will be useful to all persons involved in mycotoxin analysis is *Modern Methods in the Analysis and Structural Elucidation of Mycotoxins* (1986) edited by R. J. Cole (3). Also of interest are an in-depth book on *Fusarium Species: Their Biology and Toxicology* (1986) by A. Z. Joffe (4), a report on *Mycotoxins* prepared by the United Kingdom Ministry of Agriculture, Fisheries and Food (5), and, of more general interest, books on *Mycotoxins: Formation, Analysis and Significance* (1985) by J. E. Smith and M. O. Moss (6) and *Mycotoxicology* (1987) by W. F. O. Marasas and P. E. Nelson (7).

With the present coverage of individual mycotoxins or groups of mycotoxins by the Associate Referees, it is apparent that recent developments in other areas are omitted in their reports. Here are a few highlights: *Fusarium* mycotoxins other than the trichothecenes and zearalenone are active research topics: 2 new LC methods for moniliformin in grains have been reported (8, 9), its derivatization for GC/MS has been investigated (10), and both moniliformin and fusarin C have been detected in U.S. corn screenings associated with a field outbreak of equine leukoencephalomalacia (LEM) (11). It is unlikely, however, that either toxin is responsible for LEM or hepatocarcinogenicity of *Fusarium moniliforme* cultures (11-13), and the mycotoxins that cause these diseases remain unknown. LC methods for citreoviridin (in corn), cyclosporin A (in rice), and phomopsin A (in lupin stubble) have also been reported recently (14-16); phomopsin A has, in fact, been detected in Australian lupin seed (17, 18). Progress in the area of multimycotoxin methodology, which is also not a current Associate Referee topic, includes an LC method for patulin, penicillic acid, zearalenone, and sterigmatocystin in cocoa beans (19); and, although not applied to foodstuffs, the reverse-phase LC analysis of 134 mycotoxins and other secondary fungal metabolites has been performed (20).

Associate Referees are encouraged to keep abreast of the actions of the AOAC Committee on Interlaboratory Studies, whose most recent report (21) includes 11 recommendations for harmonization of protocols for the design, conduct, and interpretation of collaborative studies on analytical chemical methods.

Two collaborative studies (on aflatoxins) have been carried out during the last year. Reports of the Associate Referees are as follows:

Aflatoxin M.—Associate Referee Robert D. Stubblefield (USDA, Peoria, IL) notes several papers published during the last year that deal with methodology for aflatoxin M_1 (22-28). Carisano and Della Torre (22) described a method for dry milk that is sensitive to ≤ 2 ng/L (ppt). The method utilizes a C_{18} Sep-Pak cartridge to extract M_1 and basic and acidic acetonitrile-water washes to clean up the extract. Aflatoxin B_2 is used as an internal standard in the reverse-phase LC determination step. Recoveries of 92% at 10 ng/L levels were reported. Serralheiro and Quinta (24) reported a thin-layer chromatographic (TLC) confirmation method for M_1 that uses *p*-anisaldehyde either as an overspotting solution or as a spray reagent. A green fluorescent derivative is produced that has an R_f value lower than M_1 after TLC development. The limit of detection is 0.3 ng with this technique. Dominguez et al. (27) used an alkaline extraction system in a new TLC method for milk and dairy products, and Bijl et al. (28) described a 2-dimensional TLC or LC method for cheese with a detection limit in the range of 10 ng/kg.

Van Egmond et al. (25) reported on a study of four methods of analysis of aflatoxin M_1 in milk powders. Twenty-four Dutch Government laboratories tested 2 TLC methods and 2 LC methods with milk powder samples containing 0.02-0.40 ng M_1/g. One of the methods included in the study was the AOAC method II (**26.095-26.099**). The 4 methods differed little from each other with regard to accuracy and precision. Reproducibility requirements were satisfactory. It was noted that a large number of outliers were present with the AOAC method which has been under discussion by the International Dairy Federation Group E33 Mycotoxins Committee to eliminate some of the problems that are characteristic of the methodology. They are also investigating another TLC method (29). The Associate Referee has not investigated this method but has chosen to investigate newer methodology that utilizes enzyme-linked immunosorbent assay (ELISA). Both affinity columns and an ELISA kit for aflatoxin M_1 are under investigation. The investigation will be reported at the 1987 AOAC Annual International Meeting. Should the data merit it, a collaborative study will be contemplated.

Additional recent papers of interest concern an assessment of liver cancer risk posed by aflatoxin M_1 (30); heat stability of M_1 in milk (31); degradation of M_1 by UV energy (32, 33); presence of aflatoxin M_1 in Italian consumer milk (34, 35) and cheeses imported to the U.S. (36); aflatoxin residues in milk from naturally contaminated grain (37); and comparative carcinogenicity of aflatoxin M_1 and B_1 in male Fischer rats (38). A newly characterized hydroxy derivative of aflatoxin B_1, designated aflatoxin M_4, has been detected in commercial milk samples in France using a TLC method (39).

The Associate Referee recommends that the official AOAC methods for the determination and confirmation of identity of aflatoxin M_1 in milk and cheese (**26.095-26.100**) and for LC determination of aflatoxins M_1 and M_2 in fluid milk (**26.B02-26.B06**) be retained in first action status; that the procedure for preparation of standards for TLC (M_1) (**26.008-26.011**) and the TLC methods for determination of aflatoxin M_1 in dairy products (**26.090-26.094**) and for determination and confirmation of aflatoxins B_1 and M_1 in liver (**26.101-26.109**) be made official final action; and that the alternative confirmatory method for aflatoxin M_1 in liver (**26.110**) be declared as surplus.

Aflatoxin Methods.—Associate Referee Douglas L. Park (University of Arizona, Tucson, AZ) reports that 2 collab-

orative studies were completed during the past year and that 2 more are underway.

A collaborative study was conducted to evaluate an LC method for the determination and quantitation of aflatoxins B_1, B_2, G_1, and G_2 in corn, peanut butter, and raw peanuts (40). Ten laboratories from the United States, Canada, Switzerland, and South Africa participated in the study. Statistical analysis of the data was performed to determine or confirm outliers and compute repeatability and reproducibility of the method. The data support a recommendation that this method be adopted as official first action. A complete report of the study and results obtained is being prepared.

A collaborative study was also conducted to evaluate a solvent-efficient TLC method for the determination and quantitation of aflatoxins B_1, B_2, G_1, and G_2 in corn, peanut butter, and raw peanuts (41). Fourteen laboratories from the United States, Canada, France, Denmark, and Tunisia participated in the study. Statistical analysis of the data was performed to determine or confirm outliers and compute repeatability and reproducibility of the method using either visual or densitometric techniques for the determinative step. The data support the use of this method for these commodities at the higher levels of contamination (20–100 ng/g of total aflatoxins). The use of a densitometer resulted in greater precision than visual comparison to standards. A complete report of the study and results obtained is being prepared.

The Associate Referee has revised his recommendation of last year concerning the ELISA screening method for aflatoxin B_1 to include applicability to cottonseed products and mixed feed only. He recommends the method for adoption as official first action as a screening method, i.e., to determine the presence or absence of aflatoxin B_1 at a given concentration in cottonseed products and mixed feed, using visual determination or an ELISA reader. A full report on the collaborative study has been prepared for publication.

A follow-up collaborative study of the ELISA screening method for aflatoxin B_1 is underway. Samples have been distributed to 14 laboratories in the United States, Canada, The Netherlands, Japan, Switzerland, Tunisia, France, and the United Kingdom. Naturally and artificially contaminated samples of corn, peanut butter, and raw peanuts are included in the study. Results are currently being returned to the Associate Referee's laboratory. A collaborative study to evaluate a second ELISA screening method (rapid screening card) is also underway. Samples will include naturally and artificially contaminated corn, peanut butter, raw peanuts, cottonseed meal, and poultry feed. The method will detect aflatoxins B_1, B_2, and G_1. Approximately 15 laboratories will be selected to participate in the study, and samples are currently being prepared.

The following official first action methods have been recommended for adoption as official final action: aflatoxins in food (Romer minicolumn method) (**26.014-26.019**); aflatoxins in corn and peanuts (Holaday-Velasco minicolumn method) (**26.020-26.025**); aflatoxins in peanuts and peanut products (CB method) (**26.026-26.031**); aflatoxins in peanuts and peanut products (BF method) (**26.032-26.036**); aflatoxins in coconut, copra, and copra meal (TLC method) (**26.044-26.048**); aflatoxins in corn (TLC method) (**26.049-26.051**); aflatoxins in cottonseed products (including sample preparation incorporated under **26.003**) (TLC and LC methods) (**26.052-26.060**); aflatoxins in green coffee (TLC method) (**26.061-26.066**); aflatoxins in pistachio nuts (TLC methods) (**26.067** and **26.068**); aflatoxins in soybeans (TLC method) (**26.069**); aflatoxins in eggs (TLC method) (**26.070-26.075**); identification of aflatoxin by derivative formation on TLC plate (**26.083**); and confirmation of identity of aflatoxin B_1 by negative chemical ionization MS (**26.A01-26.A08**). The TLC method for aflatoxins in green coffee (**26.061-26.066**), which has been proposed for adoption as official final action, and the TLC method for identification of aflatoxin B_1 (**26.076-26.082**) are recommended for declaration as surplus. The section on preparation of aflatoxin standards should be amended under section **26.007** to advise that stock solutions should not be stored in methanol following UV measurements. Also the section on TLC determination of aflatoxins (**26.031**) should be revised to allow the use of mixed reference standards in multiaflatoxin methods.

Recent publications on aflatoxin methodology include papers on a high performance TLC method for screening aflatoxins in poultry feed using silica Sep-Pak cartridges (42), the estimation of aflatoxin in corn by measurement of BGY fluorescence in aqueous extracts (43), measurement of femtomoles of aflatoxins, their metabolites, and DNA adducts by synchronous fluorescence spectrophotometry (44), LC determination of aflatoxins in cattle feed using bromine for postcolumn derivatization (45), application of ELISA to determine and survey aflatoxin B_1 in peanut butter (46, 47), an ELISA method for aflatoxin B_1 in corn and cottonseed (48), and application of monoclonal antibodies to monitor human exposure to aflatoxins (49).

Alternaria Toxins.—Associate Referee Edgar E. Stinson (USDA, Philadelphia, PA) reports no new analytical methods for *Alternaria* mycotoxins, apart from the use of TLC and LC to determine alternariol, alternariol methyl ether, altenuene, and tenuazonic acid in olives (50). Examination of fresh, visibly moldy tomatoes from catsup processing plants revealed that California tomatoes were generally infected with *Geotrichum candidum, Aspergillus,* and *Penicillium,* whereas tomatoes from the Midwest and East U.S.A. were predominantly infected with *Alternaria* (51). Tenuazonic acid was found in 73 out of 146 tomato samples at an average level of 4.9 μg/g of moldy tissue (range 0.4–70 μg/g), but it should be noted that *Alternaria* spp. were not detected in 35 of these positive samples (51). Toxigenic *Alternaria alternata* isolates from tomato in Iraq have also been reported (52).

Characterization of phytotoxins from *Alternaria* spp. continues. Pyrenocines A and B (53) are among the latest of these to be reported. The Associate Referee notes that the main possibility of *Alternaria* phytotoxins affecting humans and livestock would be through *Alternaria* contamination of tobacco and pasture foliage, respectively. The hazard associated with pyrolysis products of *Alternaria* (as would be encountered in *Alternaria*-contaminated tobacco) was established over 20 years ago (54) but has never been thoroughly investigated. There would seem to be little chance of hazard from *Alternaria* phytotoxins in citrus, strawberry, Kikuchiana pear, or radish leaves.

The Associate Referee recommends that further work be done on the identification of toxic *Alternaria* metabolites likely to occur in foods and feedstuffs and that analytical methods be developed.

Citrinin.—Associate Referee David M. Wilson (University of Georgia, Tifton, GA) reports that the planned collaborative study on a citrinin method was delayed but hopes to initiate this study in the near future. No new methods have been published for citrinin. It was included in a study on TLC determination of 107 metabolites of *Penicillium* and other fungi (55). Interest in citrinin seems to be high, however, because several new papers on the production and biological effects of citrinin have appeared (56–62).

Cyclopiazonic Acids.—Associate Referee John Lansden

(USDA, Dawson, GA) reports that there is little or no interest in the commercial sector in analysis of commodities for cyclopiazonic acid. There are, however, indications that cyclopiazonic acid may be a more common metabolite of *Aspergillus flavus* than aflatoxins (63, 64). The effect of some nutrients on the production of cyclopiazonic acid by *Penicillium verrucosum* var. *cyclopium* has also been studied recently (65).

Emodin and Related Anthraquinones.—This topic is still vacant.

Ergot Alkaloids.—Associate Referee George M. Ware (FDA, New Orleans, LA) reports that he plans to confirm the separation of 8 ergot alkaloids and their isomers achieved by Magg and Ballschmiter (66) using a column of LiChrosorb RP-18 (5 μm) or Nucleosil-5 C_{18} with a mobile phase of acetonitrile–0.02M phosphate buffer (pH 7) (1 + 1). Retention indices for the ergot alkaloids were calculated by using alkan-2-ones as standards. New developments in analytical methodology for ergot alkaloids include use of a Kel-F graphite electrode for electrochemical detection of ergonovine maleate (67) and a method for determination of ergot alkaloids in cereal products that uses LC, TLC, and GC/MS for determination (68). Tandem mass spectrometry was used to separate and compare the ergot alkaloids occurring in the sclerotium of *Claviceps purpurea* parasitic on tall fescue with those found in this fungus parasitic on wheat and barley (69).

The effect of feeding ergot-contaminated grain to pregnant and nursing sows was reported (70). The Associate Referee recommends that a collaborative study on the method developed in his laboratory (71) for ergot alkaloids be completed. With respect to future research, the Associate Referee recommends that research on the stability of ergot alkaloids be continued. The Associate Referee also recommends that research on a better chromatographic system be conducted during the next year.

Ochratoxins.—Associate Referee Stanley Nesheim (FDA, Washington, DC) reports that work is still in progress to determine the reasons for low and variable recoveries of ochratoxin A from stored samples of barley, corn, and swine tissue that delayed the collaborative study last year. The method (72) has now become somewhat outdated. Several methods have recently been published which offer apparent improvements. It is important that these be checked because they contain features that could be included in a method to be collaboratively studied. One of the recent methods (73) reports 90.6 ± 3.6% recovery of ochratoxin A from several animal feeds. This method employs a new extraction solvent (ethanol–chloroform–5% aqueous acetic acid) and cleanup using a Sep-Pak silica cartridge followed by a cyano cartridge. Another method includes the use of a C_{18} Sep-Pak cartridge (74).

In other areas of methodology for ochratoxins, LC/MS has been used for barley analysis (75), and radioimmunoassays have been applied to determination of ochratoxin A in serum and pig kidneys (76, 77). Development of ochratoxin A in 2 granary-stored barley cultivars has been studied (78). Other recent research has shown that, contrary to previous experiments, little or no ochratoxin A is destroyed when contaminated green coffee beans are roasted and coffee decoction is prepared (79). Finally, fungi of the *Aspergillus glancus* group isolated from cereals have been shown to form ochratoxin A (80).

The Associate Referee recommends that the TLC/LC method for ochratoxin A in barley, corn, and swine tissue be modified to incorporate the latest state-of-the-art methodology and then be collaboratively studied. He also recommends that the official first action TLC methods for ochratoxins in barley (**26.111**-**26.118**) and ochratoxin A in green coffee (**26.119**-**26.125**) be adopted as official final action.

Penicillic Acid.—Associate Referee Charles W. Thorpe (FDA, Washington, DC) reported last year on the variable and low recoveries of penicillic acid added to freshly ground samples of corn and dried beans which were then stored for up to 2 months. It has now been found that when the ground samples either are air dried for 1 week or are exposed to radiation prior to the addition of penicillic acid the recoveries are satisfactory. The collaborative study on the method for penicillic acid (81) is now planned for the fall of 1987.

Some biological research on penicillic acid was conducted during the past year. Penicillic acid was one of 4 mycotoxins found to give a positive response in the SOS spot test using *Escherichia coli* (82). Bacterial tests have also been used to demonstrate detoxification of penicillic acid by ammonia (83). Penicillic acid (and sterigmatocystin) induced DNA single-strand breaks and inhibition of DNA synthesis in cell cultures (84). In a study of the toxicity of penicillic acid for rat alveolar macrophages in vitro, the results were similar to those observed for patulin in an earlier study, and the data suggest the possibility of a respiratory hazard to workers exposed to contaminated grains (85). In a microbiological study of mold-spoiled foods obtained from individuals and bakeries, 6 fungi, all identified as *Penicillium expansum*, produced patulin or penicillic acid (86).

The Associate Referee repeats his earlier recommendation that a collaborative study of the method for the determination of penicillic acid in corn and dried beans (81) be conducted.

Penicillium islandicum Toxins.—This topic is still vacant.

Secalonic Acids.—This topic is still vacant.

Sterigmatocystin.—Associate Referee Octave J. Francis, Jr, (FDA, New Orleans, LA) reports that the collaborative study of the method for the determination of sterigmatocystin in cheese which was summarized in the last report is scheduled for publication (87). In that report, the Associate Referee deferred recommending the method for official first action approval pending development of a more satisfactory determinative system. Since the last report, an LC multitoxin method has been published that allows the determination of several specified toxins, including sterigmatocystin, in cocoa beans (19). The method reports a lower limit of detection of 1 ng which equates to 13 ng sterigmatocystin per g of cocoa beans. Recoveries of sterigmatocystin added to sample matrix were 101 and 108% for the spiked levels of 30 and 60 ng/g, respectively. The method utilizes reverse-phase liquid chromatography, a cyano column, and UV detection at 245 nm. The Associate Referee plans to evaluate combination of this LC determinative step with the extraction and cleanup procedure of the cheese method.

An ELISA method for sterigmatocystin in barley (88), 2 articles discussing the production of sterigmatocystin on commodities such as corn (89) and sunflower (90), and at least 4 reports dealing with toxicological studies (91–94) were published recently.

The Associate Referee recommends continued study on this topic. He also recommends that the official first action TLC method for sterigmatocystin in barley and wheat (**26.132**-**26.138**) be adopted as official final action.

Tree Nuts.—Associate Referee Vincent P. DiProssimo (FDA, Brooklyn, NY) reports no further work on this topic. The TLC methods for aflatoxins in pistachio nuts (**26.067** and **26.068**) are recommended for adoption as official final action.

Trichothecenes.—Associate Referee Robert M. Eppley (FDA, Washington, DC) reports that no new collaborative studies were planned for this year. Improvements and modifications to existing and newer methods need to be evaluated further before additional studies are initiated. The next study should also include a procedure for the determination of deoxynivalenol (DON) in corn. There have been recent developments in immunoassay procedures for DON and 3-acetyl DON (95, 96), and commercial development of immunoassay kits is pending. A limited validation study of one or more of these kits is possible within the next year or so.

With regard to the other trichothecenes, only a few have so far been detected as natural contaminants in cereal grains, i.e., (in addition to DON) 3- and 15-acetyl DON, nivalenol, T-2 toxin, HT-2 toxin, diacetoxyscirpenol, and trichothecin (97–100); recently, scirpentriol and 15-monoacetoxyscirpenol, in addition to nivalenol, were detected in sorghum from Thailand (101). Most of these toxins have been reported occasionally in isolated situations. The reported occurrences of the acetyl DONs and nivalenol in association with the presence of DON warrants consideration of validation of a method or methods to detect these toxins in cereal grains.

Review of recent literature revealed several new and modified approaches for the determination of trichothecenes, including macrocyclic trichothecenes (1, 2, 101–117). Several methods have been reported for the detection of trichothecenes and their metabolites present in body fluids and tissues (102, 103, 110, 111, 118). Additional studies have been conducted on detoxification of DON (119, 120).

The Associate Referee recommends that studies be continued on the improving, developing, and evaluating of methods for known, naturally occurring trichothecenes, including screening methods and procedures for confirmation of identity, which could be selected for collaborative study in the near future.

Xanthomegnin and Related Naphthoquinones.—Associate Referee Allen S. Carman, Jr (FDA, New Orleans, LA) reports that work planned on the development of screening methodology for xanthomegnin, viomellein, and ochratoxin was not completed due to other duties, but it will be continued next year. In the past year, there have been no new additions to the literature; only one literature citation is to be added (121) and it is from 1985. It reports the identification and screening of *Penicillium* isolates from Indiana shelled corn for the production of 6 mycotoxins (citrinin: 0/20 positive; cyclopiazonic acid: 0/496; ochratoxin: 0/496; patulin: 0/158; penicillic acid: 3/350; xanthomegnin: 11/87); xanthomegnin was produced by *P. viridicatum.*

Zearalenone.—Associate Referee Glenn Bennett (USDA, Peoria, IL) reports an increased awareness of zearalenone as demonstrated by the large number of publications on occurrence, animal studies, and methodology for this mycotoxin during the last year.

Numerous recent surveys have confirmed that zearalenone is present in cereal grains in many areas of the world and that it usually coexists with other trichothecenes, primarily nivalenol and/or deoxynivalenol. Wheat rejected in New Zealand contained 0.04–0.35 μg zearalenone/g (122). The authors concluded that the zearalenone contamination did not preclude using the grain for feeds. Nivalenol (0.02–0.77 μg/g) and deoxynivalenol (1.5–10.8 μg/g) were also present in these samples. Eight of 20 imported corn samples in Korea contained >0.5 μg zearalenone/g with 2 samples containing 1 μg/g (123). These authors concluded that these 2 samples would be hazardous for pigs. Zearalenone was also detected in maize and maize products imported into Sweden (124). Cereals grown in the United Kingdom (4/31 samples) contained an average of 1 μg zearalenone/kg (125). In Korea, 42/51 samples contained zearalenone (126); whereas in Poland, only one of 63 samples of wheat contained detectable levels (127). Zearalenone has also been found in cereals from China and Taiwan (128), in preharvest corn in Spain (129), in corn grown in Queensland, Australia (130) and in Italy (131), in stored sorghum in India (132), and in heavily damaged soybeans in the United States (133). A survey of 395 samples of grains and animal feeds stored on U.S. farms in 4 states indicated that 16% of the corn samples, 23% of feed samples, and 35% of other grain samples contained zearalenone (134).

Processing of contaminated cereals does reduce or redistribute zearalenone to some degree. Milling of naturally contaminated wheat (2.05 μg/g) resulted in a 48–66% reduction of zearalenone in flour fractions intended for human consumption, while those fractions intended for animal feed incurred a 1.9- to 2.3-fold increase (135). Another milling study showed that less than 10% of the zearalenone in the starting wheat (8 ng/g) remained in the flour fractions (136). The baking process did not affect levels of toxin in the flour. Although the potential exists for zearalenone to be present in foods prepared from contaminated cereals, a limited survey of baby foods on the market in Japan showed no zearalenone in the 27 samples assayed (137). A simulated commercial process for refining vegetable oils effectively removed zearalenone (10 μg/g) from spiked corn oil (138). The preparation of tortillas from corn samples naturally contaminated with zearalenone (0.23–4.23 μg/g) resulted in a 59–100% reduction of toxin (139). These naturally contaminated corn samples had been in storage since 1972 and demonstrated the stability of zearalenone in cereal grains in storage (99). The carryover of zearalenone into native beer (burukutu) prepared in villages in Nigeria was high (51%), only 12% being removed with the discarded solid residues (140).

Studies on the production of zearalenone by *Fusarium graminearum* strains indicated that a food-acceptable strain of *F. graminearum,* A-3/5, lacks the structural genes for zearalenone production and probably would not produce the mycotoxin under any environmental condition (141). Other investigations demonstrated that high levels of zearalenone are produced (along with deoxynivalenol) by *F. graminearum* strains isolated from corn in the United States and Germany (142, 143). A study on the microbial conversion of zearalenone by various species of fungi showed that *Rhizopus* sp. was able to convert zearalenone to a glycoside (144).

Zearalenone has been implicated as a contributing agent, via contaminated feed, to disorders in cows and buffaloes in Egypt (145), high mortality in chickens in Israel (146), and to estrus and abortions in pigs in Italy (147) and Yugoslavia (148). However, controlled studies with purified zearalenone, at levels much higher than those found in naturally contaminated feed, demonstrate that zearalenone by itself does not seem to be an important factor in dairy cow health (149). Feeding studies using low levels of zearalenone (3, 6, and 9 μg/g) in the diets of mature gilts showed that the most apparent effect is increased pseudopregnancy (150). In a related study, the same levels (3, 6, and 9 μg/g) of zearalenone were fed to boars (151). This study suggested that the higher concentration may affect testicular epididymal function because a trend toward lower testicular weight and reduced total mobile spermatozoa was observed. The estrogenic effect of a single dose of zearalenone (10 μg/g body weight, intraperitoneally) in female rats resulted in a significant increase in uterine acetyl cholinesterase activity (152). Another report

showed that, when it was orally administered, zearalenone was 650 times less potent than 17β-estradiol in female rats (153).

New reports on screening methods for zearalenone described TLC detection of toxigenic *Fusarium* isolates (154) and the screening of cereals for zearalenone by feeding contamined cereals to infant mice (155). More specific LC methods were developed to discern estrogens, used as anabolic drugs, from zearalenone and zearalenol in avian tissue (156) and to detect the fraudulent use of zeeranol and the natural occurrence of zearalenone in cattle urine (157). Thermospray LS/MS has been applied to the analysis of a wide range of mycotoxins, including zearalenone (2). ELISAs continue to be of interest. A specific and sensitive monoclonal antibody for zearalenone and α-zearalenol has been produced and was used to detect zearalenone at 0.5 ng/mL; it showed a lower degree of variability than polyclonal antibodies (158). This antibody reacted as well with α-zearalenol as with zearalenone. The applicability of ELISA for detection of zearalenone in milk was also evaluated (159). The detection limit was 10 ng/mL. An important and critical risk assessment of zearalenone has been completed by the Health Protection Branch, Health and Welfare Canada (160). This extensive report evaluates the potential human exposure to zearalenone from corn cereals and recommends studies to more fully assess the effects of zearalenone in humans, especially children.

The Associate Referee recommends that the ELISA procedure for zearalenone be validated by collaborative study. He also recommends that the official first action TLC method for zearalenone in corn (**26.139-26.147**) and the official first action LC method for α-zearalenol and zearalenone in corn (**26.A09-26.A16**) be adopted as official final action.

Recommendations

(1) Adopt as official first action the ELISA screening method for aflatoxin B_1 in cottonseed products and mixed feed.

(2) Adopt as official final action the official first action methods for preparation of standards for TLC (M_1) (**26.008-26.011**); aflatoxins in food (Romer minicolumn method) (**26.014-26.019**); aflatoxins in corn and peanuts (Holaday-Velasco minicolumn method) (**26.020-26.025**); aflatoxins in peanuts and peanut products (CB method) (**26.026-26.031**); aflatoxins in peanuts and peanut products (BF method) (**26.032-26.036**); aflatoxins in coconut, copra, and copra meal (TLC method) (**26.044-26.048**); aflatoxins in corn (TLC method) (**26.049-26.051**); aflatoxins in cottonseed products (including sample preparation incorporated under **26.003**) (TLC and LC methods) (**26.052-26.060**); aflatoxins in green coffee (TLC method) (**26.061-26.066**); aflatoxins in pistachio nuts (TLC methods) (**26.067** and **26.068**); aflatoxins in soybeans (TLC method) (**26.069**); aflatoxins in eggs (TLC method) (**26.070-26.075**); identification of aflatoxin by derivative formation on TLC plate (**26.083**); confirmation of identity of aflatoxin B_1 by negative chemical ionization MS (**26.A01-26.A08**); aflatoxin M_1 in dairy products (TLC) (**26.090-26.094**); aflatoxins B_1 and M_1 in liver (TLC) (**26.101-26.106**); confirmation of aflatoxins B_1 and M_1 in liver (**26.107-26.109**); ochratoxins in barley (TLC) (**26.111-26.118**); ochratoxin A in green coffee (TLC) (**26.119-26.125**); sterigmatocystin in barley and wheat (TLC) (**26.132-26.138**); zearalenone in corn (TLC) (**26.139-26.147**); and α-zearalenol and zearalenone in corn (LC) (**26.A09-26.A16**).

(3) Declare as surplus the official first action (proposed for final action) TLC method for aflatoxins in green coffee (**26.061-26.066**) as well as the official first action methods for identification of aflatoxin B_1 by TLC (**26.076-26.082**) and for confirmation of aflatoxin M_1 in liver (**26.110**).

(4) Amend section **26.007** to advise that stock solutions of aflatoxin standards should not be stored in methanol. Modify the section on TLC of aflatoxins (**26.031**) to allow use of the mixed "resolution reference standard" (**26.012**) for quantitation of aflatoxins B_1, B_2, G_1, and G_2.

(5) Create a new Associate Refereeship on Immunochemical Methods for Mycotoxins.

(6) Continue study on all topics.

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This report of the General Referee was presented at the 101st Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. 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 edition, 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).

Oils and Fats

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Antioxidants.—Associate Referee B. D. Page continued investigation of the diode array detector for confirmation of antioxidants detected by the LC method (1, 2). Performance of various C_{18} reverse-phase columns was also investigated, concentrating on the separation of dodecyl gallate (DG) and butylated hydroxy toluene (BHT). A significant difference in the separation of DG and BHT was observed with 6 different column packing materials (separation varied from coelution to baseline separation). In addition, it was found that the newer Supelcosil RP-18 packing (5 μm, spherical shape) provided excellent peak shape without the use of 5% acetic acid in the mobile phase. Apparently, suppression of ionization is not a factor in reducing peak tailing. The acetic acid appears to mask unreacted silanol groups of the silica gel packing materials which cause tailing of the polar antioxidants.

The IUPAC Commission on Oils, Fats and Derivatives conducted an interlaboratory study of the liquid chromatographic (LC) method to obtain recovery data on octyl and dodecyl gallate as well as to affirm recoveries of BHA, BHT, PG, TBHQ and NDGA obtained in the original AOAC collaborative study. Three vegetable oil samples and one animal fat sample, all containing various antioxidants, were sent to 28 participants with instructions to conduct 2 separate analyses and 2 LC injections per analysis for each sample. Although the method (in IUPAC style) submitted to participants included a solvent system identical to that specified in AOAC **20.011**, instructions to collaborators included a sample solvent system (mobile phase program) and a washing program that were different from those specified in the AOAC method. The U.S. participant observed that the AOAC solvent system produced better shaped LC peaks than did the sample solvent system. The Commission adopted the method during its July 1987 meeting.

Emulsifiers.—There was no Associate Referee activity on this topic during the past year. Riisom and Hoffmeyer (3) described an LC method for separation of mono-, di-, and triglycerides on a diol column (diol phase chemically bonded to silica gel). Christopoulou and Perkins (4) reported the separation of fatty acids, mono-, di-, and triglyceride mixtures by size exclusion chromatography (HPSEC) on columns packed with 5 μm styrene/divinylbenzene copolymer. Takano and Kondoh (5) used reverse-phase liquid chromatography for analysis of monoglycerides in mixtures and commercial products. The use of various LC modes for glyceride analysis was reviewed by Aitzetmüller (6).

Hydrogenated Fats.—Associate Referee R. A. DePalma conducted a preliminary interlaboratory study of a capillary column GC method for determination of C-18 *trans* monoene and *cis-cis* methylene interrupted unsaturated diene and triene levels in vegetable oils and margarines. A 60 m × 0.25 mm fused silica capillary column coated with SP-2340 (cyano-silicone stationary phase) was specified. The interlaboratory study was successful and a protocol was developed for a forthcoming collaborative study of the method, expanded to include determination of total *trans* and *cis* monoene and total *cis-cis* methylene interrupted polyunsaturated fatty acids (*cis*-PUFA). Athnasios et al. (7) demonstrated that *cis*-PUFA in fats and oils could be determined with good precision using a 60 m SP2340 capillary column. Also, the GC results agreed well with those obtained by the enzymatic method (8).

Lower Fatty Acids.—Associate Referee G. Bigalli conducted additional studies of methodology for GC determination of lower fatty acids. Iverson and Sheppard (9) described the determination of fatty acids in butter by temperature-programmed gas chromatography of the butyl esters. Fleming et al. (10) developed a GC method using a 25 m fused-silica capillary column coated with Carbowax 20M for determination of volatile fatty acids in biological samples. The IUPAC Commission on Oils, Fats and Derivatives completed collaborative study of 2 methods for determination of butyric acid in fats containing butterfat (11) and adopted one of the methods (12). The results of collaborative study of the 2 methods (Phillips and Sanders and Kuzdzal-Savoie procedures) as well as the Phillips and Sanders method which was adopted (IUPAC Method 2.310) were published in *Pure Appl. Chem.* (13). It is recommended that IUPAC method 2.310 be adopted as official first action.

Marine Oils.—Associate Referee R. G. Ackman completed a preliminary interlaboratory evaluation of a capillary column GC method for determination of the fatty acid composition of marine oils (14). Results of the analysis of a cod liver oil sample were satisfactory. Relative standard deviations for 20:5 n-3 and 22:6 n-3 (each, 7% of total fatty acids determined as methyl esters) were 8.0 and 13.7%, respectively. The Associate Referee, in association with Jeanne D. Joseph, Southeast Fisheries Center, National Marine Fisheries Service, Charleston, SC, is planning a joint AOAC-AOCS collaborative study of the method.

Olive Oil Adulteration.—Associate Referee E. Fedeli has initiated studies on determination of vegetable oil sterols by capillary gas chromatography, waxes in vegetable oils, and quantitative determination (absolute amount) of sterols in vegetable oils. Kapoulas and Andrikopoulos (15) proposed use of second-derivative spectrophotometry for detection of virgin olive oil adulteration with refined oils. Flor (16) used LC to determine olive oil grades as well as to detect the presence of other vegetable oils in olive oil. It was found that 6 main triglycerides (LOO, LOP, OOO, POO, POP, and SOO) could be used to distinguish virgin olive oil from other grades of olive oil. The area before LLL (trilinolein) and the ratios of OOO/POO and LOO/LOP were also used for grade determination. More than a very low level of LLL indicated the presence of another vegetable oil (soybean, sunflower, etc.).

Oxidized Fats.—Associate Referee M. M. Blumenthal participated in an IUPAC collaborative study of a method for LC measurement of triglyceride polymers (17). Six samples with a triglyceride polymer content (as determined by gel permeation liquid chromatography) of 0–10% were sent to 17 laboratories. Relative standard deviations (RSD_R) of 5–12% were obtained for samples with 5–10% triglyceride polymers (RSD_R was 24% for a sample containing 1.8% triglyceride polymers). Wu and Nawar (18) evaluated 9 analytical methods (measurement of viscosity, polymers, dielectric constant, polar compounds, dimers, free fatty acids, smoke point, carbonyls, and cyclic monomers) as well as some combinations of these methods for monitoring oxidation of frying fats. These workers pointed out that no single test could be expected to reflect the total decomposition pattern of frying fats or determine the precise point at which a frying oil should be rejected. However, it was suggested that measurement of the polymer/FOS ratio (polymers determined by Peled's procedure [19] and change in dielectric constant as measured by the Foodoil Sensor [Northern Instruments Co., Minneapolis, MN]) was most suitable for monitoring the quality of used frying fats. Wyatt (20) developed a purge and trap procedure to measure volatiles associated with vegetables oil autoxidation. Volatiles are purged from the oil, concentrated onto an external trap, and then backflushed onto a benchtop GC/MS for subsequent analysis. Pongracz (21) investigated the use of headspace GC detection of pentane to determine rancidity of edible fats.

Pork Fat in Other Fats.—There was no Associate Referee activity on this topic during the past year. Saeed et al. (22) proposed that 11,14-eicosadienoic acid (20:2) can be used as an indicator for the presence of pork or lard in beef and mutton or their fats. However, Pocklington (personal communication) presented evidence that 20:2 does occur in beef and mutton fat and cannot be used as an indicator of the presence of pork fat in beef and mutton mixtures.

Sterols and Tocopherols.—There was no Associate Referee activity on this topic during the past year. The United Kingdom proposed a revision of International Standardization Organization (ISO) method 6799 (23) for determination of sterol content of vegetable fats and oils as absolute values (expressed as mg sterol/100 g oil or fat). The revised method involves saponification of the oil or fat, addition of betulin (internal standard) prior to extraction of the unsaponifiable matter containing the sterols, separation of the sterol fraction by TLC, silylation of the isolated sterols, and quantitative analysis of the silylated sterols by capillary column GC. Bukovits and Lezerovich (24) described a method for direct determination of individual tocopherols in mixtures by means of second derivative UV spectrophotometry.

Other Topics.—In the absence of adverse comments, the General Referee recommends that the following official first action methods be adopted official final action: **28.104-28.109**, Beta-Sitosterol in Butter Oil; **28.120-28.123**, 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.

The General Referee recommends that **28.B01-28.B05** (Triglycerides in Fats and Oils, Gas Chromatographic Method) (25) 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 results obtained by use of the capillary or packed columns (26).

Commission on Oils, Fats and Derivatives, Applied Chemistry Division, IUPAC

The Commission met on July 7–9, 1987, in Münster, Federal Republic of Germany, and on August 23–24, in Boston, MA. The Commission discussed 16 projects and topics including methods for determination of polycyclic aromatic hydrocarbons (PAHs), mineral oil residues, color in lecithins, acetone-insoluble materials in lecithins, polyenoic (n-3 and n-6) fatty acids in food fats, tocopherols by liquid chromatography, determination of heavy metals by graphite furnace atomic absorption spectrophotometry (AAS), triglycerides according to their equivalent chain number (ECN) by reverse-phase liquid chromatography, phospholipids by liquid chromatography, polymerized triglycerides by gel permeation (GP) liquid chromatography, antioxidants by liquid chromatography, *trans* unsaturation in fats and oils, fatty acid composition of unhydrogenated and hydrogenated animal fats and marine oils, mono- and diglycerides by liquid chromatography, extraction of fats from foods, and guidelines for conducting and evaluating the results of collaborative studies.

Study was proposed of 2 methods for PAHs, one a rapid method for benzo(a)pyrene (BaP) in fats and oils, the other a method for total PAHs in fats and oils. The method for BaP involves passage of a petroleum ether solution of the sample through a column of neutral aluminum oxide followed by additional reverse-phase LC cleanup (fluorimetric detection) and optional confirmation by GC/MS. The method for total PAHs involves complexation-extraction with caffeine, silica gel column purification, and reverse-phase LC separation and quantitation with a diode array (UV) detector.

A single method is not capable of determining all the substances which might be considered as mineral oil residues. Conventional GC would be applicable to the middle fraction of petroleum products, generally termed mineral oil. Headspace gas chromatography would be suitable for analysis of the light fraction, and liquid chromatography could be considered for the heavy fraction. Study of an alumina column-capillary column GC method was proposed for determination of the middle fraction.

Capillary column gas chromatography was evaluated for determination of n-3 and n-6 polyunsaturated fatty acids in vegetable oils with different degrees of hydrogenation. Five samples of soybean oil were submitted to collaborators for capillary column GC analysis (most participants used CP Sil 88 columns). Resolution of individual fatty acids presented a problem with highly hydrogenated samples. Variations in reported results were also attributed to difficulties in identifying individual components. It was decided that (1) a revised method should be drafted in which interpretation of the GC chromatogram is standardized, and (2) a capillary column GC method should be proposed for determining the complete fatty acid composition (including *trans* components) of unhydrogenated and hydrogenated animal fats and marine oils and blends of marine and vegetable oils.

A collaborative study was conducted to check the applicability of the IUPAC LC method for tocopherols to products such as margarine which contain added tocopherol esters (added α-tocopherol acetate). Four samples (2 pairs of blind duplicates) were sent to 10 collaborating laboratories. One of the pairs contained 10 μg α-tocopherol/g and a quantity of added α-tocopherol acetate equivalent to 85 μg α-tocopherol/g. A significant number of laboratories reported poor results for the samples with added α-tocopherol acetate, apparently due to incomplete saponification before LC analysis. The method was deemed suitable for free tocopherols, but it requires revision to define the saponification time, temperature, and degree of vigorous shaking required to achieve complete saponification of tocopherol esters.

A method was proposed for determination of lead in fats and oils by graphite furnace atomic absorption spectrometry (AAS). Use of a matrix modifier (lecithin) is required to prevent loss of lead at the ashing temperature. A collaborative study will be conducted beginning in late 1987.

A collaborative study was completed of a reverse-phase LC method for determination of ECNs of triglycerides. Soybean oil, almond oil, sunflower oil, and mixtures of almond and sunflower oil were examined by 18 laboratories. The results were satisfactory, except for ECN 50 (present at 1–2% level). It was proposed that a 1987–1988 study of the method include analysis of palm oil, rapeseed oil, and olive oil. Determination of low levels of trilinolein would allow detection of small amounts of undeclared seed oils in olive oil (olive oil contains only a very low or undetectable level of trilinolein). It was also suggested that the method could be extended to determination of mono- and diglycerides.

A method for determination of polymerized triglycerides by gel permeation liquid chromatography was subjected to collaborative study. The method was unsatisfactory for levels less than 5%. A new collaborative study was planned for levels of 1–5% following validation of a revised procedure.

Excellent results were obtained in an interlaboratory study of AOAC method **20.009-20.013** for determination of antioxidants in oils and fats, and the method was adopted by the Commission. New topics for consideration included methods for extracting lipids from foods and methods for detecting refining and determining minor components of fats and oils.

The Commission issued a set of guidelines for design, conduct, and interpretation of collaborative studies representing the main recommendations agreed upon at a joint ISO/IUPAC/AOAC Harmonization Workshop held in May 1987 in Geneva. The guidelines state that the minimum number of materials to be used in most collaborative studies is 5, the minimum number of participating laboratories is 8 (absolute minimum 5 laboratories). The best estimates of repeatability are to be obtained using a split-level design (single values from each of 2 closely related materials); a combination of split levels and blind duplicates in the same study; or alternatively, blind duplicates. Analysis of known (parallel) duplicates should be discouraged. Precision estimates should be calculated with no outliers removed and with outliers removed, using the Cochran and Grubbs outlier tests. The Grubbs test should only be applied to laboratory means. Outlier removal should be halted when more than 22% (i.e., >2 of 9 labs) would be removed as a result of sequential application of the outlier tests.

Methods for emulsifiers, thiobarbituric acid, thin-layer chromatography of phospholipids, and metals (iron, copper, and nickel) in vegetable oils by AAS were prepared for publication in *Pure Appl. Chem.* The 7th revised and enlarged edition of the Commission's "Standard Methods for the Analysis of Oils, Fats and Derivatives" (Blackwell Scientific Publications, Boston, MA) was published in 1987. The new chairperson and vice-chairperson of the Commission are J.

Beare-Rogers and A. Dieffenbacher, respectively. D. Pocklington continues as secretary. The next meeting of the Commission will be held August 16–18, 1988, at the University of St. Andrews, St. Andrews, Scotland.

Recommendations

(1) Adopt as official first action IUPAC method 2.310, Determination of Butyric Acid.

(2) 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.)"

(3) Adopt as official final action the following official first action methods: **28.104-28.109**, beta-sitosterol in butter oil; **28.120-28.123**, 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.

(4) Continue study on all other topics.

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Plant Toxins

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Recent reports have focused increased attention on the health hazards that may be associated with the ingestion of intrinsic plant toxins (1, 2). In addition, the EURO Food Tox II Meeting, October 15–18, 1986, in Zurich, Switzerland, addressed topics of concern in this area (3).

Substantial progress has been made in the development and evaluation of methodology in the Associate Refereeships for Glucosinolates and for Steroidal Alkaloids. Collaborative studies are being organized in these areas. On the basis of growing concern about the presence of hydrazine derivatives in mushrooms, the establishment of an Associate Refereeship in this area is recommended. Other subjects under active consideration with regard to official methods requirements are cyanogenic glucosides/cyanide in cassava, hypoglycin in ackee fruit, and lathyrogens.

Glucosinolates (D. Ian McGregor, Agriculture Canada, Saskatoon, Saskatchewan, Canada).—Over the past years, progress internationally on improvement of methods for glucosinolate analysis has been closely followed. This was facilitated by a workshop organized on analytical aspects of glucosinolates in rapeseed sponsored by the European Economic Community (EEC) and held at Gembloux, Belgium, October 1–3, 1986, and the 7th International Rapeseed Congress held at Poznan, Poland, May 11–14, 1987.

In addition to AOAC, the International Standards Organization (ISO) and EEC currently have an interest in establishing standard methods for glucosinolate analysis. The latter organizations are interested in establishing a standard method of analysis for *Brassica* crops, in particular, rapeseed. Over the past couple of years, this interest has intensified because of the imminent conversion of commercial rapeseed production in Europe to the low glucosinolate (canola) type. Such a conversion took place in Canada over a decade ago. However, whereas commercial rapeseed production in Canada is almost exclusively of the spring (annual) type, most of the rapeseed production in Europe is of the winter (biennial) type. Production of the superior low glucosinolate (canola) rapeseed has been held up due to difficulties encountered by plant breeders in introducing the low glucosinolate characteristic into the winter type.

This past year has seen 2 interlaboratory studies conducted in Europe, one by ISO and the other by EEC. The ISO study should really be classified as a comparison of methodology rather than as a collaborative study because participants were allowed to use one or more of several methods of analysis. Indeed, not only were participants given a choice, but use of more than one method was actively encouraged. An array of diverse methods was used, which included gas chromatography of trimethylsilyl derivatives, enzymatic release and colorimetric measurement of glucose, colorimetric measurement of palladium complexing, liquid chromatography (LC) of desulfo glucosinolates, and near infrared reflectance spectrophotometry. The exercise merely showed the variety of methods currently in use for determining the glucosinolate content of *Brassica* crops, specifically rapeseed, and gave little

insight into the potential for agreement between laboratories. Results of this study are to be presented and discussed at an ISO meeting to be held in London, England, September 29–October 1, 1987.

The EEC study, on the other hand, was limited to 2 methods of analysis, LC analysis of intact glucosinolates and of desulfo glucosinolates. Of the 6 participating laboratories, only one performed LC analysis of intact glucosinolates, whereas all 6 performed LC analysis of desulfo glucosinolates. It was originally intended that the EEC study would be completed before and discussed at the meeting held at Gembloux, Belgium; however, this timetable was not met. Instead, the meeting dealt in depth with the problems encountered in optimizing both LC methods and also considered other possible approaches that could be of value in monitoring the conversion of the European crop from high to low glucosinolate quality. In addition to an accurate and precise method of analysis suitable for adoption as an official or standard method of analysis, a need was expressed for a rapid and simple method suitable for screening the commercial crop throughout the production and marketing system.

From the deliberations of EEC on the determination of the glucosinolates of rapeseed by LC analysis, the following may be concluded:

(1) Extraction: Water, as opposed to water alcohol (methanol) mixtures, is a better solvent for extraction of glucosinolates rapeseed meal. Water–methanol mixtures do not completely extract indole glucosinolates.

(2) Ion exchange purification: Washing the column after addition of the sample with acidic buffer, preferably acetate buffer pH 4.0, and incubating with desulfatase at elevated temperature, preferably 39°C, improves glucosinolate recovery.

(3) Column separation: Elevating column temperature can improve resolution but also can result in the loss of indole glucosinolates, in particular, 4-hydroxy-3-indolylmethyl glucosinolate.

(4) Quantitation: Storage of aqueous extracts or purified desulfo glucosinolates can result in losses, particularly of 4-hydroxy-3-indolylmethyl glucosinolate.

Although 2-hydroxy-2-phenylethyl glucosinolate (glucobarbarin) is preferred over benzyl glucosinolate (glucotropaeolin) as an internal standard which can be added early in the analysis procedure, neither is as yet readily (commercially) available, and *o*-nitrophenylgalactoside internal standard is better resolved than either of the glucosinolates.

A second collaborative study is planned by EEC to further optimize the LC method. Of particular interest will be the determination of the extent to which variability between laboratories is due to differences in equipment as opposed to influences in sample-dried extract prepared in one laboratory which need only be dissolved and injected into the LC instruments. It has also been proposed to EEC that a larger collaborative study be performed once the method has been further optimized.

The Associate Referee intends, over the next few methods, to verify that the proposed changes to LC analysis of desulfo glucosinolates will improve quantitative analysis, particularly as it applies to the seed of rapeseed and mustard. If no further changes appear warranted, a decision will then have to be made as to whether AOAC should adopt the EEC method or should consider an independently conceived method.

Steroidal Alkaloids (Allen S. Carman, Natural Toxins Research Center, Food and Drug Administration, New Orleans, LA.)—This report will be limited to those steroidal alkaloids commonly known as glycoalkaloids. Those alkaloids and their glycosides elaborated by the genera *Solanum* and *Lycopersicum* will be covered. Those elaborated by the genus *Veratrum* will not. Because of the relative abundance of literature on the subject (more than 50 publications since 1986), the review presented herein will be limited to the aspects of analysis and determination with emphasis on the literature since 1985. For broader coverage, the reader is referred to several comprehensive reviews (4–6).

Historically, glycoalkaloids were most commonly analyzed by colorimetry (7) or titrimetry (8). Thin-layer chromatography (TLC) (9) and gas chromatography (GC) (10) have also been used. With the maturation of liquid chromatography (LC) as an analytical technique, approaches utilizing it began to appear in the literature (11, 12). The above brief survey by no means includes all of the citations for any particular approach. Those given are only cited as examples.

Since 1985, chromatographic and immunoassay procedures have dominated. Van Gelder (13) described a procedure using capillary GC that resolves the aglycones solanidine, demissidine, solasadiene, tomatidine, and solasodine without derivatization. A novel feature of the method was a 2-phase technique using dilute hydrochloric acid and carbon tetrachloride for the hydrolysis of the glycoalkaloids. Morgan and coworkers (14) reported an enzyme-linked immunosorbent assay (ELISA) for total glycoalkaloids (TGA) in potatoes. They later compared this method to 2 others which use colorimetry as the determinative step (15). Agreement of the ELISA method with the colorimetric methods is good, and overall reproducibility for replicate assays is as good or better than for the 2 colorimetric methods. This was one of the first complete assay protocols to appear that does not require precipitation of the glycoalkaloids by base as part of the cleanup procedure, a tedious and time-consuming step. A radioimmunoassay (RIA) procedure reported earlier by others (16) does not appear to have gained wide acceptance due to cross-reactivity of the antiserum and the requirement of using a relatively small antiserum dilution. However, an RIA for the determination of TGA in serum (17) does not appear to suffer from these problems. Recently, 2 similar LC procedures have appeared which should have broad appeal because of the rapidity of analyses and requirement of only standard LC equipment or expertise. The method developed by the Associate Referee (18) uses an ion-pairing reagent in the presence of aqueous acetic acid to extract the glycoalkaloids and retain them on a disposable C-18 cartridge column for cleanup. The analytes are eluted with aqueous acetonitrile and injected directly onto a C-8 LC column for analysis using UV absorbance at 202 nm for detection. The method offers a TLC procedure for confirmation of identity.

A modification of this method has also been published (19). The other method by Hellenas (20) uses an almost identical approach except that no ion-paring reagent is utilized and a sample enrichment column replaces the sample loop on the LC injector. Separation is accomplished on a C-18 LC column. Recoveries, sensitivities, and reproducibility of the 2 methods are comparable. A novel departure from the above approaches has been taken by Price and coworkers (21) in the use of fast atom bombardment mass spectrometry (FAB-MS) for the determination of tomatine in tomatoes. The freeze-dried fruit was extracted with an aqueous-organic solvent mixture, filtered, evaporated to a small volume, and mixed with ion-exchange resin. The supernatant was freeze-dried and mixed with dilute acetic acid, and an aliquot of the solution was analyzed by FAB-MS.

The method is offered as an example of the potential of FAB-MS for quantitative minor component analysis.

A collaborative study of the method described in (18) was planned for the past year but was delayed due to the difficulty in stabilizing the analytes in samples for shipment to collaborators. The problem is being resolved, and the study is scheduled for completion next year.

Pyrrolizidine Alkaloids.—Progress in this area continues to be delayed by difficulties in obtaining reference standards.

Phytoestrogens.—A published (22) procedure for the analysis of genistein, biochanin A, daidzein, formonetin, and coumestrol in several commodities is being evaluated (S. S. Kuan, private communications). Initial studies gave poor recoveries. Modifications of the extraction solvent systems are being investigated.

Recommendations

(1) *Glucosinolates:* Coordinate collaborative study with EEC; continue study in other areas.

(2) *Hydrazines:* Appoint Associate Referee; continue study.

(3) *Pyrrolizidine Alkaloids:* Continue study.

(4) *Steroidal Alkaloids:* Complete collaborative study for potato glycoalkaloids; continue study in other areas.

(5) *Phytoestrogens:* Complete pre-collaborative study of LC method for isoflavones and coumestanes; continue study in other areas.

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Seafood Toxins

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Cyanobacterial Peptide Toxins.—The Associate Referee is Harold W. Siegelman of Brookhaven National Laboratory, Biology Department, Upton, NY.

The cyclic heptapeptide toxins called cyanoginosins of *Microcystis aeruginosa* are the primary cause of poisoning correlated with blooms of freshwater cyanobacteria. The toxin cyanoginosin-LR was found to be the principal toxin of specific clones of a Norwegian and a Canadian strain of *M. aeruginosa,* and the same toxin was found in a Canadian strain of *Anabaena flos-aquae* (1). This is the first report of a cyanoginosin in a cyanobacteria other than *M. aeruginosa.* Identifications were based on liquid chromatography (LC), amino acid analyses, and mass spectrometry. Small-scale purification and identification of the cyanoginosins by liquid chromatographic and thin-layer chromatographic methods have been described (1, 2).

A monoclonal antibody for cyanoginosin-LA has been obtained (3) that cross reacts with equal efficiency with 6 other variants (-LB, -LR, -LY, -AY, -FR, and -YR) of cyanoginosin. When cyanoginosin-YM labeled with ^{125}I was administered intraperitoneally to male mice, the liver was the principal target, with minor radioactivity found in the kidneys (4). The ultrastructural changes in mouse liver induced by either lethal or sublethal doses of cyanoginosin-LR have been described (5). A general review of algal toxins, including the cyanobacterial toxins, has appeared (6).

Diarrhetic Shellfish Poisoning (DSP).—The Associate Referee is Takeshi Yasumoto of the Faculty of Agriculture, Tohoku University, Tsutsumidori-Amamiyamachi, 1-1 Sendai 980, Japan.

Toxins: The chemical structure of yessotoxin (YTX) has been determined by NMR techniques (7). YTX, which has a molecular formula of $C_{56}H_{80}O_{21}S_2Na_2$, resembles the brevetoxins in having 11 ether rings contiguously fused in a *trans* configuration, but it is distinct from the brevetoxins in having a longer backbone of 47 carbons, a terminal side chain of 9 carbons, and 2 sulfate esters and in lacking carbonyl groups.

A new member of pectenotoxin analogs has been isolated and named pectenotoxin-6 (PTX6). Unlike the other pectenotoxins, PTX6 was obtained from an acidic fraction. Elucidation of its chemical structure is underway.

The principal toxin in European mussels had been reported to be okadiac acid (8). However, in a recent study, the toxin composition of mussels collected from Sognefjord in Norway was comparable to that of Japanese mussels, i.e., dinophysistoxin-1 was the major toxin with a pectenotoxin-like toxin and a yessotoxin-like toxin as minor constituents. This finding is the first indication of the presence of more than one toxin in a European mussel.

Source of the toxins: Production of okadaic acid by *Prorocentrum lima* collected from tropical coral reefs was previously established (9). However, the toxigenicity of the same species growing in temperate waters has not been determined. In collaboration with researchers in Vigo, Spain, both oka-

daic acid and dinophysistoxin-1 were detected in a strain of *P. lima* collected from Vigo. Production of both toxins by a temperate water-strain of *P. lima* suggests the possible involvement of this species in DSP.

Assay: The LC/fluorometric method being developed for the detection of the DSP toxins has been found useful in determining okadaic acid and dinophysistoxin-1 in shellfish (10). Efforts are underway to expand the method to determine the other toxins.

Effects on human health: Okadaic acid appears to be a tumor promoter possessing a non-TPA type of activity (11). This report calls for a careful investigation to assess the tumor-promoting activity of okadaic acid and its derivatives present at concentrations below the current quarantine level for shellfish.

Neurotoxic Shellfish Poisoning.—The Associate Referee is Daniel G. Baden of the Rosenstiel School of Marine and Atmospheric Sciences, University of Miami, FL.

Florida's red tide dinoflagellate *Ptychodiscus brevis* produces at least 8 membrane-depolarizing agents (12, 13). All of the known toxins are based on 2 distinct multiring polyether backbones (12). Several different detection methods are under development including (a) radioimmunoassay, (b) enzyme-linked immunosorbent assay, (c) molecular pharmacological assays using voltage-dependent sodium channel preparations from rodent brain, and (d) liquid chromatography.

Liquid chromatography of brevetoxins using reverse-phase C-18 columns and an isocratic elution solvent of 85% aqueous methanol resolves the toxins in order of increasing hydrophobicity (PbTx-3, PbTx-6, PbTx-2, PbTx-7, PbTx-5, and PbTx-1) (14). Toxins eluted are detected by UV absorbance at 215 nm.

Two separate radiometric assays have been developed to detect brevetoxins. Brevetoxin PbTx-3 has been produced in tritiated form by reductive tritiation of brevetoxin PbTx-2. Tritiated PbTx-3 (sp. act. = 10 Ci/mmole) has been used as a specific probe in competitive radioimmunoassays developed to detect brevetoxins in food sources and in body fluids (15). This probe has also been utilized to characterize the brevetoxin binding site in rat brain synaptosomes (14). Detection sensitivity using each of the assays is in the nanomolar to picomolar concentration ranges (16). Preliminary experiments indicate that PbTx-1 can be readily reduced in a fashion similar to PbTx-2 to produce tritiated PbTx-7 at similar specific activities (unpublished data).

Four brevetoxins (PbTx-2 and -3 and PbTx-1 and -7), 2 possessing the same structural backbone as PbTx-3 and 2 brevetoxins possessing a second structural backbone, have been compared quantitatively in their abilities to competitively displace tritiated PbTx-3 from its specific site in each assay. In radioimmunoassay, the ability to displace appeared to be based solely on each toxin's structural similarity to PbTx-3, the hapten against which the antibodies were raised. In the synaptosome assay, the ability to competitively displace labeled PbTx-3 correlated in a positive fashion with each toxin's potency in whole animals (16).

Further work is underway (a) to produce antibodies to each of the 2 structural backbone brevetoxins and evaluate their respective cross-reactivities, and (b) to examine further the feasibility of converting the sodium channel assay to a type of enzyme-linked assay. This latter endeavor is undertaken with the new knowledge that in the sodium channel assay binding affinity parallels potency.

The radioimmunoassay has been converted to a competitive sandwich ELISA assay, which uses microtitre plates, with adsorbed toxin followed by specific gamma-globulin, and visualized using Protein A-urease conjugate. Assay sensitivity is currently similar to radioimmunoassay, and further refinement promises pico- to femtomolar sensitivity by late 1987. Antibodies to each of the brevetoxin backbones will be used in the ELISA. Further work is underway to produce PbTx-3- and PbTx-7-linked enzymes to permit competitive ELISA to be evaluated.

With the increasing frequency and intensity of *Ptychodiscus brevis* red tides along Florida coasts and the coasts of Texas through Mexico, it is imperative (a) that specific assays for the subject toxins be developed, and (b) that the assays developed reflect the potency of the materials being assayed. It is useless to have at our disposal assays which detect precisely the amount of toxin present, when that detection has no element of potency measurement within it. In other words, a small amount of a highly potent material is much more dangerous from a public health point of view than is a larger amount of a low potency material. Neurotoxic shellfish research is addressing "composite potency" measurements in a much more rigorous manner than ever before.

Paralytic Shellfish Poisons (PSP)—Electrochemical Detection.—The Associate Referee is William L. Childress, FDA, Boston, MA.

An investigation is underway to determine the PSP toxins by electrochemical detection after LC separation and photolytic derivatization of the toxins.

Paralytic Shellfish Poisons—LC Determination.—The Associate Referee is John J. Sullivan, FDA, Seattle, WA.

Work has progressed in several areas relating to the development of an LC method for the PSP toxins. One area involved an interlaboratory comparison of the method on approximately 100 samples and a more intensive single laboratory comparison of the LC method with the standard mouse bioassay procedure. The data from these studies are being evaluated and, when evaluation is complete, should indicate the scope of application of the LC method and whether any refinements in the procedure need to be made.

Progress continues on preparation for the LC/PSP collaborative study. The laboratory recruiting process was started by surveying those laboratories worldwide that might be interested in participating. The survey is intended to identify laboratories that have the proper instrumentation available and are willing to commit the necessary time. Based on the results of the survey, laboratories will be selected for participation in the collaborative study which will probably be initiated in the fall of 1987. Between now and when the study is actually started, refinements in the methodology will be made if required and work will be started in collecting the necessary samples and standards.

Paralytic Shellfish Poisons—Immunoassays.—The Associate Referee is Patrick Guire, Bio-Metric Systems, Inc. (BSI), Eden Prairie, MN.

Results have been published (17) from the FDA study comparing the BSI radioimmunoassay kit and liquid chromatography with the mouse bioassay for PSP in Connecticut and Maine shellfish. Findings indicate usefulness of the immunoassay system based on the BSI antibody preparation for the rapid economical detection of saxitoxin in those samples not requiring detection of neosaxitoxin or its derivatives. Researchers at the University of Wisconsin–Madison (F. S. Chu) have resumed their work towards a PSP enzyme immunoassay, but the results are still in the prepublication stage. A manuscript has been submitted for publication from

Woods Hole, MA, (D. M. Anderson) using the BSI polyclonal antibody against saxitoxinol for studies of the intracellular synthesis and localization of PSP toxins in toxic algal cells.

Tetrodotoxin. — Associate Referee is Yuzuru Shimizu, College of Pharmacy, University of Rhode Island, Kingston, RI. Prof. Shimizu has no report.

Evaluation of the LC procedure of Yasumoto and Michishita (18) is currently underway at FDA (E. Calvey, private communication).

Ciguatoxin—Biochemical Methods. — The Associate Referee is Yoshitsugi Hokama of the John. A. Burns School of Medicine, University of Hawaii at Manoa, Honolulu, HI.

The preliminary, practice trial of the 9-laboratory collaborative study to evaluate the rapid enzyme immunoassay stick test for the detection of ciguatoxin and related polyethers has been concluded. Eight of the 9 laboratories obtained results that were within acceptable limits for each of the 3 fish cake samples homogenized with ciguatoxin. The RSD (reproducibility, CV) among the laboratories was 23–30%. The full collaborative study will commence in the fall of 1987.

Recommendations

(1) Complete the collaborative study on the enzyme immunoassay "stick test" as a screening method to detect ciguatoxin and related polyethers.

(2) Initiate collaborative study of the fluorometric procedure for paralytic shellfish poisons.

(3) Continue study on other topics.

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GENERAL REFEREE REPORTS: COMMITTEE ON FOODS II

Alcoholic Beverages

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Sam Blittman, chemist at the Bureau of Alcohol, Tobacco and Firearms Laboratory in San Francisco, has been appointed the Associated Referee for Alcohol Content by Oscillating U-Tube Density Meter. He reports no additional collaborative studies are planned at this time; however, he is closely following the literature for data and maintaining contact with laboratories using the density meter for alcohol determinations.

Tom Peffer of Beringer Vineyards and Jeff Kasavan of Taylor California Cellars have been appointed as co-Associate Referees for the topic Sugars in Wine by LC. They report that a survey of methods and instruments and of current applications being used in various laboratories, a literature search, and inquiries for possible collaborators are in progress, and a collaborative study is being planned.

Associate Referee Barry Gump at Fresno State University has sent a report with the preliminary data collected from the sulfur dioxide in wine collaborative study conducted in May 1987. The completed data were presented along with his recommendation at the AOAC Annual International Meeting in September at San Francisco. His initial review indicates a good response from collaborators and favorable results for blind duplicates and the recovery of added sulfur dioxide. He anticipates presenting a comprehensive paper showing comparative results with other methods and experimental data indicating the ruggedness of this method. After reviewing the preliminary report, the General Referee also anticipates the data will substantiate a recommendation for adoption as interim official first action.

The method described by Arthur Caputi in the manuscript "Titrimetric Determination of Carbon Dioxide in Wine" has been granted interim official first action. Since the paper had already been presented in poster form at an Annual Meeting, it was not presented at the 1987 meeting.

David Chia, the Associate Referee for Malic Acid Wine, reports continued problems with the analysis; nevertheless, work is progressing slowly, although no new collaborative experiment is planned.

The American Society of Brewing Chemists' (ASBC) method, Specific Gravity of Beer and Wort Using a Digital Density meter, which was presented by the AOAC-ASBC Liaison Officer Peter Gales, has been granted interim official first action, was presented in poster form at the Annual International Meeting in September 1987, and will be submitted to the *Journal of AOAC*.

Recommendations

(1) Adopt as official first action the interim official action methods for carbon dioxide in wine and for specific gravity of beer and wort.

(2) Continue study on all topics.

This report of the General Referee was presented at the 101st Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. The recommendations were reviewed by the Committee on Foods II. See the report of the committee, this issue.

Cereals and Cereal Products

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Fat Acidity in Flour.—A study was conducted to compare fat acidity values between the method of the American Association of Cereal Chemists (AACC), which is similar to the AOAC method (**14.069-14.071**), and the method of the International Standardization Organization (ISO) using 8 commercially available flours. AACC/AOAC methodology yielded values significantly lower than those obtained by the ISO method. As the fat solvent, AACC/AOAC methodology utilized petroleum ether, which extracted fatty acids only, whereas the ISO method utilized ethyl alcohol, which extracted amino acids and acid phosphates as well as fatty acids. This study was undertaken to establish comparative data as requested by the working group of the Codex Alimentarius Committee on Cereals and Legumes.

A minimum collaborative study also was conducted to determine if a difference in fatty acid values occurred when benzene was used as part of the titration medium as prescribed in **14.069-14.071**, compared to values derived when toluene was substituted for benzene. Ralph Lane served as Associate Referee for the study and participated with Dick Erickson and Frank Ebert as collaborators. Data from the study indicated no differences in fat acidity values with respect to use of these 2 solvents. It appears that toluene, which is reported to be less toxic than benzene, may be used in place of benzene without influencing the outcome of the fat acidity analysis.

Iron in Flour.—Collaborators for the study on reducing the 11-point standard curve for iron (**14.012**) to a 4-point standard curve has been selected from the AOAC Talent File. The study under the direction of Jim Martin, Associate Referee, is currently underway.

Phytate in Foods.—Associate Referee Barbara Harland continues to be active in the area of phytate analysis. She served as Associate Referee for the phytate method, **14.B01-14.B05**, which is due for consideration as official final action.

Recommendations

(1) Consider substitution of toluene for benzene in method **14.069-14.071**, based on data from a minimum collaborative study.

(2) Continue collaborative study of rapid analysis of iron in cereal based products.

(3) Adopt as official final action the official first action method for phytate in foods, **14.B01-14.B05.**

This report of the General Referee was presented at the 101st Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. 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 edition, and "Changes in Official Methods," *J. Assoc. Off. Anal. Chem.* (1986) **69**, 349–390 (B methods).

Chocolate and Cacao Products

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Because of the General Referee's involvement with Working Group 18 of the International Office of Cocoa, Chocolate and Sugar Confectionery (IOCCC), "Revision and Updating of IOCCC Standard Methods of Analysis," little progress has been made on AOAC collaborative studies. However, as a result of that involvement, several methods of analysis, new ones and revised old ones, are under consideration for adoption by both AOAC and IOCCC.

In a followup to last year's report (*J. Assoc. Off. Anal. Chem.* [1987] **70,** 289), the General Referee plans to establish an expert group to deal with the problem of revising the existing IOCCC method for determination of moisture in cocoa and cocoa products. Another urgent item being considered is a method for determination of total fat in cocoa products. It is hoped that this project will be conducted with close cooperation between AOAC and IOCCC.

Recommendations

Continue study on all topics.

This report of the General Referee was presented at the 101st Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. The recommendations were reviewed by the Committee on Foods II. See the report of the committee, this issue.

Color Additives

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A new Associate Referee for the Analysis of Color Additives for Arsenic, Barium, and Heavy Metals, William McShane, has been appointed. He is with Kraft, Inc., Technical Center, Glenview, IL.

The Associate Referee for Color in Candy and Beverages, Mary Young, reports that she is preparing a poster presentation and a final draft of her paper, "Rapid Determination of Color Additives, Using the C_{18} Cartridge: Collaborative Study." The method has been granted interim first action status.

The Associate Referee for Colors in Other Foods, Nicholas Adamo, reports publication of "Survey of Color Usage in Food," by the United Kingdom Ministry of Agriculture, Fisheries and Food (Food Surveillance Paper No. 19 [1987] Ministry of Agriculture, Fisheries and Food, 86 pp.).

The Associate Referee for Color by Liquid Chromatography, Elizabeth Cox, reports that her work in this area was published in the November 14, 1986, FDA Laboratory Information Bulletin No. 3093 ("Liquid Chromatographic Determination and Thin Layer Chromatographic Identification of Synthetic Colors in Unsweetened Powdered Soft Drink Mixes" by Elizabeth A. Cox and Naomi Richfield-Fratz, Food and Drug Administration, Division of Colors and Cosmetics, Washington, DC 20204). Elizabeth Cox has recently transferred within FDA and is no longer working in the field of analysis for color additives. We expect to continue the topic and recommend appointment of another Associate Referee.

The Associate Referee on Colors in Cosmetics, Sandra Bell, reports that LC methods for the separation of cosmetic color additives have been published: "Identification of Cosmetic Dyes by Ion-Pair Reversed-Phase High Performance Liquid Chromatography" by L. Gagliardi, G. Cavazzutti, A. Amato, A. Basili, and D. Tonelli (*J. Chromatogr.* [1987] **394,** 345–352) and one newly found in a recent literature search, "A Combined HPLC-VIS Spectrophotometric Method for the Identification of Cosmetic Dyes" by J. Weneger, H. Greenbauer, R. Fordham, and W. Karcher (*J. Liq. Chromatogr.* [1984] **4,** 809–821).

The Associate Referee for Uncombined Intermediates of Water-Soluble Azo Colors, Daniel Marmion, reports that he is no longer working in this field. We recommend that the topic be continued and a new Associate Referee be appointed. A potential candidate has been located. We are waiting for a final decision. Activities under this topic include a paper accepted for publication in the *Journal of Chromatography* entitled "Determination of Cresidine in FD&C Red No. 40," by Naomi Richfield-Fratz and John Bailey. A poster is being presented at the 101st AOAC annual meeting entitled "Isolation and Characterization of Trace Organic Constituents in FD&C Red No. 40" by Naomi Richfield-Fratz, William Baczynskyj, John Bailey, and George Miller.

The Associate Referee for Subsidiary Colors in Certifiable Color Additives, John Bailey, reports that 2 papers were published under the topic area, "HPLC Determination of Subsidiary Dyes, Intermediates and Side Reaction Products of Erythrosine" by Frank Lancaster and James Lawrence (*J. Chromatogr.* [1987] **388,** 248–252) and "Characterization, Purification and Analysis of Solvent Yellow 33 and Solvent Green 3 Dyes" by William Blakemore, Larry Rushing, Harold Thompson Jr., James Freeman, Robert Levine, and Charles Nony (*J. Chromatogr.* [1987] **391,** 219–231). Solvent Yellow 33 is certifiable as D&C Yellow No. 11. Bailey reports that time constraints make it necessary to appoint a replacement as Associate Referee. Two potential candidates are being considered.

Another publication recently uncovered in a literature search and related to the analysis of color additives is "Detection and Determination of Organic Impurities in Food Coal-Tar Dyes by HPLC" by Y. Tonogai, Y. Ito, and M Iwaida of the National Institute of Hygienic Science, Osaka, Japan (*J. Food Hyg. Soc. Jpn* [1983] **24,** 275–281 [in English]).

Recommendations

(1) Continue study on all present topics.

(2) Adopt the interim official method on "Rapid Determination of Color Additives using the C_{18} Cartridge" by Mary Young, Associate Referee for Color in Candy and Beverages as official first action.

(3) Consider the need for a new topic on determination of color additives exempt from certification. The trade literature indicates increased use of the colors exempt from certification in foods. Analytical problems have arisen in the FDA district laboratories due to the lack of tested methodology in this area.

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Flavors

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In the past year, Sidney Kahan, Associate Referee for Vanilla, has submitted a rewritten collaborative study for the liquid chromatographic determination of vanillin and related flavor compounds in vanilla. The General Referee strongly recommends that this method be adopted official first action.

Recommendations

(1) Adopt official first action the liquid chromatographic determination of vanillin and related flavor compounds in vanilla.

(2) Continue study on all other topics.

This report of the General Referee was presented at the 101st Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. The recommendations were reviewed by the Committee on Foods II. See the report of the committee, this issue.

Fruits and Fruit Products

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Adulteration of Apple Juice.—Last year, Associate Referee E. R. Elkins reported that the paper "Interlaboratory Variability of Methods Used for Detection of Economic Adulteration in Apple Juice" had been published (Zyren, J, & Elkins, E. R. [1985] *J. Assoc. Off. Anal. Chem.* **68**, 672–676). The methods have been studied extensively by the National Food Processors Association (NFPA) Committee of Food Industry Analytical Chemists, and good results have been obtained. The methods were used in an AOAC collaborative study conducted by Elkins earlier this year. The study, involving the L-malic/total malic acid ratio, has been completed and the results are being statistically evaluated. The CV values ranged from 4 to 10%. The Associate Referee plans to recommend official first action. Elkins recommends continued study, and the General Referee concurs.

Adulteration of Orange Juice by Pulpwash and Dilution.—Associate Referee Don Petrus reports that his laboratory has completed the chemical and spectral characterizations of citrus products from California, Arizona, Mexico, Belize, and Brazil. He also indicated that a mini-collaborative study, using the visible and ultraviolet absorption and fluorescence spectral methodology, will be initiated soon to apply the methodology to other citrus-producing areas. The Associate Referee recommends continued study, and the General Referee concurs.

Fruit Acids.—Associate Referee E. D. Coppola reports that his organization has received many reprint requests for his collaborative study on determination of fruit acids (Coppola, E. D., & Starr, M. S. [1986] *J. Assoc. Off. Anal. Chem.* **69**, 594–597). Recently, John Heuser, NFPA, referred to this methodology in his work on apple and pineapple juices.

One caveat for the user of the AOAC method is that isocitric acid does not separate well from malic acid. Therefore, in certain juices where isocitric acid may be present at significant levels, the malic acid value may be inflated. In common juices, such as cranberry and apple, however, this potential interference does not seem to be a problem, because isocitric acid is present in trace amounts only.

Fruit Juices, Identification and Characterization.—The General Referee recommends continued study on this subject.

Limonin in Grapefruit Juice.—Associate Referee Russell Rouseff reports that 11 laboratories took part in interlaboratory research designed to study the characteristics of an immunoassay for the determination of limonin in grapefruit juice. Each laboratory received 16 randomly coded samples (8 blind duplicates) in accordance with AOAC recommendations. The results are in, and the data have been statistically analyzed. Using procedures in the AOAC Statistical Manual (Steiner's ranking test), results from 2 of the laboratories could not be used because of consistently high or low values. Average coefficients of variation for the 8 blind duplicate samples from the remaining 9 laboratories ranged from 11 to 31%, an acceptable level of precision at the low ppm level. The complete results will be submitted to *J. Assoc. Off. Anal. Chem.* in the form of a paper in the near future; therefore, the General Referee recommends continued study.

Moisture in Dried Fruits.—Associate Referee Ed Steffen reports that for many years the dried fruit moisture tester of the Dried Fruit Association (DFA) has been an AOAC official method 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 main problems in conducting a collaborative study has been in obtaining samples with uniform moisture. A few years ago, for example, the fig industry had a problem with a buyer's quality control department which could not understand why samples from different parts of the same shipment showed such wide variations in moisture content. They compared fig paste to flour which, of course, does not have this problem. With fig paste, water is added in varying amounts to keep the paste from sticking to a grinder; it therefore varied slightly within any shipment, although the processors do attempt to control the moisture as much as possible by taking a number of readings on every shipment.

DFA has worked on this problem, which seems to have been largely corrected. Under the current procedure, controlled amounts of water are added to low-moisture figs to produce a uniform moisture content. In addition, samples with various amounts of moisture can now be supplied to collaborators. A few contacts have been made, but more collaborators will have to be found before a meaningful collection of data can be made.

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 a national survey (1399 samples tested from July 1, 1986 through June 17, 1987) of commercial single-strength and reconstituted frozen concentrated orange

juices, benzoic acid in the range of 0.6–235.7 ppm was found in 36 samples; 0.1–260.3 ppm sorbic acid was found in 12 samples. Ninety samples contained <0.5 ppm benzoate, but it is uncertain whether this was the result of addition of preservative or adulteration with pulpwash or whether it was a natural presence. Eight samples showed both benzoate and sorbate. The method is now ready for a collaborative study, and it is hoped one will be started next year. The Associate Referee recommends continued study, and the General Referee concurs.

Recommendations

(1) The General Referee recommends a change in the method for acidity (titratable) of fruit products, **22.058-22.059**. For the most part, the final action AOAC method compares favorably with the international standards method, Fruit and Vegetable Products—Determination of Titratable Acidity, ISO 750, First Ed. 1981-11-15. However, the ISO method contains a provision which should be included in the AOAC entry. Section 5.3 of the ISO method provides a means to express the titratable acidity in g acid per 100 g or 100 mL of product by using an established factor. Accordingly, the General Referee recommends addition of the following material as an introductory paragraph to the AOAC method for titratable acidity: It is possible to express the titratable acidity conventionally in g acid per 100 g or per 100 mL of product, as appropriate, by using the factor appropriate to the acid, as follows: malic acid, 0.067; oxalic acid, 0.045; citric acid monohydrate, 0.070; tartaric acid, 0.075; sulfuric acid, 0.049; acetic acid, 0.060; lactic acid, 0.090.

(2) Study should continue on all topics.

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Section numbers refer to *Official Methods of Analysis* (1984) 14th edition.

Nonalcoholic Beverages

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Leonard Valenti, Associate Referee for quinine, has had delays in getting a statistical review of his collaborative study. He plans to complete a paper in the future.

The Associate Referee for caffeine and methyl xanthine in nonalcoholic beverages, John M. Newton, has done some work comparing capillary gas chromatography to liquid chromatography for the separation of caffeine, theophylline, and theobromide.

Recommendations

(1) Appoint an Associate Referee on the analysis of safrole in sassafrass root.

(2) Continue study on all topics.

This report of the General Referee was presented at the 101st Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. The recommendations were reviewed by the Committee on Foods II. See the report of the committee, this issue.

Preservatives and Artificial Sweeteners

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Aspartame, Benzoates, Saccharin, and Caffeine (Liquid Chromatography).—Associate Referee N. G. Webb and D. D. Beckman developed a method for the determination of aspartame in beverage mixes by liquid chromatography (*J. Assoc. Off. Anal. Chem.* [1984] **67,** 510–513). The conditions used in this method represent a modification of the official final action method, **12.018-12.021**, for benzoates, saccharin, and caffeine. Aspartame was quantitated in the presence of these additives as were artificial colors and flavors. The Associate Referee had planned collaborative study of this method but other commitments have necessitated that she relinquish her refereeship. Initially, it was contemplated that one of Associate Referee Webb's colleagues might continue her work, but this has not proven feasible. It is recommended that a new Associate Referee be appointed and that work be continued on this topic.

Organic Preservatives (Chromatography).—Associate Referee Rosella Bigornia is pursuing the objective of a general qualitative liquid chromatographic method for common preservatives. Preliminary studies have resulted in a promising reverse phase, gradient elution procedure. The preservatives being studied are: benzoic acid, sorbic acid, and the parabens, ranging from the methyl to the heptyl ester. Several foods spiked with these additives have been successfully chromatographed using this approach. A protocol will be developed for a collaborative study, which it is hoped may be completed during the coming year.

Sulfites in Foods (Ion-Chromatographic Methods).—Darryl M. Sullivan and Hie-Joon Kim have been appointed co-Associate Referees. They, together with their respective colleagues, have developed different approaches to the analysis of sulfite by ion chromatography.

Each of these researchers is interested in conducting collaborative studies of their respective methods. Kim has submitted a collaborative study protocol which is presently being evaluated.

Sulfites in Foods (Flow Injection Methods).—Associate Referee John Sullivan has completed a collaborative study for sulfites in foods, using a flow injection analyzer (FIA). The commodities tested included shrimp, potato flakes, dried pineapple, and wine (red and white). The FIA method is based on a *J. Assoc. Off. Anal. Chem.* publication ([1986] **69,** 542–546). However, several minor improvements/modifications were made aimed at simplifying the extraction procedure and improving accuracy for those samples containing interfering substances. The study involved the determination of "total sulfite" in all samples in addition to "free sulfite" in the wines. The results of the study are being evaluated.

Sulfites in Foods (Polarographic Methods).—Associate Referee Walter Holak and coworker Bhailel Patel have developed a differential polarographic method which is capable of determining sulfur dioxide in various food products down to ca 10 ppm. The method was adopted last year as official first action for total sulfites in the products tested (freeze-dried shrimp, orange juice, dried apricots, strained peas and dehydrated potatoes). Some total sulfite recoveries were low, and although these were attributed to sulfite-sample matrix interactions, a study was initiated of the means by which recoveries might be improved. As a first step, a 4-laboratory mini-study is underway using sulfite-stabilized samples in-

stead of spikes. The samples—clams, instant potatoes, wine cooler, and grape juice—were shipped frozen. The Associate Referee is waiting for the participating laboratories to submit their results.

Sulfites in Shrimp (Screening Methods).—Associate Referee M. Hudak-Roos conducted a collaborative study of a colorimetric qualitative screening method for total sulfite in shrimp. The method is a modified Monier-Williams approach in which the liberated SO_2 is passed into a potassium permanganate solution of known concentration. It is designed as a pass/fail test at the 100 ppm level.

Participating laboratories were required to run the samples by the Monier-Williams method as well as by the modified method. No false positives were obtained; however, a moderate number of false negatives (as defined by the corresponding Monier-Williams results) were obtained at the critical 100 ppm level. Variability in the Monier-Williams results, which were being used as the standards, also compromised the evaluation. Although the results of this study did not support AOAC adoption of the modified Monier-Williams method, the possibility of another study using a recently developed instrumental method is being considered. It is recommended that study on this topic be continued.

Recommendations

(1) Appoint Associate Referee for Aspartame, Benzoates, Saccharin, and Caffeine (Liquid Chromatography).

(2) Continue study on all other topics.

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Section numbers refer to *Official Methods of Analysis* (1984) 14th edition.

Processed Vegetable Products

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pH Determination.—The collaborative study of the method for pH of acidified foods was reported in *J. Assoc. Off. Anal. Chem.* (1981) **64**, 332–336. The method received official final action status in 1983. Study of the suitability of new electrode and instrument developments as well as the suitability of this method for low-acid and acid foods has been suggested. Interested persons are invited to submit comments and participate in continued study of this topic. The Associate Referee recommends that work continue on this topic.

Sodium Chloride.—The Associate Referee retired with the recommendation that a review of these methods be performed to identify current needs pertaining to processed vegetable products. Interested persons are invited to submit comments and participate in continued study of this topic. Appointment of a new Associate Referee is recommended.

Total Solids By Microwave Moisture Methods.—The method for the determination of solids in processed tomato products by microwave drying oven was adopted as official first action in 1984. A technical communication titled "Microwave Oven Drying Method for Total Solids Determination In Tomatoes—A Collaborative Survey" by Samuel L. Wang was recently published (*J. Assoc. Off. Anal. Chem.* [1987] **70**, 758–759). This report indicates that the reliability of this method is closely related to the performance of the microwave solids analyzer and recommends certification of the microwave solids analyzer for use in the method. The Associate Referee is considering development and addition of a calibration procedure into this method. Interested persons are invited to submit findings and comments on this issue. Continued study of this topic is recommended.

Water Activity in Foods.—The method for water activity determination was adopted official final action in 1982. A manuscript titled "Evaluation of Precision Estimates for Fiber-Dimensional and Electrical Hygrometers for Water Activity Determination" by William H. Stroup, James T. Peeler, and Kent Smith of the Food and Drug Administration has been published in *J. Assoc. Off. Anal. Chem.* (1987) **70**, 955–959. Studies are recommended to investigate product-instrument sensor interactions and to determine how often calibration is needed and the means whereby calibration can be performed in significantly less time. Interested persons are invited to submit comments and participate in continued study of this topic.

LC Determination of Sugars in Processed Vegetables.—No response was received 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.

Recommendations

(1) Appoint Associate Referees on Sodium Chloride and on LC Determination of Sugars in Processed Vegetables.

(2) Continue study on all topics.

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Spices and Other Condiments

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Bulk Index of Spices.—Associate Referee Tom Haney is preparing a report on the collaborative study.

Ethylene Oxide Residue in Spices.—Associate Referee Lynn Theiss reports a revised method is under evaluation. A collaborative study will follow.

Moisture in Spices.—Associate Referee Lou Sanna reports that the official first action method for moisture in spices (distillation method) will be recommended for adoption as official final action at the 1987 AOAC Annual Meeting.

Piperine in Black Pepper.—Associate Referee Ted Lupina, Kalsec, Inc., reports that his manuscript "UV Spectrophotometric Determination of Piperine in Pepper Preparations: "Collaborative Study" was published (*J. Assoc. Off. Anal. Chem.* [1987] **70**, 112–113). The method is official first action.

Pungency of Capsicums and Their Oleoresins.—Patrick Hoffman, McCormick and Co., reports that comments and suggestions for reducing variation between laboratories at very low and very high pungency levels are being solicited from collaborators prior to beginning a second collaborative study. The goal is to provide a simple and reliable method of analysis at *all* pungency levels.

Vinegar.—Associate Referee Dana Krueger reports that he

is writing a report of the collaborative study on the SIRA (carbon stable isotope ratio analyses) method for detection of adulteration of apple cider vinegar.

Recommendation

(1) Adopt as official final action the official first action method for moisture in dried spices, **30.B01-30.B03**.

(2) On the basis of the results of a collaborative study completed in 1986, adopt the Codex Alimentarius method for determination of soluble solids in vinegar to replace AOAC method **30.063**.

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Sugars and Sugar Products

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Color, Turbidity, and Reflectance—Visual Appearance.—Associate Referee M. A. Clarke reports no new developments.

Corn Syrup and Sugars.—Associate Referee Raffaele Bernetti reports no new developments.

Enzymatic Methods.—Associate Referee Marc Mason reports no new developments.

Gas Chromatographic Methods.—Associate Referee Mary An Godshall reports no new developments.

Honey.—Associate Referee Jonathan W. White, Jr, submits the following report, which is included in its entirety because some of the information therein is not generally available; the General Referee agrees with both of White's recommendations:

The Associate Referee pointed out at the 1986 AOAC Annual International meeting (1) that the U.S. Department of Agriculture requires honey offered under their loan-purchase program to be tested for adulteration by using the isotope ratio method (2, 3). Unfortunately, they regard any $\delta^{13}C$ value less negative than −23.4‰ as unequivocally adulterated, contrary to AOAC recommendations in the isotope ratio method in the 13th edition of *Official Methods of Analysis* (1980), which originally set this limit at −21.5‰ (4).

Soon after the method was adopted, it was realized that −21.5‰, set for statistical reasons, was too liberal a limit, allowing up to 15% of added corn syrup in honeys with the average $\delta^{13}C$ of −25.4‰. Shortly, a TLC method for high-fructose corn syrup in honey (5) became available, which was considerably more sensitive for corn products than was the isotope ratio method. In 1980, at the recommendation of the Associate Referee, AOAC adopted the TLC method, intended for use when the $\delta^{13}C$ value is in the so-called "grey area" between −23.4 (2s) and −21.5‰ (4s) (6–8). No indication of the conditions requiring use of the TLC method was included in the isotope ratio method (**31.150-31.153**) in the 13th edition of *Official Methods of Analysis* (3). However, such conditions were added to the method (**31.158-31.161**) in the 14th edition of *Official Methods of Analysis* (4).

In response to protests from the beekeeping industry and others regarding the use of the −23.4‰ limit, USDA-Agricultural Stabilization and Conservation Service (ASCS) now permits producers receiving allegations of adulteration resulting therefrom to request a TLC test of the honey at their expense to confirm or deny the charge of adulteration. An additional problem with the ASCS limit of −23.4‰ is that certain honey types normally have $\delta^{13}C$ at or lighter than this value, for example citrus honey, which has been shown (9) to average −23.8‰, s = 0.96, significantly less negative than the average of all honeys ($\delta^{13}C$ = −25.4‰, s = 0.98) (9). This means that many samples of pure citrus honey placed in the ASCS program have been mistakenly branded "adulterated," to the great distress of the producer. In addition, there is some evidence that honey from mesquite (*Prosopis* spp.) and related plants may have values even less negative than that of citrus honey and are similarly discriminated against. It would be prudent, therefore, to extend the interpretative language in **31.161** requiring the TLC test to include these limits at least for citrus honey. The limiting value of −21.5‰ given in the method for all honey should be changed, for citrus honey only, to −20.0‰ (9), the 4s value for this type of honey.

Recent information (J. Bowden, personal communication) indicated that approximately 93% of the TLC tests requested by producers from ASCS have been negative. However, several instances of apparently positive results have occurred in which the producers of the honey are convinced that no corn syrup could be present, even though the test was run according to the specified procedure and was visually positive, i.e., showed streaks or spots at R_f 0.35 or less, and with a 50% alcohol eluate residue of <15 mg. Circumstances of production of 2 of these samples brought to the attention of the Associate Referee strongly indicate that they probably contain honeydew.

In the paper describing the development of the TLC test (published subsequent to the adoption of the isotope ratio test) Kushnir (5) described an additional step to eliminate false positives caused by honeydew found in some honeys. She proposed that samples with 50% alcohol residues of over 15 mg and found positive be rerun with the addition of an elution by 25% alcohol before the 50% alcohol elution. This eliminates the natural material responsible for the false positives without affecting the material from high-fructose corn syrup. This step is included in method **31.157**.

Kushnir (5) noted that about 40% of honeys were found earlier (10) to contain >1.5% higher sugars, corresponding to a 15 mg residue in the TLC test. Most of these honeys do not contain honeydew (11); thus, the requirement of weighing all 50% alcohol residues is unnecessarily burdensome to the analyst. Furthermore, it is conceivable that some honeydew-containing honeys may have less than 15 mg residue. It is, therefore, proposed that the requirement for weighing the residue be deleted and that the 25% alcohol step be required only when a positive result is obtained, as a confirmatory step to eliminate possible honeydew interference.

Recommendations

(1) Insert the following sentence before the last sentence in sec. **31.161**: "Corresponding $\delta^{13}C$ values for predominately citrus honey are −21.9‰ and −20.0‰." Insert "(−20.0‰ for citrus honey)" after "PDB" in last sentence of **31.161**.

(2) Modify sec. **31.157** as follows: Under (**a**) *Preparation of sample,* line 6, delete "tared"; lines 7 and 8, delete everything following "bath in current of air or N" to end of paragraph and substitute "or by other appropriate means."; and

line 10, insert "-0.2" after "0.1," insert period after "H_2O," and delete "for each 10 mg material." Under (c) *Interpretation,* lines 2 and 3, delete "When wt of isolated carbohydrate fraction is < 15 mg (1.5%)" and capitalize the following word "Any"; line 4, replace "conclusive" by "presumptive"; and line 7, delete "If wt is > 15 mg" and substitute "In this event."

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(1) White, J. W., Jr (1987) *J. Assoc. Off. Anal. Chem.* **70,** 181–189
(2) White, J. W., Jr, & Doner, L. W. (1978) *J. Assoc. Off Anal. Chem.* **61,** 746–750
(3) *Official Methods of Analysis* (1984) 14th Ed., AOAC, Arlington, VA, secs **31.158-31.161**
(4) *Official Methods of Analysis* (1980) 13th Ed., AOAC, Arlington, VA, secs **31.150-31.153**
(5) Kushnir, I. (1979) *J. Assoc. Off. Anal. Chem.* **62,** 917–920
(6) White, J. W., Jr (1980) *J. Assoc. Off. Anal. Chem.* **63,** 1168
(7) *Official Methods of Analysis* (1980) 13th Ed., AOAC, Arlington, VA, secs **31.148-31.149**
(8) Official Methods of Analysis (1984) 14th Ed., AOAC, Arlington, VA, secs **31.156-31.157**
(9) White, J. W., Jr, & Robinson, F. A. (1983) *J. Assoc. Off Anal. Chem.* **66,** 1–3
(10) White, J. W., Jr, Riethof, M. L., Subers., M. H., & Kushnir, I. (1962) *Composition of American Honeys,* Tech. Bull. 1261, Agricultural Research Service, U.S. Department of Agriculture, Washington, DC
(11) White, J. W., Jr (1980) *J. Assoc. Off. Anal. Chem.* **63,** 11–18

Lactose.—The study by Associate Referee Janice R. Saucerman and Charles E. Winstead, "Liquid Chromatographic Determination of Lactose Purity: Collaborative Study," published in *J. Assoc. Off. Anal. Chem.* (1984) **67,** 899–901, is recommended for official final action.

Liquid Chromatographic Methods.—Associate Referee W. S. Charles Tsang reports that his collaborative study on liquid chromatography (LC) of sucrose in cane, beet, and high test molasses (reported as a poster at the 1986 AOAC meeting) showed results rather widely dispersed. The study is being redesigned, with more specific sample pretreatment instructions and LC conditions.

Maple Saps and Syrups.—Associate Referee MariaFranca Morselli recommends a change in the name of this topic to "Maple Sap, Maple Syrup, and Maple Syrup Products," because considerable work in this topic relates to products made with maple syrup. The General Referee supports this recommendation.

Dr. Morselli and the General Referee recommend that the method for detection of adulteration by cane and corn syrup in maple syrup, using the $^{13}C/^{12}C$ ratio, which was granted official first action in 1984 and was published as "Mass Spectrometric Determination of Cane Sugar and Corn Syrup in Maple Syrup by Use of $^{13}C/^{12}C$ Ratio: Collaborative Study," by Maria Franca Morselli and Kelly L. Baggett, *J. Assoc. Off. Anal. Chem.* (1984) **67,** 22–24, be adopted as official final action.

Dr. Morselli recommends that method **31.191**, Canadian lead number of maple products, and method **31.192-31.194**, Winton lead number of maple products, be deleted from the 15th edition of *Official Methods of Analysis* because they have fallen into disuse. They are obsolete and have been replaced by the carbon ratio mass spectrometric method (**31.185-31.190**). The General Referee concurs with this recommendation.

A collaborative study on methodology for sodium and chloride analysis for maple sap and syrup is planned.

Oligosaccharides in Sugars and Sugar Products.—Associate Referee George Steinle reports that he is organizing a collaborative study on the analysis of raffinose in molasses by liquid chromatography.

Polarimetric Methods for Measurement of Sugars.—Associate Referee Ronald Plews reports no new developments.

Stable Carbon Isotope Ratio Analysis.—Associate Referee Landis Doner reports that the collaborative study on $^{18}O/^{16}O$ measurements to detect beet invert syrup in orange juice is now complete and will be submitted as a manuscript later this year. Further action awaits statistical evaluation.

Standardization of Methods of Sugar Analysis.—Associate Referee Mary An Godshall recommends that the method for determination of dextran in raw cane sugar by the Roberts copper dextran method be granted interim official first action. The General Referee supports this recommendation.

Sugars in Cereals.—Associate Referee Lucien Zygmunt reports that a study on longevity of amino-bonded, cartridge-type LC columns has shown: (*1*) The manufacturing process for these columns is not always reproducible; several had to be returned to the manufacturer. (*2*) On a 100 × 4.6 mm id column packed with 5 μm particles, analysis of mono- and disaccharides, with heated 50% alcohol–water extraction, was possible for 240 injections before column resolution failed. For analysis of gluco-oligosaccharides up to maltopentose, about 250 injections were possible. Both systems used a 15 × 3.2 mm id guard column packed with 7 μm particles.

Sugars in Licorice Products.—Associate Referee Raymond Tuorto is no longer active in this area. The General Referee recommends that this topic be discontinued.

Sugars in Syrups.—Associate Referee Rose Goff reports that a study of sucrose in cane, maple, and table syrups, by LC, is in progress.

Sulfites in Sugars and Syrups.—Associate Referee Richard Riffer reports no new developments. He has compiled a literature survey of this subject that is available upon request.

Weighing, Taring, and Sampling.—Associate Referee Melvin Lerner reports no new developments. Dr. Lerner retired in July 1987; a new Associate Referee is under consideration.

Recommendations

(1) Consider for adoption the non-substantive changes in methods **31.157** and **31.161**.

(2) Consider for adoption as official final action the LC method for determination of lactose purity (**31.064-31.071**).

(3) Consider a change in title for topic "Maple Sap and Syrups" to "Maple Sap, Maple Syrup, and Maple Syrup Products."

(4) Consider for adoption as official final action method **31.185-31.190** for mass spectrometric determination of cane and corn syrup in maple syrup.

(5) Consider deletion of methods **31.191**, Canadian lead number of maple products and **31.192-31.194**, Winton lead number of maple products.

(6) Adopt as official first action the interim official first action copper method for determination of dextran in raw cane sugar.

(7) Discontinue the topic Sugars in Licorice Products.

(8) Continue study on all other topics.

This report of the General Referee was presented at the 101st Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. 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 edition.

Vitamins and Other Nutrients

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Fifteen papers in the topic area have been published in the *Journal of the AOAC* since the 1986 AOAC Annual Meeting. In addition, parts of 2 issues were devoted to papers that comprised the symposium on amino acids presented at the 1985 AOAC Annual Meeting.

Sixteen poster session papers were presented at the 101st AOAC Annual Meeting. A forum for intended amino acid collaborative studies titled "Amino Acid Collaborative Studies and Chromatography Techniques: IE, GC, LC," was conducted as part of this meeting.

Walter Holak, the Associate Referee for iodine, was awarded AOAC's Harvey W. Wiley Award.

A. J. Sheppard, the Associate Referee for vitamin E in pharmaceuticals (gas chromatography), and his coworkers published the results of a study titled "Food Chemical Codex Gas Chromatographic Method for Mixed Tocopherols Concentrate—A Collaborative Study" (*Pharmacop. Forum* [Jan.–Feb. 1987] 2155–2162).

Abdel-Gawad Soliman of the Atlanta Center for Nutrient Analysis was appointed Associate Referee for a new topic, Vitamins A, D, E, and K by Gel-Permeation and Liquid Chromatography.

J. T. Tanner and S. A. Barnett, co-Associate Referees for infant formulas, received the Associate Referee of the Year Award of the Committee on Foods II.

Recommendations

(1) Adopt as official final action the following official first action methods for nutrient analysis in ready-to-feed milk-based infant formula: (*1*) **43.A21**-**43.A40**—for vitamin B_6, calcium, magnesium, potassium, sodium, vitamin C, riboflavin, niacin, manganese, copper, iron, and zinc; and (*2*) **43.B01**-**43.B39**—for chloride, phosphorus, thiamine, vitamin B_{12}, ash, fat, protein, total solids, and carbohydrate.

(2) Adopt as official first action the interim first action methods (*1*) Food Chemical Codex Method for Mixed Tocopherols Concentrate, and (*2*) Determination of Sulfur Amino Acids and Tryptophan.

(3) Continue study on all topics.

This report of the General Referee was presented at the 101st Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. 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) and *J. Assoc. Off. Anal. Chem.* (1986) **69**, 349–390 (B methods).

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Metals and Other Elements

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Arsenic in Animal Tissues.—Associate Referee Randy Simpson reports no progress on this topic and regretfully resigns as the Associate Referee because of his reassignment to another area of research (robotics).

Atomic Absorption Spectrophotometry.—Associate Referee Milan Ihnat reports the development of agriculture-food biological reference materials for monitoring performance of AAS methods and others. Eleven candidate reference materials cover a range of food products: hard red spring wheat flour, soft winter wheat flour, durum flour, corn starch, potato starch, sugar, whole milk powder, wheat gluten, corn bran, whole egg powder, and cellulose. Chemical characterization of the products is under way. Ihnat has published a high reliability flame AAS method (*Fres. Zeit. Anal. Chem.* (1987) **326,** 739–741) which is part of the basis for a multielement method for foods to be collaboratively studied.

Cadmium and Lead in Earthenware.—Associate Referee Benjamin Krinitz did not report on this topic. John Gould and coworkers at the U.S. Food and Drug Administration (FDA) have reported some effort in this topic area. Gould has developed a "quick-test" for lead release from earthenware/ceramicware, using citric acid to leach a small area of the ware for 5–10 min. The small amount of leach solution contained on filter paper is treated with a chromatogenic solution which quickly becomes rose/red if lead is present. The "quick-test" is being applied to a variety of types of ware and the results will be compared to the AOAC method for lead release, **25.024-25.027**.

In addition, Susan Hight at FDA reported on initial investigations of using an inductively coupled plasma (ICP) emission spectrometer to determine lead, cadmium, and other elements leached from earthenware. Using the AOAC method for leaching earthenware, **25.024-25.027**, Hight established analytical parameters for determining the following elements by using ICP: Al, Ba, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Sn, Sr, Ti, V, Zn, and Zr. A collaborative study on some or all of these elements will be considered if there is sufficient need and interest.

The final action "Cadmium and Lead in Earthenware—Atomic Absorption Spectrophotometric Method" was developed jointly by AOAC and the American Society for Testing and Materials (ASTM). Comparison of each organization's publication of the method indicates a discrepancy in the level of the lead standard solutions. The AOAC method uses the standards 0, 5, 10, 15, 20 μg Pb/mL as tested in the collaborative study (*J. Assoc. Off. Anal. Chem.* [1973] **56,** 869–875). ASTM Method C 738-81 (*Annual Book of ASTM Standards* [1987] **15.02,** 309–311) uses a slightly lower range of standards—0, 1, 3, 5, 10, 15 μg Pb/mL. Because many lead levels of interest are found between 1 and 10 μg Pb/mL and because the 20 μg Pb/mL standard may be beyond the linear range, calibration of the atomic absorption spectrophotometer at the lower range of lead standards is preferred.

Emission Spectrochemical Methods.—Associate Referee Fred Fricke reports no progress on the topic for the past year. He still intends to perform the pre-study trial and then a full collaborative study of a method for the multielement analysis of foods by inductively coupled plasma emission spectroscopy as described in a previous report (*J. Assoc. Off. Anal. Chem.* [1986] **69,** 260).

Fluorine.—Associate Referee Robert Dabeka reported no progress on this topic for the past year.

Graphite Furnace–Atomic Absorption Spectrophotometry.—Associate Referee Robert Dabeka reports that he has prepared samples for an interlaboratory trial for a coprecipitation graphite furnace AAS method for lead, cadmium (*Can. J. Spectrosc.* (1986) **31,** 44–52), and arsenic (*Can. J. Spectrosc.* (1985) **30,** 154–157) in infant formula, peas, beef with broth, potatoes, and apples.

Hydride Generating Techniques.—Associate Referee Stephen Capar reports no progress on this topic but is still planning to study continuous flow hydride generation techniques for the determination of arsenic, selenium, and other elements in foods.

Lead in Calcium Supplements.—Patricia Maroney-Benassi has been appointed the Associate Referee to this new topic. Lead is known to be a potential contaminant in calcium supplements. Any efforts to control the amount of lead in these products require a reliable analytical method. She is currently studying perchloric acid digestion of the sample followed by determination of lead by differential pulse anodic stripping voltammetry (a modification of the method reported in *J. Assoc. Off. Anal. Chem.* [1979] **62,** 1054–1061). Analytical problems that need to be solved are the occasional formation of precipitates and organic electrochemical interferences. The quantitation limit is about 0.4 μg Pb/g.

Mercury.—Associate Referee Walter Holak's work on determining methyl mercury by liquid chromatography is being reported under the newly acquired topic *Organometallics in Fish.* No work is planned on the analysis of total mercury.

Methyl Mercury in Fish and Shellfish.—Associate Referee Susan Hight reports that the rapid method for determining methyl mercury (*J. Assoc. Off. Anal. Chem.* [1987] **70,** 24–30) was granted interim official first action and will be recommended for official first action at the Annual International Meeting. The official first action "Mercury (Methyl) in Fish and Shellfish—Gas Chromatographic Method," **25.146-25.152**, was recommended for official final action since there have been no adverse comments reported on the method.

Multielement Determination After Closed System Digestion.—Associate Referee Walter Holak did not report on this topic. The official first action "Arsenic, Cadmium, Lead, Selenium, and Zinc in Food—Multielement Method," **25.001-25.007**, was recommended for official final action since there have been no adverse comments reported on the method.

Neutron Activation Analysis.—This topic is now being reported under Metals and Other Elements instead of Radioactivity because neutron activation analysis (NAA) is used to determine the level of trace elements and not the level of radioactivity. William C. Cunningham has been appointed the new Associate Referee to this topic. He is reviewing an AOAC collaborative study on determining the level of sodium in foods by NAA, initiated a few years ago but never completed. He will decide whether to continue the study or initiate a new study. Cunningham will also be working closely with the American Society for Testing Materials (ASTM)

Task Group on Nuclear Methods of Chemical Analysis which is developing standard methods for performing NAA. He will coordinate ASTM method development with AOAC method protocols to enable endorsement of the methods by both organizations.

Organometallics in Fish.—This topic was transferred to Metals and Other Elements from the Committee on Foods I to consolidate AOAC efforts on the determination of methyl mercury. Associate Referee Walter Holak is in the process of collaboratively studying a method titled "Determination of Methyl Mercury in Seafood by Liquid Chromatographic and Atomic Absorption Spectrophotometric Detection." He submitted his analytical method and collaborative study protocol to AOAC for review; the General Referee and Residues Committee statistician both returned their comments to Holak for consideration; he successfully responded to all comments, and the collaborative study method and protocol was approved. The collaborative study will begin after collaborative study samples are prepared and tested. The method specifies a special LC/AAS interface apparatus. The Associate Referee has constructed a number of these interface apparatuses which will be shared by the participating laboratories. Holak anticipates sending the collaborative study samples to collaborators in fall 1987.

Organotin in Foods.—Allen D. Uhler has been appointed the Associate Referee to this new topic. He has been exploring the development of a multiresidue analytical method for organotin compounds in foods and has evaluated a number of methods presented in the literature. Currently, his work is centered on determining tributyltin and its degradation products in fish by using a relatively rapid, sensitive gas chromatographic method with a flame photometric detector. Quantitation limits of about 10 ng/g have been achieved for tributyltin and dibutyltin.

Polarography.—Associate Referee Susan Hight recommends that parameters for determination of lead and cadmium by square wave voltammetry (SWV) be studied for use with the dry ash sample preparation procedure in the official first action method, **25.008-25.015**. The SWV technique is much more rapid than differential pulse anodic stripping voltammetry. The official first action "Cadmium and Lead in Food—Anodic Stripping Voltammetric Method," **25.008-25.015**, was recommended for official final action since there have been no adverse comments reported.

Tin—Vacant. The official first action "Tin in Canned Foods—Atomic Absorption Spectrophotometric Method," **25.A01-25.A04**, was recommended for official final action since there have been no adverse comments reported.

Recommendations

(1) Adopt as official final action the official first action multielement method, **25.001-25.007**, for arsenic, cadmium, lead, selenium, and zinc in food. Discontinue topic.

(2) Adopt as official final action the official first action ASV method, **25.008-25.015**, for cadmium and lead in food. Study the application of SWV to the determination of lead and cadmium in foods.

(3) Adopt as official final action the official first action method for methyl mercury in fish, **25.146-25.152**. Adopt as official first action the rapid method for methyl mercury in fish (*J. Assoc. Off. Anal. Chem.* [1987] **70**, 24–30).

(4) Adopt as official final action the AAS official first action method for tin in canned foods, **25.A01-25.A04**. Discontinue topic.

(5) Modify method **25.024-25.027**, *Cadmium and Lead in Earthenware*, by changing **25.025(*c*)** (*2*) *Working Solns* from "Dil. 0.0, 5.0, 10.0, 15.0, and 20.0 mL stock soln to 1 L with 4% HOAc (0, 5, 10, 15, and 20 μg/mL)" to "Dil. 0.0, 1.0, 3.0, 5.0, 10.0, and 15.0 mL stock soln to 1 L with 4% HOAc (0, 1, 3, 5, 10, and 15 μg/mL)."

(6) Discontinue topics *Arsenic in Animal Tissues*, and *Mercury*.

(7) Continue study on all other topics.

Multiresidue Methods (Interlaboratory Studies)

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Comprehensive Multiresidue Methodology.—(Associate Referee Darryl E. Johnson, FDA, Minneapolis, MN.) The Associate Referee, newly appointed this year, has made significant progress toward developing a multiresidue analytical method applicable to the synthetic pyrethroid class of pesticide chemicals in fruits and vegetables. Working with extracts obtained from both **29.011** and **29.A03** and 6 pyrethroids (tetramethrin, permethrin, cypermethrin, fenvalerate, and decamethrin), he has developed a quantitative Florisil cleanup procedure that is based on **29.046** and includes an additional elution with 5% acetone in hexane. The acetone–hexane eluate is cleaned up further with a bonded phase extraction column. The procedure is described to provide adequate cleanup for electron capture detection at 0.05 ppm for each pyrethroid from fortified samples of kiwi fruit, apples, potatoes, and peppers. Elevated temperature chromatography, i.e., 230–240°C, was used for the determinations; both wide bore capillary and packed columns were included. The Associate Referee reports that he is currently investigating the optimum chromatographic conditions that will give reproducible responses and proper isomeric resolution of the individual pyrethroids. He plans on investigating the temperature programming of a wide bore capillary column equipped with a 5 m retention gap; the retention gap is believed to be needed to stabilize response. To date, all chromatographic determinations have been done at isothermal conditions, and responses have not been reproducible with the wide bore capillary system.

Extraction of Low Moisture-High Fat Samples.—(Associate Referee Leon D. Sawyer, FDA, Washington, DC.) During the past year, the Associate Referee continued monitoring the results of an investigation using semipreparative LC silica gel cleanup for fats and oils as an alternative to acetonitrile partitioning. No progress was made beyond that reported last year with regard to poor separation of some organophosphate pesticides from lipid materials. The investigating laboratory attempted to use a reverse-phase separation with a C-18 column and acetonitrile mobile phase but encountered other recovery problems with the organochlorinated pesticides. The Associate Referee has reinitiated the study of the Unitrex system which was abandoned 2 years ago as a cleanup alternative. Previous attempts with vegetable oils and this technique may have been unsuccessful because "oxidized" or "degraded" oils were used in the initial investigations. Results to date with refined oils have shown excellent cleanup with approximately 80–90% recoveries for 6 of 8 organochlorinated pesticides tested. Endrin and *p,p'*-DDT recoveries have been much more varied, presumably because of their susceptibility to degradation under certain chromato-

graphic conditions. Three BHC isomers, chlorpyrifos, dieldrin, and heptachlor epoxide, were the other chemicals tested from olive, safflower, sunflower, and soybean oils.

Fumigants.—(Associate Referee James Daft, FDA, Kansas City, MO.) The Associate Referee reported that the modified AOAC whole grain acetone soak procedure (reported last year) for multifumigant and related volatile halocarbons was published in the *Journal of the AOAC* (1987) **70,** 734–739. In addition to the 19 chemicals to which the method is applicable as reported last year, he now includes 3 additional ones, *ortho-* and *para*-dichlorobenzene and 1,2-dibromo-3-chloropropane. Plans are under way to collaboratively study the method during the next year.

Additional fumigant work by the Associate Referee involved analysis of 548 food samples (fat-containing and nonfat-containing) for residues of the 22 chemicals to which the above-referenced method is applicable. However, in this work, a modification of the proposed method was used for low fat samples and a Florisil cleanup step was added for samples containing over 20% fat. The modifications were used for expediency since the goal was to survey, nonquantitatively, for the presence of residues. He reported that 60% of the samples had one or more of the chemicals present as a residue.

Miniaturization of Multiresidue Methodology.—(Associate Referee D. Ronald Erney, FDA, Detroit, MI.) No work on this topic was accomplished this year. The Associate Referee reported that he still plans on repeating an interlaboratory study that was conducted 2 years ago.

Organophosphorus Pesticide Residues.—(Associate Referee Ronald Laski, FDA, Buffalo, NY.) The Associate Referee has determined the gas chromatographic characteristics of 11 organophosphorus chemicals on DEGS, OV-101, and OV-17 columns with FPD-P detection. Six (6) of the chemicals—carbophenothion sulfoxide/sulfone, fenamiphos, fensulfothion oxygen analog, and phorate sulfoxide/sulfone—chromatographed on all 3 GC systems. Their recoverability through the applicable sections of methods **29.001-29.018**, **29.054-29.058**, and **29.A01-29.A04** will be determined. No chromatographic responses were obtained with coumaphos, fenamiphos sulfoxide/sulfone, and oxydemeton methyl sulfone.

The Associate Referee plans to initiate a study of the applicability of determining selected organophosphorus pesticides and their metabolites as "total sulfones." This procedure has been proposed by Hill et al. (*Analyst* (1984) **109,** 483).

Sweep Codistillation.—(Associate Referee Barry G. Luke, State Chemistry Laboratory, East Melbourne, Victoria, Australia.) The Associate Referee relayed results from a limited interlaboratory study (5 laboratories) on the recoveries of chlorpyrifos, bromophos ethyl, and ethion from meat fat by using a Unitrex system with beadless fractionization tubes. The study indicated that interlaboratory variability (RSD_x = 10–17%) was typical of what is expected for residue methods. Recoveries also appeared acceptable. The Associate Referee acknowledged that these results would not be equivalent to an AOAC collaborative study because of the limited number of samples and use of certain protocols, e.g., participants fortified their own samples. He did feel encouraged about the results and still plans on conducting an AOAC collaborative study with organophosphate pesticides in meats.

The Associate Referee briefly mentioned 3 other planned interlaboratory studies involving the Unitrex system: (*1*) PCBs in meat fat (4 laboratories); (*2*) DDE/DDT in an unspecified commodity (9 laboratories); and (*3*) a repeat of organophosphate pesticides in meat fat. In addition, he reported on the successful application of the technique for analyzing breast milk for PCBs. No details on any of these studies have been received by the General Referee at the time of this report.

Recommendations

(1) Proceed with investigation of temperature-programmed wide bore capillary chromatography with electron capture detection and attempt to resolve the response variability that is reported to exist. Attempt to establish conditions that will resolve as many pyrethroid chemicals/isomers as possible. Continue investigations of pyrethroid recoveries from various food commodities, using both **29.011** and **29.A03** extractions with Florisil and bonded phase extraction cleanup procedures.

(2) Continue investigating and evaluating oil cleanup chromatographic techniques such as Unitrex, semipreparative LC, GPC, and solid phase extraction columns that appear to provide advantages over **29.014** (partitioning) in cleanup efficiency and/or time and reagent usage.

(3) Draft a specific method, applicable only to representative fumigants in whole grains and milk products, for review and comment before continuing with collaborative study plans, based on the approach in *J. Assoc. Off. Anal. Chem.* (1987) **70,** 734–739. The proposed method must include recovery and method performance data for all chemicals tested.

(4) Continue with previous plan of designing an interlaboratory trial of a miniaturized version of **29.001-29.008** as outlined in last year's Committee recommendation for this topic.

(5) Complete recovery studies of the 6 organophosphorus chemicals that have been determined to provide a chromatographic response through the applicable sections of **29.001-29.018**, **29.054-29.058**, and **29.A01-29.A04**. Continue with plans to study the applicability of converting organophosphorus chemical residues to sulfones prior to determination.

(6) Reconsider plans in designing a collaborative study for organophosphate pesticides in meat fat. Since more recovery and cleanup experience exists with the organochlorinated pesticides (including PCBs) with the Unitrex cleanup of meat fats, it is recommended that they be included in the first AOAC collaborative study of this analytical approach. It is further recommended that the Associate Referee design a collaborative study protocol for evaluation by the General Referee and Statistical Consultant within the next year. Continued evaluation of advantages and disadvantages of "beaded" vs "beadless" fractionization tubes for the Unitrex system is also recommended.

Organohalogen Pesticides

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Chlordane.—(Wilbur Saxton, FDA, Seattle, WA.) The Associate Referee has had no opportunity to work on this topic in the past year. He is uncertain whether or not he will be able to complete the testing of the methods under consideration in the upcoming year.

Chlorinated Dioxins.—(David Firestone, FDA, Washington, DC.) The Associate Referee reported that developmental work in the field of dioxin analysis continues and that there is also effort underway to prepare purified dioxin and furan congeners. Interest centers on development of rapid extrac-

tion and cleanup procedures and on automation of instrumentation that can be used for cleanup.

Some researchers are attempting to measure interlaboratory reproducibility of methods for dioxin analysis by conducting "round robin" studies among several laboratories. Results of one such study are being presented at the 1987 Annual Meeting by Norbert Fehringer and Stephen Walters of FDA's Pesticides and Industrial Chemicals Research Center (PICRC), Detroit. Study participants were the 4 FDA laboratories that perform analysis for 2,3,7,8-TCDD, i.e., PICRC, the Detroit and Chicago District Laboratories, and the Division of Contaminants Chemistry, Washington, DC. Five fish samples, including 2 which were duplicates of one another and one which was a fortified portion of another, were analyzed by 6 analysts in the 4 laboratories, each using the method normally used in that laboratory.

As the report by Fehringer and Walters indicates, reproducibility among laboratories is considered excellent for the low levels of analyte involved (9–56 parts per trillion), but this cannot be considered a measure of reproducibility of any one method since each laboratory chose to use different steps of the basic analytical approach. Enough similarities exist in the various procedures, however, that it appears a single method could be developed from the various steps used.

Interest is also high in the use of monoclonal antibodies for immunoassay analysis of dioxins. Other in vitro techniques being considered include receptor binding assays using a suitable radioligand.

The Associate Referee recommends continued monitoring of methods for dioxins and furans and development of uniform methods subjected to interlaboratory validation.

Chlorophenoxy Alkyl Acids.—(Vacant.) An effort has been made to find an Associate Referee for this topic. Marvin Hopper, FDA Total Diet Research Center, Kansas City, MO, has developed a method that is in regular use in FDA's Total Diet Program. The method involves extraction of the residues from samples acidified with sulfuric acid, using different techniques for different sample types. The extract is cleaned up by gel permeation chromatography (GPC), and the acids are methylated by ion pair alkylation. The methylated extract is then cleaned up on a mini-Florisil column, and the methyl esters are determined by gas chromatography with electron capture and electroconductivity detection.

The extraction steps of this method have been described in detail in FDA's *Pesticide Analytical Manual,* Volume I (PAM I), Section 221.1. The ion pair chromatography methylation step, which replaces the diazomethane methylation of the PAM I version, is described by Hopper in *J. Agric. Food Chem.* (1987) **35,** 265–269.

The method limit of detection for most commodities is 0.001 ppm. Recovery tests routinely accompany its use in FDA's Total Diet Program. Recoveries of compounds fortified at levels of 0.01–0.04 ppm routinely range from 30 to 100%, with an overall average of 60–70%. These recoveries have been considered acceptable, because of the wide range of food products and low levels of residues involved. A decision by the Methods Committee is needed, however, on whether these results reflect a method of adequate accuracy and precision for collaborative study.

Hopper has expressed a willingness to become the Associate Referee for this topic and to collaborate this method, if these recovery results are considered adequate. He is not in a position to continue to test other methods for CPAs nor to do further work to improve the recoveries of this method. He is also concerned that the automated GPC equipment might be considered specialized and that, therefore, it may be difficult to find enough laboratories to participate in a collaborative study.

The Methods Committee has indicated in the past that it would require any method for CPAs to be supported by evidence of efficacy in extraction of bound residues. Hopper has not yet tested his method for extraction efficiency, but he is willing to undertake such tests if he can obtain samples containing incurred residues, especially 2,4-D.

Ethylene Oxide and Its Chlorohydrin.—(Vacant). The person being considered as Associate Referee for this topic last year made no further contact with the General Referee. No other likely candidate has been found.

Gel Permeation Chromatography (GPC) Cleanup for Organochlorine Residues.—(Tim Spurgeon, ABC Laboratories, Columbia, MO).The Associate Referee has not had an opportunity to work on this topic in the past year. His position in his organization has changed since last year, and he does not expect to be able to be directly involved. However, new employees in his organization may be able to do so. He is willing to continue as Associate Referee, with the expectation that someone in his organization could manage a future collaborative study on the GPC method, as applied to the analysis for polychlorinated biphenyls (PCBs) in meats and fish, if the Methods Committee still believes it should be done.

Methyl Bromide.—(Joseph Ford, USDA, Gulfport, MS, and Richard DePalma, Procter and Gamble, Cincinnati, OH.) A renewed interest in collaborating a method for methyl bromide residues has arisen because the Environmental Protection Agency (EPA) has requested data on residues of this fumigant. EPA has expressed a willingness to accept data obtained using the head space methodology described by J. R. King et al. (1981) *J. Agric. Food Chem.* **29,** 1003–1005, and so this method has been chosen for collaborative study.

The co-referees on this topic will first study the stability of the residues of methyl bromide, a highly volatile compound. They have a −80°F freezer available, and they believe that residues will be stable if stored in such a freezer. The co-referees will work together to study ways to prepare, store, and ship samples for use in a collaborative study, and, if this is successful, will then develop a protocol for collaborative study. Members of the Grocery Manufacturers of America organization are particularly interested in the success of such a study and can be expected to assist in finding collaborators.

Pentachlorophenol.—(George Yip, FDA, Washington, DC.) The Associate Referee has not had time to devote to this project in the past year. No work on this project has been scheduled in his plan for next year either, because other topics have higher priority.

Polychlorinated Biphenyls.—(Leon D. Sawyer, FDA, Washington, DC.) The Associate Referee has reviewed several proposed individual congener analytical approaches in the past year. He has also reviewed the results of interlaboratory trials of 4 studies using these approaches. He has concluded that individual congener analysis is still a highly specialized technique that cannot at this point be uniformly adopted and applied to give consistent quantitative or qualitative values.

In the coming year, Sawyer intends to compare the results obtained by the current official method and those obtained using an individual congener approach. In anticipation of this study, he has obtained the appropriate gas chromatographic equipment and the necessary standards.

Polychlorinated Biphenyls (PCBs) in Blood.—(Virlyn Burse, Centers for Disease Control, Atlanta, GA.) The Associate Referee was able in the past year to get an additional 2 laboratories to complete the collaborative study begun in

Table 1. Recoveries of compounds added to specified commodities and analyzed per 29.044–29.049[a]

Commodity	Recovered, % (fortification level, ppm)				
	Tetrasul	Endosulfan I	Endosulfan II	Endosulfan S04	Tetradifon
Blueberries	95.4 (0.110)	91.2 (0.250)	99.7 (0.312)	92.2 (0.475)	105.2 (0.600)
	96.8 (0.220)	92.0 (0.500)	105.1 (0.623)	94.5 (0.950)	105.8 (1.20)
Broccoli	102.7 (0.110)	87.6 (0.250)	98.1 (0.312)	89.3 (0.475)	105.3 (0.600)
Cabbage	NA[b]	101 (NR)[c]	100 (NR)	103 (NR)	NA
	NA	103 (NR)	101 (NR)	104 (NR)	NA
Cantaloupe	90.9 (0.110)	83.2 (0.250)	94.6 (0.312)	85.9 (0.475)	100.3 (0.600)
	90.0 (0.220)	85.6 (0.500)	98.4 (0.623)	91.0 (0.950)	101.7 (1.20)
Carrots	101.8 (0.110)	95.2 (0.250)	107.4 (0.312)	94.7 (0.475)	111.0 (0.600)
Cauliflower	94.5 (0.110)	90.0 (0.250)	109.0 (0.312)	106.1 (0.475)	119.2 (0.600)
Celery	95.5 (0.110)	93.0 (0.242)	100.6 (0.328)	98.6 (0.440)	98.5 (0.537)
	NA	93.0 (0.484)	101.4 (0.656)	98.7 (0.879)	99.1 (1.07)
Collard greens	113.6 (0.110)	96.0 (0.250)	106.7 (0.312)	98.7 (0.475)	109.0 (0.600)
Eggplant	85.4 (0.110)	93.2 (0.250)	110.6 (0.312)	99.2 (0.475)	107.7 (0.600)
Endive	115.5 (0.110)	90.8 (0.250)	103.2 (0.312)	97.1 (0.475)	107.0 (0.600)
Grapes	105.5 (0.091)	101.7 (0.242)	103.4 (0.328)	111.4 (0.440)	107.4 (0.537)
	94.0 (0.183)	90.9 (0.484)	94.8 (0.656)	102.0 (0.879)	99.1 (1.07)
Green beans	106.4 (0.110)	90.4 (0.250)	103.5 (0.312)	98.3 (0.475)	106.7 (0.600)
	89.5 (0.220)	86.4 (0.500)	100.0 (0.623)	89.3 (0.950)	100.8 (1.20)
Green peppers	97.3 (0.110)	88.8 (0.250)	107.4 (0.312)	102.3 (0.475)	112.2 (0.600)
Lettuce	95.6 (0.091)	92.6 (0.242)	82.9 (0.328)	95.0 (0.440)	97.0 (0.537)
	101.1 (0.183)	96.7 (0.484)	100.2 (0.656)	106.7 (0.879)	104.7 (1.07)
Mustard greens	89.1 (0.110)	97.6 (0.250)	116.3 (0.312)	104.8 (0.475)	116.8 (0.600)
Peaches	95.4 (0.110)	92.8 (0.250)	105.1 (0.312)	91.6 (0.475)	108.3 (0.600)
	100.4 (0.220)	92.8 (0.500)	106.3 (0.623)	93.8 (0.950)	106.7 (1.20)
Pears	104.5 (0.110)	84.0 (0.250)	106.1 (0.312)	105.7 (0.475)	110.0 (0.600)
Peas	109.1 (0.110)	84.8 (0.250)	101.6 (0.312)	93.1 (0.475)	104.3 (0.600)
Plums	110.9 (0.110)	94.4 (0.250)	109.3 (0.312)	101.9 (0.475)	110.0 (0.600)
Potatoes	92.7 (0.110)	85.2 (0.250)	97.4 (0.312)	85.3 (0.475)	101.8 (0.600)
	85.9 (0.220)	81.4 (0.500)	92.4 (0.623)	83.9 (0.950)	96.7 (1.20)
Radishes	94.5 (0.110)	84.4 (0.250)	97.1 (0.312)	91.6 (0.475)	112.2 (0.600)
Radish tops	100.9 (0.110)	90.4 (0.250)	106.7 (0.312)	94.1 (0.475)	113.3 (0.600)
Spinach	97.3 (0.110)	93.6 (0.250)	106.4 (0.312)	101.5 (0.475)	112.3 (0.600)
Squash	NA	98 (NR)	98 (NR)	97 (NR)	NA
	NA	104 (NR)	104 (NR)	98 (NR)	NA
Strawberries	100.0 (0.091)	99.2 (0.242)	101.8 (0.328)	107.3 (0.440)	105.6 (0.537)
	101.6 (0.183)	101.0 (0.484)	102.7 (0.656)	109.3 (0.879)	107.5 (1.07)
Sweet potatoes	108.2 (0.110)	93.6 (0.250)	113.8 (0.312)	106.3 (0.475)	107.3 (0.600)
Tomatoes	95.4 (0.110)	92.8 (0.250)	105.2 (0.312)	95.2 (0.475)	100.3 (0.600)
	100.4 (0.220)	92.8 (0.500)	106.3 (0.623)	93.8 (0.950)	106.7 (1.20)
Turnips	110.0 (0.110)	86.8 (0.250)	100.0 (0.312)	93.7 (0.475)	113.3 (0.600)
Turnip greens	113.6 (0.110)	89.2 (0.250)	94.9 (0.312)	90.0 (0.475)	105.8 (0.600)

[a] Data collected and reported by Lawrence Mitchell.
[b] NA = Not applicable; sample not run.
[c] NR = Not reported.

1985 on the CDC method for PCBs in blood serum. These additional collaborators brought the total to 6, to fulfill the requirements for a complete collaborative study.

The statistician for the Committee on Residues, Richard Albert, and Ann Wilson of his branch performed the statistical evaluation of the results according to accepted AOAC procedures. These have been submitted to the Associate Referee.

The Associate Referee is now in the process of writing the report of this collaborative study and will submit it to the General Referee and the Methods Committee for interim approval when it is complete.

Tetradifon, Endosulfan, and Tetrasul.—(Lawrence Mitchell, FDA, Atlanta, GA.) The Associate Referee for this topic ran a successful collaborative study in 1975 on the method, **29.044-29.049**, for the 5 compounds of interest. Because the method is related to the general method for nonfatty foods, **29.001-29.018**, using only a different elution system for the Florisil cleanup step, it seemed desirable at that time to extend the official status for these chemicals to all the commodities that have official status under the general method.

In 1977, the referee reported to then-General Referee Jerry Burke on the results of recovery experiments for these 5 compounds added to 10 different fruits and vegetables. He also pointed out that recoveries for 3 of the compounds on 2 additional commodities had been mentioned in the collaborative study report. He agreed to collect the remainder of the commodities and perform additional recovery studies. This year, he sent data that he had developed on another 17 commodities. Of these 29 commodities, only one, blueberries, does not have official status for **29.001-29.018**.

Unfortunately, the referee has not been able to complete the studies with the final 12 commodities that do have official status for **29.001-29.018**. Instead, all the results currently available have been included in this report as Table 1. These results, plus the evidence provided by many years of FDA experience with this method, support its wide applicability and reliability.

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 cleanup 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 of the Associate Referee.

Such studies should help the referee decide on the most practical method for residues of chlordane in butter, fish, and poultry fat, and at the same time, test the compatibility of the capillary GC with sample extracts. The decision on which method should be subjected to collaborative study can then be based on this comparison.

(2) Continue to monitor progress on the development of methods for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and for hexachloro-, heptachloro-, and octachloro-substituted dibenzo-*p*-dioxins and dibenzofurans in foods. Continue to evaluate methods toward the ultimate goal of establishing one as an official method through AOAC collaborative procedures.

(3) Appoint Marvin Hopper as new Associate Referee for Chlorophenoxy Alkyl Acids, if the committee considers the recoveries of the method he has developed to be adequate for collaborative study. Hopper should then study the extraction efficiency of the method, using samples containing incurred residues, especially products of interest to EPA such as grains, asparagus, and citrus. If the method extraction efficiency is adequate, he should develop a plan to study the method collaboratively.

(4) Continue in the effort to find an Associate Referee for Ethylene Oxide and its Chlorohydrin. The new referee should study the method of Scudamore and Heuser (1971) *Pestic. Sci.* **2**, 80–91, for determining ethylene oxide, ethylene chlorohydrin, and ethylene bromohydrin in foods.

(5) Continue to plan for eventual collaboration of the GPC method, **29.037-29.043**, for PCBs in fish, meat and poultry fat. In the meantime, provide assistance to other Associate Referees who may wish to employ GPC as a technique to be applied to other compounds (e.g., chlordane, chlorophenoxy alkyl acids).

(6) Complete the preliminary work on the method of King et al. for methyl bromide in foods in which residues are most likely to occur and in which EPA is requesting residue data; develop handling procedures for samples containing methyl bromide residues, and test the reliability of the procedures by interlaboratory study. Develop a protocol for collaborative study, obtain approval of the General Referee and Statistical Consultant, and complete the study.

(7) Continue work on developing a method for analysis of pentachlorophenol in eggs, fish, milk, and gelatin; plan and execute a limited interlaboratory study of the method before planning a collaborative study.

(8) Study the application of the individual congener analysis of PCB residues and report on the comparison of results produced by that technique and by the AOAC official technique.

(9) Complete the report on the collaborative study of the method for PCBs in blood serum; submit it to the General Referee and the Committee on Residues for interim approval.

(10) Adopt as official first action method **29.044-29.049** for tetrasul, tetradifon, endosulfan I, endosulfan II, and endosulfan sulfate on the following crops: blueberries, broccoli, cantaloupe, carrots, cauliflower, celery, collard greens, eggplant, endive, grapes, green beans, green peppers, lettuce, mustard greens, peaches, pears, peas, plums, potatoes, radishes, radish tops, spinach, strawberries, sweet potatoes, tomatoes, turnips, and turnip greens.

(11) Adopt as official first action method **29.044-29.049** for endosulfan I, endosulfan II, and endosulfan sulfate on cabbage and squash. Discontinue this topic.

Other Recommendations

(1) Continue the first action status of the method for pentachlorophenol in gelatin, **29.A14-29.A18**. Questions still exist on the gas chromatography step of this method.

(2) Richard Ellis, USDA, has indicated that he has no resources available to pursue the completion of the collaborative study report on the method for pentachlorophenol in animal and poultry tissue in a form that would answer the objections raised in 1986. He is willing for someone else to be appointed to this topic if the Committee on Residues wishes to pursue collaboration of such a method.

This report of the General Referee was presented at the 101st AOAC Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. 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 edition, and to "Changes in Official Methods," *J. Assoc. Off. Anal. Chem.* (1985) **68**, 369–411 (A methods).

Organonitrogen Pesticides

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Reports were received from Associate Referees on 7 topics. Associate Referees are required for an additional 11 topics.

Anilazine.—An Associate Referee is required to select a method for anilazine residues and conduct a collaborative study.

Benzimidazole-type Fungicides.—Associate Referee Mikio Chiba reports that he has been unable to conduct accuracy, precision, and ruggedness testing of his method for benomyl and carbendazim in food crops.

Captan and Related Fungicides.—Associate Referee Dalia Gilvydis reports that the protocol for her method to determine captan, folpet, and captafol has been modified to employ a wide bore fused silica capillary column (20 m × 0.53 mm id, DB-1) for the gas chromatographic determinative step, replacing the 6 ft packed column specified previously. The revised method will be tested in a 2–3 laboratory interlaboratory trial before the collaborative study is initiated.

Carbamate Herbicides.—An Associate Referee is required to select and test a method for carbamate herbicides.

Carbamate Insecticides.—Associate Referee Richard Krause reports that he has developed a method for confirmation of the phenolic carbamate residues determined by **29.A05-29.A13**. Residues extracted, cleaned up, and separated by liquid chromatography in **29.A05-29.A13** are hydrolyzed in the postcolumn reactor, then measured with an electrochemical detector operating in the oxidative mode at 0.60 V. Detector response was 99% of theoretical (SD 2.8%) for extracts of apple, grape, cabbage, or tomato spiked with 0.5–0.1 ppm bufencarb, carbaryl, carbofuran, 3-hydroxy carbofuran, methiocarb, and isoprocarb.

Carbofuran.—An Associate Referee is required to study and test collaboratively a method for carbofuran phenolic metabolites and 3-hydroxy carbofuran glucoside in crops. A method is also required for carbofuran and its carbamate and phenolic metabolites in milk and meat.

Chlorothalonil.—An Associate Referee is required to conduct a collaborative study of an existing multiresidue method as applied to chlorothalonil.

Daminozide and 1,1-Dimethylhydrazine (UDMH).—Associate Referee M. D. Parkins reports that a method for daminozide involving alkaline degradation to UDMH, formation of the salicylaldehyde dimethylhydrazone, and quantitation of the latter by gas chromatography/mass spectrometry is the subject of an interlaboratory study. The method involves the use of a standard curve prepared from blank matrix and an internal standard to compensate for variation in instrument response. The Associate Referee is examining the effect of these procedures on the quantitation results.

A method for UDMH involving the derivatization with 4-nitro salicylaldehyde and determination by gas chromatography with nitrogen/phosphorus detection is being studied in 2 laboratories. A collaborative study will be initiated by the Associate Referee if satisfactory data are generated.

Organonitro Pesticides.—Associate Referee Richard Krause reports that the chromatography of most nitrophenol pesticides has been improved through the use of a chiral LC column. Dinoseb and DNOC still produce broad peaks, and it is planned to investigate the use of a mobile phase buffered near pH 3 to alleviate the problem.

Diquat and Paraquat.—Associate Referee Brian Worobey reports that samples for analysis have been sent to 11 collaborators. Two laboratories have withdrawn from the study, 2 have completed the analyses, and the remaining 7 have yet to report.

Dithiocarbamate Fungicides.—An Associate Referee is required to study methods for the determination of dimethyldithiocarbamates and ethylenebis(dithiocarbamates) in foods.

Maleic Hydrazide.—An Associate Referee is required to study a GC or LC method for maleic hydrazide.

Organotin Fungicides.—An Associate Referee is required to study a method for fenbutatin, triphenyl tin, and cyhexatin residues.

Sodium o-Phenylphenate.—An Associate is required to select a method for *o*-phenylphenol residues and conduct a collaborative study.

Substituted Ureas.—Associate Referee Ronald Luchtefeld reports that recovery data have been developed for 6 phenylurea herbicides in 6 foods at spiking levels of 0.05 and 0.50 ppm (*J. Assoc. Off. Anal. Chem.* [1987] **70,** 740–745). Recoveries were 91% or greater with a maximum CV of 14 at 0.05 ppm, and at least 94% with a maximum CV of 9.1 at 0.50 ppm. An intralaboratory study indicated a similar range of CV values but recoveries often exceeded 100%. An interlaboratory study involving 2 other laboratories is being conducted prior to initiation of a collaborative study.

Thiolcarbamate Herbicides.—An Associate is required to select a method for thiolcarbamates and conduct a collaborative study.

s-Triazines.—An Associate Referee is required to study a method for atrazine and cyanazine residues.

Trifluralin.—An Associate Referee is required to study the applicability of existing multiresidue procedures to the determination of trifluralin residues.

Recommendations

(1) Appoint Associate Referees to study anilazine, carbamate herbicides, carbofuran and metabolites, chlorothalonil, dithiocarbamate fungicides, maleic hydrazide, organotin fungicides, sodium *o*-phenylphenate, thiolcarbamate herbicides, *s*-triazines, and trifluralin.

(2) Conduct accuracy, precision, and ruggedness testing of the Associate Referee's alkaline degradation method for benomyl and carbendazim.

(3) Conduct interlaboratory trial of the Associate Referee's modified method for captan, folpet, and captafol and submit recovery and precision data to the General Referee for review and comment. If data are satisfactory, initiate collaborative study.

(4) Conduct an interlaboratory study to assess the effect of different detector types on the response obtained from various carbamate/coextractive combinations and submit the data to the General Referee for review and comment.

(5) Conduct ruggedness testing on the alkaline hydrolysis–GC/MS method for daminozide to determine sources of variation and to define the best approach to quantitation. Prepare a revised procedure and supporting statistical data for review and comment by the General Referee.

Submit details of the revised method for UDMH and data from the interlaboratory study to the General Referee for review and comment. If satisfactory data are obtained, initiate a collaborative study.

(6) Analyze data obtained from the collaborative study of the method for diquat and paraquat in potatoes and submit a report and recommendation.

(7) Continue development of a multi-residue method for organonitro pesticides in foods.

(8) Obtain performance data from interlaboratory trial, and if satisfactory, prepare protocol for collaborative study for review by General Referee. Upon concurrence by General Referee and Statistical Consultant, initiate collaborative study.

This report of the General Referee was presented at the 101st AOAC Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. The recommendations were reviewed by the Committee on Residues. See the report of the committee, this issue.

Organophosphorus Pesticide Residues

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Disulfoton.—(S. Szeto, Agriculture Canada, Vancouver.) The Associate Referee reports the proposed residue method, which consists of an organic extraction, charcoal column cleanup, oxidation of parent compound and metabolites to sulfones, and determination by GC/AFID, has undergone additional evaluation within his laboratory. Several types of samples have been analyzed and recoveries greater than 90% for disulfoton and its oxidative metabolites have been attained.

Fenvalerate.—(T. D. Spittler, New York State Agricultural Experiment Station, Geneva.) The Associate Referee reports his laboratory has continued to use the Shell (Modesto) fenvalerate method successfully for esfenvalerate determinations. Esfenvalerate is the isomerically pure (*S,S*) form of fenvalerate and is rapidly replacing it in the marketplace. The Associate Referee's informal poll of analysts in other laboratories indicates that existing methods for fenvalerate are being used, without modification, for determination of esfenvalerate. Although the methods in use have never undergone AOAC collaborative study, he feels there is little interest among users in devoting the time required to conduct such a study. He does not recommend a collaborative study at this time.

Phorate.—The Associate Referee resigned this year.

Phosphine.—(B. Puma, FDA, Washington, DC.) The Associate Referee reports he has conducted some initial studies covering techniques for handling gaseous phosphine standards, phosphine behavior on GC, and the applicability of the solvent soaking procedure for the extraction of fumigants in grains (**29.072**).

Recommendations

(1) Appoint an Associate Referee to evaluate and collaboratively study analytical methods for determining residues of azinphos-methyl in foods.

(2) Choose sample matrix and prepare protocol for collaborative study of Associate Referee's method for determination of disulfoton and its oxidative metabolites (*J. Agric. Food Chem.* [1982] **30,** 1082–1086). Submit protocol for review and comment by General Referee and Statistical Consultant to Committee on Residues. Upon approval, begin collaborative study.

(3) 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.

(4) Discontinue Fenvalerate as a topic.

(5) Appoint an Associate Referee to evaluate gel permeation chromatography (GPC) as cleanup technique for organophosphorus pesticides and their metabolites in extracts of high fat samples.

(6) Appoint an Associate Referee to evaluate and collaboratively study analytical methods for determining residues of methamidophos and its metabolites in foods.

(7) Appoint an Associate Referee to evaluate and collaboratively study analytical methods for determining residues of monocrotophos in foods.

(8) Appoint an Associate Referee to evaluate and collaboratively study analytical methods for determining residues of permethrin in foods.

(9) Appoint an Associate Referee to fill the recently vacated topic of phorate and its metabolites in foods.

(10) Continue study on phosphine.

(11) Appoint an Associate Referee to evaluate and collaboratively study analytical methods for determining residues of resmethrin in foods.

Other Recommendations

(1) Initiate a topic for terbufos and its oxidative metabolites. (This pesticide has been classified in the FDA Surveillance Index as Class II.) Appoint an Associate Referee to evaluate and study methodology.

(2) Combine the synthetic pyrethroid pesticides under one topic and appoint an Associate Referee to evaluate the need for methodology and study the possibility of extending or developing a multiresidue method.

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Section numbers refer to *Official Methods of Analysis* (1984) 14th edition.

Radioactivity

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Cesium-137.—The method for the determination of cesium-137 in milk and other foods by gamma-ray spectroscopy has been accepted as official. A candidate for a new Associate Referee has been found, and the appointment is pending approval by the candidate's supervisor. The first order of business will be evaluation of methods for determining cesium-137 at lower levels in milk, foods, and biological materials.

Iodine-131.—The method for the determination of iodine-131 in milk and other foods by gamma-ray spectroscopy, has also been accepted as official. The collaborative study protocol for the 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 at 3 levels of activity is being conducted as a prelude for the study for iodine-131 in milk. The test is being conducted to determine the feasibility of a low-level study for iodine-131.

Neutron Activation Analysis.—Associate Referee William Stroube resigned this year, and the Associate Refereeship has been transferred to the General Referee for Metals and Other Elements.

Plutonium.—Alfred Robinson, U.S. Testing Co., Richland, WA, has been appointed Associate Referee. He is in the process of investigating the method of the Department of Energy for determining plutonium in foods, biological materials, and water (HASL-300-Ed 25, *EML Procedures Manual* (1982), pp. E-Pu-01-01) and related procedures. Further studies are planned.

Radium-228.—The collaborative study was completed, and the data are being evaluated by the Associate Referee; a report is expected later this year.

Strontium-89 and Strontium-90.—The Associate Referee reported that little progress has been made this year because of pressures from other activities. However, he is still preparing a collaborative study protocol 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 contact was made for a candidate for Associate Referee for this subject, but the person was not available. A search is now in process for an Associate Referee.

Recommendations

(1) Await the official appointment of Associate Referee for cesium-137; begin studying methods for determining lower levels of cesium-137 in milk, food, and biological materials.

(2) Await the results of the study for iodine-131 in water before proceeding with collaborative study for iodine-131 in milk.

(3) Submit the study for radium-228 to the Methods Committee when completed.

(4) Appoint a new Associate Referee for Tritium.

(5) Continue study on all other topics.

This report of the General Referee was presented at the 101st AOAC Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. The recommendations were reviewed by the Committee on Residues. See the report of the committee, this issue.

GENERAL REFEREE REPORTS: COMMITTEE ON MICROBIOLOGY

Analytical Mycology and Microscopy of Foods and Drugs

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The General Referee has developed a qualitative method for identifying mixtures of dried peach and apple fragments. A regulatory sample was received with labeling indicating that it contained a mixture of dried peach and apple tissue fragments. A common whole mount technique for soft tissues was used on the sample to confirm the presence of both fruits. In this procedure, a dried fruit fragment, 0.5 sq. mm or less, is placed on a microscope slide along with a drop of glycerine and water (1 + 1). The slide is heated until the fragment is rehydrated, and a cover glass can be pressed down. Then the rehydrated fragment is examined microscopically for the presence of characteristic thick-walled peach epidermal hairs or apple starch. In addition to the bright-field microscope, the polarizing microscope equipped with a first-order red plate is used in the identification of starch grains and other cellular components. When apple and peach are present in roughly equal proportions, this method results in a rapid confirmation of both components. However, no peach fragments were found and each sample contained 100 or more fruit fragments. A fragment-by-fragment examination becomes time-consuming and tedious when one component is present in a trace amount. The new method is based on the presence of characteristic peach epicarp hairs. In this method, all of the dried fruit tissue fragments are combined and placed in a single 100 mL blender cup. Water is added and the mixture is blended 1 min at high speed. One-half milliliter portions of this mixture are placed on a large capacity rot fragment slide and are microscopically scanned at 30–40× magnification using a bright-field microscope. Because of their unique appearance, the presence of any peach hairs can be determined and the identification of peach can be confirmed. This method would be applicable to any fruit product combinations where the components have unique, easily recognized microscopic characteristics. A report on this new method is being prepared for the 1988 AOAC Annual International Meeting.

The General Referee has developed a new technique for teaching the basic concepts of the Becke line method, which is routinely used in determining the refractive indices of transparent material. The Becke line test is a microscope method used to determine the refractive index (RI) of transparent particles such as glass. The RI is a valuable property used in identifying and confirming glass or other transparent materials. The Becke line is a bright halo, near the boundary of a transparent particle, that moves with respect to that boundary as the microscope is focused away from the particle. The halo will always move into the material with the higher RI (either the particle or the mounting oil). The RI of the particle is determined by matching the RI of the particle to the known RI of a calibrated oil. When the RIs match, the Becke line disperses into equally intense blue and orange lines. Particles of the unknown material are mounted on microscope slides in a series of oils each with a different RI. The first mounting oil is chosen almost at random out of a possible 200 oils. It is very important for an analyst being trained in this technique to gain experience in interpreting the direction of movement and the degree of relief (intensity) of the Becke line so that the next oil that is chosen will be as close as possible to the matching liquid, and to therefore minimize the number of different mounts needed. Teaching beginners the Becke method can be a long, oily process with much trial and error.

A new method, developed for a recent workshop, eliminates the oil selection and slide-making process by using permanent slides made up in advance. In the new method, a single permanent mounting medium with a known refractive index is used to prepare slides of glass samples with different RIs varying around the mounting medium. The glass samples are taken from a commercial set of 60 reference standards. The mounting medium is a plastic resin that melts at 60–70°C and is solid at room temperature. The resin, "Cargille Melt Mount," has been recently developed and marketed. Using this procedure, several sets of slides can be made up well before a training session and can be kept by the trainees for future reference. Any number of slides showing high, medium, and low relief, and near or exact matches can be randomly identified within a set, and any number of sets can be presented as unknowns to a class.

The General Referee has reported on the use of the video microscope in Howard mold count training. Traditionally, the best instruction method for Howard mold count training has been a one-on-one, field-by-field microscopic examination of reference samples by instructor and trainee using a dual-head microscope. The hand-carried traveling microscope is limited to one training head because of its size and weight. This limits the number of trainees an instructor can work with during a training session. A color video camera attached to the compound microscope increases the number of trainees an instructor can work with. The use of the next higher magnification objective and color contrast within the sample material has helped eliminate the resolution and contrast problems found in earlier black and white systems.

The General Referee has also reported on the use of image analysis in direct microscopic counts of tomato catsup. In the Howard mold count (HMC) method, **44.207**, microscopic fields are scored positive if the aggregate lengths of no more than 3 hyphae exceed $\frac{1}{6}$ of the field diameter of 1.382 mm. No provisions are made in this method for hyphal fragment size and number resulting from variable degrees of product comminution. With the image analyzer, a computer coupled to a compound microscope, it is possible to count and measure all mold observed in each microscopic field regardless of size and numbers. This makes possible a total mold count based on the aggregate lengths of all mold hyphae. Four HMC slides of 25 fields each (100 fields per sample) were measured for each of 40 reference samples prepared with known levels of comminution. Using standard sample preparations, the number of mold segments ranged from 38 to 682, the total length ranged from 6.47 to 62.41 mm, and the average mold segment length ranged from 0.058 to 0.270 mm. The coarsely comminuted samples had fewer and larger hyphae than the more highly comminuted samples. The coarsely comminuted samples tended to have a lower total mold segment length than the finer samples, possibly due to better breaking and distribution of clumps. High-speed blending of samples re-

duced variation of average segment length but failed to reduce average total length disparity between coarse and finely comminuted samples. Direct microscopic counting techniques involving the image analyzer are being investigated in the General Referee's laboratory.

Recommendations

(1) Adopt as official final action the following official first action methods: Mold in Fruit Nectars, Purees, and Pastes—Howard Mold Count, **44.201-44.203**; Mold in Tomato Powder (Dehydrated)—Howard Mold Count, **44.211**; Mold in Soft Drinks—*Geotrichum* Mold Count, **44.217**; Mold in Citrus Juices—*Geotrichum* Mold Count, **44.218**; Mold in Vegetables, Fruits, and Juices (Canned)—*Geotrichum* Mold Count, **44.219**; Mold in Comminuted Fruits and Vegetables—*Geotrichum* Mold Count, **44.220-44.222**. Mold in Cream Style Corn—*Geotrichum* Mold Count, **44.223**.

(2) Discontinue the following topics and terminate the refereeships: Baseline Mold Counts by Blending; Fluorescence Microscopy of Molds, Yeasts, and Spores; *Geotrichum candidum* Morphology; *Geotrichum* Mold in Frozen Fruits and Vegetables; Howard Mold Counting, Use of Wide-Field Microscope; Howard to Viable Mold Counts of Frozen Fruits and Vegetables, Comparison; Microscopic Mold Counts, Effects of Interfering Plant Material; Mold in Spices; Molds and Yeasts in Beverages.

(3) Continue study on the remaining topics.

This report of the General Referee was presented at the 101st AOAC Annual International Meeting, Sept. 14-17, 1987, at San Francisco, CA. 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 edition.

Extraneous Materials in Foods and Drugs

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The TLC method for the determination of coprostanol as an indicator of mammalian feces and the method for light filth in botanicals received interim official first action approval. Both methods will be recommended for adoption as official first action.

Collaborative studies of a chemical test for mammalian feces in grain products and a method for light filth in tofu were conducted.

Collaborative studies of methods for filth in cheese, the saturated brine–olive oil extraction of light filth from tomato products, and light filth in canned fish and fish products are planned for next year.

The following Associate Refereeships are being discontinued: (1) Filth in Botanicals—Joseph A. McDonnell; (2) Insect Excreta in Flour—Raymond Galacci; (3) Filth in Dried Mushroom Products—Jack Boese; (4) Filth in Shrimp—Alan R. Olsen; (5) Filth in Chocolate Products—Donald A. Mastrorocco, Jr.

The following Associate Refereeships are being recommended: (1) Filth in Spirulina—Marvin Nakashima; (2) Filth in Soybean Curd—Marvin Nakashima.

The topics Insect Excreta in Flour and Filth in Shrimp are being discontinued. Methods development research will continue on all other topics.

Recommendations

(1) Adopt as official first action the interim official first action TLC method for the determination of coprostanol as an indicator of mammalian feces.

(2) Adopt as official first action the interim official first action method for extraction of light filth from whole leaves of alfalfa, papaya, and spearmint. Extension of the method to lemon balm is not recommended.

(3) Adopt as official final action the following official first action methods: Filth in Cocoa, Chocolate, and Press Cake, **44.006-44.007**; Filth in Ground Coffee and Coffee Substitutes, **44.008**; Aphids in Hops, **44.009-44.011**; Light Filth in Tea, **44.014-44.016**; Sediment in Dairy Products, **44.021**; Filth in Shelled Nuts, **44.028-44.029**; Filth in Pecans, **44.030-44.031**; Filth in Coconut (Shredded), **44.032**; Filth and Extraneous Material in Peanut Butter, **44.033-44.038**; Insect Infestation (Internal) of Wheat, **44.040-44.042**; Light Filth (Pre- and Post-Milling) in Flour (White), **44.052**; Insect Excreta in Flour, **44.054**; Light Filth in Rice Flours (Powders), Extruded Rice Products, and Rice Paper, **44.055-44.057**; Light Filth in Flour (Soy), **44.060**; Light Filth in Wheat Gluten, **44.061**; Light Filth in Starch, **44.062**; Light Filth in White Breads and High-Fat Products, **44.067**; Light Filth in Breading of Frozen Food Products, **44.068**; Light Filth in Alimentary Pastes, **44.069**; Light Filth in Cereals (Corn and Rice) and Corn Chip Products, **44.070**; Light Filth in Cereals (Whole Wheat), **44.071**; Light Filth in Barley, Oatmeal, and Mixed Dry Infant Cereal, **44.072-44.073**; Light Filth in Crabmeat (Canned), **44.080**; Light Filth in Fish (Canned) and Fish Products, **44.082**; Light Filth in Shrimp (Canned), **44.083**; Light Filth in Pork Sausage (Uncooked) and Ground Beef or Hamburger, **44.084-44.085**; Filth in Apple Butter, **44.086**; Thrips and Other Insects in Frozen Blackberries and Frozen Raspberries, **44.089-44.090**; Filth in Jam and Jelly, **44.094**; Filth in Citrus and Pineapple Juices (Canned), **44.095-44.096**; Light Filth in Raisins, **44.097**; Filth in Potato Chips, **44.101**; Filth in Corn Chips, **44.102**; Filth in Candy, **44.103**; Filth in Sirups, Molasses, and Honey, **44.105**; Filth in Sugars, **44.106**; Foreign Matter in Corn (Canned), **44.109**; Filth in Green Leafy Vegetables, **44.110-44.111**; Filth in Pureed Infant Food, **44.113-44.114**; Light Filth in Potato Products (Dehydrated), **44.117**; Filth in Sauerkraut, **44.118**; Filth in Tomato Products, **44.119-44.120**; Light Filth in Spices and Condiments, **44.125-44.126**; Filth in Ground Annatto, **44.129**; Heavy Filth in Capsicums (Ground), **44.130**; Light Filth in Capsicums (Ground), **44.131-44.132**; Light Filth in Nutmeg (Ground), **44.140-44.141**; Light Filth in Nutmeg (Reconditioned), **44.142**; Filth in Paprika, **44.145-44.147**; Light Filth in Pepper, **44.148-44.149**; Filth in Horseradish (Prepared), **44.155**; Light Filth in Mustard (Prepared), **44.156**; Light Filth in Gums (Plant, Crude), **44.158**; Light Filth in Papain (Crude and Refined), **44.161**; Insect Penetration through Packaging Materials, **44.162**.

This report of the General Referee was presented at the 101st AOAC Annual International Meeting, Sept. 14-17, 1987, at San Francisco, CA. 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 edition.

Dairy Microbiology

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As a result of the increasing number of microbiological methods for detecting and enumerating microorganisms in milk and milk products submitted for AOAC evaluation and approval, in 1986 the AOAC Official Methods Board established the General Refereeship on Dairy Microbiology by separating dairy microbiology topics from food topics. All food microbiology topics will continue under the direction of W. H. Andrews, AOAC General Referee for Food Microbiology.

At present, 6 dairy microbiology topics are under review:

(1) *Petrifilm Method for Aerobic Plate Count.*—This method currently has official first action status for raw and pasteurized milk (**46.B05-46.B07**). Studies are under way to extend the use of the method to a wider spectrum of milk and milk products.

(2) *Bactoscan Method for Determination of Total Number of Bacteria in Raw Milk.*—This topic, established in 1984, is currently inactive.

(3) *Hydrophobic Grid Membrane Filter (HGMF) Method for Aerobic Plate Count and Coliform Count.*—This method (**46.B01-46.B04**) has received official final action status for determining the aerobic plate count of raw milk, 2% skim milk, and skim milk powder, and for determining the coliform count in cheddar cheese.

(4) *Listeria monocytogenes—Rapid Detection of Listeria in Dairy Products by Using a DNA Probe.*—This is a new topic and is progressing through method validation studies prior to collaborative study.

(5) *Listeria monocytogenes—Detection in Dairy Products by Using Cultural Methods.*—This topic was established in 1985 and is progressing through method development and validation for collaborative study.

(6) *Applicability of Alkaline Phosphatase Test to Detect Raw Milk Used in Cheese Manufacture.*—A method validation study is nearing completion, and a collaborative study is planned for the near future.

Development of rapid methods for detecting disease-causing organisms in foods is the current area of greatest need. In addition to methods for *Listeria*, methods for *Campylobacter* and *Yersinia*, are needed.

Recommendation

Continue study on all topics.

This report of the General Referee was presented at the 101st AOAC Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. 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.) A collaborative study was conducted to evaluate the variability of biological indicator evaluator resistometer (BIER) vessels used for testing steam biological indicator (BI) resistance. The effect of media on the D-value of BIs is also being evaluated. A paper will be presented.

Chemical Indicators.—(Marvin Hart, 3M Co., St. Paul, MN.) A collaborative study of methodology for evaluating temperature-specific chemical indicators used to monitor steam sterilizer performance has commenced. The methodology was written and approved with comments by AOAC. Collaborators are being selected, and the experimental work is being conducted. The report on the completed study is scheduled for presentation at the 101st AOAC Annual International Meeting.

Electronic Particle Counters.—(Gordon S. Oxborrow.) Five electronic particle counters from 5 different manufacturers were compared and evaluated for ease of calibration and use. Data indicated that any of the instruments could be used for testing particulate content in small volume parenterals. A paper will be presented at the 101st AOAC International Annual Meeting.

Limulus Amebocyte Lysate Test for Presence of Endotoxin.—(Christine W. Twohy, FDA, Minneapolis, MN.) A 1984 collaborative study was undertaken to determine the efficacy of an extraction procedure for bacterial endotoxin. Twelve laboratories from government and industry participated in the study of 3 samples of 8 medical devices contaminated in duplicate with various amounts of endotoxin derived from 3 sources: a raw *Pseudomonas cepacia* (ATCC 25416) endotoxin, the USP Reference Standard Endotoxin EC-5, and Difco *Escherichia coli* endotoxin 055B5 (Lot 504089). The extraction was performed on the devices, and the eluates were tested for endotoxin content by using the limulus amebocyte lysate gel tube test. The recovery of endotoxin from the devices ranged from 42 to 120%.

These results were presented in a poster session at the 1986 AOAC meeting and were published in the *Journal of Parenteral Science and Technology.*

Packaging Integrity for Medical Devices.—(Ana M. Placencia, FDA, Minneapolis, MN.) A study was conducted comparing microbial penetration of medical grade packaging materials and porosity readings. The 14 samples tested included materials in the 20 to 50 lb weights and polyolefins. No correlation was found between microbial penetration and porosity. The microbial test methods used, the membrane agar plate method and the exposure chamber method, were developed at the FDA Sterility Analysis Research Center (SARC). The porosity readings were done using an ARO tester.

A collaborative study was distributed to 15 participants to test the 2 SARC methods. Results of the study will be presented at a future AOAC meeting.

Sterility Testing.—(Michael Palmerei, FDA, Brooklyn, NY.) No work has been accomplished on this project this year.

Recommendations

Continue study on all topics.

This report of the General Referee was presented at the 101st AOAC Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. 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.—Glucuronidase is present in most strains of *Escherichia coli* but is absent in most other enteric bacteria. Thus, an assay for this enzyme may be useful for determining the presence of *E. coli.* The substrate 4-methylumbelliferyl-beta-D-glucuronide (MUG) is incorporated into lauryl tryptose (LT) broth. The inoculated tubes are incubated under specified conditions and examined under longwave UV light for the presence of a fluorogenic glucuronidase end product. Fluorescent tubes are considered presumptively positive for *E. coli.*

Associate Referee Lloyd Moberg conducted a collaborative study which compared the AOAC 10-day most probable number (MPN) method with the LT-MUG procedure in which the LT-MUG medium was incubated 24, 48, and 72 h. Seventeen laboratories participated in the study but not all laboratories analyzed each of the chilled (cheese, ground beef, and pork sausage) or frozen (dairy topping and entree sauce/gravy) foods.

A major objective of this study was to validate the optimal incubation period for the LT-MUG medium. If potential fluorescence at 72 h is considered 100%, then Table 1 shows percentages of tubes fluorescing at incubation periods of 24, 48, and 72 h. The increase in the percentage of tubes fluorescing at a 24 h incubation period, compared with those fluorescing at a 48 h incubation period, varied from 0.1 to 12.5%. In all cases, however, the resultant MPN values were not significantly different for these 2 incubation periods. Accordingly, the Associate Referee recommends that the LT-MUG procedure be adopted official first action as an alternative rapid method to **46.016** for the enumeration of *E. coli* in chilled and frozen foods. Inoculated LT-MUG tubes would be incubated for 24 ± 2 h at 35°C and fluorescent tubes would be streaked onto EMB agar and suspect *E. coli* colonies confirmed as outlined in **46.016**. Additional studies would be needed to expand the applicability of the LT-MUG method to foods other than chilled and frozen foods.

The General Referee concurs with this recommendation, provided the LT-MUG test is not used for the analysis of chilled or frozen shellfish. It has been reported (*J. Food Prot.* [1985] **48,** 244–245) that the presence of an endogenous glucuronidase in oysters interferes with the fluorogenic detection of *E. coli* in this particular food. Modification of this assay by incorporating MUG into EC medium, rather than into LT broth, has been reported to eliminate this interference. The General Referee recommends that an additional collaborative study be undertaken to validate the efficiency of EC-MUG medium for the enumeration of *E. coli* in shellfish.

Rapid Methods for the Enterobacteriaceae.—Associate Referee Russell Flowers conducted 3 collaborative studies during the past year. The first of these studies was an evaluation of the MICRO-ID system (General Diagnostics, Division of Organon Teknika, Durham, NC), a diagnostic kit requiring only 4 h for the biochemical identification of *Salmonella, E. coli,* and other enteric bacteria. A total of 78 enteric isolates, representing 11 genera, was furnished to each of 9 participating laboratories. This system correctly identified 98.8% of the *Salmonella* isolates, 97.7% of the *E. coli* isolates, and 88.1% of the other enteric isolates. The Associate Referee recommends that the MICRO-ID system be adopted official first action and be used (*1*) 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; (*2*) as an alternative to conventional biochemicals, **46.016**, for the identification of foodborne *E. coli* isolates; and (*3*) for the presumptive generic identification of other *Enterobacteriaceae* isolates from foods.

Table 1. Percent of potential fluorescence in LT-MUG medium for incubation periods of 24, 48, and 72 h

	Tubes fluorescing, %		
Food	24 h	48 h	72 h
Cheese	95.6	99.5	100.0
Dairy topping	93.1	100.0	100.0
Entree sauce	99.5	99.6	100.0
Pork	88.8	97.4	100.0
Ground beef	83.4	95.9	100.0

The second study was concerned with a collaborative evaluation of the *Salmonella* 1-2 TEST (BioControl Systems, Inc., Kent, WA). This procedure is a rapid screening test for detecting motile *Salmonella* organisms in food and is performed in a disposable plastic device that contains a nonselective motility medium and a selective enrichment broth in 2 separate chambers. Just prior to adding the preenriched sample aliquot, a plug separating the 2 chambers is removed. A small portion of the preenriched sample is then added to the compartment containing the selective enrichment. Following an 8–10 h incubation period, a positive *Salmonella* reaction is indicated by the presence of an immobilization band that forms in the motility medium from the reaction of motile *Salmonella* bacteria and flagellar antibodies. It should be emphasized that this method will not detect nonmotile *Salmonella* organisms.

Twenty-three collaborators analyzed one or more of 6 foods (ground pepper, dry whole egg, nonfat dry milk, soy flour, milk chocolate, and raw ground turkey) by the conventional culture method and the *Salmonella* 1–2 TEST. Each of these food types was inoculated with one of 2 levels of *Salmonella.* Overall, there was a 94.9% agreement between the 2 methods. False negative rates for the culture method and the *Salmonella* 1-2 TEST were 0.2 and 5.1%, respectively. Due to analytical problems, the Associate Referee intends to repeat the collaborative study analyses of at least one of the food types. Pending successful completion of that segment, the Associate Referee will recommend the adoption of this method official first action for the *Salmonella* analysis of all foods.

The third study was a collaborative evaluation of the TECRA *Salmonella* Visual Immunoassay (Bioenterprises Pty Ltd, Roseville, New South Wales, Australia). In addition to providing presumptively positive results for *Salmonella* on the second day after initiation of analyses, this particular enzyme immunosorbent assay offers the distinct advantages of not requiring any expensive equipment and of being adaptable for laboratories that have relatively small workloads, as well as for laboratories that need to analyze a large number of samples. Fourteen laboratories analyzed one or more of 6 food types (ground pepper, dry whole egg, nonfat dry milk, soy isolate, milk chocolate, and raw ground turkey) by the conventional culture method and the TECRA method. Statistical analysis of the data indicated no significant differences

between the 2 methods for the recovery of *Salmonella* from each of the food types. Overall agreement was 92.3% between the 2 methods. False negative rates for the culture method and the TECRA method were 1.7 and 1.4%, respectively. Accordingly, the Associate Referee recommends that the TECRA *Salmonella* Visual Immunoassay be adopted as official first action for *Salmonella* analysis of all foods.

Because all 3 collaborative study manuscripts were submitted after the deadline, the General Referee recommends that all 3 procedures be considered for interim official first action.

Redigel Media.—Except for the substitution of pectin for agar as a solidifying agent, the components of the pectin-based Redigel media (RCR Scientific, Inc., Goshen, IN) are the same as their agar-based counterparts. Use of the pectin-based media offers several advantages: media are presterilized so that heating and autoclaving are unnecessary; in a pour plate method, the inoculum is added to the Redigel medium at room temperature, thereby avoiding thermal shock to cells; and media are especially adaptable for field testing.

Associate Referee Jonathan Roth conducted a collaborative study in which 10 laboratories compared the pectin-based Redigel plate count medium with the agar-based Standard Methods plate count medium for determining total microbial counts in 9 foods (flour, spices, cheese, cream, homogenized milk, raw milk, raw chicken, raw oysters, and frozen broccoli). Plate count values were higher with the pectin-based medium than with the agar-based medium for 8 of the 9 foods, but these counts were significantly different only for homogenized milk. The repeatability and reproducibility standard deviations favored the pectin-based medium with 6 and 7 of the 9 foods tested, respectively. Thus, the Associate Referee recommends that the pectin-based medium be used as an alternative for the agar-based medium in **46.005(g)** in determining standard plate count values of foods, and the General Referee concurs.

Vibrio cholerae.—*V. cholerae* gastroenteritis in this country has been associated with the consumption of raw oysters. The American Public Health Association (APHA) recommends a procedure in which two 25 g sample duplicates are enriched in 225 ml alkaline peptone water and in gelatin phosphate salt broth, incubated for 6–8 h at 35°C, and streaked to thiosulfate–citrate–bile salts–sucrose (TCBS) agar and gelatin phosphate salt agar for isolation. In a preliminary investigation (*Appl. Environ. Microbiol.* [1987] **53,** 1181–1182), Associate Referee Angelo DePaola demonstrated increased recovery and specificity for *V. cholerae* from the Pacific oyster, *Crassostrea gigas,* enriched in alkaline peptone water at 42°C and streaked to TCBS agar.

Accordingly, the Associate Referee conducted a collaborative study to compare the APHA procedure and the elevated temperature procedure for the isolation of *V. cholerae* from the Eastern oyster, *Crassostrea virginica.* Twelve laboratories participated in the study. The elevated temperature and APHA procedures recovered 98.3 and 72.5%, respectively, of 120 samples inoculated with one of 2 levels of an 0 Group 1 strain and a non-0 Group 1 strain of *V. cholerae.* In addition to higher recovery, the elevated temperature procedure was more specific than the APHA procedure. Specificity was defined as the ratio of colonies confirmed as *V. cholerae* to the total number of picked suspect colonies. These values for the elevated temperature and APHA procedures were 86.8 and 21.2%, respectively. Thus, the Associate Referee recommends that the elevated temperature procedure for the isolation of *V. cholerae* from oysters be adopted official first action, and the General Referee concurs.

Associate Referee Reports

Bacillus cereus, Isolation and Enumeration.—Co-Associate Referee Gayle Lancette reports that typical isolates on mannitol–egg yolk–polymyxin (MYP) agar are almost always identified by the biochemical tests in **46.111** as *Bacillus cereus.* During the past year the co-Associate Referee has compared **46.111** with a rapid staining technique recently developed by Holbrook and Anderson (*Can. J. Microbiol.* [1980] **26,** 753–759) for confirmation of *B. cereus* isolates. This staining technique involves the microscopic identification of lipid globules in the vegetative cells of *B. cereus.* In addition to reducing the reporting time for the identification of *B. cereus* to 2 days, this staining technique could make biochemical testing unnecessary.

All of the 243 *B. cereus,* 13 *B. thuringiensis,* and 4 *B. cereus* var. *mycoides* isolates from foods were positive for the presence of lipid globules by the staining technique. Spore formation on MYP agar was variable and strain dependent. Of 14 other *Bacillus* species tested, lipid globules were observed only in some cells of *B. aneurnolyticus* and *B. thiaminolyticus.* However, these cultures were atypically mannitol and lecithinase negative after 24 h on MYP. On the basis of these results, the co-Associate Referee has concluded that staining typical isolates on MYP agar for lipid globules is comparable to the biochemical tests in **46.111** for confirmation of *B. cereus* isolates.

Clostridium perfringens.—Associate Referee Stanley Harmon previously evaluated a reverse passive latex agglutination (RPLA) test kit (Denka Seiken Ltd, Tokyo, Japan) for detecting and quantitating *Clostridium perfringens* enterotoxin (PET) in culture fluids and in extracts from feces of food poisoning patients. Although less sensitive than a double antibody enzyme-linked immunosorbent assay or counter immunoelectrophoresis, the PET-RPLA procedure is easier to perform, specific, and able to detect nanograms of PET.

During the past year, the Associate Referee conducted a preliminary evaluation involving 5 laboratories, of this test kit. The results of that study will be a subject of a poster presented at the 1987 AOAC meeting.

Genetic Methods for Bacterial Pathogens.—Associate Referee Walter Hill reports that considerable progress has been made in developing a gene probe for *Listeria monocytogenes.* A procedure for lysing cells on colony hybridization filters has been perfected. This gene probe is able to detect hemolytic strains when more than 10 viable cells per g of food are present. The next step will be to optimize conditions of enrichment of food samples, thereby increasing sensitivity of the procedure. It is anticipated that a collaborative study of the refined procedure will be conducted during 1988.

Hydrophobic Grid Membrane Filter Methods.—As a prelude to a collaborative study of the hydrophobic grid membrane filter (HGMF) procedure for yeasts and mold counts of foods, Associate Referee Phyllis Entis compared 5 media for the growth of 20 mold species. These media were: (*1*) potato dextrose agar (PDA) with trypan blue and antibiotics at concentrations different from those recommended in the *Bacteriological Analytical Manual* (BAM) 6th edition; (*2*) PDA with antibiotics as recommended in BAM; (*3*) PDA with antibiotics, as recommended in BAM, and 7.5% NaCl; (*4*) same as first medium but with 7.5% NaCl; and (*5*) a new medium, provisionally referred to as YM-5 agar, which contains, per L, 10 g soytone (phytone), 5 g yeast extract, 40 g glucose, 0.1 g trypan blue, 2.0 mL dichloran solution (0.2 g dissolved in 800 mL 95% ethanol), 1.0 mL rose bengal solution (1.25% aqueous solution), and 17.0 g agar.

The growth rate studies demonstrated that PDA with 7.5% NaCl greatly retarded the growth of most of these mold cultures. Some strains were unable to initiate growth in the presence of 7.5% NaCl over a 5-day incubation period. Growth rates and gross morphology on PDA and PDA with trypan blue were virtually identical over the 5-day incubation period. YM-5 agar produced both denser growth and, in some instances, slightly larger colonies over 5 days than did PDA.

With respect to mold identification on the HGMF, most of the 20 cultures sporulated after 72 h and demonstrated typical microscopic morphology. The remaining cultures sporulated within 5 days.

Following additional in-house testing of naturally contaminated samples, the Associate Referee is planning to conduct a collaborative study to compare the HGMF procedure and the traditional plate count method for the enumeration of yeasts and molds in foods.

Petrifilm Methods.—The Petrifilm system (Medical-Surgical Division, 3M, St. Paul, MN) is a "sample ready" technique, requiring no media preparation, that was adopted official first action at the 1985 AOAC meeting for the enumeration of total aerobic microflora and total coliforms in raw and pasteurized milk. Since there have been no adverse comments reported to the co-Associate Referees, it has been recommended that the official first action method, **46.B05-46.B07**, be adopted to official final action. The General Referee concurs.

Rapid Methods for the Enterobacteriaceae.—At the 1985 AOAC meeting, an enzyme immunoassay screening procedure, **46.B21-46.B29** (Organon Teknika, Durham, NC) was adopted as an official first action method for the detection of *Salmonella* organisms in high-moisture and low-moisture foods. The method was subsequently modified. The first modification involved reducing the preenrichment incubation period from 24 ± 2 h to 18–24 h. Second, the incubation period for the selective enrichments was reduced from 18–24 h to 6–8 h. Third, the postenrichment period was increased from 6 h to 14–18 h. Finally, the centrifugation step to concentrate the *Salmonella* organisms from the incubated postenrichments was eliminated. These 4 modifications were embodied in a procedure and proposed as an alternative to **46.B21-46.B29** for the analysis of low-moisture foods only. This procedure was subjected to a successful collaborative study and was adopted as an official first action method, **46.C17-46.C25**, at the 1986 AOAC meeting. Thus, **46.B21-46.B29** may be used for the *Salmonella* analysis of both high-moisture and low-moisture foods, whereas **46.C17-46.C25** may be used for low-moisture foods only.

Associate Referee Russell Flowers reports that no adverse reports relative to method **46.B21-46.B29** have been received. Accordingly, it has been recommended that the official first action method, **46.B21-46.B29**, be adopted official final action. The General Referee concurs. The method described in **46.C17-46.C25** remains in official first action status.

Redigel Media.—Associate Referee Jonathan Roth reports that in addition to his own in-house validation study and collaborative study, 2 other evaluations in independent laboratories have confirmed the usefulness of Redigel media, and these results have been incorporated into manuscripts which are currently in press. Immediate plans of the Associate Referee call for the development and evaluation of Redigel media for the microbiological determinations other than standard plate count values.

Yeasts, Molds, and Actinomycetes.—At the invitation of Marcel Dekker, Inc., New York, NY, Associate Referee Philip Mislivec is serving as book editor of a reference publication entitled *Analytical Mycology of Foods.* In addition, he has indicated that a proposal for collaboratively studying recently introduced mycological media is being developed.

Recommendations

(1) Adopt as official first action the MUG procedure for the enumeration of *E. coli* in chilled and frozen foods, exclusive of chilled and frozen shellfish.

(2) Adopt as official first action the use of a pectin-based medium as an alternative to the agar-based medium in **46.005(g)** for determining standard plate count values of foods.

(3) Adopt as official first action the elevated temperature procedure for the isolation of *V. cholerae* from oysters.

(4) Adopt as official final action the official first action dry rehydratable film method, **46.B05-46.B07**, for determining aerobic plate count and total coliform values in raw and pasteurized milk.

(5) Adopt as official final action the official first action enzyme immunoassay screening method, **46.B21-46.B29**, for the detection of *Salmonella* in foods. Official first action method, enzyme immunoassay screening method (revised), **46.C17-46.C25**, remains in that status.

(6) Continue study on all other topics.

This report of the General Referee was presented at the 101st AOAC Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. 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 edition, and to "Changes in Official Methods," *J. Assoc. Off. Anal. Chem.* (1986) **69**, 349–390 (B methods) and *J. Assoc. Off. Anal. Chem.* (1987) **70**, 385–403 (C methods).

GENERAL REFEREE REPORTS: COMMITTEE ON FEEDS, FERTILIZERS, AND RELATED MATERIALS

Antibiotics

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The previously reviewed and recommended collaboratively studied procedure for neomycin in feeds is recommended again. The data from the collaborative study were analyzed statistically according to the recommendations of the Committee statistician. The data in the collaborative study indicated that this procedure would yield results typical of those obtained using microbiological diffusion systems.

The current official method, **42.277-42.280**, is poor. It is difficult, is neither accurate nor precise, and, for the most part, is not being used. The procedure of Stahl et al. is the best available; it is easily performed, is theoretically sound, and is as accurate, precise, and reproducible as the vast majority of microbial diffusion procedures. It is therefore recommended for adoption.

The Charm receptor assay for screening for several families of antibiotics in milk has achieved interim approval.

Future collaborative studies planned for the coming year include a study on a simplified design for diffusion assays for antibiotics in feeds, a procedure for the analysis of bacitracin in feeds, and an immunologically based screening procedure for sulfonamide residues in tissues.

Recommendations

(1) Adopt as interim official action the Stahl et al. procedure for determining neomycin in feeds.

(2) Adopt as official first action the interim official action method for screening antibiotics in milk.

(3) Continue study on all topics.

This report of the General Referee was presented at the 101st AOAC Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. 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 edition.

Drugs in Feeds

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Chapter 42 of *Official Methods of Analysis* was reviewed for appropriate action on those official first action methods listed. Those drugs in feeds not presently listed as active topic areas are presented first:

Aprinocid.—This drug in complete feed (**42.021-42.026**) and in premix (**42.027-42.032**) has apparently been withdrawn from the market in Europe and is not listed in the *Feed Additive Compendium* as registered for use in the United States. Therefore, leave the method as first action and consider deleting it from the 15th edition.

Diethylstilbestrol.—This drug is not listed as allowed in the United States; therefore leave method (**42.059-42.062**) as first action and consider deleting it from the 15th edition.

Dimetridazole.—Consider this spectrophotometric method (**42.063-42.068**) for final action after correcting dilution factors by substituting 66.67 for 1.335×10^{-3}, 250 for 5×10^{-3}, 333.3 for 6.66×10^{-3} in **42.068**. The calculation errors were brought to the attention of the General Referee by Isilda Andrade of the Ministerio da Industria e Comercio in Lisbon, Portugal. Two papers have recently been published on the topic of dimetridazole and ipronidazole (not allowed) in swine feeds: "Rapid Liquid Chromatographic Determination of Dimetridazole and Ipronidazole in Swine Feed," J. E. Roybal et al. (1987) *J. Assoc. Off. Anal. Chem.* **70**, 626–630, and "Quantitative Confirmation of Dimetridazole and Ipronidazole in Swine Feed by Capillary Gas Chromatography/Mass Spectrometry with Multiple Ion Detection," W. J. Morris et al. (1987) *J. Assoc. Off. Anal. Chem.* **70**, 630–634.

Ipronidazole.—The method for the analysis of ipronidazole in complete feeds (**42.082-42.087**) was reported to be unsatisfactory when bentonite is present in the feeds (M. Osadca et al. [1974] *J. Assoc. Off. Anal. Chem.* **57**, 29–31). The authors recommended a new collaborative study, but apparently such a study was never conducted. Leave the method as first action. (See references under dimetridazole above.)

Sulfadimethoxine.—In this colorimetric method (**42.168-42.170**) the specified ficin (Calbiochem, fig latex) is no longer available. It is not possible to correlate without study this material with the several types offered by other suppliers. Leave the method as first action and consider appointing an Associate Referee to investigate this problem.

Sulfaguanidine.—This drug is not listed in the *Feed Additive Compendium* and, therefore, apparently is not allowed in feeds in the United States. Leave method **42.171** as first action.

Sulfaquinoxaline.—This colorimetric method I (**42.179-42.183**) has limitations, but they are listed. Consider for final action.

Sulfonamides (sulfathiazole, sulfamethazine, sulfamerazine, and sulfaquinoxaline).—This spectrophotometric method (**42.185-42.188**) is applicable to premixes and concentrates. The method measures sulfaquinoxaline alone or it measures total sulfonamides. Leave the method as first action until tested on present-day stabilized premixes. The companion method for sulfonamides (**42.189-42.191**) should be left as first action.

The following is a list of Associate Referees and the current active topics assigned to them:

Amprolium (Elzbieta J. Kentzer, Associate Referee).—The LC method for determination of amprolium in feeds and premixes is scheduled for publication in the March 1988 issue of the *Journal of the AOAC*. Some precollaborative testing will be carried out in 1988 with a full collaborative study scheduled for 1989.

Carbadox.—Associate Referee Virginia Thorpe completed evaluation of a collaborative study for the determination of carbadox and pyrantel tartrate in complete feeds by LC and submitted the method to the Methods Committee for consideration. The Committee has recommended that this method be further studied and has expressed the feeling that with a little more preparation the method could be successfully collaborated.

Ethopabate.—Associate Referee Joseph Hillebrandt is solving some "extraneous peak" problems in his adaptation

of the Thorpe LC method prior to sending out a familiarization sample to interested laboratories. He is planning a full collaborative study in late 1987 or early 1988. The Associate Referee reports that a coworker has developed an LC method for the analysis of thiabendazole in complete feeds that will possibly be published later in the year.

Ethylenediamine Dihydroiodide.—Associate Refereeship open.

Furazolidone and Nitrofurazone.—Associate Referee Robert L. Smallidge recommends final action status for the official first action liquid chromatographic method (**42.A01-42.A06**). There have been no negative reports on the use of the qualitative test for furazolidone and zoalene (**42.077-42.080**) in feeds; therefore, it is also recommended for final action.

Melengestrol Acetate. (Raymond Davis, Associate Referee).—The present first action gas chromatographic method (**42.088-42.096**) has been recommended for final action.

Morantel Tartrate.—Linda Werner has been appointed Associate Referee. She reports that an in-house LC method for the analysis of morantel tartrate has been developed and validated for the sample concentration range of 0.44–4.4 g/lb. Some recent extraction concerns with this method will be resolved in 1988, and a collaborative study will be initiated. An LC method for morantel tartrate in premixes (88 g/lb) was developed and validated in the Associate Referee's laboratory in 1987.

Phenothiazine and Related Drugs.—Associate Refereeship open.

Pyrantel Tartrate.—Joyce Konrardy has been appointed Associate Referee. Her plans are to develop a reliable analysis for pyrantel tartrate in the next several months and to follow this with a collaborative study.

Roxarsone. (Associate Referee Glenn M. George).—Two methods are official for roxarsone: a spectrophotometric method (**42.160-42.167**) and a furnace atomic adsorption method (**42.B01-42.B10**). The latter method should be adopted final action in 1988.

Sulfa Drug Residues.—Associate Referee Robert K. Munns is currently preparing the report on a completed collaborative study on the GC determination of sulfamethazine residues in swine feed (R. K. Munns & J. E. Roybal [1982] *J. Assoc. Off. Anal. Chem.* **65**, 1048–1053).

An LC method for the analysis of sulfa drug residues in swine finishing feeds has been submitted for publication from the General Referee's laboratory.

Sulfamethazine and Sulfathiazole.—Associate Referee Dwight M. Lowie reports that he has developed a satisfactory cleanup procedure for use in the analysis of sulfonamides in medicated feeds, but he still has some extraction problems to resolve. He expects to make good progress in the coming months.

The colorimetric method for sulfamethazine (**42.172-42.174**) is commonly used, but it is not quantitative with some of the premix matrixes currently used in preparing complete feeds. It is also apparently not quantitative when certain ingredients are present in the feed. The method should remain as first action.

Recommendations

(1) Appoint Associate Referees for the following drugs in feeds: Arsanilic Acid, Ethylenediamine Dihydroiodide, Phenothiazine (and related drugs), and Sulfadimethoxine.

(2) Declare as surplus the official first action aprinocid methods, **42.021-42.026** and **42.027-42.032**.

(3) Declare as surplus the official first action diethylstilbestrol method, **42.059-42.062**.

(4) Adopt as official final action the official final action spectrophotometric method for dimetridazole, **42.063-42.068**), after making the suggested corrections.

(5) Adopt as official final action the official first action qualitative method for furazolidone and zoalene **42.077-42.080**.

(6) Adopt as official final action the official first action LC method for furazolidone, **42.A01-42.A06**.

(7) Adopt as official final action the official first action GC method for melengesterol acetate **42.088-42.096**.

(8) Adopt as official final action the official first action spectrophotometric Method I, **42.179-42.183**, for sulfaquinoxaline.

(9) Initiate the topic Sulfadimethoxine.

(10) Continue study on all other topics.

This report of the General Referee was presented at the 101st AOAC Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. 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 edition, and 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).

Feeds

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Amino Acids.—Associate Referee Wayne Stockland reported that 5 laboratories have submitted completed results on his amino acid study; however, 8–10 laboratories have yet to submit data.

Calcium Salts of Isobutyric Acid and Mixed 5-Carbon Volatile Fatty Acids.—John Rodgers is surveying existing methodology in the area.

Carotenoid Pigments in Feeds.—D. E. McNaughton of the Agriculture and Fisheries Ministry of New Zealand reports a high degree of interest in carotenoid pigments in Australia and New Zealand. He is in the process of beginning a collaborative study.

Microbial Additives and Enzymes.—William Y. Cobb has investigated the applicability of AOAC method **10.155-10.158**, alpha-amylase in malt, to alpha-amylase in feeds. He reports that the method appears satisfactory if problems with the availability of "Lintner" starch and beta-amylase can be resolved. He has also reviewed literature methods for beta-glucanase. Because of the broad scope of the topic, the Associate Referee recommends splitting his topic into the separate topics of microbial additives and enzymes. Dr. Cobb has regretfully submitted his resignation as Associate Referee.

Minerals in Feeds.—Joel Padmore responded to questions about methodology. Because of the broad scope of the topic he recommends splitting the topic into separate area mineral premixes and finished feeds. He continued his study of ICP methods.

Crude Fiber.—David Holst reports responding to questions. No studies are in progress or planned.

Mixed Feeds—Infrared Reflectance Techniques.—Associate Referee Frank Barton has completed a "Preliminary Study

on the Use of Near Infrared Reflectance Spectroscopy (NIRS)" and has submitted his manuscript to the *Journal of the AOAC* for publication. W. R. Windham, in conjunction with the Associate Referee, has begun a collaborative study on moisture analysis by near infrared reflectance spectroscopy.

Feed Microscopy.—Patricia Ramsey of the California Department of Feed and Agriculture has been appointed Associate Referee. She is working with the American Association of Feed Microscopists to determine areas most in need of new methodology. She plans to begin a collaborative study in the next few months.

Fat.—Paul Guenther of the Colorado Department of Agriculture was appointed Associate Referee. No activity was reported in this area.

Sampling.—Associate Referee Darrel Sharpe of the Missouri Department of Agriculture is planning to initiate a collaborative study comparing tube to probe samplers.

Iodine.—No activity was reported in this area.

Non-Nutritive Residues.—No activity was reported in this area.

Crude Protein.—Associate Referee Peter Kane has completed his collaborative study on the comparison of mercury oxide and copper sulfate/titanium dioxide catalysts for manual Kjeldahl digestion. The manuscript has been approved for publication and the method has received interim official first action approval. He is currently evaluating the Technicon Traacs channel autoanalyzer for use in determining protein, calcium, and phosphorus. He is also working on a block digestion method for crude protein, which substitutes copper sulfate for mercury oxide. Rose Sweeney and Paul Rexroad of the University of Missouri have had their manuscript, "Comparison of the LECO FP-2828 Nitrogen Determinator with AOAC Kjeldahl for Crude Protein," accepted for publication in the *Journal of the AOAC.*

Water in Feeds by Karl Fischer.—Raffaele Bernetti is completing his collaborative study on determination of moisture in corn by the Karl Fischer method. He plans to present the study at the 1988 AOAC meeting in San Francisco. He is also planning to present a paper comparing methods for the determination of water in corn grain.

Recommendations

(1) Split the topic Minerals in Feeds into 2 topics: (a) Mineral Premixes and (b) Complete Feeds. Appoint new Associate Referees.

(2) Split the topic Microbial Additives and Enzymes into 2 topics: (a) Microbial Additives and (b) Enzyme Methods. Appoint new Associate Referees.

(3) Establish the topic Selenium in Feeds and appoint an Associate Referee.

(4) Establish the topic Vitamins A and E in Feeds and appoint an Associate Referee.

(5) Continue study in all other topics.

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Section numbers refer to *Official Methods of Analysis* (1984) 14th edition.

Fertilizers and Agricultural Liming Materials

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Due to early retirements, job changes, deaths, and resignations, the ranks of associate refereeships have been decimated during the past year. Volunteers are being sought to fill several vacated positions. Methods validation studies that should receive attention include micronutrients by ICP and several new slow-release materials.

Iron.—Associate Referee James R. Silkey continues to investigate methods to differentiate chelated from nonchelated iron when added as a micronutrient to fertilizer.

Melamine.—The collaborative study completed last year was returned to the Associate Referee by the Committee on Feeds, Fertilizers, and Related Materials for major revision. Billy Arcement, the Associate Referee, is revising the paper.

Phosphorus.—Joe R. Trimm, Tennessee Valley Authority, National Fertilizer Development Center, Muscle Shoals, AL, was appointed Associate Referee to fill a vacancy.

Sampling.—In 1982, AOAC, TFI, and AAPFCO each appointed 2 members to a Task Force to investigate the official bag-sampling method, **2.001**(a). A comprehensive study was completed and a poster presentation was made at the 1987 AOAC meeting. A contributed paper will be submitted to the *Journal of the AOAC* for publication.

Recommendations

(1) 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, **2.177-2.181**; atomic absorption spectrophotometric method for aluminum in aluminum sulfate-type soil acidifiers, **2.194-2.197**.

(2) Continue study on all other topics.

This report of the General Referee was presented at the 101st AOAC Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. 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 edition.

Plants

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No reports were received from Associate Referees on the topics Ashing Methods; Atomic Absorption Spectroscopy; Fluoride; Starch; Selenium; Sulfur.

Recommendations

(1) Adopt as official final action the following official first action methods, with the concurrence of the authors: metals in plants by direct reading spark emission spectroscopy, **3.006-3.010**; metals in plants by atomic absorption spectroscopy, **3.013-3.016**; fluoride in plants by potentiometry, **3.075-3.080**; glycerol, propylene glycol, and triethylene glycol in cased cigarette filler and ground tobacco by gas chromatography,

3.155-3.158; metals and other elements in plants by inductively coupled plasma spectroscopy, **3.A01-3.A04**.

(2) Continue study on all topics.

This report of the General Referee was presented at the 101st AOAC Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. 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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Tobacco

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Nicotine Alkaloids in Tobacco.—This new topic was approved for study last year; I recommend the appointment of Kenneth L. Rush as Associate Referee under the auspices of the Tobacco Chemist's Research Conference. He has conducted a collaborative study on an automated colorimetric method for the determination of nicotine alkaloids in tobacco.

Polyphenols in Tobacco.—This new topic was also approved for study last year; I recommend the appointment of Maurice E. Snook as Associate Referee. He has extensive experience in the LC analysis of tobacco polyphenols.

Tar and Nicotine in Cigarette Smoke.—Harold C. Pillsbury, Associate Referee, has resigned. FTC will no longer be determining tar and nicotine levels in cigarette smoke.

Recommendations

(1) Adopt as official first action the automated colorimetric method for the determination of nicotine alkaloids in tobacco.

(2) Determine current status of method for determination of tar and nicotine in cigarette smoke and, if needed, appoint new Associate Referee.

(3) Continue study on polyphenols.

This report of the General Referee was presented at the 101st AOAC Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. The recommendations were reviewed by the Committee on Feeds, Fertilizers, and Related Materials. See the report of the committee, this issue.

Veterinary Analytical Toxicology

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The method for determining liver copper (**49.A01-49.A05**) was adopted official final action at the 1986 Annual Meeting. Adoption was based on previously reported collaborative study results and positive feedback from method users.

The Fourth Annual Workshop on Veterinary Analytical Toxicology was held at the AOAC Midwest Regional Section Meeting, June 16, 1987, Fargo, ND. The program was one of the most comprehensive yet. Numerous scientists attended the combination lecture, demonstration, and poster session. Participants from all regions of the United States and several from Canada were kept busy answering questions and demonstrating techniques. The workshop topics included: GC/MS demonstrations, analysis of animal ingesta for cantharadin, feed microscopy in diagnostic toxicology, ion chromatography for serum nitrate, ergot analysis by LC/fluorescence, selenium determination by noncolumn LC fluorescence, remote diagnostic toxicology database demonstration, variation in brain cholinesterase results, antibiotic residues, and determination of various ions in rumen contents. Through the efforts of concerned diagnostic scientists, the workshop has gained national attention and has become an important part of the Midwest Regional Section of AOAC. The fifth workshop is planned for the Midwest Regional Section Meeting, Columbia, MO, June 1988.

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 AAVLD, October 1986, Louisville, KY, and its recommendations have been reported. At that same meeting, the annual session on Veterinary Analytical Toxicology was held with topic discussion on cyclopiazonic acid, ion chromatography, furazoladone determination in serum, and problems with dip vat samples. An additional technical session was also held at the Annual AOAC International Meeting in Scottsdale, AZ, on September 17, 1986.

Several laboratories were, and continue to be, involved with the massive heptachlor contamination incident in Missouri and Arkansas, starting in the spring of 1986 (1–3). Diagnostic laboratory personnel spent hundreds of hours monitoring milk and fat biopsy samples for heptachlor epoxide and oxychlordane. These efforts were somewhat off the normal path taken by diagnostic laboratories. In general, they are not certified to do chlorinated hydrocarbon residue analyses.

The incident also pointed to the lack of any organized system for reporting such contaminations when they are encountered. Considerable discussion has taken place in an effort to relieve this communication problem (3). Efforts must be stronger in the reporting area to avoid widespread contaminations. Programs such as the AAFCO-FEEDCON Feed Contamination Prevention Awareness Program are a step in the right direction. Information gained through FEEDCON is a valuable tool to laboratories involved in all aspects of residue work. Too often, incidents become widespread before the proper authority is aware. A comprehensive communications network among state, federal, and private laboratories should be established. Nonregulatory laboratories involved with day-to-day testing need to have a quick and effective way of communicating directly to regulatory agencies. Other incidents (4, 5) of the same nature have occurred in the past and will continue to occur.

No changes in topic assignments occurred during the past year. Interest has been expressed in the topics of cyanide and diagnostic field kits but no assignments have been made. Activity has varied in the areas of study with considerable constraints on budgets and time limiting the activity of some Associate Referees.

Communications among analysts in diagnostic laboratories continue to improve, and contacts gained at sessions such as those mentioned above have proved to be very valuable. Significant strides have been made in improved quality of data; consequently, more accurate diagnoses are obtained. The continued support of 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 activity that has occurred during the past year.

Antibiotic Screening Methods. — Wynne Landgraf (National Veterinary Services Laboratories, Ames, IA) and Stephen C. Ross (Animal Disease Laboratory, Centralia, IL) report on results of a collaborative study on monensin in feeds that was conducted last year. Considerable variation occurred at the high level (interlaboratory CV = 63% at 1300 ppm), whereas variation at a lower level (interlaboratory CV = 26% at 16 ppm) was less. Results for the external control sample ranged from 1.8 to 43 ppm with 36% CV and 75% recovery. The external control sample was the same sample used for a preliminary interlaboratory study in which variation was less than 18% with 105% recovery. Problems were also encountered with blank samples containing less than 4 ppm monensin; 8 of the 11 participating laboratories reported false positives of 6–9 ppm on one of the 2 blind duplicate blanks.

Some of the problems with the study were probably due to incomplete instructions concerning the charcoal columns used for cleanup. However, it is difficult to attribute all of the variation to this. The variation at the high concentration was most likely due to incorrect use of the spectrophotometer and/or reagent depletion. The problem with the blank samples can be attributed to the presence of alfalfa in the feed. Collaborators failed to provide all raw data in some cases, making it impossible to determine the reason for the wide variation in results for the control sample.

The Associate Referees recommend that the study be repeated with a smaller range of concentrations for the samples and more detailed instructions on the determination step.

The Associate Referees also recommend continued study of the topic. The deregulation of antibiotics in feeds has opened the door for a greater potential of exposure to nontarget species.

Atomic Absorption Methods. — Stephen Kasten (Animal Disease Laboratory, Centralia, IL) reports progress toward determining the concentrations of molybdenum in corn silage, shelled corn, soybean meal, and alfalfa hay samples that will be used in a study of methods for molybdenum, primarily AA furnace techniques. Currently, work on a dry ash technique has given reproducible results. An interlaboratory study is planned. The Associate Referee recommends continued study.

Copper in Animal Tissue. — David L. Osheim (National Veterinary Services Laboratories, Ames, IA) reports that the liver copper method (**49.A01-49.A05**) was adopted official final action at the 1986 Annual Meeting. This was the second copper method adopted official final action. Both methods are currently being used by the Associate Referee's laboratory and other laboratories with satisfactory results.

Fluoride in Animal Tissue. — David L. Osheim (National Veterinary Services Laboratories, Ames, IA) reports on continued efforts to formulate a suitable spiked bone sample for use in a collaborative study. Previously reported data (6) on the ion electrode method for determining fluoride in bone provided justification for a collaborative study. No suitable certified material is available to use for determination of accuracy. Repeated attempts have failed to produce a uniform spiked sample. The Associate Referee recommends continued study. Literature values for normal and affected fluoride need to be supported by an official method.

Lead in Animal Tissues. — Robert J. Everson (Purdue University, West Lafayette, IN) reports that a collaborative study of lead in blood is currently being conducted. The study involves determination by furnace atomic absorption spectroscopy of concentrations from 0.1 to 1.0 ppm. Results will be reported upon completion of the study. The Associate Referee recommends continued study.

Lipid-Soluble Vitamins. — Associate Referee Karen Harlan (University of Illinois, Centralia, IL) reports that an extensive review of analytical methods for vitamins A, E, D, and K is underway. A nonaqueous reverse phase LC/UV method (7) has potential for study. Vitamin E in serum will be studied first. The Associate Referee recommends continued study.

Multielemental Analysis by ICP. — W. Emmett Braselton (Michigan State University, East Lansing, MI) reports on continued development of a database on the frequency distribution of element concentrations in serum, liver, and kidney of animal species. The work on major domestic farm animals and dogs was completed and reported previously (8–10). Work is continuing on minor species (cats, goats, poultry) and zoo animals. In addition, a hydride generator has been added to the Associate Referee's system, and developmental work with arsenic in water has been started with plans also to study animal tissue.

During the past year, 2 more diagnostic toxicology laboratories have obtained ICP equipment. With the technique now being used in more than one location, the Associate Referee will have the opportunity to conduct interlaboratory studies on ICP analyses. The Associate Referee recommends continued study of the use of ICP as a diagnostic tool in veterinary toxicology.

Multiple Anticoagulant Screening. — Associate Referee Jim Stedelin (Animal Disease Laboratory, Centralia, IL) reports on work to improve recovery and simplicity of the basic tissue anticoagulant extraction procedure. Acetonitrile extraction on small liver samples (1–10 g) has improved extraction recovery up to 40% over the previously reported (11) chloroform/methanol extraction. Work has been primarily with positive tissue from field samples containing bromodiolone and brodifacoum.

Improvements have been also made in the LC mobile phase. Use of a borate buffer (pH 10) in place of the ammonium hydroxide has improved day-to-day reproducibility. The Associate Referee recommends continued study.

Natural Products. — Associate Referee George Rottinghaus (University of Missouri, Columbia, MO) reports on completion of work on the method for determining ergot alkaloids in seed, screenings, and dried plant material that was reported here 1 year ago. The lack of appropriate standards is still a constant problem, but new sources are being investigated. Work has also centered on development of a method for detecting ergovaline in fresh fescue plant material at low levels. A series of 400 samples is currently being analyzed to test the applicability of the method on field samples. The method involves extraction with basic chloroform, silica gel minicolumn cleanup, and LC analysis. The detection limit is below 50 ppb with recoveries on spiked samples of greater than 80%.

The Associate Referee also reports on work with determination of ergovaline in plasma. Limited study on spiked samples has yielded 95% recovery at the 1 ppb level with a detection limit of 0.1 ppb. Work is continuing to assess the method on a day-to-day basis. The Associate Referee recommends continued study.

Nitrates/Nitrites. — Michael P. Carlson and Norman D. Schneider (University of Nebraska, Lincoln, NE) report on the development of positive and negative forage nitrate control samples. The positive sample contains 2.9% nitrate (in-house CV = 2.6%, $n = 25$) and the negative sample contains less than 0.2% nitrate (CV = 9.5%, $n = 25$). Ten cooperating

laboratories will assay both samples using the nitrate electrode method (**49.B06-49.B13**) and will provide results for evaluation. If the results are satisfactory, the control samples will be available in 50 g lots upon request from any interested laboratory.

Numerous laboratories have expressed interest in the nitrate electrode method since its adoption as official first action. State regulatory, diagnostic, service, plant, and biochemistry laboratories have found the method to be quick, accurate, and precise.

The Associate Referees continue to address the need for a collaborated method for nitrate/nitrite in biological fluids (ocular fluid and serum). Problems with reproducibility of the cadmium reduction-diazo coupling method (12) make it labor-intensive and costly. Discussion (Boermans, private communication, 1986) and a report of ion exchange chromatographic applications (13) for determining nitrate and nitrite in serous fluids strongly support further study of the method. Several laboratories are now using IEC procedures for routine screening (N. Weiser and R. Smith, private communications, 1987). Data reported here 1 year ago clearly justify an interlaboratory study.

The Associate Referees recommend continued official first action status for the forage nitrate method (**49.B06-49.B13**).

Pesticides in Toxicological Samples.—H. Michael Stahr (Iowa State University, Ames, IA) reports that a collaborative study will be done in the coming year with 6 collaborators analyzing freeze-dried blood. The goal is to complete the study in time to be reported by next year's meeting.

Sodium Monofluoroacetate (1080).—H. Michael Stahr (Iowa State University, Ames, IA) reports on continued work with the indirect fluoride method for 1080 screening. A second laboratory is studying the method; results will be compared.

A method was evaluated during that past year that involves derivatizations and direct extraction. It will be applied to tissues to determine if it can be used on a routine basis.

Zinc in Animal Tissues.—Dana Perry (University of Arizona, Tucson, AZ) reports that a collaborative study of zinc in animal serum is currently being completed. The method under study is a direct dilution atomic absorption spectroscopy technique. Using direct dilution, the potential for contamination is minimized. In general, rubber stoppers contain zinc. Extreme care must be exercised in collection of specimens and handling in the laboratory. The collaborative study will be reported after results have been analyzed.

Recommendations

(1) Continue official first action status of the method for arsenic in animal tissues, **49.B01-49.B05**, and the method for nitrite in forage, **49.B06-49.B13**.

(2) Continue study on all other topics.

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This report of the General Referee was presented at the 101st AOAC Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. 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

Soils and Sediments

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Two Associate Referees have reported on the status of their topics. It is recommended that both of these topics be continued.

Soil Distribution Coefficients.—Danny Jackson, formerly of Battelle-Columbus Laboratories is now with Radian Corp., Austin, TX. Because of his recent move, Dr. Jackson temporarily suspended his work on testing a draft standard method he has authored. It is anticipated that he will resume his work in the near future.

Volatile Organic Chemicals in Soil and Sediment—Adsorption Isotherm Test.—Leverett Smith, Kennedy/Jenks/Chilton, San Francisco, CA, has continued developing a revised draft method in his laboratory. His progress was significantly hindered during the past year; he oversaw a rapid and substantial expansion of his laboratory, which now includes 2 GC/MS units and supporting equipment and staff. He intends to subject the method to ruggedness testing; on the basis of those results, the draft will be revised and subjected to an interlaboratory collaborative study.

Termiticides in Soil.—The General Referee has recommended to AOAC that Wayne Pask, Office of the Indiana State Chemist and Seed Commissioner, be appointed Associate Referee for Termiticides in Soil. First, Dr. Pask will evaluate existing methods for chlordane in soils. Then, he intends to thoroughly test the most promising method for ruggedness. Afterwards, he will proceed to the collaborative study stage. At some time in the future, after establishing a method for chlordane, he intends to extend his work to other termiticides, including chlorpyrifos, isofenfos, permethrin, and fenvalerate.

Liquid Chromatographic Method for Determination of Explosives Residues in Soils.—The General Referee is now discussing his interest in conducting a collaborative study of a liquid chromatographic method for the determination of explosives residues in soils with Thomas Jenkins, Department of the Army, Hanover, NH.

AOAC Liaison With ISO Technical Committee 190.—The International Organization for Standardization Technical Committee 190, Soil Quality, has become increasingly involved in the development of test methods of interest to this General Refereeship. The General Referee receives and reviews this Committee's documents on an ongoing basis. Also, the General Referee furnishes documents of immediate interest to this Committee.

Recommendations

(1) Continue developing adsorption isotherm test for volatile organic chemicals in soils and sediments.

(2) Continue developing standard method for distribution coefficients of nonvolatile organics in soils.

(3) Continue developing standard methods for termiticides in soil.

(4) Pursue the potential development of standard methods for explosives residues in soil.

(5) Monitor progress made in method development in 150 participating countries of ISO.

(6) Continue recruiting Associate Referees and collaborators who can study and develop methods in the topic areas discussed in the 1984 report.

This report of the General Referee was presented at the 101st AOAC Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. The recommendations were approved by the Committee on Hazardous Substances in Water and the Environment. See the report of the committee, this issue.

Waste Materials

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Revitalization of the activities in the Industrial Process Waste refereeship is continuing.

Conventional Analytical Methods

Inorganic Analytes.—Several studies to evaluate solid waste testing methods have been completed during the past year, and will be recommended for interim approval sometime during the next 12 months. These methods are detailed below.

An inductively coupled plasma atomic emission spectroscopic (ICP) method was evaluated in the EPA round-robin Phase 1 study by 7 laboratories. Fourteen metals were determined in acidified aqueous matrixes. In addition, a single laboratory evaluation of the method was conducted with wastes. The method was used to determine 22 elements in 7 different wastes. The relative standard deviation from triplicate analyses for all elements and wastes was 9%, and the mean recovery of spiked elements for all wastes was 93%.

Organic Analytes.—A new study was conducted on the evaluation of the U.S. EPA gas chromatographic/mass spectrometric methods: method 8250 utilizing a packed column technique and method 8270 utilizing a capillary column technique for determination of semivolatile organic compounds. The method is used for analysis of extracts prepared from solid wastes, soils, and ground water. In limited applications, direct injection of a sample may be used. Method 8250 was tested by 15 laboratories using reagent water, drinking water, surface water, and industrial wastewater spiked at 6 concentrations over the 5–1300 $\mu g/L$ range. Single-operator accuracy and precision were found to be directly related to the analyte concentration and were essentially independent of the sample matrix. The average standard deviation for a number of extractables is about 30%.

An interim protocol was developed at EPA for automated determination of semivolatile organic compounds by gas chromatography/Fourier transform infrared (GC/FT-IR) spectrometry in wastewater, soils, sediments, and solid wastes.

The protocol is designed for the high throughput, automated analysis of multicomponent environmental and hazardous waste extracts. Wastewater analysis for semivolatile organic compounds is based on extracting a 1 L sample with methylene chloride and concentrating the extract to 1 mL.

The analysis of the semivolatile fraction derived from solid waste analysis is based on extracting a 50 g sample and concentrating the sample extract to 1.0 mL. A gel permeation option is included to purify further those extracts that cannot be concentrated to the specified final volume. Using capillary GC/FT-IR techniques, wastewater identification limits of 10 to 60 ppb can be achieved with this method, whereas the corresponding identification limits for solid samples are 200 ppb to 1 ppm. The most frequent obstacle to achieving these identification limits is expected to be the presence of large quantities of interfering high boiling coextractants. These coextractants would raise the identification limits by preventing the concentration of extracts to the desired final volume, thereby necessitating gel permeation cleanup, and by decreasing the spectra signal-to-noise ratio of GC-volatile analytes by raising the spectral background intensity. The developed protocol is unique because, for those cases where complete identification is impossible, the functionality or compound class of the analyte may be determined. This may be accomplished by utilizing the group frequency capability of modern Fourier transform infrared spectrometers. A knowledge of chemical functionality coupled with available biological toxicity data may lead to an assessment of the risks associated with these partially identified analytes.

Bioassay Methods

Associate Referee Llew Williams (EMSL/LV) reports completion of the first phase study of monoclonal antibody assay method for pentachlorophenol. The next step, the multilaboratory collaborative testing, is now in the organizational stage to start this year.

Recommendation

Continue study on all topics.

This report of the General Referee was presented at the 101st AOAC Annual International Meeting, Sept. 14–17, 1987, at San Francisco, CA. The recommendations were approved by the Committee on Hazardous Substances in Water and the Environment. See the report of the committee, this issue.

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REPORTS AND TRANSACTIONS

THE 101st AOAC ANNUAL

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AT THE CATHEDRAL HILL HOTEL • SAN FRANCISCO, CALIFORNIA
SEPTEMBER 14–17, 1987

Since 1884
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TRANSACTIONS: ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS

The one-hundred-first annual international meeting of the Association of Official Analytical Chemists was held at The Cathedral Hill Hotel, San Francisco, California, on September 14, 15, 16, and 17, 1987. The following reports, along with the actions of the Association, were given at the business meeting, held Thursday, September 17, 1987, Frank J. Johnson, 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*;
ALAN R. HANKS (Office of the Indiana State Chemist, Purdue University, Department of Biochemistry, West Lafayette, IN 47907);
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 C. 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 September 13 and 15, 1987, in San Francisco, CA. The meeting was attended by several General Referees and Associate Referees whose topics are assigned to the Committee and by several visitors.

William Horwitz discussed his recent review and analysis of over 1200 results from collaborative studies of pesticide formulation methods. His analysis revealed the same dependence of precision on concentration found in his earlier work on methods for drug analysis. Also similar to the drug methods, the precision of pesticide formulations analyses is independent of analyte, matrix, and method. Dr. Horwitz recommended that the Committee review the results of a few methods for which precision is poorer than might be expected.

The Committee discussed items for the agenda of the Methods Board/Volunteer Workshop held on Thursday, September 17, 1987. Included were the following: (*1*) Application of Harmonization Guidelines to Pesticide Formulation Methods; (*2*) Role of Committee Statistician in Collaborative Studies; (*3*) Cooperative Efforts with CIPAC; (*4*) Deletion of Inactive Topics.

The Committee also discussed the methods approval process. The consensus was that the process worked reasonably well this year. However, concern was expressed that the process involves too many people in reviews of an editorial nature.

The Committee discussed the low output of collaborative studies over the last few years. Most committee members feel the economic decline of the agricultural chemicals industry is the primary reason for the decrease in new methods development. The Committee feels that the Association must improve the "selling" of itself in the industrial sector, particularly at the research director and vice president of research levels. The need is not just for sustaining membership, but for Associate Referees to conduct and scientists to participate in collaborative studies—a much more expensive proposition. The Committee will work through AOAC administrative channels to develop more effective approaches to enlisting broader support from the agricultural chemicals industry.

The Committee discussed and is developing a position paper on the needs for methods of analysis for adjuvants and inert ingredients in pesticide formulations. This paper is being prepared for submission to the U.S. Environmental Protection Agency through the appropriate channels.

The Committee also discussed the need for a generally approved method for determining theoretical plates in chromatographic systems.

The Committee discussed the CIPAC method for bentazon (Basagran). This method has been accepted by CIPAC but the collaborative study did not use sufficient numbers of samples to meet AOAC standards. The Committee will ask the General Referee to request that the Associate Referee repeat the study with an appropriate number of samples. If that is not possible, a co-Associate Referee will be sought to conduct such a study. Further, the Committee will request that CIPAC review the adequacy of the existing collaborative study in view of the IUPAC harmonization recommendations.

The Committee discussed the recommendation of the General Referee for Herbicides II that the topic on metribuzin be deleted. The Committee chose to recommend continued study because the current methods have not been validated for formulations containing a mixture of active ingredients.

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.

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 edition, and 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), and *J. Assoc. Off. Anal. Chem.* (1987) **70**, 385–403 (C methods).

* An asterisk before a recommendation indicates that official action on that recommendation resulted in its appearance in "Changes in Official Methods."

Table 1. Recommendations for General Referees

Current designation	Disposition	General Referee
Herbicides I	Transfer topics to Herbicides II	—
Herbicides II	No change	Arthur Hofberg
Herbicides III	Change title to Herbicides I	Peter Bland
Fungicides and Disinfectants	No change	Peter Bland
Organohalogen Insecticides	No change	David Tomkins
Other Insecticides, Synergists, and Insect Repellants	No change	David Tomkins
Organothiophosphorus Insecticides	No change	Open
Other Organophosphorus Insecticides	No change	Marshall Gentry
Carbamate and Substituted Urea Insecticides	No change	Marshall Gentry
Rodenticides and Miscellaneous Pesticides	No change	Marshall Gentry
CIPAC Studies	No change	James Launer
Microbial Pesticides	No change	Open

The General Referee has recommended continued study of the topic.

Eugene Cole, Associate Referee for the Use-Dilution Test, presented the results of his investigations of the existing method, the identification of 31 modifications to the method, and collaborative studies of both the existing and the modified method. The results indicate that both the existing and the modified methods produce highly variable results. It should be noted that the original collaborative study of 1953 also produced highly variable results that were not subjected to statistical analysis. The method appears to perform satisfactorily in some laboratories but poorly in others. Cole indicated that he plans to conduct a collaborative study on a suspension method as a potential replacement for the existing carrier-based method. The Associate Referee recommended that the existing method be repealed when a suitable replacement method becomes available. This issue has been studied by an EPA Task Force, which has also recommended pursuit of the suspension method. The General Referee has recommended continued study of the topic.

The Committee recommends that Aram Beloian be reappointed as GR for Disinfectants, and that the General Referee subject areas in Pesticide Formulations be realigned as shown in Table 1.

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 first action the official final action methods (**4.036-4.041**). (b) Continue study with emphasis on suspension methods.
*(5) *Use-Dilution Test:* (a) Repeal first action the official final action methods for *Staphylococcus aureus* and *Salmonella choleraesuis* (**4.007-4.010**). (b) Continue study with emphasis on suspension methods.
(6) *Virucide Tests:* Continue study.

PESTICIDE FORMULATIONS: FUNGICIDES AND DISINFECTANTS

*(1) *Anilazine (Dyrene):* (a) Adopt as official first action the interim first action liquid chromatographic method described by the Associate Referee. (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:* Appoint Associate Referee; topic open.
(6) *Dinocap:* Appoint Associate Referee; topic open.
(7) *Dioxins in Pentachlorophenol:* Appoint Associate Referee; topic open.
(8) *Dithiocarbamate Fungicides:* Continue study.
(9) *Oxythioquinox (Morestan):* Continue official first action status of the liquid chromatographic method (**6.B34-6.B39**). (b) Continue study.
(10) *o-Phenylphenol:* Appoint Associate Referee; topic open.
(11) *Quaternary Ammonium Compounds:* Appoint Associate Referee; topic open.
(12) *Thiram:* Appoint Associate Referee; topic open.
(13) *Triadimefon (Bayleton):* Continue study.
(14) *Triphenyltin (Fentin):* Continue study.
(15) *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) *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 (**6.372-6.377**); 2,4,5-trichlorophenoxyacetic acid (**6.391-6.395**); 2,4-dichlorophenoxyacetic acid esters and amine salts (**6.288-6.292**). (b) Continue study.
(2) *Dicamba:* Continue study.
(3) *Pentachlorophenol:* Appoint Associate Referee; topic open.
(4) *Plant Growth Regulators:* Appoint Associate Referee; topic open.

PESTICIDE FORMULATIONS: HERBICIDES II

(1) *Benefin, Trifluralin, Pendimethalin, and Ethafluralin:* Continue study.
(2) *Bensulide (Betasan):* Continue study.
(3) *Benzoylprop-ethyl:* Appoint Associate Referee; topic open.
(4) *Bromacil:* Continue study.
(5) *Chlorsulfuron (Glean):* Continue study.
(6) *Dimethyl Tetrachloroterephthalate:* Continue study.
(7) *Dinoseb:* Appoint Associate Referee; topic open.
(8) *Fluometuron:* Continue study.
(9) *Metsulfuron-methyl (Ally):* Continue study.
(10) *Methazole:* Continue study.
(11) *Naptalam (Alanap):* Continue study.
(12) *Oryzalin (Surflan):* Appoint Associate Referee; topic open.
(13) *Substituted Urea Herbicides:* Appoint Associate Referee; topic open.
(14) *Sulfometuron-methyl (Oust):* Continue study.
*(15) *Thiocarbamate Herbicides:* (a) Adopt as official final

action the official first action gas chromatographic method (**6.581-6.585**). (b) Discontinue topic.

PESTICIDE FORMULATIONS: HERBICIDES III

*(1) *Alachlor, Butachlor, and Propachlor:* (a) Adopt as official first action the interim first action gas chromatographic method for alachlor microencapsulated formulations described by the Associate Referee. (b) Adopt as official final action the official first action gas chromatographic methods for butachlor (**6.B12-6.B16**), and propachlor (**6.B17-6.B21**). (c) Continue study.
(2) *Alachlor/Atrazine Mixtures:* Continue study.
(3) *Amitrole:* Appoint Associate Referee; topic open.
(4) *Bentazon:* Continue study.
(5) *Bromoxynil:* Continue study.
(6) *Cacodylic Acid, MSMA, and DSMA:* Appoint Associate Referee; topic open.
(7) *Cyanazine (Bladex):* Continue study.
(8) *Dalapon:* Discontinue topic.
(9) *Dichlobenil:* Continue study.
(10) *Fluazifop-butyl (Fusilade):* Discontinue topic.
(11) *Fomesafen:* Continue study.
*(12) *Metolachlor:* (a) Adopt as official final action the official first action gas chromatographic method (**6.A14-6.A18**). (b) Discontinue topic.
(13) *Metribuzin (Lexone or Sencor):* Continue study.
(14) *Pesticides in Fertilizers:* Continue study.
(15) *Propanil (3',4'-Dichloropropionanilide):* Appoint Associate Referee; topic open.
(16) *Sodium Chlorate:* Appoint Associate Referee; topic open.
(17) *s-Triazine Herbicides:* Discontinue topic.

PESTICIDE FORMULATIONS: CARBAMATE INSECTICIDES AND SUBSTITUTED UREA INSECTICIDES

(1) *Aldicarb:* Continue study.
(2) *Aminocarb:* Discontinue topic.
(3) *Bendiocarb:* (a) Continue official first action status of the liquid chromatographic method (**6.B40-6.B44**). (b) Continue study.
(4) *Carbaryl:* Continue study.
(5) *Carbofuran and Carbosulfan:* (a) Continue official first action status of the liquid chromatographic method for carbofuran (**6.B45-6.B48**). (b) Continue study.
(6) *Methiocarb:* Discontinue topic.
(7) *Methomyl:* Continue study.
(8) *Mexacarbate (Zectran):* Continue study.
(9) *Oxamyl:* Continue study.
(10) *Pirimicarb:* (a) Continue official first action status of the gas chromatographic method (**6.560-6.564**). (b) Appoint Associate Referee; topic open.
(11) *Propoxur (Baygon):* Discontinue topic.
(12) *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:* Continue study.
(3) *Chlordimeform:* Discontinue topic.
(4) *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.
(5) *Ethylan:* Continue study.
(6) *Fenvalerate:* Appoint Associate Referee; topic open.
(7) *Heptachlor:* Continue study.
(8) *Methoxychlor:* Continue study.
(9) *Methyl Bromide:* Continue study.
(10) *Toxaphene:* Continue study.
(11) *Trichlorfon (Dylox):* Continue study.

PESTICIDE FORMULATIONS: ORGANOTHIOPHOSPHORUS INSECTICIDES

(1) *Acephate (Orthene):* Continue study.
(2) *Azinphos-methyl (Guthion):* Continue study.
(3) *Coumaphos:* Appoint Associate Referee; topic open.
(4) *Demeton (Systox):* Delete topic.
(5) *Demeton-S-methyl (Metasystox-I):* Discontinue topic.
*(6) *Diazinon:* (a) Adopt as official final action the official first action gas chromatographic method for diazinon (**6.425**). (b) Discontinue topic.
(7) *Dimethoate:* Continue study.
(8) *Dioxathion:* Continue study.
(9) *Encapsulated Organophosphorus Pesticides:* (a) Continue official first action status of the gas chromatographic method for encapsulated diazinon (**6.426-6.429**). (b) Continue study.
(10) *EPN:* Continue study.
(11) *Ethoprop:* Continue study.
(12) *Fenitrothion:* (a) Continue official first action status of the gas chromatographic method (**6.A19-6.A24**). (b) Continue study.
(13) *Fensulfothion:* (a) Continue official first action status of the gas chromatographic method (**6.B28-6.B33**). (b) Continue study.
(14) *Fenthion:* Continue study.
(15) *Fonofos:* Continue study.
(16) *Isofenphos:* (a) Continue official first action status of the gas chromatographic method (**6.C01-6.C06**). (b) Continue study.
(17) *Malathion:* Continue study.
(18) *Methamidophos (Monitor):* Continue study.
(19) *Methidathion (Supracide):* Continue study.
(20) *Oxydemeton-methyl (Metasystox-R):* Continue study.
(21) *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.
(22) *Phorate:* Appoint Associate Referee; topic open.
(23) *Pirimiphos-methyl:* Continue study.
(24) *Temephos:* Continue official first action status of the CIPAC-AOAC liquid chromatographic method (**6.509-6.515**).
(25) *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) Appoint Associate Referee; topic open.

(3) *Fenamiphos (Nemacur):* Continue study.
(4) *Mevinphos:* Appoint Associate Referee; topic open.
(5) *Monocrotophos:* Appoint Associate Referee; topic open.
(6) *Naled:* Continue study.
(7) *Tetrachlorvinphos (Gardona, Rabon):* Appoint Associate Referee; topic open.

PESTICIDE FORMULATIONS: OTHER INSECTICIDES, SYNERGISTS, AND INSECT REPELLANTS

(1) *Allethrin:* (a) Continue official first action status of the titrimetric method for technical allethrin (**6.165-6.170**). (b) Continue study.
(2) *Aluminum Phosphide:* Appoint Associate Referee; topic open.
(3) *2,3,4,5-Bis(2-butylene)tetrahydro-2-furfural (MGK Repellant 11):* Continue study.
*(4) *Cyhexatin (Plictran):* Adopt as official first action the interim first action CIPAC–AOAC liquid chromatographic method described by the General Referee.
(5) *Cypermethrin:* (a) Continue official first action status of the CIPAC–AOAC gas chromatographic method (**6.B01-6.B05**). (b) Continue study.
(6) *Cyromazine (Larvadex):* Continue study.
(7) *Dipropyl Isocinchomeronate (MGK Repellant 326):* Continue study.
(8) *Fumigants:* Appoint Associate Referee; topic open.
(9) *Nicotine:* Continue study.
*(10) *Permethrin:* (a) Adopt as official final action the official first action CIPAC–AOAC gas chromatographic method (**6.B06-6.B11**). (b) Continue study.
(11) *Piperonyl Butoxide and Pyrethrins:* Continue study, especially low levels and mixed formulations.
(12) *Resmethrin:* Continue study.
(13) *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) Continue official first action status of the liquid chromatographic method (**6.597-6.601**). (b) Continue study.
(2) *Chlorophacinone:* Appoint Associate Referee; topic open.
(3) *Diphacinone:* Appoint Associate Referee; topic open.
(4) *Naphthylthiourea:* (a) Continue official first action status of the surplus method (**6.157**). (b) Appoint Associate Referee; topic open.
(5) *Sampling:* (a) Continue official first action status of the sampling procedures for fertilizers (**6.002**) as applied to pesticide-fertilizer mixtures. (b) Appoint Associate Referee; topic open.
(6) *Strychnine:* Appoint Associate Referee; topic open.
(7) *Warfarin:* Appoint Assoicate Referee; topic open.

Committee on Drugs and Related Topics: Recommendations for Official Methods

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The Committee on Drugs and Related Topics met on August 27, 1987, in Rockville, MD, and took the following actions:

1. Reviewed the draft of the new form "Guidelines for AOAC Reviewers." The members were informed that they should vote on all manuscripts submitted to them. If a member feels unqualified to comment on the manuscript, an "abstain" vote should be sent to the AOAC office; if no reply is received, it will be counted as "accepted." The Committee discussed the flow of paper work during the report approval process that was established at last year's meeting. The Committee decided to change the procedure for handling manuscripts that had been modified on the basis of members' comments. It was agreed that the revised manuscript should be sent to all Committee members, not only to those who had made substantive comments in the first round of review.

2. Discussed the document on "Methods Validation and Approval Process" approved by the Official Methods Board. It was suggested that the word "validation" in the first line should be clarified by adding the words "by collaborative study" after it, to distinguish it from validation of a method that is performed by the Associate Referee before a collaborative study is begun.

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 edition, and 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), and *J. Assoc. Off. Anal. Chem.* (1987) **70**, 385–403 (C methods).
* An asterisk before a recommendation indicates that official action on that recommendation resulted in its appearance in "Changes in Official Methods."

3. Agreed that plans for proposed collaborative studies need not be sent to all Committee members, but may be sent if requested by the General Referee or the Committee.

4. Requested the AOAC office to notify all Committee members of new Associate Referee appointments, not just the General Referee and the Secretary. The Committee requested the AOAC office to send a copy of the Committee's "Performance Parameters" (*J. Assoc. Off. Anal. Chem.* [1987] **70,** 321) to all new Associate Referees.

5. Discussed the proposed format of a model Associate Referee report, and recommended that the model should be in the form of a typewritten manuscript instead of a final typeset paper.

6. Voted to recommend that the Association investigate the possibility of describing equipment in AOAC official methods in generic terms, instead of specifying a particular model, or equivalent. Some models that are specified in *Official Methods of Analysis* have been discontinued and are no longer available.

7. Voted to recommend that the Association recognize the acceptability of designs for collaborative studies of methods for drug residues in animal tissues that include only 2 or 3 collaborators but considerably more than the currently required 30 data points.

8. Voted to recommend the reappointment of the following General Referees: James Fitzgerald, Drugs I; Edward Smith, Drugs II; Ronald Yates, Cosmetics (all for 2 years), and Charlie Barnes, Drug Residues in Animal Tissues (1 year).

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.**

DIAGNOSTICS AND TEST KITS

(1) *Automated Microbial Identification Systems—VITEK:* The topic was established and an Associate Referee has been appointed. Continue study.
(2) *Automated Microbial Identification Systems—HP5898A:* The topic was established and an Associate Referee has been appointed. Continue study.
(3) *Gene Probes for TB and Enteric Infections:* The topic was established and an Associate Referee has been appointed. Continue study.
(4) *Immunological and Diagnostic Assay of Pesticides, Hormones, and Enzymes:* The topic was established and an Associate Referee has been appointed. Continue study.
(5) *Multicomponent Analysis of Clinical Specimens:* The topic was established and an Associate Referee has been appointed. Continue study.
(6) *Oxalates—Enzymatic Procedures:* Discontinue topic.

DRUG RESIDUES IN ANIMAL TISSUES

(1) *Benzimidazoles:* Continue study.
(2) *Dimetridazole:* Discontinue topic.
(3) *Estrogenic Compounds:* Discontinue topic.
(4) *Ipronidazole in Turkey and Swine:* Continue study.
(5) *Levamisole:* Discontinue topic.
(6) *Screening Methods:* Continue study.
(7) *Tiamulin (Screening Method):* Discontinue topic.

DRUGS I

(1) *Acetaminophen in Drug Mixtures:* Continue official first action status of the liquid chromatographic method 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) *Aspirin and Caffeine with Other Drugs:* Discontinue topic.
*(5) *Diethylpropion Hydrochloride:* Adopt as official first action the interim official first action method for determination of diethylpropion hydrochloride in drug substance and tablets. Continue study.
(6) *Phenothiazines and Tricyclic Antidepressants in Formulations by LC:* Discontinue topic.
(7) *Phenothiazine-Type Drugs by TLC:* Continue study.
*(8) *Primidone:* Adopt as official final action the official first action method for liquid chromatographic determination of primidone in tablets, **37.A17-27.A22.** Discontinue topic.
(9) *Sulfamethoxazole and Trimethoprim:* Discontinue topic.

DRUGS II

(1) *Aminacrine:* Continue study.
(2) *Atropine in Morphine and Atropine Tablets and Injections:* Discontinue topic.
(3) *Belladonna Alkaloids:* Appoint Associate Referee. Continue study.
(4) *Colchicine in Tablets:* Continue study.
(5) *Curare Alkaloids:* Continue study.
(6) *Dicyclomine Capsules:* Continue study.
(7) *Epinephrine–Lidocaine Combinations:* Discontinue topic.
(8) *Epinephrine and Related Compounds by LC-Electrochemical Detectors:* Continue study.
(9) *Ergot Alkaloids:* Continue study.
(10) *Homatropine Methyl Bromide in Tablets:* Discontinue topic.
(11) *Morphine Sulfate in Morphine Injection:* Continue study.
(12) *Neostigmine:* Discontinue study.
(13) *Phenethylamine Drugs, Semiautomated Individual Unit Analysis:* Discontinue topic.
(14) *Pheniramine with Pyrilamine, Phenylpropanolamine, and Phenylephrine:* Appoint Associate Referee. Continue study.
(15) *Physostigmine and Its Salts:* Continue study.
(16) *Pilocarpine:* Continue study.
(17) *Rauwolfia Alkaloids:* Appoint Associate Referee. Continue study.
(18) *Rauwolfia serpentina:* Continue study.

DRUGS III

(1) *Ampicillin and Amoxicillin:* Discontinue topic.
*(2) *Coumarin Anticoagulants:* Adopt as official first action the interim official first action method for liquid chromatographic determination of coumarin anticoagulants in dosage forms. Continue study.
*(3) *Flucytosine:* Adopt as official first action the interim

first action method for liquid chromatographic determination of flucytosine in capsules. Continue study.

(4) *Fluoride:* Discontinue topic.

(5) *Halogenated Hydroxyquinoline Drugs:* Continue study.

(6) *Haloperidol:* Continue study.

(7) *Hydralazine:* Continue study.

(8) *Insulin by Liquid Chromatography:* Discontinue topic.

*(9) *Levodopa:* Adopt as official first action the interim official first action method for liquid chromatographic determination of levodopa-carbidopa in tablets. Continue study.

(10) *Medicinal Gases:* Continue study.

(11) *Meprobamate Tablets:* Discontinue topic.

(12) *Mercury-Containing Drugs:* Discontinue topic.

(13) *Metals in Bulk Drug Powders:* Continue study.

*(14) *Microchemical Tests:* Adopt as official final action the official first action microchemical identification tests for promethazine, triflupromazine, perphenazine, and thiethylperazine in pure drug substances, **36.A07-36.A10**. Discontinue topic.

(15) *Penicillins:* Continue study.

(16) *Salts of Organic Nitrogenous Bases:* Continue study.

*(17) *Trimethobenzamide:* Adopt as official final action the official first action method for ion-pair column chromatographic determination of trimethobenzamide, **36.A01-36.A06**. Discontinue topic.

DRUGS IV

*(1) *D- and L-Amphetamines—LC Separations:* Adopt as official first action the interim official first action method for liquid chromatographic determination of D- and L-amphetamines. Continue study.

(2) *Benzodiazepines:* Continue study.

*(3) *Diazepam:* Adopt as official final action the official first action method for liquid chromatographic determination of diazepam, **40.A01-40.A06**. Continue study.

(4) *Heroin:* Continue study.

DRUGS V

(1) *Betamethasone:* Continue study.

(2) *Chlorpropamide:* Continue official first action status of the liquid chromatographic method for the determination of chlorpropamide in tablet dosage forms, **37.B01-37.B06**. Continue study.

(3) *Digitoxin, Automated Individual Tablet Analysis:* Discontinue topic.

*(4) *Estrogens (Conjugated) by LC:* Surplus the official final action colorimetric method for conjugated estrogens in drugs, **39.001-39.005**. Continue study.

*(5) *Hydrocortisone:* Adopt as official final action the official first action methods for liquid chromatographic determination of hydrocortisone in drug substances and tablets, infrared identification of hydrocortisone in drug substances and tablets, and thin-layer chromatographic identification of hydrocortisone in drug substances and tablets, **39.047-39.055**. Discontinue topic.

*(6) *Methocarbamol:* Adopt as official final action the official first action method for liquid chromatographic determination of methocarbamol in pharmaceutical dosage forms, **36.212-36.218**. Discontinue topic.

(7) *Miconazole:* Discontinue topic.

(8) *Pentaerythritol Tetranitrate:* Continue study.

(9) *Prednisolone:* Continue official first action status of the liquid chromatographic method for determination of prednisolone in drug substance and in tablets, **39.B01-39.B06**. Continue study.

(10) *Progestins in Tablets—Automated Methods:* Continue study.

*(11) *Steroid Acetates:* Adopt as official first action the interim official liquid chromatographic method for determination of dexamethasone acetate and cortisone acetate in drug substances and in dosage forms. Continue study.

*(12) *Steroid Phosphates:* Adopt as official first action the interim official first action methods for liquid chromatographic determination of dexamethasone, thin-layer identification of dexamethasone in drug substances and elixirs, infrared identification of dexamethasone phosphate, and gas chromatographic determination of alcohol in dexamethasone elixirs. Continue study.

*(13) *Other Topics:* Continue official first action status of and surplus the enzymatic hydrolysis-spectrophotometric method for determination of dexamethasone in creams, ointments, and solutions, **39.056-39.060**.

IMMUNOCHEMISTRY

(1) *Heparin by Non-IRA Procedures:* Continue study.

(2) *Hybridoma-Monoclonal Antibodies:* Continue study.

FORENSIC SCIENCES

(1) *Chromatographic Methods for Forensic Characterization of Paints and Other Polymeric Materials:* Continue study.

(2) *Electrophoretic Methods:* Continue study.

(3) *Enzyme-Linked Immunosorbent Assays for Forensic Characterization of Body Fluid Stains:* Continue study.

(4) *Explosives and Explosives Residues:* Continue study.

(5) *Grouping Tests for Blood and Other Body Fluids:* Continue study.

(6) *Gunshot Residues:* Continue study.

(7) *Isoelectric Focusing Methods for Forensic Characterization of Body Fluid Stains:* Continue study.

(8) *Screening and Confirmatory Tests for Dried Blood Stains:* Continue study.

(9) *Soils, Geological Analysis:* Continue study.

Committee on Foods I: Recommendations for Official Methods

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The Committee discussed information from the Official Methods Board, including the new review process, awards, terms of reference for the Committee, statistical parameters, effect of biotechnology on method development and approval process, and 3 new task forces (Methods Outreach, Sampling/Sample Preparation, and Validation of Methods in Crisis Situations).

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:* Appoint Associate Referee; continue study.

DAIRY CHEMISTRY

(1) *Adulteration of Dairy Products with Vegetable Fat:* Continue study.
*(2) *Babcock Test and Babcock Glassware:* Correct **16.178(a)** to read ". . . centf. 5 min. Fill bottle to neck with hot H_2O and centf. 2 min. Add hot . . . ." Continue study.
(3) *Babcock, Mojonnier, and Kjeldahl Tests:* Review collaborative study results of the Babcock and Mojonnier methods for fat in milk; continue study.
(4) *Calcium, Phosphorus, and Magnesium in Cheese:* Review collaborative study results for the IDF method for phosphorus in processed cheese; continue study.
(5) *Casein and Caseinates:* Discontinue topic.
(6) *Chloride Meters:* Continue study.
(7) *Chocolate Milk, Fat Test:* Discontinue topic.
(8) *Cryoscopy of Milk:* Review collaborative study results of the IDF thermistor method for the determination of the freezing point of milk; continue study.
(9) *Fat in Milk (AutoAnalyzer):* Discontinue topic.
(10) *Fat in Milk:* Continue study.
*(11) *Infrared Milk Analyzer (IRMA):* Revise title of topic to *Mid-Infrared Instrumentation*. Incorporate the use of A, B, and A + B filters for the measurement of fat in milk (**16.083-16.092**); continue study.
(12) *Iodine:* Continue study.
(13) *Lactose in Dairy Products (Chromatographic Determination):* Continue study.
(14) *Lactose in Dairy Products (Enzymatic Method):* Continue study.
(15) *Moisture in Cheese:* Appoint Associate Referee; continue study.
(16) *Nitrates in Cheese:* Continue study.
(17) *Nonfat Milk Solids:* Continue 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 by Microwave Drying:* Appoint Associate Referee; continue study.
(25) *Tyramine:* Continue study.
(26) *Whey Proteins in Nonfat Dry Milk:* Review collaborative study results; continue study.
(27) *Other Topics:* Review collaborative study results of the IDF methods for lactic acid and lactates in dried milk and sodium, potassium, and calcium contents of dried milk. Initiate the new topics *Mojonnier Method (Robotic), Fat in Dairy Products (Udy Turbidity Test),* and *Moisture in Cheese (Microwave).* Appoint Associate Referees.

SEAFOOD PRODUCTS

The General Referee areas of Decomposition and Filth in Foods (Chemical Methods) and Fish and Other Marine Products have been combined; appoint General Referee.

(1) *Ammonia in Seafood:* Conduct collaborative study; continue study.
(2) *Coprostanol:* Continue study.
(3) *Crabmeat:* Continue study.
(4) *Determination of Fish Content in Coated Products (Breaded or in Butter):* Continue study.
(5) *Drained Weight of Block Frozen, Raw Peeled Shrimp:* Conduct collaborative study; continue study.
(6) *Ethanol in Seafoods:* Continue study.
(7) *Gas and Liquid Chromatography:* Conduct collaborative study on cadaverine and putrescine; continue study.

The recommendations submitted by the Committee on Foods I were adopted by the Association.

Section numbers refer to *Official Methods of Analysis* (1984) 14th edition, and to "Changes in Official Methods," *J. Assoc. Off. Anal. Chem.* (1985) **68**, 369–411 (A methods).

* An asterisk before a recommendation indicates that official action on that recommendation resulted in its appearance in "Changes in Official Methods."

(8) *GC Determination of Volatile Amines:* Continue study.
*(9) *Minced Fish in Fish Fillet Blocks:* Adopt as official first action the interim first action method for the determination of minced fish in mixed fillet–minced cod blocks; continue study.
(10) *Nitrites in Smoked Fish:* Appoint Associate Referee; continue study.
(11) *Organometallics in Fish:* Topic transferred to General Referee for Metals and Other Elements.
(12) *Shellfish Decomposition:* Appoint Associate Referee; continue study.
(13) *TLC Determination of Amines in Fishery Products:* Continue study.

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) *Dilauryl Thiodipropionate:* Appoint Associate Referee; continue study.
(6) *Dressings:* Continue study.
(7) *EDTA in Food Products:* Continue study.
(8) *Gums:* Appoint Associate Referee; continue study.
(9) *Indirect Additives from Food Packages:* Continue study.
(10) *Monier-Williams Modification:* Continue study.
(11) *Nitrates and Nitrites:* Continue study.
(12) *Nitrates (Selective Ion Electrode Titration):* Appoint Associate Referees; 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:* Appoint Associate Referee; continue study.
(20) *Sodium Lauryl Sulfate:* Appoint Associate Referee; continue study.
(21) *Sulfiting Agents in Foods:* Appoint Associate Referee; continue study.

MEAT, POULTRY, AND MEAT AND POULTRY PRODUCTS

(1) *Automated Methods:* Appoint Associate Referee; Continue study.
(2) *Bioassay Methods for Meat and Poultry Products:* Revise topic title to *Immunological Methods for Meat and Poultry Products.* Appoint Associate Referee; continue study.
(3) *Chemical Antibiotic Methods:* Continue study.
(4) *Fat in Meat Products:* Continue study.
(5) *Histologic Identification Methods:* Discontinue topic.
(6) *Identification of Meats, Serological Tests:* Continue study.
(7) *Immunochemical Identification of Additives:* Continue study.
(8) *LC Methods for Meat and Poultry Products:* Continue study.
(9) *3-Methyl Histidine:* Continue study.
(10) *Microwave Digestion of Meat Samples for Total Protein Analysis:* Continue study.
(11) *Nitrates and Nitrites:* Appoint Associate Referee: continue study.
(12) *Nitrosamines in Bacon:* Continue study.
(13) *NMR Systems for Meat Analyses:* Discontinue topic.
*(14) *Nonmeat Protein in Meat:* Adopt as official first action the interim first action enzyme immunoassay method for the determination of soy protein in meat products; continue study.
(15) *Protein (Crude):* Continue study.
(16) *Proteins in Meat and Meat Products:* Continue study.
(17) *Species Identification Methods:* Continue study.
(18) *Specific Ion Electrode Applications:* Continue study.
(19) *Temperature, Minimum Processing:* Conduct collaborative study; continue study.
(20) *Total Fat:* Continue study.
(21) *Total Solids and Moisture by Microwave Drying:* Continue study.
(22) *Other Topic:* Initiate a new topic *Sample Preparation Techniques for Meat Analysis*; appoint an Associate Referee.

MYCOTOXINS

*(1) *Aflatoxin M:* Adopt as official final action the official first action method for preparation of standards for TLC, **26.008-26.011**, and the TLC methods for aflatoxin M_1 in dairy products, **26.090-26.094**, and for determination and confirmation of alfatoxins B_1 and M_1 in liver, **26.101-26.109**. Declare as surplus the alternative confirmatory method for aflatoxin M_1 in liver, **26.110**. Continue study.
*(2) *Aflatoxin Methods:* Adopt as official final action the official first action methods for aflatoxins in food (Romer minicolumn method) (**26.014-26.019**); aflatoxins in corn and peanuts (Holaday-Velasco minicolumn method) (**26.020-26.025**); aflatoxins in peanuts and peanut products (CB method) (**26.026-26.031**); aflatoxins in peanuts and peanut products (BF method) (**26.032-26.036**); aflatoxins in coconut, copra, and copra meal (TLC method) (**26.044-26.048**); aflatoxins in corn (TLC method) (**26.049-26.051**); aflatoxins in cottonseed products (including sample preparation incorporated under **26.003**) (TLC and LC methods) (**26.052-26.060**); aflatoxins in green coffee (TLC method) (**26.061-26.066**); aflatoxins in pistachio nuts (TLC methods) (**26.067** and **26.068**); aflatoxins in soybeans (TLC method) (**26.069**); aflatoxins in eggs (TLC method) (**26.070-26.075**); identification of aflatoxin by derivative formation on TLC plate (**26.083**); and confirmation of identity of aflatoxin B_1 by negative chemical ionization MS (**26.A01-26.A08**). Declare as surplus the TLC methods for aflatoxin in green coffee, **26.061-26.066**, and identification of aflatoxin B_1, **26.076-26.082**. Amend **26.007** to advise that stock solutions of aflatoxin standards should not be stored in methanol; modify **26.031** to allow use of mixed "resolution reference standard," **26.012**, for quantification of aflatoxins B_1, B_2, G_1, and G_2.
(3) *Alternaria Toxins:* Continue study.
(4) *Citrinin:* Continue study.
(5) *Cyclopiazonic Acids:* Continue study.
(6) *Emodin and Related Anthoquinones:* Appoint Associate Referee; continue study.
(7) *Ergot Alkaloids:* Continue study.
*(8) *Ochratoxins:* Adopt as official final action the TLC methods for ochratoxins in barley, **26.111-26.118**, and ochratoxin A in green coffee, **26.119-26.125**.

(9) *Penicillic Acid:* Continue study.
(10) *Penicillium islandicum Toxins:* Appoint Associate Referee; continue study.
(11) *Secalonic Acid:* Appoint Associate Referee; continue study.
*(12) *Sterigmatocystin:* Adopt as official final action the TLC method for sterigmatocystin in barley and wheat, **26.132-26.138**; continue study.
*(13) *Tree Nuts:* Adopt as official final action the TLC methods for aflatoxins in pistachio nuts, **26.067** and **26.068**; continue study.
(14) *Trichothecenes:* Continue study.
(15) *Xanthomegnin and Related Naphthoquinones:* Continue study.
*(16) *Zearalenone:* Adopt as official final action the TLC method for zearalenone in corn, **26.139-26.147**, and the LC method for α-zearalenol and zearalenone in corn, **26.A09-26.A16**.
(17) *Other Topic:* Initiate the new topic *Immunochemical Methods for Mycotoxins*; appoint an Associate Referee.

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; continue study.
(5) *Marine Oils:* Continue study.
(6) *Olive Oil Adulteration:* Continue study.
(7) *Oxidized Fats:* Continue study.
(8) *Pork Fats in Other Fats:* Continue study.
(9) *Sterols and Tocopherols:* Continue study.

PLANT TOXINS

(1) *Glucosinolates:* Coordinate with EEC on the collaborative study on glucosinolates; continue study.
(2) *Phytoestrogens:* Appoint Associate Referee; continue study.
(3) *Pyrrolizidine Alkaloids:* Appoint Associate Referee; continue study.
(4) *Steroidal Alkaloids:* Complete collaborative study for potato glycoalkaloids; continue study.
(5) *Other Topics:* Initiate new topics *Hydrazines* and *Hypoglycine in Ackee*; appoint Associate Referees.

SEAFOOD TOXINS

(1) *Ciguatoxins, Biochemical Methods:* Complete preliminary collaborative study for ciguatera toxins; 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 Method):* Continue study.
(6) *Paralytic Shellfish Poisons (LC Methods):* Initiate collaborative study for LC method for PSP toxins. Continue study.
(7) *Tetrodotoxins:* Continue study.
(8) *Other Topics:* Initiate new topic *Paralytic Shellfish Poisons (Immunoassays)*; appoint an Associate Referee.

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. Associate Referee appointed.
(2) *Alcohol Content of High Solids Distilled Spirits:* Continue study.
*(3) *Carbon Dioxide in Wine:* Adopt as official first action the interim official first action method for titrimetric determination of carbon dioxide in wine as described by the Associate Referee; continue study.
(4) *Citric Acid in Wine:* Continue study.
(5) *Color Intensity for Distilled Alcoholic Products:* Continue study.
(6) *Ethanol in Wine by GC:* Continue study.
(7) *Flavor Compounds in Malt Beverages:* Continue study.
(8) *Glycerol in Wine:* Continue study.
(9) *Hydrogen Cyanide:* Continue study.
(10) *Malic Acid in Wine:* Continue study.

The recommendations submitted by the Committee on Foods II were adopted by the Association.

Section numbers refer to *Official Methods of Analysis* (1984) 14th edition, and 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); *J. Assoc. Off. Anal. Chem.* (1987) **70**, 385–403 (C methods).

* An asterisk before a recommendation indicates that official action on that recommendation resulted in its appearance in "Changes in Official Methods."

*(11) *Malt Beverage and Brewing Materials:* Adopt as official first action the American Society of Brewing Chemists' (ASBC) method for specific gravity of beer and wort using a digital density meter as described by the Associate Referee.
(12) *Sorbic Acid in Wine:* Continue study.
(13) *Sugars in Wine by Enzymatic Methods:* Continue study.
(14) *Sugars in Wine by LC:* Collaborative study planned.
(15) *Sulfur Dioxide in Wine (Ripper Method):* Collaborative study in progress.
(16) *Synthetic Colors:* Continue study.
(17) *Tartrates in Wine:* Continue study.
(18) *Thujone in Alcoholic Beverages:* Continue study.
(19) *Vanillin and Ethyl Vanillin:* Continue study.
*(20) *Other Topics:* Initiate a new topic, *Ethyl Carbamate in Wine.* Adopt as official final action the following official first action methods: **9.135**, acids in cordials and liqueurs (total acidity); **10.028-10.033**, ethanol in beer (GC method); **10.098-10.106**, *N*-nitrosodimethylamine in beer (GC method I); **10.120-10.125**, extract of barley for malting; **10.182**, aphids in hops (flotation method); **10.222-10.224**, solids (total) in liquid and pressed yeast (16 h drying method); **11.008-11.011**, Kirk modification to alcohol in wines (by dichromate oxidation); **11.012-11.014**, alcohol in wines (GC method); **11.022**, caloric content of wines; **11.046**, acidity (volatile) of wines exclusive of SO_2 (by barium hydroxide treatment); **11.052**, citric and malic acids in wines; **11.A05**, carbohydrate content in wine. Continue official first action status of the following methods: **9.013**, artificial colors; **9.113-9.115**, cyanide; **9.139**, total malic acid; **9.145**, thujone; **10.215-10.218**, sampling of yeast; **11.072**, cyanide; **11.062-11.066** and **11.067-11.069**, respectively, manometric and volumetric methods for carbon dioxide in wines; **10.C01-10.C05** and **10.C06-10.C10**, respectively, potassium and sodium in beer.

CEREAL AND CEREAL PRODUCTS

*(1) *Fat Acidity in Flour:* Adopt non-substantive change of toluene for benzene in the titration medium in method **14.069-14.071** as proposed by Associate Referee, based on mini-collaborative study.
(2) *Iron:* Collaborative study in progress.
*(3) *Phytates:* Adopt as official final action the official first action method for phytate in foods, **14.B01-14.B05**. Continue study.
(4) *Other Topics:* Continue official first action status of the following methods: **14.048-14.052**, α-amylase; **14.108-14.110**, lactose; **14.112-14.115**, mineral oil; and **14.144-14.147**, sterols.

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) *Triglyceride Composition in Cocoa Butter and Fat from Chocolate:* Continue study.
(6) *Total and Solid Fat Content in Chocolate Products by NMR:* Continue study.
(7) *Other Topics:* Continue official first action status of the following methods: **13.002**, moisture; **13.040**, unsaponifiable matter in cacao butter; **13.045**, lecithin; **13.055**, glucose.

COLOR ADDITIVES

(1) *Arsenic, Barium, and Heavy Metals:* New Associate Referee appointed.
*(2) *Color in Candy and Beverages:* Adopt as official first action the interim official first action method for rapid determination of color additives using the C_{18} cartridge as described by the Associate Referee.
(3) *Color in Cosmetics:* Two papers have been published (*J. Chromatogr.* [1987] **397**, 345–352 and *J. Liq. Chromatogr.* [1984] **7**, 809–821).
(4) *Color in Nonfrozen Dairy Desserts:* Continue study.
(5) *Color in Other Foods and Drugs:* Continue study.
(6) *Inorganic Salts:* Continue study.
(7) *Intermediates, Uncombined, in Certifiable Water-Soluble Azo Colors:* New Associate Referee to be appointed.
(8) *Intermediates in Other Certifiable Colors:* Continue study.
(9) *Liquid Chromatography:* New Associate Referee to be appointed.
(10) *Subsidiary Colors in Certifiable Color Additives:* New Associate Referee to be appointed.
(11) *X-Ray Fluorescence Spectroscopy:* Continue study.
(12) *Other Topics:* Initiate new topic, *Determination of Color Additives Exempt from Certification.*

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:* Continue study.

FRUIT AND FRUIT PRODUCTS

(1) *Adulteration of Apple Juice:* Continue study.
(2) *Adulteration of Orange Juice by Pulpwash and Dilution:* Petrus method is being evaluated for use on citrus products from sources other than Florida. Mini-collaborative study is planned for the coming year. Continue study.
(3) *Fruit Acids:* Continue study.
(4) *Fruit Juices, Identification and Characterization:* Continue study.
(5) *Limonin in Grapefruit Juice:* Continue study. An immunoassay is being evaluated.
(6) *Moisture in Dried Fruits:* Continue study. Collaborative study is planned to expand the use of the dried fruit moisture tester to dried fruit products other than raisins and prunes.
(7) *Orange Juice Content:* Continue study. New Associate Referee to be appointed.
(8) *Sodium Benzoate in Orange Juice:* Continue study. Collaborative study of the method is planned for 1988.
*(9) *Other Topics:* Adopt the addition of the following material as an introductory paragraph to the method for acidity (titratable) of fruit products, **22.058-22.059**, as recommended by the General Referee: "It is possible to express the titratable acidity conventionally in g acid per 100 g or per 100 mL of product, as

appropriate, by using the factor appropriate to the acid, as follows: malic acid, 0.067; oxalic acid, 0.045; citric acid monohydrate, 0.070; tartaric acid, 0.075; sulfuric acid, 0.049; acetic acid, 0.060; lactic acid, 0.090."

NONALCOHOLIC BEVERAGES

(1) *Caffeine and Methyl Xanthines:* Continue study. Associate Referee is comparing LC and capillary GC methods.
(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:* Statistical review of collaborative study is in progress. Continue study.
(6) *Safrole in Sassafras:* Appoint Associate Referee; continue study.

PRESERVATIVES AND ARTIFICIAL SWEETENERS

(1) *Aspartame, Benzoates, Saccharin and Caffeine by LC:* Planned collaborative study of aspartame in beverage mixes was not conducted. New Associate Referee to be appointed.
(2) *Organic Preservatives:* Collaborative study of benzoic acid, sorbic acid, and parabens in foods is planned.
(3) *Sulfites (Flow Injection Methods):* Results of collaborative study are being evaluated.
(4) *Sulfites (Polarographic Methods):* Mini-collaborative study is in progress using sulfite-stabilized samples.
(5) *Sulfites in Shrimp (Screening Methods):* Collaborative study designed as pass/fail test at 100 ppm total sulfite in shrimp was not successful. Another study is being considered.
(6) *Sulfites (Ion Chromatographic Methods):* Collaborative study protocol is being evaluated.
(7) *Other Topics:* Continue official first action status of the following methods: **20.043-20.048**, preservatives in ground beef; **20.029-20.033**, benzoic acid by TLC; **20.034-20.042**, benzoic and sorbic acids by GC; **20.062**, boric acid by atomic absorption spectrophotometry; **20.073-20.074**, soluble fluorides by fluorescence quenching of aluminum 8-hydroxyquinolate; **20.079-20.081**, formaldehyde; **20.090-20.092**, nitrites; **20.094**, qualitative test for quaternary ammonium compounds (QAC); **20.107-20.109**, eosin yellowish method for QAC; **20.115-20.118**, sorbic acid oxidation method; **20.138-20.139**, thiourea in frozen peaches; **20.174-20.178**, identification of nonnutritive sweeteners; **20.179**, qualitative method for cyclamates; **20.181-20.184**, cyclamates in canned fruit; **20.185-20.189**, cyclohexylamine in cyclamates; **20.190**, **20.191**, and **20.193**, dulcin; **20.194**, P-4000; **20.210**, saccharin by sublimation.

PROCESSED VEGETABLE PRODUCTS

(1) *pH Determination:* Continue study.
(2) *Sodium Chloride:* New Associate Referee to be appointed. Continue study.
(3) *Sugars in Processed Vegetables by LC:* Associate Referee to be appointed.
(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. Collaborative study is being evaluated.
(3) *Ethylene Oxide and Ethylene Chlorohydrin Residues:* Continue study. Collaborative study is planned.
(4) *Extractable Color in Capsicum Spices and Oleoresins:* Continue study.
*(5) *Moisture in Dried Spices:* Adopt as official final action the official first action method **30.B01-30.B03**, moisture in spices (distillation method).
(6) *Monosodium Glutamate:* Appoint Associate Referee. Continue study.
(7) *Piperine in Black Pepper:* Continue study.
(8) *Pungency of Capsicums and Oleoresins:* Collaborative study is planned on a method to determine all pungency levels.
(9) *Vinegar:* Continue study.

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:* Adopt non-substantive change in sec. **31.161** by inserting the following sentence before the last sentence: "Corresponding $\delta^{13}C$ values for predominantly citrus honey are −21.9‰ and −20.0‰." in last sentence of **31.161**, insert "(−20.0‰ for citrus honey)" after "PDB." Adopt non-substantive change in sec. **31.157** by modifying as follows: Under (**a**) *Preparation of sample,* line 6, delete "tared"; lines 7 and 8, delete everything following "bath in current of air or N" to end of paragraph and substitute "or by other appropriate means."; and line 10, insert "–0.2" after "0.1," insert period after "H_2O," and delete "for each 10 mg material." Under (c) *Interpretation,* lines 2 and 3, delete "When wt of isolated carbohydrate fraction is <15 mg (1.5%)" and capitalize the following word "Any"; line 4, replace "conclusive" by "presumptive"; and line 7, delete "If wt is >15 mg" and substitute "In this event."
*(7) *Lactose:* Adopt as official final action the official first action method **31.064-31.071**, liquid chromatographic determination of lactose purity.
(8) *Liquid Chromatographic Methods:* Continue study. Collaborative study is planned based on results of 1986 study using more specific sample pretreatment and LC conditions.
*(9) *Maple Saps and Syrups:* Change the topic title from "Maple Sap and Syrups" to "Maple Sap, Maple Syrup, and Maple Syrup Products." Adopt as official final action the official first action method, **31.185-31.190**, mass spectrometric determination of cane sugar and corn syrup in maple syrup by $^{13}C/^{12}C$ ratio. Repeal, official first action, methods **31.191**, Canadian lead number of maple products, and **31.192-31.194**, Winton lead number of maple products. Collaborative study planned on methodology of sodium and chloride in maple sap and syrup.
(10) *Oligosaccharides in Sugars and Sugar Products:* Col-

laborative study on raffinose in molasses by LC is planned.

(11) *Polarographic Methods for Measurement of Sugars:* Continue study.

(12) *Stable Carbon Isotope Ratio Analysis:* Collaborative study, $^{18}O/^{16}O$ measurements to detect beet invert syrup in orange juice, is being evaluated.

*(13) *Standardization of Methods of Sugar Analysis:* Adopt as official first action the interim official first action method for dextran in raw cane sugar by Roberts' copper dextran method.

(14) *Sugars in Cereals:* Continue study. Amino-bonded, cartridge-type LC columns have been evaluated.

(15) *Sugars in Licorice Products:* Discontinue topic.

(16) *Sugars in Syrups:* Continue study.

(17) *Sulfites in Sugars and Syrups:* Continue study.

(18) *Weighing, Taring and Sampling:* Continue study. New Associate Referee to be appointed.

VITAMINS AND OTHER NUTRIENTS

*(1) *Amino Acids:* Adopt as official first action the interim official first action method for determination of sulfur amino acids and tryptophan for the analysis of protein-bound cysteine and methionine in processed foods and for determination of tryptophan in foods and food and feed ingredients.

(2) *Automated Nutrient Analysis:* Continue study.

(3) *Biotin:* Continue study.

(4) *Carotenoids:* Continue study.

(5) *Dietary Fiber:* Continue study. Collaborative study under review.

(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:* Adopt as official final action the official first action method **43.A21-43.A40** for nutrient analysis in ready-to-feed milk-based infant formula for vitamin B_6, calcium, magnesium, potassium, sodium, vitamin C, riboflavin, niacin, manganese, copper, iron, and zinc. Adopt as official final action the official first action method **43.B01-43.B39** for nutrient analysis in ready-to-feed milk-based infant formula for chloride, phosphorus, thiamine, vitamin B_{12}, ash, fat, protein, carbohydrate, and total solids.

(10) *Pantothenic 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:* Associate Referee has been appointed.

(16) *Vitamin D:* Continue study.

*(17) *Vitamin E:* Adopt as official first action the interim official first action Food Chemicals Codex gas chromatographic method for mixed tocopherols concentrate.

(18) *Vitamin E in Pharmaceuticals (Gas Chromatography):* Continue study.

(19) *Vitamin K:* Continue study.

Committee on Residues: Recommendations for Official Methods

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The minimum requirement to add new chemicals to official multiresidue methods is to be a 3-laboratory collaborative study (laboratories must be from different organizations but may include the Associate Referee's laboratory) and an adequate "bank" of historical supporting data (data from FDA, EPA, states, industry, etc.). If adequate supporting data are not available, a full collaborative study is to be required.

Associate Referees and General Referees are to examine and evaluate official multiresidue methods as to applicability to an individual chemical before developing, studying, evaluating, or collaborating methods for an individual chemical.

METALS AND OTHER ELEMENTS

(1) *Arsenic in Animal Tissues:* Discontinue topic.

(2) *Atomic Absorption Spectrophotometry (AAS):* Continue study to develop 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 a unified AAS scheme for multielement analysis of foods and other biological substrates.

*(3) *Cadmium and Lead in Earthenware:* (a) Revise method **25.024-25.027** by changing **25.025(c)** *(2) Working solns* from "Dil. 0.0, 5.0, 10.0, 15.0, and 20.0 mL stock soln to 1 L with 4% HOAc (0, 5, 10, 15, and 20 μg/mL)" to "Dil. 0.0, 1.0, 3.0, 5.0, 10.0, and 15.0 mL stock soln to 1 L with 4% HOAc (0, 1, 3, 5, 10, and 15 μg/mL)." (b) Continue study on the use of inductively coupled plasma (ICP) emission spectrometry for lead, cadmium, and other elements leached from earthenware with the AOAC method for leaching earthenware, **25.024-25.027**. Determine if a collaboratively studied method is required for elements other than cadmium and lead.

(4) *Emission Spectrochemical Methods:* Prepare protocol for collaborative study of method combining wet acid digestion of plant or animal matrixes with inductively coupled plasma (ICP) emission spectroscopic 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 a pre-study test with trial sample solutions.

(5) *Fluorine:* Continue study to improve the 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).

(6) *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 the 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 an improved version of the coprecipitation GF-AAS method for arsenic in foods (*Can. J. Spectrosc.* [1985] **30,** 154–157).

(7) *Hydride Generating Techniques:* Continue study of the continuous flow hydride generation procedure of Panaro and Kroll (*Anal. Lett.* [1984] **17,** 157–172) for determining arsenic and selenium in foods.

(8) *Lead in Calcium Supplements:* Continue study to solve problems of occasional precipitate formation and organic electrochemical interferences in method to determine lead in calcium supplements by differential pulse anodic stripping voltammetry after perchloric acid digestion of the sample.

(9) *Mercury:* Discontinue topic.

*(10) *Methyl Mercury in Fish and Shellfish:* (a) Adopt as official first action the interim first action method for rapid determination of methyl mercury in fish and shellfish (*J. Assoc. Off. Anal. Chem.* [1987] **70,** 667–672). (b) Adopt as official final action the official first action method for methyl mercury in fish, **25.146-25.152.**

*(11) *Multielement Determination After Closed System Digestion:* (a) Adopt as official final action the official first action multielement method for arsenic, cad-

The recommendations submitted by the Committee on Residues were adopted by the Association.

Section numbers refer to *Official Methods of Analysis* (1984) 14th edition, and 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).

* An asterisk before a recommendation indicates that official action on that recommendation resulted in its appearance in "Changes in Official Methods."

mium, lead, selenium, and zinc in food, **25.001-25.007**. (b) Discontinue topic.

(12) *Neutron Activation Analysis:* Complete review of uncompleted collaborative study on determining sodium in foods to decide whether to continue study or initiate a new study; continue coordinating American Society for Testing and Materials method development with AOAC method protocols to enable endorsement of the methods by both organizations.

(13) *Organometallics in Fish:* Continue collaborative study on method for determination of methyl mercury in seafood by liquid chromatographic and atomic absorption spectrophotometric detection.

(14) *Organotin Compounds in Foods:* (a) Change name of topic to Organotin Compounds. (b) Continue development of a multiresidue method for organotin compounds in foods. (c) Expand topic to include residues of the organotin fungicides cyhexatin, triphenyltin hydroxide, and fenbutatin oxide.

*(15) *Polarography:* (a) Adopt as official final action the official first action anodic stripping voltammetric method for cadmium and lead in food, **25.008-25.015**. (b) Study the application of square wave voltammetry in place of differential pulse anodic stripping voltammetry for the determination of lead and cadmium, **25.008-25.015**.

*(16) *Tin:* (a) Adopt as official final action the official first action nitrous oxide–acetylene flame–AAS method for determining tin in canned foods, **25.A01-25.A04**. (b) Discontinue topic.

MULTIRESIDUE METHODS (INTERLABORATORY STUDIES)

(1) *Comprehensive Multiresidue Methodology:* Continue study of multiresidue method for the synthetic pyrethroid class of pesticides in food commodities, using both **29.011** and **29.A03** extraction procedures with Florisil and bonded phase extraction cleanup procedures.

(2) *Extraction of Low Moisture-High Fat Samples:* Continue evaluation of chromatographic cleanup techniques such as Unitrex, semipreparative LC, GPC, and solid phase extraction columns as alternatives to acetonitrile partitioning, **29.014**, for removing fats and oils from extracts of oil seeds and other low moisture-high fat foods for the 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 comprehensive approach published in *J. Assoc. Off. Anal. Chem.* (1987) **70,** 734–739, for review and comment by the General Referee. Recovery and method performance data concerning all chemicals tested must be included with the 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.

(4) *Miniaturization of Multiresidue Methodology:* Conduct second interlaboratory trial of Associate Referee's miniaturized multiresidue method for nonfatty foods, consisting of acetonitrile extraction (as in **29.001**(a)), transfer of residues to petroleum ether (as in FDA Laboratory Information Bulletin 2592), Florisil cleanup (as in miniaturized method for fish, **29.029-29.036**), and electron capture GC (as in **29.018**); require participants to follow instructions as in complete description of analytical procedure written in official method format. If results are satisfactory, prepare protocol for collaborative study to assess method performance for approval by General Referee and Statistical Consultant to Committee on Residues.

(5) *Organophosphorus Pesticide Residues:* Continue studies on the recovery of the organophosphorus chemicals (carbophenothion sulfoxide/sulfone, fenamiphos, fensulfothion oxygen analog, and phorate sulfoxide/sulfone) through applicable sections of **29.001-29.018**, **29.054-29.058**, and **29.A01-29.A04**; study the applicability of converting organophosphorus chemical residues to sulfones prior to determination.

(6) *Sweep Codistillation:* (a) Continue study to further evaluate "beaded" vs "beadless" fractionation tubes for the Unitrex system. (b) Prepare a protocol for a collaborative study of the method for determining organochlorine pesticides in meat fats with the Unitrex cleanup for approval by the General Referee and Statistical Consultant to Committee on Residues; initiate collaborative study.

ORGANOHALOGEN PESTICIDES

(1) *Chlordane:* Perform method comparison studies on the 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, G.G., et al. [1984] *J. Assoc. Off. Anal. Chem.* **67,** 295–298). Chlordane residues (*cis-* and *trans-*chlordane, *cis-* and *trans-*nonachlor, and octachlor epoxide (oxychlordane), and heptachlor epoxide in butter, eggs, fish, and poultry fat can be determined by the Associate Referee's electron capture (EC) capillary column GC system. On the basis of 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:* Appoint an Associate Referee. 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 the General Referee and Committee on Residues. Recovery and method performance data concerning all chemicals tested must be included with the proposed method. If satisfactory, conduct efficiency studies by extracting field-incurred residues from samples. If satisfactory, conduct interlaboratory trial and prepare protocol for collaborative study on commodities most likely 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:* Appoint an Associate Referee 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) *Gel Permeation Chromatography (GPC) Cleanup for Organochlorine Residues:* Transfer topic to General Referee for Organophosphorus Pesticides.

(6) *Methyl Bromide:* Draft, in AOAC official method format, a specific method for methyl bromide based on method of King et al. (*J. Agric. Food Chem.* [1981] **29,** 1003–1005) for review and comment by General Referee and Committee on Residues. Principle of the method, procedure for correcting for recovery, calculation procedures, and recovery and method performance data must be included with the 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 most likely to retain methyl bromide from fumigation and submit protocol for approval by General Referee and Statistical Consultant to Committee on Residues; initiate collaborative study.

(7) *Pentachlorophenol:* Continue as official first action the GC method for pentachlorophenol in gelatin, **29.A14-29.A18** (*J. Assoc. Off. Anal. Chem.* (1985) **68,** 388–389 and 419–421); continue study to improve GC determination of underivatized pentachlorophenol in this method or convert pentachlorophenol to derivative amenable to GC quantitation; continue study to extend official status of this method to determination of pentachlorophenol in milk, fish, and eggs.

(8) *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 recommendation by Committee on Residues for this topic in 1985 (*J. Assoc. Off. Anal. Chem.* [1986] **69,** 299).

(9) *Polychlorinated Biphenyls (PCBs):* Continue study of methods for separating PCBs from organochlorine pesticide residues that co-extract in 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.**

(10) *Polychlorinated Biphenyls (PCBs) in Blood:* Complete the report on the collaborative study of the method for PCBs in blood serum and submit to the General Referee and the Committee on Residues for interim approval.

(11) *Tetradifon, Endosulfan, and Tetrasul:* Discontinue topic.

ORGANONITROGEN PESTICIDES

(1) *Anilazine:* Appoint an 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:* Continue study to assess accuracy, precision, and ruggedness of Associate Referee's method for simultaneous determination of benomyl and its hydrolysis product methyl 2-benzimidazolecarbamate (MBC, also known as the fungicide carbendazim) in fruits and vegetables, in which hot aqueous alkali is used to convert benomyl and MBC to 1-(2-benzimidazolyl)-3-*n*-butyl urea (BBU) and 2-aminobenzimidazole (2-AB), respectively, and the resulting BBU and 2-AB are determined by LC with UV detection; prepare collaborative study protocol with description of method in official methods format for approval by General Referee and Statistical Consultant to Committee on Residues.

(3) *Captan and Related Fungicides:* Conduct interlaboratory trial of the Associate Referee's modified method for captan, captafol, and folpet, and submit data to General Referee for review and comment. If data are satisfactory, initiate collaborative study.

(4) *Carbamate Herbicides:* Appoint an 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 an interlaboratory trial on the method developed by the Associate Referee for confirmation of phenolic carbamate residues to assess the effect of different electrochemical detector types on the response obtained from various carbamate/co-extractive combinations; submit the 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; initiate collaborative study.

(6) *Carbofuran:* Appoint an 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 an 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 and data from the interlaboratory study to the General Referee for review and comment. If satisfactory, prepare protocol for collaborative study and, upon approval of protocol by General Referee and Statistical Consultant to Committee on Residues, initiate collaborative study. (b) Conduct ruggedness testing on the alkaline hydrolysis–GC/MS method for daminozide to determine sources of variation and to define the best approach to quantitation. Submit the revised procedure with data from interlaboratory study to General Referee

for review and comment. If satisfactory, prepare protocol for collaborative study and, upon approval of protocol by General Referee and Statistical Consultant to Committee on Residues, initiate collaborative study. (c) If practical, the collaborative studies for UDMH and daminozide should be conducted simultaneously.

(9) *Diquat and Paraquat:* Complete collaborative study of the method for diquat and paraquat in potatoes, submit report and recommendation to General Referee and Committee on Residues for possible interim action.

(10) *Dithiocarbamate Fungicides:* Appoint an Associate Referee to develop methods for distinguishing dimethyldithiocarbamates from ethylenebisdithiocarbamates and for determining the parent fungicides and their metabolites in foods.

(11) *Maleic Hydrazide:* Appoint an Associate Referee to develop and collaboratively study a GC or LC method for determining maleic hydrazide in crops.

(12) *Organonitro Pesticides:* Continue study to develop LC-electrochemical detection parameters for determination of nitro- and dinitro-substituted pesticides at nanogram levels, with goal of using these parameters in a multiresidue method for organonitro pesticides in foods.

(13) *Organotin Fungicides:* Transfer topics to General Referee for Metals and Other Elements to be incorporated in topic renamed Organotin Compounds.

(14) *Sodium o-Phenylphenate:* Appoint an Associate Referee to develop and collaboratively study a GC or LC method for determining *o*-phenylphenol in foods.

(15) *Substituted Ureas:* Submit performance data from interlaboratory trial 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; initiate collaborative study.

(16) *Thiolcarbamate Herbicides:* Appoint an Associate Referee to evaluate and collaboratively study methods for determining residues of thiolcarbamate herbicides in crops.

(17) *s-Triazines:* Appoint an Associate Referee to evaluate methods for determining residues of atrazine, simazine, and cyanazine in food crops and to collaboratively study method selected.

(18) *Trifluralin:* Appoint an Associate Referee to evaluate existing pesticide multiresidue methods, such as **29.001-29.018**, for use in determining trifluralin residues in crops, and to conduct collaborative study of selected method.

ORGANOPHOSPHORUS PESTICIDES

(1) *Azinphos-methyl:* Discontinue topic.

(2) *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 results are satisfactory, submit protocol for collaborative study of method for approval by General Referee and Statistical Consultant to Committee on Residues, initiate collaborative study.

(3) *Extraction Procedures:* Appoint an Associate Referee to study the efficiency of procedures for extracting field-incurred residues of organophosphorus pesticides from crops and to develop improved extraction procedures for incorporation into multiresidue methods.

(4) *Fenvalerate:* Discontinue topic.

(5) *High Fat Samples:* Change name of topic to Gel Permeation Chromatography (GPC) Cleanup. Continue study and develop plan for collaborative study to extend official status of the 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.

(6) *Methamidophos:* Discontinue topic.

(7) *Monocrotophos:* Discontinue topic.

(8) *Permethrin:* Discontinue topic.

(9) *Phorate:* Appoint an Associate Referee to evaluate and collaboratively test analytical methods for determining phorate and its metabolites in foods.

(10) *Phosphine:* Continue study to evaluate methods for determining residual phosphine in grains including the solvent soaking procedure for the extraction of fumigants in grains, **29.072**.

(11) *Resmethrin:* Discontinue topic.

(12) *Terbufos:* Initiate topic to evaluate and collaboratively study analytical methods for determining terbufos and its metabolites in foods; appoint an Associate Referee.

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 determination of Cs-137 in other foods. (b) Appoint an Associate Referee to evaluate and collaboratively study radiochemical methods for determining Cs-137 in foods and other biological matrixes 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 determination of I-131 in other foods. (b) Prepare protocol for collaborative study of more sensitive 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) *Neutron Activation Analysis:* Transfer topic to General Referee for Metals and Other Elements.

(4) *Plutonium:* Continue study of the 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.

(5) *Radium-228:* Complete evaluation of the collaborative study; submit report and recommendation to General Referee and Committee on Residues for interim action.

(6) *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.

(7) *Tritium:* Appoint an Associate Referee to evaluate and collaboratively study methods for determining tritium in foods and biological materials.

Committee on Microbiology: Recommendations for Official Methods

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The Committee on Microbiology met on September 13, 1987, in San Francisco, CA, and recommended the following items for action to the Official Methods Board:

(*1*) Change the name of the Committee on Microbiology to the Committee on Microbiological and Microanalytical Methods.

(*2*) Redefine the purposes of the Committee as: (a) To guide and supervise the development and validation of analytical methods for the detection and identification of microbiological and microanalytical analytes from a variety of matrices and to recommend to the Association appropriate action on completed collaborative studies. (b) To provide expert consultation and liaison with other committees that are involved with the application of microorganisms in bioassay systems to detect other analytes, in biological pesticides, and in evaluation of the effectiveness of sterilization and disinfection procedures.

(*3*) In view of these redefined purposes, reassign the following topics that are currently under this Committee: (a) All antibiotic methods currently under the general refereeship Dairy Microbiology. (b) The entire general refereeship topic Drug and Device Related Microbiology.

(*4*) Realign the General Referee topics. The proposed topics are: Analytical Mycology and Microscopy (Stanley Cichowicz); Extraneous Materials in Foods and Drugs (Jack Boese); Dairy Microbiology (James Messer); Environmental Sanitation Microbiology (vacant); Food Microbiology (Nondairy) (Wallace Andrews); Pharmaceuticals and Cosmetics Microbiology (vacant); Water Microbiology (vacant).

In a general discussion, the Committee took the following actions:

(*1*) Gave to all General Referees the charge to redefine Associate Referee topics on the basis of analyte, matrix, and method as recommended by the Committee and approved by the Official Methods Board in 1986. This will be completed by the 1988 Annual Meeting.

(*2*) Asked all General Referees to submit a list of current official first action methods that should be recommended for official final action adoption. The list should be submitted directly to the AOAC office for inclusion in *The Referee*, June 1988 issue.

(*3*) Endorsed the use of the new AOAC guidelines for methods reviewers.

ANALYTICAL MYCOLOGY OF FOODS AND DRUGS

(1) *Baseline Mold Counts by Blending:* Discontinue topic.
(2) *Chemical Methods for Detecting Mold:* Continue topic.
(3) *Direct Count of Molds, Yeasts, and Spores by Fluorescence Microscopy:* Discontinue topic.
(4) *Geotrichum candidum Morphology:* Discontinue topic.
(5) *Geotrichum Mold in Canned Fruits, Vegetables, and Fruit Juices:* Continue study.
(6) *Geotrichum Mold in Frozen Fruits and Vegetables:* Discontinue topic and continue study under topic 5 as canned and frozen products.
(7) *Howard Mold Counting, Use of Widefield Eyepiece:* Discontinue topic.
(8) *Howard to Viable Mold Counts of Frozen Fruits and Vegetables:* Discontinue topic.
(9) *Microscopic Mold Count Methods, Use of Compound Microscope:* Discontinue topic.
(10) *Microscopic Mold Counts, Effects of Interfering Plant Material:* Discontinue topic.
(11) *Mold in Spices:* Discontinue topic.
(12) *Molds and Yeasts in Beverages:* Discontinue topic.
(13) *Standardization of Plant Tissue Concentrations for Mold Counting:* Continue study.
*(14) *Other Topics:* (a) Change the title of the general refereeship to Analytical Mycology and Microscopy. (b)

The recommendations submitted by the Committee on Microbiology were adopted by the Association.
Section numbers refer to *Official Methods of Analysis* (1984) 14th edition, and to "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).
* An asterisk before a recommendation indicates that official action on that recommendation resulted in its appearance in "Changes in Official Methods."

Adopt as official final action the following official first action methods: mold in fruit nectars, purees, and pastes by Howard mold count, **44.201-44.203**; mold in tomato powder (dehydrated) by Howard mold count, **44.211**; mold in soft drinks by *Geotrichum* mold count, **44.217**; mold in citrus juices by *Geotrichum* mold count, **44.218**; mold in vegetables, fruits, and juices (canned) by *Geotrichum* mold count, **44.219**; mold in comminuted fruits and vegetables by *Geotrichum* mold count, **44.220-44.222**; mold in cream style corn by *Geotrichum* mold count, **44.223**.

EXTRANEOUS MATERIALS IN FOODS AND DRUGS

(1) *Baked Goods with Fruit and Nut Tissues, Light Filth in:* Continue study.
(2) *Botanical Drugs, Adulteration by Foreign Plant Materials:* Continue study.
*(3) *Botanicals, Filth in:* Adopt as official first action the interim official first action method for extraction of light filth from whole leaves of alfalfa, papaya, and spearmint. Continue study.
(4) *Canned and Dried Soups, Filth in:* Continue study.
(5) *Cheese, Filth and Mite Contamination:* Continue study.
(6) *Chocolate Products, Filth in:* Continue study.
(7) *Cocoa Powder and Press Cake, Filth in:* Continue study.
(8) *Crabmeat, Shrimp, and Tuna, Canned, Brine Extraction Techniques:* Continue study.
(9) *Fish and Fish Products, Canned, Light Filth in:* Continue study.
(10) *Grains, Whole, Cracking Flotation Methods:* Continue study.
(11) *Insect Excreta in Flour:* Discontinue topic.
(12) *Meats, Processed, Filth in:* Continue study.
(13) *Mite Contamination Profiles and Characterization of Damage to Foods:* Continue study.
(14) *Mites in Stored Foods:* Continue study.
(15) *Mushroom Products, Dried, Filth in:* Continue study.
(16) *Mushrooms, Canned, Filth in:* Continue study.
(17) *Performance Evaluation of Methods for Filth:* Continue study.
(18) *Rye Bread:* Continue study.
(19) *Shrimp, Filth in:* Discontinue topic.
*(20) *Soluble Insect and Other Animal Filth:* Adopt as official first action the interim official first action thin layer chromatographic method for determination of coprostanol as indicator of mammalian feces.
(21) *Soybean Curd, Filth in:* Establish topic.
(22) *Spirulina, Filth in:* Establish topic.
(23) *Tomatoes and Mushrooms, Canned, Filth in, Brine Extraction Techniques:* Continue study.
(24) *Urine, Methods for Detection:* Continue study.
(25) *Vegetable Products, Dehydrated, Filth in:* Continue study.
(26) *Vertebrate Excreta, Chemical Identification Tests:* Continue study.
*(27) *Other Topics:* Adopt as official final action the following official first action methods: filth in cocoa, chocolate, and press cake by flotation method, **44.006-44.007**; filth in ground coffee and coffee substitutes by sedimentation and flotation method, **44.008**; aphids in hops by flotation method, **44.009-44.011**; light filth in tea by flotation method, **44.014-44.016**; sediment in dairy products by sediment test method, **44.021**; filth in shelled nuts, **44.028-44.029**; filth in pecans, **44.030-44.031**; filth in coconut (shredded), **44.032**; filth and extraneous material in peanut butter by sedimentation/flotation methods, **44.033-44.038**; insect infestation (internal) of wheat by cracking flotation methods, **44.040-44.042**; light filth (pre- and post-milling) in flour (white) by flotation method, **44.052**; insect excreta in flour, **44.054**; light filth in rice flours (powders), extruded rice products, and rice paper by flotation method, **44.055-44.057**; light filth in flour (soy) by flotation method, **44.060**; light filth in wheat gluten by flotation method, **44.061**; light filth in starch by sieving method, **44.062**; light filth in white breads and high-fat products by flotation method, **44.067**; light filth in breading of frozen food products by flotation method, **44.068**; light filth in alimentary pastes by flotation method, **44.069**; light filth in cereals (corn and rice) and corn chip products by flotation method, **44.070**; light filth in cereals (whole wheat) by flotation method, **44.071**; light filth in barley, oatmeal, and mixed dry infant cereal by flotation method, **44.072-44.073**; light filth in crabmeat (canned) by flotation method, **44.080**; light filth in fish (canned) and fish products by flotation method, **44.082**; light filth in shrimp (canned) by flotation method, **44.083**; light filth in pork sausage (uncooked) and ground beef or hamburger by enzyme digestion method, **44.084-44.085**; filth in apple butter by flotation method, **44.086**; thrips and other insects in frozen blackberries and frozen raspberries by flotation method, **44.089-44.090**; filth in jam and jelly, **44.094**; filth in citrus and pineapple juices (canned), **44.095-44.096**; light filth in raisins by microscopic examination method, **44.097**; filth in potato chips by flotation method, **44.101**; filth in corn chips, **44.102**; filth in candy by flotation method, **44.103**; filth in sirups, molasses, and honey by filtration methods, **44.105**; filth in sugars by filtration method, **44.106**; foreign matter in corn (canned) by flotation and macroscopic methods, **44.109**; filth in green leafy vegetables, **44.110-44.111**; filth in pureed infant food, **44.113-44.114**; light filth in potato products (dehydrated) by flotation method, **44.117**; filth in sauerkraut by sieving method, **44.118**; filth in tomato products, **44.119-44.120**; light filth in spices and condiments by flotation method, **44.125-44.126**; filth in ground annatto, **44.129**; heavy filth in capsicums (ground) by sedimentation method, **44.130**; light filth in capsicums (ground) by flotation method, **44.131-44.132**; light filth in nutmeg (ground) by flotation method, **44.140-44.141**; light filth in nutmeg (reconditioned) by flotation method, **44.142**; filth in paprika, **44.145-44.147**; light filth in pepper, **44.148-44.149**; filth in horseradish (prepared) by flotation method, **44.155**; light filth in mustard (prepared) by flotation method, **44.156**; light filth in gums (plant, crude) by flotation method, **44.158**; light filth in papain (crude and refined) by flotation method, **44.161**; insect penetration through packaging materials by microscopic examination method, **44.162**.

DAIRY MICROBIOLOGY

(1) *Bactoscan Methods:* Continue study.
(2) *β-Lactam Residues in Milk–Delvo-test:* Reassign to another committee.
(3) *β-Lactam Residues in Milk–Qualitative Methods:* Reassign to another committee.
(4) *β-Lactam Residues in Milk–Quantitative Methods:* Reassign to another committee.

(5) *Listeria monocytogenes—Detection in Dairy Products Using Cultural Methods:* Continue study.
(6) *Penicillins in Milk–Affinity Quantitation:* Reassign to another committee.
(7) *Somatic Cells—Automated Optical Counting Method:* Continue study.
(8) *Somatic Cells—Fossomatic Method:* Continue study.
(9) *Other Topics:* Establish the new topics *Listeria monocytogenes–Detection in Dairy Products Using DNA Probe,* and *Detection of Raw Milk in Cheese Using Alkaline Phosphatase Test.*

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) *Endotoxin by Limulus Amebocyte Lysate:* Continue study.
(5) *Medical Devices–Packaging Integrity:* Continue study.
(6) *Medical Devices–Sterility Testing:* Continue study.

FOOD MICROBIOLOGY (NONDAIRY)

(1) *Bacillus cereus, Enterotoxin:* Continue study.
(2) *Bacillus cereus, Isolation and Enumeration:* Continue study.
(3) *Campylobacter Species:* Vacant topic.
(4) *Canned Foods:* Discontinue topic.
(5) *Clostridium botulinum:* Discontinue topic.
(6) *Clostridium perfringens:* Discontinue topic.
*(7) *Escherichia coli, Enzymatic Methods:* Adopt as official first action the interim official action MUG procedure for enumeration of *E. coli* in chilled and frozen food, exclusive of chilled and frozen shellfish.
(8) *Genetic Methods for Detection of Bacterial Pathogens:* Continue study.
(9) *Hydrophobic Grid Membrane Filter Methods:* Continue study.
(10) *Identification of Microorganisms by Biochemical Kits:* Discontinue topic.
*(11) *Petrifilm Methods:* Adopt as official final action the official first action dry rehydratable film method, **46.B05-46.B07**, for determining aerobic plate count and total coliform values in raw and pasteurized milk.
*(12) *Redigel Media:* Adopt as official first action the use of pectin-based medium as an alternative to the agar-based medium in **46.005(g)** for determining standard plate count values of foods.
*(13) *Salmonella:* Adopt as official final action the official first action enzyme immunoassay screening method, **46.B21-46.B29**, for the detection of *Salmonella* in foods. Maintain official first action status of the enzyme immunoassay screening method (revised), **46.C17-46.C25**.
(14) *Shellfish:* Continue study.
(15) *Staphylococcus:* Discontinue topic.
(16) *Staphylococcus Toxin:* Discontinue topic.
(17) *Sugars:* Open topic.
*(18) *Vibrio cholerae and Its Toxins:* Adopt as official first action the interim official action elevated temperature procedure for the isolation of *V. cholerae* from oysters. (This recommendation was inadvertently omitted at the annual business meeting.)
(20) *Virology and Animal Oncology:* Discontinue topic.
(21) *Yeasts, Molds, and Actinomycetes:* Continue study.
(22) *Yersinia enterocolitica:* Continue study.
(23) *Other Topics:* Establish the new topic *Iron Milk Test for Recovering Clostridium perfringens from the Marine Environment.*

Committee on Feeds, Fertilizers, and Related Materials: Recommendations for Official Methods

RODNEY J. NOEL (Purdue University, Department of Biochemistry, West Lafayette, IN 47907), *Chairman*;
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BILLY M. COLVIN (University of Georgia, College of Veterinary Medicine, Box 1386, Tifton GA 31794), *Secretary*;
DANIEL H. MOWREY (Lilly Research Laboratory, Division of Eli Lilly & Co., Greenfield, IN 46140), *Statistical Consultant*

ANTIBIOTICS IN FEEDS

(1) *Bacitracin:* Continue study.
(2) *Bacitracin (Chemical Method):* Continue study.
(3) *Bambermycins:* Continue study.
(4) *Chloramphenicol in Animal Tissues:* Transfer topic to another committee.
(5) *Chloramphenicol Residues in Milk:* Transfer topic to another committee.
(6) *Chlortetracycline:* Continue study.
(7) *Coban:* Continue study.
(8) *Cup Plate System for Antibiotic Analysis:* Continue study.
(9) *Design and Computerization of Microbiological Tests:* Continue study.
(10) *Erythromycins:* Continue study.
(11) *Lasalocid (LC Method):* Transfer topic to another committee.
(12) *Lasalocid (Microbiological Assay):* Continue study.
(13) *Lincomycin:* Continue study.
(14) *Monensin:* Continue study.
(15) *Narasin:* Continue study.
(16) *Neomycin:* Continue study.
(17) *Oxytetracycline:* Continue study.
(18) *Rumensin:* Continue study.
*(19) *Screening Procedures for Antibiotics:* Adopt as official first action the interim official action rapid detection and identification of 7 families of antimicrobial drugs in milk by microbial receptor assay. Continue study.
(20) *Statistics of Microbiological Assay:* Continue study.
(21) *Tetracyclines in Tissues (Chromatographic Assay):* Transfer topic to another committee.
(22) *Tetracyclines in Tissues (Microbiological Assay):* Transfer topic to another committee.
(23) *Tylosin:* Continue study.
(24) *Virginiamycin (Diffusion Assay):* Continue study.
(25) *Virginiamycin (Turbidimetric Assay):* Continue study.

DRUGS IN FEEDS

(1) *Amprolium:* Continue study.
*(2) *Arprinocid:* Declare as surplus the official first action liquid chromatographic (**42.021-42.026**) and spectrophotometric (**42.027-42.032**) methods.
(3) *Arsanilic Acid:* Continue study.
(4) *Carbadox:* Continue study.
*(5) *Diethylstilbestrol:* Declare as surplus the official first action spectrophotometric method, **42.059-42.062**.
*(6) *Dimetridazole:* Adopt as official final action the official first action spectrophotometric method, **42.063-42.068**, after correction of dilution factors by substituting 66.67 for 1.335×10^{-3}, 250 for 5×10^{-3}, and 333.3 for 6.66×10^{-3} in **42.068**.
(7) *Ethopabate:* Continue study.
(8) *Ethylenediamine Dihydroiodide:* Continue study.
*(9) *Furazolidone and Nitrofurazone:* Adopt as official final action the official first action liquid chromatographic method for furazolidone, **42.A01-42.A06**. Adopt as official final action the official first action qualitative tests for furazolidone and zoalene, **42.077-42.080**. Continue study.
*(10) *Melengestrol Acetate:* Adopt as official final action the official first action gas chromatographic method, **42.088-42.096**. Continue study.
(11) *Morantel Tartrate:* Continue study.
(12) *Phenothiazine:* Continue study.
(13) *Pyrantel Tartrate:* Continue study.
(14) *Roxarsone:* Continue study.
(15) *Sulfa Drug Residues:* Continue study.
(16) *Sulfamethazine and Sulfathiazole (Premix and Finished Feed Levels):* Continue study.
*(17) *Sulfaquinoxaline:* Adopt as official final action the official first action spectrophotometric method, **42.179-42.183**.

FEEDS

(1) *Amino Acid Analysis in Mixed Feeds:* Continue study.
(2) *Calcium Salts of Isobutyric and Mixed 5-Carbon Volatile Fatty Acids:* Continue study.
(3) *Carotenoids:* Continue study.
(4) *Enzymes and Microbial Additives:* Continue study.
(5) *Fat:* Continue study.
(6) *Fiber, Crude:* Continue study.
(7) *Infrared Reflectance Techniques in Mixed Feeds:* Continue study.
(8) *Iodine:* Continue study.
(9) *Microscopy:* Continue study.
(10) *Minerals:* Continue study.
(11) *Non-Nutritive Residues:* Continue study.

The recommendations submitted by the Committee on Microbiology were adopted by the Association.
Section numbers refer to *Official Methods of Analysis* (1984) 14th edition, and to "Changes in Official Methods," *J. Assoc. Off. Anal. Chem.* (1985) **68**, 369–411 (A methods).
* An asterisk before a recommendation indicates that official action on that recommendation resulted in its appearance in "Changes in Official Methods."

*(12) *Protein, Crude:* Adopt as official first action the $CuSO_4$/TiO_2 manual Kjeldahl digestion catalyst. Continue study.
(13) *Sampling:* Continue study.
(14) *Vitamins:* Continue study.
(15) *Water by Karl Fischer Method:* Continue study.

FERTILIZERS

(1) *Biuret:* Continue study.
(2) *Boron:* Continue study.
(3) *Dicyanodiamide:* Continue study.
(4) *Free and Total Water:* Continue study.
(5) *Iron:* Continue study.
*(6) *Melamine:* Adopt as official first action the liquid chromatographic method for triamino-*S*-triazine used as a nitrogen source in urea mixes. 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.

PLANTS

(1) *Ashing Methods:* Continue study.
*(2) *Atomic Absorption Methods:* Adopt as official final action the official first action method, **3.013-3.016**. Continue study.
(3) *Boron:* Continue study.
(4) *Chromium:* Continue study.
*(5) *Emission Spectroscopy:* Adopt as official final action the official first action methods, **3.006-3.010** and **3.A01-3.A04**. Continue study.
*(6) *Fluorine:* Adopt as official final action the official first action potentiometric method, **3.075-3.080**. Continue study.
(7) *Nitrate and Nitrite:* Continue study.
(8) *Selenium:* Continue study.
(9) *Starch:* Continue study.
(10) *Sulfur:* Continue study.

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:* Continue study.
(4) *Atomic Absorption Spectrophotometry:* Continue study.
(5) *Chlorinated Phenols in Animal Tissue:* Continue study.
(6) *Cholinesterase:* Continue study.
(7) *Copper in Animal Tissue:* Continue study.
(8) *Fluoride in Animal Tissue:* Continue study.
(9) *Lead in Animal Tissue:* Continue study.
(10) *Lipid-Soluble Vitamins:* Continue study.
(11) *Multielement Analysis by ICP:* Continue study.
(12) *Multiple Anticoagulant Screening:* Continue study.
(13) *Natural Products:* Continue study.
(14) *Neomycin:* Continue study.
(15) *Nitrates and Nitrites:* Continue study.
(16) *Pesticides in Toxicological Samples:* Continue study.
(17) *Selenium in Animal Tissue:* Continue study.
(18) *Sodium Monofluoroacetate:* Continue study.
(19) *Zinc in Animal Tissue:* Continue study.

Committee on Hazardous Substances in Water and the Environment: Recommendations for Official Methods

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The proposed Memorandum of Understanding between AOAC and the U.S. Environmental Protection Agency on the collaborative study of methods to be used in monitoring well water for pesticides was discussed. The Committee recommends that the scope be limited to 6 methods, with possible expansion to cover more of the Committee's activities. A desk review of the methods will be completed and submitted.

Under Inorganics in Ground and Drinking Water, the following items were discussed: total metals/dissolved metals, acid preservation procedures, on-site vs off-site filtration of samples, and EPA method equivalency requirements. Studies in this refereeship will have to address these issues. Metal speciation is also of continuing interest.

HAZARDOUS SUBSTANCES

(1) *Benzene in Consumer Products:* Continue study.
(2) *Nitrosamines in Infant Pacifiers:* Continue study.
(3) *Pentachlorophenol in Toy Paints:* Continue study.
(4) *Toxic Metals in Paints:* Continue study.

INORGANICS IN DRINKING AND GROUND WATER

(1) *Arsenic and Selenium Analysis:* Review a variety of methods for arsenic and selenium, including but not limited to graphite furnace and hydride generation methods. Select, test, and revise methods as necessary and prepare for collaborative testing.
(2) *Inductively Coupled Plasma:* Review EPA methods **200.7** and **5W846-6010**. Evaluate existing collaborative study data according to AOAC statistical procedures. Determine ruggedness, accuracy, and precision. If acceptable, write methods in AOAC format and recommend for official first action. If data do not meet criteria, make improvements and conduct single laboratory evaluation during the year.
(3) *Ion Chromatography:* Collect and evaluate data from various ion chromatography methods in current use, including EPA method **300.0**. Develop method to cover a broad range of anions. Conduct single laboratory testing of method during the year.

ORGANICS IN DRINKING AND GROUND WATER

(1) *Capillary Volatile Organic Analysis:* Review methods for appropriateness. Select method for single laboratory evaluation of ruggedness, selectivity, and sensitivity, and prepare for collaborative study.
(2) *EPA Methods:* Review and prioritize data from EPA collaborative studies submitted to AOAC for evaluation. Evaluate for additional collaborative testing any methods that do not meet AOAC requirements.
(3) *Phenols:* Collaboratively study the aqueous acetylation gas chromatographic method.
(4) *Quantitation of Polychlorinated Phenols:* Continue study.
(5) *Screening for Polynuclear Aromatic Compounds:* Collaboratively test the method for rapid screening of ground water extracts.
(6) *Total Organic Halogen:* Review the methods available. Select or write a method and subject to single laboratory testing, in preparation for collaborative testing.

ORGANICS IN SURFACE AND WASTE WATER

(1) *Chemical Pollutants:* Continue study.
(2) *Chlorinated Solvents:* Continue study.
(3) *Munitions:* Continue study.
(4) *Organohalogen Pesticides:* Continue study.

SOILS AND SEDIMENTS

(1) *Adsorption Isotherms for Volatile Organics:* Revise and prepare the tested method for collaborative study.
(2) *Distribution Coefficients for Nonvolatile Organics:* Continue study.
(3) *Distribution Coefficients for Volatile Organics:* Continue study.
(4) *Explosives Residues:* Continue study.
(5) *Soil Distribution Coefficients:* Continue testing draft standard method.
(6) *Termiticides:* Continue study.

WASTE MATERIALS

(1) *Bioassay Methods:* Continue study.
(2) *Inorganic Analytes:* Continue study.
(3) *Organic Analytes:* Continue study.
(4) *Physical/Chemical Properties:* Continue study.
(5) *Prescreening:* Continue study.

The recommendations submitted by the Committee on Hazardous Substances in Water and the Environment were accepted by the Association.

Executive Director's Report

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In 1987, approval and publication of official, reliable methods of analysis continued as AOAC's major activity. Implementation of the Long Range Plan and approval of amendments to the bylaws to broaden participation by nongovernment organizations and their employees in the governance and decision making of AOAC will have a great effect on the future.

Methods Approval

AOAC adopted 28 methods official first action in the second year under a new system whereby all methods are approved interim official first action before recommendation to the membership for official first action. These methods are applicable to the following areas: pesticide formulations 3, food economics and composition 6, food safety 8, residues 1, pharmaceuticals 8, and feeds, fertilizers, and other areas 2. AOAC adopted 5 new methods in food microbiology, a topic of great concern to food regulatory agencies in several countries.

The newly adopted methods mentioned above will appear in upcoming issues of the *Journal* and in the "Changes in Official Methods" supplement to the 14th Edition of *Official Methods of Analysis.* Sources of collaborative studies for these methods include: industry 12, Food and Drug Administration 11, and other organizations 5. AOAC adopted one CIPAC method in 1987. During the year, AOAC published 19 interlaboratory collaborative studies involving 207 laboratories.

Thirty-five methods are under consideration for interim official first action as of September 1987, including: pesticide formulations 5, food analysis 20, pharmaceuticals 6, and feeds 4.

Since 1981, AOAC has adopted 226 methods official first action, to bring the total since 1971 to 638.

Methods Needs

AOAC continues to recognize the need for proactive response to industry and government methods needs and to convince laboratories to participate in the required collaborative studies. Specific methods issues in 1987 included application of immunoreagents and DNA probes in food microbiology and residue analysis (veterinary drug residues in meat, aflatoxins and other mycotoxins in grain, antibiotic residues in meats, pesticide residues in foods, and identification of meat species). Many biotechnology companies have developed or are developing package test systems for use in laboratories, processing plants, and import facilities for the microbiological analysis and detection of chemical residues in feed and food products. Specific examples include numerous package test systems for *Salmonella,* 2 or 3 systems using DNA probes for *Listeria monocytogenes* in dairy products, card test systems for aflatoxin and zearalenone in corn and wheat, radioimmunoassay systems for penicillin and other antibiotic residues in milk, cholinesterase inhibition systems for cholinesterase-inhibiting pesticide residues, and specific immunoreagent methods for specific pesticides in specific commodities.

AOAC notes public concern in the United States, Canada, and the United Kingdom about pesticide residues in food, including the direction by the U.S. Congress that federal regulatory agencies develop rapid, economical methods for analysis of residues in foods for pesticides registered in the United States and other countries.

Current efforts through AOAC to develop and collaboratively study improved methods to evaluate the efficacy of hospital disinfectants reveal that current methods show a wide variability both within and between laboratories. Causes of this variability have not been completely ascertained and eliminated. Additional expensive and time-consuming effort to develop alternative methods will be necessary.

AOAC is seeking to cooperate with the U.S. Environmental Protection Agency to expedite the collaborative study of 6 multiresidue methods for 160 pesticides and their metabolites in groundwater. These methods will be used in the National Pesticide Survey in groundwater. Three methods of analysis for hazardous waste may be submitted for consideration. European approaches to the analysis of PCBs in foods and the environment are based on the analysis of 7–9 specific PCB congeners by capillary column gas chromatography.

AOAC communication and cooperation with other organizations has identified existing collaborative studies, several of which have been submitted to AOAC to support consideration of the respective methods for approval. Four methods of analysis for milk and milk products have been submitted as a result of collaborative studies carried out by the International Dairy Federation under the ISO/IDF/AOAC tripartite arrangement.

Volunteer Involvement in AOAC

In May 1986, volunteers involved as official methods committee members, General Referees, and Associate Referees with 637 associate referee topics were as follows: Associate Referees 478, General Referees 58, committee statisticians 6, and committee members 67. Distribution of these 609 volunteers among types of organizations included: industry 188, Food and Drug Administration 184, states 59, academia 56, and other 122. Industry and the Food and Drug Administration supplied 156 (33%) and 137 (29%), respectively, of the 478 Associate Referees.

Harmonization

AOAC participated with more than 30 other international organizations in an IUPAC-sponsored working conference on May 4–5, 1987, in Geneva, Switzerland, to develop protocols describing the minimum requirements for the design, conduct, and evaluation of interlaboratory collaborative studies. This conference generated recommendations for minimum standards such as number of materials, laboratories, levels of analyte, statistical analysis, symbols, and definitions of terms. Accepting organizations would agree to require their participants to adhere to these protocols as a minimum but may add additional requirements as necessary.

In January, the AOAC Board of Directors endorsed the principle of harmonization of collaborative studies and supported Richard Albert, Food and Drug Administration, as AOAC's representative at the harmonization conference. The Interlaboratory Studies Committee recommended AOAC acceptance of the protocols agreed on at the working conference, and a new AOAC document on the design, conduct, and evaluation of collaborative studies will be submitted for consideration by the Statistics Committee, and the Official Methods Board, and then for approval by the AOAC Board of Directors.

Statistical Issues

In January 1987, the Board of Directors approved the formats recommended by the Statistics Committee for the calculation of 4 precision parameters from quantitative collaborative study data, including reproducibility coefficient of variation, repeatability coefficient of variation, repeatability standard deviation, and reproducibility standard deviation. The method of calculation includes no statistical tests for outliers. The Board of Directors decided to include these 4 performance parameters in the 15th Edition of *Official Methods of Analysis* for methods approved 1984 through 1988. The Board of Directors decided to delete from the 15th Edition the existing performance parameters calculated for methods approved 1979 through 1983.

The protocols approved at the Harmonization Conference contain procedures for identification and removal of statistical outliers from valid sets of quantitative data. Tests include the Grubbs and Cochran tests, which will be included in the new AOAC document. The recommendations from the Conference prescribe minimum requirements for the statistical analysis of interlaboratory collaborative studies. These minimum requirements include the aforementioned outlier tests and one-way analysis of variance.

By the end of 1987, Associate Referees should have available a complete set of instructions for the design, conduct, and statistical evaluation of interlaboratory collaborative studies.

Official Methods Issues

In efforts to improve the methods review process, the Official Methods Board feels it is imperative that all reviewers adhere to the 2-week deadline. This will eliminate any hold-ups in the approval process, except those imposed by the Associate Referee.

To further assist Associate Referees and other methods volunteers, a series of video tapes is being developed. The first 2 in the series are (*1*) following the collaborative study process and (*2*) writing the collaborative study report. Additional tapes are anticipated on safety and statistics.

The Official Methods Board established the following policy for submitting official first action methods for consideration as official final action: After a method is in place for 2 years as an official first action method, the General Referee will recommend that the method be adopted official final action. If no adverse comments have been noted during the time the method has been official first action, the method will be announced in the May or June issue of *The Referee* as being eligible for final action status. The appropriate Methods Committee will review all recommendations and submit those for adoption as official final action to the membership at the annual business meeting.

To address the development of methods centered on proprietary products, the Official Methods Board developed the following policy: Any competent scientist in the technical area being studied can conduct a collaborative study according to AOAC-established criteria. All reagents that can be prepared in the user's laboratory must be described. For those involving trade secrets, the method must describe the principles involved in detection and quantitation.

Instrument equivalency was defined as instruments operating on the same principles and described by equivalent specifications, or instruments giving equivalent performance on actual samples.

The Official Methods Board implemented the use of forms developed by the Statistics Committee and approved by the Board of Directors for calculating the required statistical performance parameters of a method. The following must be calculated for each method approved: S_R, RSD_R, S_r, and RSD_r.

Three task forces were formed to deal with specific issues relevant to methods validation: sampling, methods outreach, and crisis methods/management.

Regarding the extension of an adopted method to commodities not included in a collaborative study, the Official Methods Board recommends that collaborative study results should apply only to the commodities included in the collaborative study. Extension of a method to other commodities requires a mini-collaborative study.

Cooperation with Other Organizations

In September, the Royal Society of Chemistry Analytical Methods Committee and AOAC signed a cooperative agreement similar to AOAC cooperative agreements with a number of other organizations. Potential areas of cooperation include nutrients in animal feed, antibiotics in animal feed, and veterinary drug residues in meat.

AOAC continues cooperation with the Nordic Committee for Food Analysis, International Office of Cacao and Chocolate, Collaborative Pesticides Analytical Council, the American Society of Brewing Chemists, the American Public Health Association, the American Association of Cereal Chemists, and the Tripartite Arrangement for Dairy Analysis Methods with the International Organization for Standardization (ISO) and the International Dairy Federation (IDF). AOAC is presently considering 4 methods for the chemical analysis of dairy products, resulting from the ISO/IDF/AOAC Tripartite arrangement. AOAC continues informal cooperative arrangements through joint committees and/or General Referees with the International Commission on Uniform Methods of Sugar Analysis, the American Oil Chemists Society, the IUPAC Commission on Fats and Oils, and the IUPAC Commission on Mycotoxins.

Publications

The poster, "Classification of Visible Can Defects" (1984), was reprinted for the fourth time. Also, the Youden and Steiner *Statistical Manual of the AOAC* (1975) was reprinted for the fifth time. The new statistical book, *Use of Statistics to Develop and Evaluate Analytical Methods* (1985), written by G. T. Wernimont and edited by W. Spendley, won the 1987 W. J. Youden Award in Interlaboratory Testing, conferred by the American Statistical Association. This annual award recognizes a publication that makes an outstanding contribution to the design and/or analysis of interlaboratory tests or describes ingenious applications to the planning and evaluation of data from interlaboratory tests.

The FDA *Food Additives Analytical Manual,* Volume II (1987), was published; it contains methods for 17 indirect food additives. The first supplement to the FDA *Bacteriological Analytical Manual* (1984) was also printed and distributed to all buyers of the manual. The supplement contains 3 new chapters: *Listeria* Isolation, Isolation and Identification of Motile *Aeromonas* Species, and Alkaline Phosphatase Methods to Detect Pasteurization. The fourth supplement to EPA's *Manual of Chemical Methods for Pesticides and Devices,* containing methods for 39 pesticides, was edited and will be published in late 1987.

A survey on the *Journal,* prepared by the ad hoc committee on evaluation and review of the *Journal,* was mailed to 400 persons, comprising respondents to a call for *Journal* readers and randomly selected *Journal* authors, reviewers, and AOAC members. The ad hoc committee on alternative forms of

publishing *Official Methods of Analysis* also ran a limited survey among persons who receive official methods supplements. The survey showed little interest in receiving methods in electronic form, a preference for maintaining the current printed volume, and an interest in availability of official methods in smaller units, such as looseleaf methods or individual chapters. As a result, the committee recommended delay in conversion of methods to electronic form but further evaluation of the economics and marketability of smaller print units.

The Editorial Board recommended acceptance of the American Chemical Society proposal to mount the *Journal of the AOAC* on STN, International (Scientific and Technical Network) under Chemical Journals Online. The *Journal* will become a member of the database that includes the 19 primary journals of ACS, the 5 polymer journals of John Wiley & Sons, the 9 primary journals of the Royal Society of Chemistry, and *Angewandte Chemie*. The *Journal of the AOAC* will become available on line in mid-1988.

In 1987, the *Journal of the AOAC* published 225 scientific papers plus the official transactions of the Association in 1130 pages. In addition to reports of research and development of analytical methodology, special reports were published on review of uric acid methodology, focus on Kjeldahl analysis, reference materials for mycotoxins and recombinant DNA-derived proteins, and the regulatory dilemma over *Listeria monocytogenes*. Papers from 2 symposia were published: "Critical Analysis of Analytical Methods for Meat Foods," and "Chromatography of Amino Acids." Copies of the *Journal* are received in 90 countries in the world. Subscription prices for 1987 will continue for 1988.

Membership and Governance

The membership approved amendments to the bylaws that establish one class of individual membership including industry, government, and academia and one class of sustaining membership for government and nongovernment organizations. Individual members vote on amendments to the bylaws, elect the officers and directors, vote on changes in dues, and vote on official first action and official final action adoption of methods. All individual members, whether from government, academia, or industry, would be eligible to serve on all AOAC boards and committees. These amendments would abolish the associate member classification. Provision for honorary membership would be retained.

The new membership section of the bylaws establishes qualifications for members, requiring a degree in science, or an equivalent if approved by the Board of Directors. Another amendment provided for a mail ballot, among the members, for voting on substantive issues, including amendments to the bylaws, election of the Board of Directors, and changes in dues.

Amendments provided that a majority of the Official Methods Board and the Board of Directors would be representatives from regulatory agencies; national, state, provincial, or local government agencies; and academia. Members employed by industry could serve on both the Official Methods Board and Board of Directors in minority numbers. Another amendment would enlarge the Board of Directors from 7 to 9 members, to provide for 5 directors instead of 3. Limitation of service on the Board of Directors to 6 consecutive years is retained.

The members approved deletion of provisions in the bylaws for adoption of methods by laboratories of national, state, provincial, and local government regulatory agencies.

Approval of these amendments implements several specific provisions of the long range strategic plan under the key issue "Improvement in Governance, Administrative Accountability and Proactive Role of AOAC." One of the goals of the Board of Directors in offering these amendments is to broaden the participation of all members in the governance, decision making, and scientific activities of the Association while retaining the credibility of the AOAC methods validation process and official status of AOAC methods. The Board of Directors asked the Membership Committee to develop recommendations regarding member benefits, status for members whose change in employment or retirement makes them ineligible to be members, and greater opportunities for members to become involved in the Association.

We are grateful to the 9 national, 44 state and 5 provincial government agencies, and the 143 industry organizations that provided financial support to AOAC in 1987. AOAC now has 3300 individual members in 79 countries, including 50 states (United States) and 9 provinces (Canada).

Long Range Planning

The Board of Directors received recommendations from AOAC staff for implementing the long range plan and directed each AOAC board, committee, and staff member to present plans for implementing their respective portions of the Long Range Strategic Plan, including enumerating tasks required to complete their section of the Long Range Plan, who is to perform these tasks, when work is to begin and end, and estimated costs, staff time, and other resources needed to complete the implementation. The Board approved the implementation plans in July 1987.

At the March meetings of the Validation Council and Governance Council, President Johnson stated that both staff and AOAC members, through their boards and committees, share responsibility for implementing the various items in the Long Range Plan. He stated that the Association belongs to the members and the members have the major responsibility and resources for implementation of all the items in the Long Range Plan.

The Board of Directors established the Governance Council, consisting of chairmen of the governing committees, and the Validation Council, consisting of chairmen of committees concerned with validation of methods, to encourage communication among committee chairmen, sharing of future plans, and identification of gaps and overlaps between and among various AOAC committees. Both councils met in March and September 1987.

Annual Meeting and Other Meeting Activities

The 101st AOAC Annual International Meeting was held at The Cathedral Hill Hotel, San Francisco, CA, September 14–17, 1987, with a total attendance of 1162. The opening session included presentations by 1987 Harvey W. Wiley Award winner, Walter Holak, and President Frank J. Johnson. Mr. Holak, who is on the staff of the Food and Drug Administration district office in Brooklyn, NY, is an expert in regulatory analytical chemistry. President Frank J. Johnson is on the staff of the National Fertilizer Development Center, Tennessee Valley Authority. The meeting included a scientific equipment and services exposition with 100 booths, which is a 15% increase over 1986 booth sales. Approximately 250 contributed papers were presented in poster sessions and workshops; the third Regulatory Roundtable addressed regulatory problems in the field of seafood toxins. The meeting program, with a focus on robotics, also offered 5 symposia: robotics; mycotoxins; industrial analytical chemistry; biotechnology: impact on present and future

methods of analysis; and cholesterol oxidation. In an effort to address topics in current regulatory crisis, AOAC included a special one-day *Listeria* Methodology Workshop. The social program was highlighted by the president's reception on Sunday evening, September 13, which hosted over 500 attendees, and also included the Tuesday evening dinner cruise attended by almost 400. Sites for the Annual International Meeting have been selected through 1990: August 29–September 1, 1988, Palm Beach, FL; September 25–28, 1989, St. Louis, MO; and September 10–13, 1990, New Orleans, LA.

Approximately 500 registrants attended the 12th Annual AOAC Spring Training Workshop which was held April 27–30, 1987, in Ottawa, Ontario, Canada. The program featured a microbiology symposium and sessions on food additives and contaminants, nutritional labeling, mycotoxins, drug residues in animal tissues, problems in drug analysis, immunoligand techniques in residues analysis, environmental contaminants, pesticide residues and micro contaminants, trace metals—data validity, and advances in laboratory robotics. The presentations also included 5 poster sessions: environmental contaminants and trace elements, drug residues and microbiology, drugs and pharmaceuticals, pesticides and mycotoxins, and food additives, contaminants, and nutrition. The scientific program was supplemented by 2 workshops: sample preparation and isolation using solid phase sorbents, presented by Analytichem International; and computer-assisted science, presented by Beckman Instruments. The scientific exposition included 21 booths. The highlight of the social program was the Tuesday evening banquet, featuring Dixieland jazz music. Attendees also enjoyed a wine and cheese mixer on Monday evening in the exhibit hall.

Infant Formula Conference II was approved by the AOAC Board of Directors. The Francis Marion Hotel in Charleston, SC, has been selected as the site for the conference, which is scheduled for April 26–28, 1988. The conference committee, which will again be co-chaired by Stephen Barnett, Bristol Myers, and James Tanner, FDA, has issued a call for papers. The Infant Formula Council will cosponsor this second conference with AOAC, and the Food and Drug Administration has expressed an interest in cosponsoring.

Short Courses

AOAC offered the short course on "Quality Assurance for Analytical Laboratories" 3 times in 1987, in April and July in Arlington, VA, and in September in San Francisco, CA, in conjunction with the 1987 AOAC Annual International Meeting. In addition, AOAC supplied course manuals and handout materials for 2 courses at the Laboratory of the Government Chemist in London, England. A one-day modified version of the short course was presented under contract with the Environmental Protection Agency as part of the program of its Solid Waste Testing and Quality Assurance Symposium, July 16, 1987. Standard proposals have been developed which offer the short course as a package to government agencies, other organizations, and industry for a set fee. Under this arrangement, the contracting organization could use the course as a means of fund-raising, as an attraction for its members, or as a training program. The Committee on Meetings, Symposia, and Educational Programs and the Committee on Laboratory Quality Assurance are coordinating efforts to develop a short course on laboratory quality assurance for the bench chemist. The Board of Directors approved the concept of a sampling short course, and preliminary steps have been taken to ensure its development in time to be offered in December 1988. Another topic area under consideration for new course development is statistics for methodology.

Awards

The 1987 Harvey W. Wiley Award winner is Walter Holak of the Food and Drug Administration, New York district office. Mr. Holak also celebrated his 25th year as a member of AOAC and as an employee of FDA.

The following 7 scientists were named 1987 Fellows of the AOAC: Patricia Bulhack, Food and Drug Administration, Washington, DC; David Fink, Merck, Sharpe, and Dohme, Inc., Rahway, NJ; Robert Isaac, University of Georgia, Athens, GA; James J. Karr, Pennwalt Technology Center, King of Prussia, PA; Dick Kleyn, Rutgers University, New Brunswick, NJ; Gerald Myrdal, Wisconsin Department of Agriculture, Madison, WI; and Douglas Park, University of Arizona, Tucson, AZ.

P. Frank Ross of the National Veterinary Services Laboratories, USDA, in Ames, IA, was named 1987 General Referee of the Year. Dr. Ross is the General Referee for Veterinary Analytical Toxicology. The 1987 Collaborative Study of the Year Award went to Elaine Bunch of the Food and Drug Administration, Seattle, WA, for her work in the areas of aminacrine and steroid phosphates. Associate Referee of the Year Awards were given to David F. Tomkins, Monsanto Co., Muscatine, IA, Associate Referee for Alachlor, Butachlor, Propachlor, Atrazine/Alachlor Mixtures; Douglas L. Park, University of Arizona, Tucson, AZ, Associate Referee for Aflatoxin Methods; James T. Tanner, Food and Drug Administration, Washington, DC, and Stephen A. Barnett, Bristol Myers-US Pharmaceutical and Nutrition Group, Evansville, IN, Associate Referees for Infant Formula and Nutrient Assay; Leon D. Sawyer, Food and Drug Administration, Washington, DC, Associate Referee for Comprehensive Multiresidue Methodology, Low Moisture/High Fat Sample Extraction, Ethylene Dibromide (EDB), Polychlorinated Biphenyls (PCBs); Russell S. Flowers, Silliker Laboratories, Inc., Chicago Heights, IL, Associate Referee for Rapid Methods for the Enterobacteriaceae; and David L. Osheim, National Veterinary Services Laboratory, Ames, IA, Associate Referee for Copper in Tissues, Fluoride.

The 1987 Certificates of Appreciation were awarded to James Lawrence, Health and Welfare Canada, Ottawa, Ontario, Canada, and Graham MacEachern, Agriculture Canada, Ottawa, Ontario, Canada, Co-Chairmen of the 1987 AOAC Spring Training Workshop. AOAC Employee Service Awards went to Marjorie D. Fuller, Assistant Business Manager, for 20 years service, David B. MacLean, Executive Director, for 10 years service, Vernora R. Petty, Administrative Assistant/Editorial, for 10 years service, and Margaret R. Ridgell, Administrative Manager, for 5 years service.

The Harvey W. Wiley Scholarship was awarded to Jo Yeargin of the University of California at San Diego.

AOAC Europe

AOAC Europe is scheduled to meet October 20–21, 1987, in Paris, France, in a joint symposium on methods of analysis in food microbiology and nutrients in foods.

Regional Sections

The Board of Directors approved awarding a charter to the Mid-Canada Regional Section, constituting the province of Manitoba. This is the seventh AOAC regional section.

Seven AOAC regional sections met since October 1, 1986: New York-New Jersey Regional Section, November 18 in East Brunswick, NJ, and May 14 in New York, NY; Eastern

Ontario-Quebec Regional Section, November 4 in Ottawa, Ontario, and again at the Spring Training Workshop, April 27–30 in Ottawa, Ontario; Mid-Canada Regional Section, November 18 in Winnipeg, Manitoba (organization meeting), March 25 in Winnipeg, Manitoba, and June 20 in Portage la Prawirie, Manitoba; Southeast Regional Section, June 2 in Atlanta, GA; Mid-West Regional Section, June 15–17 in Fargo, ND; Northeast Regional Section, June 22–24 in Aurora, NY; and Pacific Northwest Regional Section, June 25–26 in Olympia, WA.

Organizational meetings of new regional sections will be held for the following three areas: (*1*) California, Arizona, Hawaii, Nevada, Utah; (*2*) Texas, New Mexico, Oklahoma, Louisiana, Arkansas; and (*3*) Indiana, Ohio, Kentucky, Michigan.

As part of the long range plan, the Board of Directors has asked the Regional Section Committee and other AOAC committees to recommend actions to strengthen ties between the AOAC regional sections and the AOAC membership as a whole.

Financial

AOAC's sources of funds still include publication sales, meetings and exhibits, short courses, government support, and industry and individual memberships. Fiscal year 1987 income was $1,777,648 and expenses were $1,893,559, as of September 30, 1987.

The Board of Directors approved a change in the AOAC fiscal year from October 1 through September 30, to January 1 through December 31. The transition year will be 1988.

Individual member dues will remain at $45.00 US annually and Sustaining member dues will remain at $750.00 US annually for 1988.

Board of Directors

At the 1987 AOAC Annual International Meeting and Exposition in San Francisco, CA, AOAC chose the following officers and members to serve on the Board of Directors in 1987–1988: *officers*, President, Robert C. Rund, Office of the Indiana State Chemist and Seed Commissioner; President-Elect, Odette L. Shotwell, U.S. Department of Agriculture, Agricultural Research Service; Secretary/Treasurer, Thomas G. Alexander, U.S. Food and Drug Administration; *members*, Thomas P. Layloff, U.S. Food and Drug Administration; Albert W. Tiedemann, Virginia Consolidated Laboratories; H. Michael Wehr, Oregon Department of Agriculture; and immediate past president Frank J. Johnson, Tennessee Valley Authority.

The Board of Directors met on January 28–29, 1987, in Arlington, VA, and took the following actions:

(1) Approved schedule to implement the board-approved long range strategic plan before the July 1987 board meeting.
(2) Amended and accepted the strategy presented by staff for the long range plan. Under this strategy, AOAC boards, committees, and staff were assigned respective parts of the long range plan. Each group is to present to the Board of Directors in July 1987 the actions necessary with attached estimates of personnel needs, costs, and schedule for completion.
(3) Accepted the Finance Committee's recommendation that Bissell and Meade, the AOAC auditor, be hired for fiscal year 1987.
(4) Dissolved the Subcommittee on Automated Methods and Equipment, a subcommittee of the Instrumental Methods and Data Handling Committee.
(5) Concurred with the suggestion of the Committee on Quality Assurance to develop media options including a loose-leaf manual/handbook, videotapes on specific topics, PC disk or 35 MM slides with accompanying text on separate topics, pamphlets containing information on separate topics for presenting quality assurance information.
(6) Endorsed the principle of harmonization of protocols for the design, conduct, and evaluation of interlaboratory collaborative studies among international organizations.
(7) Approved financial support for Richard Albert as the AOAC representative at the IUPAC Working Conference on Harmonization of Collaborative Studies in Geneva, Switzerland on May 4–5, 1987.
(8) Accepted the recommendation of the Statistics Committee for a uniform format for the calculation and presentation of collaborative study precision data in methods submitted for approval.
(9) Commended the Statistics Committee for a job well done on developing a computerized statistical program for calculation of performance parameters from collaborative study data.
(10) Deferred inclusion of performance parameters for methods in the "C" set of changes in *Official Methods of Analysis*, 14th edition, to be published in March 1987.
(11) Reaffirmed April 1986 instructions to the Constitution Committee to draft amendments for preservation to the Board of Directors, implementing the January 1986 board approval of recommendations of the Membership Committee on classes, rights, and privileges of individual members, establishment of a single class of sustaining members of AOAC, enlargement of the Board of Directors, and composition of the Official Methods Board and Board of Directors.
(12) Approved charter for the Mid-Canada Regional Section which includes the Province of Manitoba.
(13) Approved $65,000 for new disk drive and accessories for the computer in conversion of the database system from Oracle version 4 to Oracle version 5.
(14) Approved registration fees for the 1987 Annual International Meeting as follows:

	Member	*Nonmember*
Pre-registration Fees		
Full day meeting	$105.00	$130.00
One day meeting	$ 80.00	$ 90.00
On-site Registration Fees		
Full day meeting	$120.00	$145.00
One day meeting	$ 95.00	$105.00

(15) Approved bank resolutions of the Mid-Canada Regional Section, Southeast Regional Section, and the 1987 Spring Training Workshop.
(16) Approved indemnification of regular AOAC headquarters staff (greater than 30 hours per week) from liabilities incurred in the prudent exercise of their duties and responsibilities as employees of the association.
(17) Accepted the concept in planning for Infant Formula Conference II, tentatively scheduled for April 1988 in Charleston, SC.

(18) Elected Thomas Alexander as treasurer of AOAC through September 17, 1987, to fill the vacancy created by Prince Harrill's retirement from FDA and resignation.

The AOAC Board of Directors met April 25–26, 1987, in Ottawa, Ontario, Canada, and took the following actions:

(19) Authorized the Executive Director to prepare written answers to specific questions in a press release from the House Ways and Means oversight subcommittee which is looking at possible changes in tax status of earned and unearned income from currently tax-exempt 501C(6) and 501C(3) organizations.
(20) Approved insertion of performance parameters—reproducibility coefficient of variation (RSD_R), repeatability coefficient of variation (RSD_r), repeatability standard deviation (S_r), reproducibility standard deviation (S_R)—in *Official Methods of Analysis,* 15th edition, for methods approved official first action from 1984 through the fall of 1987, with the provision that these parameters be calculated and placed in the 15th edition for methods included in the "D" set of supplements (Changes in Methods) to *Official Methods of Analysis,* 14th edition.
(21) Deleted all performance parameters listed for methods approved 1979–1983 in *Official Methods of Analysis,* 14th edition, from *Official Methods of Analysis,* 15th edition.
(22) Encouraged each regional section to develop and maintain a manual of operations following the AOAC regional section operations guidelines available from the AOAC office.
(23) Instructed regional sections as follows: forward regional section annual budget to AOAC comptroller before commencement of the regional sections fiscal year; obtain specific approval of the AOAC comptroller before finalizing any comittments of expenditures totaling more than $5000 for a single event or purchase.
(24) Approved recommendation of the Editorial Board that subscription rates for the *Journal of the AOAC* for 1988 remain the same as for 1987.

The Board of Directors met on July 14–15, 1987, in Arlington, VA, and took the following actions:

(25) Abolished the Committee on Instrumental Methods and Data Handling, effective September 18, 1987.
(26) Recommended amendments to the bylaws for consideration at the Annual International Meeting on September 17, 1987.
(A) Amend Article III, Sections 1 and 2, to establish classes of individual membership in the association, individual members, sustaining member organizations, and honorary members, with individual members eligible to hold office, serve on AOAC boards and committees, and vote on amendments to bylaws, election of officers, approval of analytical methods, and all matters not otherwise excluded.
(B) Establish a provision to allow mail balloting on all substantive matters requiring a vote by the membership, including amendments to the AOAC bylaws, election of officers, and dues.
(C) Amend Article VIII, Section 3, Paragraph A, to provide that a majority of the Official Methods Board shall be from one or more of the following: government agencies, regulatory agencies, or academic institutions.
(D) Amend Article VI, Section 1, to enlarge membership on the AOAC Board of Directors to nine (9) members, the majority of whom shall be representatives from one or more of the following: a national, state, provincial, or municipal government, a regulatory agency, or academia, with a provision that no member of the board may be elected for more than six (6) consecutive years.
(E) Deleted paragraphs A, B, and C from Article IX, Section 1. Provided for members instead of agencies voting on approval of methods of analysis.
(27) Commended the chairman and Committee on the Constitution for the work on the bylaw amendments.
(28) Supported the president's interpretation of Article XIV of the bylaws that individual members of the association vote on amendments to the AOAC bylaws.
(29) Amended previous action, that of inserting the previously designated performance parameters for methods in the 15th edition of *Official Methods of Analysis,* to provide for inclusion for these performance parameters for all methods approved official first action from 1984 (published in March 1985) through 1988 (published in January 1989). Required that performance parameters applicable to methods to be published in the "D" set of supplements (Changes in Methods) be provided with the methods that are approved September 17, 1986.
(30) Approved registration fees for the 1988 AOAC Annual International Meeting as follows:

	Member	*Nonmember*
Advance registration	$115.00	$160.00
One-day	90.00	100.00
On-site registration	130.00	175.00
On-site one-day	105.00	115.00

(31) Elected the following persons Fellows of the AOAC: Patricia Bulhack, Food and Drug Administration, Washington, DC; David W. Fink, Merck Sharp, and Dohme, Inc., Rahway, NJ; James J. Karr, Pennwalt, King of Prussia, PA; Dick R. Kleyn, Rutgers University, New Brunswick, NJ; Robert A. Isaac, University of Georgia, Athens, GA; Gerald R. Myrdal, Wisconsin Department of Agriculture, Madison, WI; Douglas L. Park, Food and Drug Administration, Washington, DC.
(32) Approved the cooperative agreement between the Royal Society of Chemistry Analytical Methods Committee and AOAC.
(33) Approved the bank resolution for the Southeast Regional Section.
(34) Reviewed and concurred with proposals from AOAC boards, committees, and staff to implement approximately 85 different items in the draft long range strategic plan. Each proposal described actions to be taken, identified the group to take the actions, established starting and completion dates, and estimated staff time and other costs of the action. Completion dates for the long range plan ranged from fall 1987 to the fall of 1990 and in some cases beyond. The board noted that the plan assumes AOAC's purposes in the bylaws are still valid, that some items were more important than others, and that each AOAC volunteer group should set its own priorities with board review of resource needs and accomplishments in implementing their parts of the long range plan.

The Board of Directors met on September 13, 1987, at the Cathedral Hill Hotel in San Francisco, CA, and took the following actions:

(35) Affirmed a mail ballot of August 14, 1987, relating to approval of recommendation to the membership of housekeeping amendments to the bylaws.
(36) Modified the language of an August 28, 1987, mail ballot and approved supplements in the amount of $15,298 to the fiscal year 1987 budget of 2 AOAC representatives.
(37) Approved in concept the point rating system to identify candidates for Fellows of the AOAC, after deleting points for publication in the scientific media.
(38) Approved establishment of an ad hoc committee to review the proposal for a short course on sampling.
(39) Approved changing the AOAC accounting period from the fiscal year October 1 through September 30 to a calendar year January 1 through December 31, effective October 1, 1987.
(40) Directed the staff to compile all policies adopted by the Board of Directors since early 1982 through September 18, 1987, and transmit this compilation with appropriate documentation to a board committee by November 15, 1987.
(41) Directed the staff to submit a draft employee handbook (personnel policy) describing benefits, duties, responsibilities, rights and so on, for submission to the board at the March 1988 meeting.
(42) Set dates for the 1988 meetings of the Board of Directors as follows: January 6–8, 1988, Arlington, VA; March 8–10, 1988, Arlington, VA; June 8–10, 1988, Arlington, VA; August 28 & September 2, 1988, Palm Beach, FL.
(43) Sources of financial support through October 1987 were:

Government:

Agriculture Canada
Association of Public Analysts
Environmental Protection Agency
  Office of Pesticide Programs
Food and Drug Administration
Health and Welfare Canada
  Health Protection Branch
State Laboratory (Ireland)
Laboratory of the Government Chemist
Ministry of Agriculture, Fisheries & Food
U.S. Department of Agriculture
  Agricultural Research Service

Alabama Department of Agriculture and Industries
Alberta Agriculture
Alberta Dairymen's Association Research Unit
Arizona State Agriculture Laboratory
Arkansas State Plant Board
Assiut University
California Department of Food and Agriculture
Colorado Department of Agriculture
Delaware Department of Agriculture
Florida Department of Agriculture and Consumer Services
Georgia Department of Agriculture
Hawaii Department of Agriculture
Hawaii Department of Health
Illinois Department of Agriculture
Indiana Office of the State Chemist
Indiana State Board of Health
Instituto di Tecnica e Sperimentazione
  Lattiero-Caseari di Thiene
Iowa Department of Agriculture
Iowa State Veterinary Diagnostic Laboratory
Kansas State Board of Agriculture
Kentucky Agricultural Experiment Station
  Division of Regulatory Services
Kentucky Department of Agriculture
Maryland Department of Agriculture
Massachusetts Department of Food and Agriculture
Michigan Department of Agriculture
Minnesota Department of Agriculture
Mississippi State Chemical Laboratory
Missouri Experiment Station Chemical Laboratory
Montana Department of Agriculture
Nebraska Department of Agriculture
New Jersey Department of Agriculture
New Mexico Department of Agriculture
New York Department of Agriculture and Markets
North Carolina Department of Agriculture
North Dakota State Laboratories Department
Oklahoma Department of Agriculture
Ontario Ministry of Agriculture and Food
Oregon Department of Agriculture
Pennsylvania Department of Agriculture
Quebec Department of Agriculture
South Carolina Department of Agriculture
South Dakota State Chemical Laboratories
Tennessee Department of Agriculture
Texas Agriculture Experiment Station
University of Vermont
  Agriculture Testing Laboratory
Utah State Department of Agriculture
Virginia Division of Consolidated Laboratory Services
Wisconsin Department of Agriculture Trade/Consumer
  Affairs
Wyoming Department of Agriculture

Industry:

ABC Research Corp.
Agway, Inc.
Alcon Laboratories, Inc.
American Burdick & Jackson
American Council of Independent Laboratories, Inc.
American Crystal Sugar Co.
American Cyanamid Co.
American Maize Products
The Andersons
Archer-Daniels-Midland
A/S N. Foss Electric
Avon Products, Inc.
BASF Corp. Chemicals Division
Beckman Instruments, Inc.
Beech-Nut Nutrition Corp.
Biochem Laboratorium BV
Bio-Rad Laboratories
Boehringer Mannheim
Borden, Inc.
Bristol-Myers Co.
Bristol-Myers U.S. Pharmaceutical & Nutritional Group
Cacaofabriek De Zaan BV
California Almond Growers Exchange
Calreco, Inc.
CAMCO
Campbell Institute for Research & Technology

Campbell Taggart, Inc.
Cargill, Inc.
Carrington Laboratories, Inc.
Castleton Beverage Corp.
CEM Corp.
Chef Reddy Foods Corp.
Chemical Waste Management Inc.
Chevron Chemical Co.
CIBA-GEIGY Corp. Agricultural Division
The Coca-Cola Co.
Comibassal International
Compu-Chem Laboratories, Inc.
ConAgra Consumer Frozen Foods Co.
Continental Baking Co.
Corn Refiners Association, Inc.
CPC International, Inc.
DFA of California
Digital Equipment Corp.
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 Co. Division
Environmental Testing & Certification Corp.
Express Foods, Inc.
FMC Corp. Agricultural Chemicals Group
FMC Corp. Food & Pharmaceutical Products Division
The Fertilizer Institute
Fisher Scientific Co.
Foss Food Technology Corp.
GAF Corp.
Galbraith Laboratories, Inc.
E & J Gallo Winery
Gama Foods, Inc.
  Division of ITC
General Foods Corp.
General Mills, Inc.
GENE-TRAK Systems
Gerber Products Co.
Gist Brocades USA
Golden State Foods Corp.
Griffith Laboratories USA, Inc.
Haarman & Reimer Corp.
Hazleton Laboratories America, Inc.
Heinz USA
Hershey Foods Corp.
Heublein Wines
Hoechst-Roussel Pharmaceuticals, Inc.
Hoffmann-La Roche, Inc.
ICI Americas, Inc.
International Minerals & Chemical Corp.
S. C. Johnson & Son, Inc.
Kellogg Co.
Kemira OY
Kraft, Inc.
The Kroger Co.
Laboratorio di Chimica Analitica Applicata SNC
Laboratory Specialists, Inc.
Lancaster Laboratories, Inc.
Lehn & Fink Products Co.
Thomas J. Lipton, Inc.
Loma Linda Foods, Inc.
McCormick & Co., Inc.
McKee Baking Co.
MCLAS Technologies, Inc.
McLaughlin Gormley King Co.
McNeil Consumer Products Co.
Marion Laboratories, Inc.
Merck Sharp & Dohme Research Laboratories
Mettler Instrument Corp.
Miles Laboratories, Inc.
3M Company
Mobay Corp.
Monsanto Agricultural Co.
Moorman Manufacturing Co.
Nabisco Brands, Inc.
National Food Processors Association
National Starch & Chemical Corp.
Nestec Ltd
New Zealand Milk Products, Inc.
Novo Biochemicals
Ocean Spray Cranberries, Inc.
O.M. Scott & Sons Co.
Organon Teknika Corp.
Orion Research, Inc.
Ortho Pharmaceutical Corp.
Oxoid USA, Inc.
Pennwalt Corp.
Pepsico
Perdue, Inc.
Pfizer Inc.
Pharmacia AB
Philip Morris USA
The Pillsbury Co.
The Procter & Gamble Co.
The Quaker Oats Co.
Ralston-Purina
Rhone-Poulenc Chemical Co.
R. J. Reynolds Industries, Inc.
R. J. Reynolds Tobacco Co.
Ross Laboratories
Sandoz Crop Protection Corp.
Schenley Distillers
Joseph E. Seagram & Sons, Inc.
Schenley Distillers
Shasta Beverages, Inc.
ShriRam Institute for Industrial Research
Silliker Laboratories, Inc.
SmithKline Animal Health Products
E. R. Squibb & Sons, Inc.
Technicon Industrial Systems
Technological Institute
The Upjohn Co.
Wallace Laboratories
  Division of Carter-Wallace, Inc.
Warner Lambert Co.
Waters Chromatography
  Division of Millipore
Welch Foods, Inc.
Zoecon Corp.

Secretary/Treasurer and the Finance Committee

THOMAS G. ALEXANDER, *Secretary/Treasurer*
Food and Drug Administration, National Center for Drugs and Biologics, Washington, DC 20204

Other Members: E. R. Elkins; J. E. McNeal

Recommendations for the Board of Directors

The Finance Committee recommends that Bisselle, Meade & Company again be selected to serve as the Association's auditors. In our opinion, the company has served the Association well with professional accounting. It is only fitting that they be reselected.

Other Discussion

The committee met 4 times during fiscal year 1987. Each quarterly financial statement was carefully reviewed. The proposed fiscal year 1988 budget was reviewed and commented on. The bank vault contents were examined and found to be in order. The fiscal year 1987 audited financial report was published (*J. Assoc. Off. Anal. Chem.* (1987) **70,** 344–345). Copies are available for those interested.

Other items acted on include policies with respect to petty cash fund, non-U.S. checking accounts, and regional section financial practices. Also the committee acted on, or is acting on, 3 issues of the Strategic Action Plan. Finally, the committee prepared, and is presenting to the Governance Council, a proposed rewriting of the committee's Terms of Reference.

STATEMENT OF FINANCIAL CONDITION—SEPTEMBER 30, 1987

Assets

Current Assets:		
Cash, Signet Bank*		$ 4,653
Cash, 1st American Bank*		69,638
Cash, 1st American Bank, payroll*		13,884
Cash, Office fund		500
Cash, Regional sections		26,799
Accounts Receivable		
Books and publications		34,333
Contracts and support		72,706
Sustaining Members		14,500
Membership		1,640
Contributions–Annual Meeting		3,000
Annual Meeting		2,815
Other		7,806
Accrued interest receivable		7,779
Inventory, books and publications—at cost		180,675
Prepaid expenses		9,776
Deposits		850
Total Current Assets		$451,354
Investments:		
Securities	$ 65,956	
Certificates of Deposit	1,267,431	$1,333,387
Fixed Assets:		
Office furniture, fixtures, and equipment	$ 65,551	
Computer and computer software	351,421	
	$ 416,972	
Less: accumulated depreciation	−217,308	$ 199,664
Deferred Costs:		
Official Methods of Analysis—15th Edition	$ 14,951	
Annual Meeting—1988	5,714	
Annual Meeting Exhibit Booths—1988	1,484	
EPA Update	7,246	
Sampling Manual	2,712	
QA Prin Update	1,623	
Infant Formula	3,801	
Statistical Manual Text	1,578	
Principles of Food Analysis	83	
Q/A Short Courses	1,881	$ 41,073
Total Assets		$2,025,478

* Interest bearing account

Liabilities and Fund Balances

Current Liabilities:		
Accounts payable		$ 80,371
Accrued and withheld payroll taxes		1,593
Total Current Liabilities		$ 81,964
Deferred Income:		
Journal subscriptions	$ 87,608	
Sustaining Members	47,438	
Membership	22,140	
Annual Meeting 1988—Exhibit booths	575	
Infant Formula	4,000	
Q/A Short Courses	6,600	$ 168,361
Reserve for Publications		$ 200,000
Restricted Reserve for FAAM		$ 5,155
Restricted Reserve for MAM		$ 2,267
Restricted Reserve for ADAM		$ 3,387
Restricted Fund—Harvey Wiley		$ 46,521
Restricted Fund—15th Edition		$ 618,139
Restricted Fund—Liability Insurance		$ 4,016
Fund Balance:		
Balance, October 1, 1986	$ 995,678	
Less: excess of expenses over income for the twelve months ended September 30, 1987	−115,911	
Balance, September 30, 1987	$ 879,767	
Add: Income—Regional Sections	15,901	
Adjusted balance, September 30, 1987		$ 895,668
Total Liabilities and Fund Balances		$2,025,478

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; H. B. S. Conacher; D. J. Dube; T. L. Jensen; R. J. Noel; R. Schmitt; E. Sheinin; L. L. Zaika

Recommendations for the Board of Directors

During this past year the Official Methods Board has taken a position on several important issues and recommends the following for adoption as AOAC official policy by the Board of Directors.

A. Studies proposed for official first action approval must be presented in a poster or other designated form at an Annual International Meeting and before a vote of the membership for official first action. If an Associate Referee is unable to attend an Annual International Meeting, the presentation may be made by the General Referee or a fellow scientist familiar with the method.

B. An official first action method may not be adopted official final action before the second Annual International Meeting after it was first adopted. Notice of the General Referee's intent to recommend a method for adoption as official final action must be published in *The Referee* in advance of the Annual International Meeting.

C. Any competent scientist in the technical field of a method to be studied, regardless of his/her level of vested interest in proprietary products/reagents used in the method, may conduct a collaborative study (usually as the Associate Referee) according to established AOAC criteria. Furthermore, all reagents which can be prepared in the user's laboratory must be described; proprietary reagents to be protected as trade secrets must be described so as to specify the principles involved in detection and quantitation.

Rationale for Recommendations to the Board of Directors

A. Currently, methods approved interim official first action are listed in *The Referee* before the Annual International Meeting at which they may be adopted official first action. A copy of the method is also made available for review by interested parties. However, unless a presentation is made in some other form, there is inadequate opportunity for public view of the method and direct interaction on questions with the Associate Referee.

B. A method approved official first action is not published as a part of "Changes in Methods" until 4–6 months after the date of approval, and such a method actually becomes official 30 days after publication. Thus, the policy recommendation is made to give interested users adequate time to obtain, use, and evaluate a method before it is adopted official final action.

C. Recent developments in biotechnology have resulted in a rapid increase in the use of proprietary products in the detection of many analytes such as aflatoxins, drugs, antibiotics, pesticide residues, and animal tissues as economic adulterants of meat products. It is not unusual for a scientist directly involved or closely associated with the development of a proprietary biotechnology product/reagent to wish to conduct a collaborative study. These scientists may at times appear to place such high value on an AOAC-approved method using their products that their objectivity is seemingly blurred, and it is difficult to avoid the impression of an intent to take advantage of AOAC. Because the Official Methods Board has not observed any distortions or scientific biases in the methods employing proprietary biotechnology product/reagents; because the developers of proprietary products have the right to protect trade secrets; because the possession of vested interests stimulates the conduct of collaborative studies; and because the users of AOAC methods should, if so inclined, be able to prepare nonproprietary reagents, while having knowledge of the scientific principles behind the performance of proprietary products or reagents; the Official Methods Board submits the relevant recommendations for adoption as AOAC policy.

Meetings

The Official Methods Board continued in its primary responsibility to administer the AOAC collaborative study and approval process through 3 meetings during the year: December 10–12 in St. Louis, MO, June 4–6 in Toronto, Ontario, Canada, and September 12 in San Francisco, CA, at the AOAC Annual International Meeting. The timeliness of the December and June meetings allowed many actions and decisions to guide all persons involved in the method study and approval processes to be incorporated in informational letters sent in January and June of 1987 to General and Associate Referees.

Educational Forum Feedback

For the last few years the Official Methods Board has devoted considerable time and effort not only to the Volunteer Educational Forum, but also to evaluation and responses with feedback received on the new interim review process as a result of the forum. The Official Methods Board plans to continue the Volunteer Educational Forum and would be pleased to receive suggestions on form, format, and content to meet the needs of method volunteers. That portion of the 1987 educational session devoted to method committees of the whole is in direct response to method volunteer requests. Further, during this past year, the following actions, responses, or decisions were made as a result of feedback from the session in Scottsdale:

A. Associate Referees can and should do their own statistical calculations, using the forms (and statistical programs) to be provided by the AOAC Committee on Statistics, with oversight provided by methods committee statistical consultants.

B. It is the consensus of the Official Methods Board that one statistical consultant per methods committee is sufficient, as conflicts may arise when 2 statistical consultants are asked to review the same manuscript. In the event any Methods Committee has 2 statistical consultants, the committee chairman may make the decision as to which one to use.

C. In the event of conflicts between the educational packet distributed at the AOAC Annual International Meeting in Scottsdale, AZ, and the *Handbook for AOAC Members,* 5th edition, the guidelines contained in the educational packet should be followed.

D. The Official Methods Board believes that all General Referee and statistical consultant comments should be included with a collaborative study when it is distributed to a methods committee for review and that General Referees should be included in any correspondence concerned with studies by their Associate Referees.

E. A suggestion to place a "seal of approval" stamp on study designs and protocols which have been reviewed and approved by General Referees and statistical consultants has been rejected. The new review process is still too young to

penalize Associate Referees for failure to submit their plans for advance review.

F. The Official Methods Board encourages preliminary interaction between an Associate Referee and General Referee before submission of a study protocol.

G. General Referees should make certain that Associate Referees send complete methods as they are to be used and not just instructions to collaborators or mixed statements including the method plus collaborator instructions.

Awards

The Official Methods Board annually coordinates the selection process for recognition of excellence in performance by Associate and General Referees.

A. In 1987, the methods committee nominees for General Referee of the Year were: Committee on Pesticides and Disinfectants—James Launer, retired, Oregon Department of Agriculture; Committee on Drugs and Related Topics—James Fitzgerald, Food and Drug Administration; Committee on Foods I—David Firestone, Food and Drug Administration; Committee on Foods II—Randolph Dyer, Bureau of Alcohol, Tobacco and Firearms; Committee on Residues—no nominee; Committee on Microbiology—Wallace Andrews, Food and Drug Administration; Committee on Feeds, Fertilizers, and Related Topics—P. Frank Ross, U.S. Department of Agriculture: and Committee on Hazardous Substances in Waste and the Environment—no nominee. During its June meeting in Toronto, the Official Methods Board voted to select P. Frank Ross, U.S. Department of Agriculture, General Referee for Veterinary Analytical Toxicology, as the General Referee of the Year for 1987.

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—"HPLC Method for Anilazine in Formulated Products: Collaborative Study," by Stephen C. Slahck, Mobay Corp.; Committee on Drugs and Related Topics—"Analysis and Identification of Dexamethazone in Bulk Drugs and Elixirs: Collaborative Study," by Elaine E. Bunch, Food and Drug Administration; Committee on Foods I—no nominee; Committee on Foods II—"Titrimetric Determination of Carbon Dioxide in Wine: Collaborative Study," by Arthur Caputi (Associate Referee) and Durwood R. Walker, E.&J. Gallo Winery; Committee on Residues—"Rapid Determination of Methyl Mercury in Fish and Shellfish: Collaborative Study," by Susan C. Hight, Food and Drug Administration; Committee on Microbiology—"Identification of Mammalian Feces by Coprostanol TLC: Collaborative Study," by George P. Hoskin, Food and Drug Administration; Committee on Feeds, Fertilizers, and Related Topics—"Comparison of HgO and $CuSO_4/TiO_2$ as Manual Kjeldahl Digestion Catalysts in the Determination of Crude Protein: Collaborative Study," by Peter F. Kane, Purdue University; and Committee on Hazardous Substances in Waste and the Environment—no nominee. The study conducted by Elaine E. Bunch was selected for the Collaborative Study of the Year award for 1987.

C. The recipients of the methods committee Associate Referee of the Year awards in 1987 were: Committee on Pesticides and Disinfectants—David Tomkins, Monsanto Agricultural Products Co.; Committee on Drugs and Related Topics—Elaine E. Bunch, Food and Drug Administration; Committee on Foods I—Douglas L. Park, University of Arizona (formerly with the Food and Drug Administration); Committee on Foods II—Stephen A. Barnett, Mead Johnson & Co., and James T. Tanner, Food and Drug Administration; Committee on Residues—Leon D. Sawyer, Food and Drug Administration; Committee on Microbiology—Russell S. Flowers, Silliker Laboratories, Inc.; Committee on Feeds, Fertilizers, and Related Topics—David Osheim, National Veterinary Services Laboratory; and Committee on Hazardous Substances in Waste and the Environment—no nominee. The methods committee Associate Referee awards recognize outstanding work and service by Associate Referees in their topic areas.

Task Forces

Three new task forces have been initiated to address the AOAC strategic plan and other issues assigned to the Official Methods Board.

A. An ad hoc Sampling Task force has been charged to "assess the extent that AOAC should become involved in the development of sampling guidelines and sample preparation guidelines for the analytical (both chemical and biological) examination of foods, drugs, pesticides, water, air, and other materials or matrices of interest to AOAC." The Official Methods Board is most pleased to have Douglas L. Park, University of Arizona (formerly with the Food and Drug Administration), serving as the task force chairman.

B. A Methods Outreach Task Force has been organized to address the AOAC Strategic Plan assignment to "develop quicker response to methodology needs." The primary charge for this task force is the identification of method sources and laboratory resources: What groups, agencies, organizations, individual laboratories, and fields of study are likely, or potential, sources of methods that can be, or already have been, subjected to collaborative study, and/or may meet the continually growing need for new validated methods. What laboratories and groups with common/like interests or goals related to those of AOAC, but not necessarily involved in AOAC activities currently, can be attracted to participate in the AOAC methods process? Also, the Official Methods Board is asking for a recommendation on a structure that would provide continual updating of the task force outputs to place AOAC in a position to stay current in the identification of new methods, method areas, resources, sources, and clientele. The Official Methods Board is extremely pleased to have H. Michael Wehr, Oregon Department of Agriculture, as chairman of the Methods Outreach Task Force.

C. Another AOAC Strategic Plan assignment to "develop a statement for methods needed in crisis situations" has prompted the establishment of the Crisis Methods Task Force. During the past few years, several situations have developed rapidly and evoked immediate demands for analytical methods: aldicarb in watermelons, EDB in grain products and other foods, sulfite on foods, and ethylcarbamate in wine and other liquors. Regulatory and industry laboratories may be fortunate and find an existing, validated method available such as the LC postcolumn carbamate pesticide method (**29.A05**) useful for aldicarb; however, there may not be a method, or existing methods may require multilaboratory validation in order for users, both industry and government, to have adequate confidence in their use in a regulatory environment. The Crisis Methods Task Force is charged to evaluate existing emergency validation procedures and then to outline and recommend a protocol for evaluation of a method for use in a crisis situation, including the role of

AOAC. The Official Methods Board is very fortunate to have H. B. S. Conacher, Health and Welfare Canada, serving as the Crisis Methods Task Force chairman.

D. As indicated in the 1986 Official Methods Board report (*J. Assoc. Off. Anal. Chem.* (1987) **70**, 346-349), the recommendations of the final report of the ad hoc Committee on Biological and Pass/Fail Methods have been considered fully during this past year. The following actions were taken on these recommendations:

1. *Substitution of Expert's Counts in Extraneous Materials Collaborative Studies/Addition to the Preamble of Chapter 44.*—The Official Methods Board accepted the use of expert counts as a mode for evaluation of extraneous material pass/fail collaborative study data and accepted and recognized the value of the proposed addition to the preamble of Chapter 44.
2. *Samples Used in Collaborative Studies.*—The Official Methods Board accepted the recommended statements on samples for use in biological and pass/fail collaborative studies as material for incorporation in the *Handbook for AOAC Members.* During this year, additional work was completed on "Preparation of Test Samples for AOAC Microbiological Collaborative Studies," by Wallace Andrews (*J. Assoc. Off. Anal. Chem.* (1987) **70**, 931-936). The Official Methods Board has recommended it be referenced in the next edition of the Handbook.
3. *Statistical Parameters and Study Design Considerations for Biological and Pass/Fail Collaborative Studies.*—The Official Methods Board accepted and supports the ad hoc committee's statement on the subject and passed the recommendations on to the AOAC Committee on Statistics.
4. *False Positives and False Negatives in Biological and Pass/Fail Collaborative Studies.*—The Official Methods Board agreed to refer the proposed definitions and treatment of false positives and false negatives to the AOAC Committee on Statistics for their review and consideration.
5. *Application of a Method to Different Commodities.*—The ad hoc committee's position was not resolved on this issue; however, the Official Methods Board reached a conclusion and recommends that collaborative study results should be applied only to commodities included in actual studies. Extension of a method to new or different commodities requires a mini-collaborative study.

Again the Official Methods Board wishes to recognize the outstanding work of the ad hoc Committee on Biological and Pass/Fail Methods and to give special thanks for the leadership provided by Donald A. Mastrorocco, Hershey Chocolate Co.

Other Actions, Activities, and Decisions

Many actions and decisions made this year may be of varying interest to different segments of the membership; these included but were not limited to the following:

1. Reviewed a proposed agreement for cooperative efforts with the Analytical Methods Committee of the Royal Society of Chemistry for method validation and a proposed memorandum of agreement with the U.S. Environmental Protection Agency for the collaborative study of groundwater methods.
2. Responded to the Board of Directors' requests for comments and recommendations on a proposal by the laboratory computers and robotics subcommittee of Instrumental Methods and Data Handling Committee and proposals from the Committees on Statistics, Safety, and Membership.
3. Developed a collaborative study review guide and consolidated various Official Methods Board position statements, guidelines, and other significant decisions into an Official Methods Board policy and operations manual.
4. Made recommendations on inclusion of statistical parameters in *Official Methods of Analysis* and accepted in principle, with comments and questions, recommendations contained in the IUPAC Workshop on the Harmonization of Collaborative Analytical Studies.
5. Concluded the use of outside experts for method reviews by official methods committees was acceptable, if needed, but in such situations the collaborative study manuscripts must be treated like unpublished papers in peer review.
6. Reviewed the use of critical steps in selected methods from *Official Methods of Analysis* and identified a need to highlight these steps in published methods.
7. Moved the topic areas covering cosmetics and drug residues from the Committee on Microbiology to the Committee on Drugs and Related Topics.
8. Reviewed the performance of Associate Referees, General Referees, and methods committee members.
9. Made recommendations on by-law changes to the Board of Directors.

Interim Official First Action and Official First Action Methods

The users of AOAC official methods should recognize that the designation interim does not relegate a method to an inferior class. Methods approved as interim official first action are but a vote of the Association away from adoption as official first action. The AOAC's best scientific minds, as represented by members of its various methods committees, have collectively reviewed and approved a method before it is designated interim official first action by the Official Methods Board. Those without the scientific background and knowledge to judge methods should accept the evaluation of the qualified experts involved in approving AOAC official methods.

The Official Methods Board would like to conclude this report by first thanking all of the volunteers involved in the methods validation process this year. Thanks are also in order for a job well done by 2 methods committee chairmen who conclude their terms of service: "Harry" B. S. Conacher, chairman of the Committee on Foods I; and Douglas J. Dube, chairman of the Committee on Hazardous Substances in Waste and the Environment.

The progress of the Official Methods Board and management of the collaborative study process would not be possible without the excellent staff support of Rita Bahner and her assistant, Michelle Glass. My many thanks for their tireless efforts and enduring patience.

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; I. H. Pomerantz; O. L. Shotwell; A. W. Tiedemann; C. H. Van Middelem

Recommendations for the Board of Directors

The Editorial Board recommends that the Board of Directors approve the American Chemical Society's proposal and contract to AOAC for mounting the *Journal of the Association of Official Analytical Chemists* on STN, International, under Chemical Journals Online, and according to the Business Plan presented by ACS to AOAC on Aug. 10, 1987.

Discussion

In 1987, the Editorial Board held a meeting in Washington, DC, on March 9–10, and a meeting in San Francisco, CA, on September 12. The following report highlights both the March and September meetings.

K. W. Boyer presented a report on alternative means of publishing *Official Methods of Analysis,* proposing that publication of AOAC methods by magnetic or optical disc media be postponed until compact disks become more widely available and used in personal computers. Publishing *Official Methods of Analysis* both in one volume and in separate sections (e.g., microbiological methods) was recommended. Peripheral chapters (e.g., on safety) would be included in each separate section. Sections would be made available only to purchasers of the complete volume; the shorter versions are intended to encourage use by laboratories pursuing specific areas of interest. The sections would include chapters arranged in accordance with the responsibilities of the Methods Committees. The AOAC publishing staff will work with the editor of the 15th edition and the Editorial Board committee to define chapter(s) to be grouped in sections and to evaluate economic impact.

The status of 13 publications was reviewed. Reviewers' comments were received on a revised text of Garfield's *Quality Assurance Principles for Analytical Laboratories,* and completion of a final manuscript is feasible by next spring. Garfield's new book, "Guide to Sampling Food, Drug and Agricultural Commodities," will be offered to reviewer(s) in its final draft in a few weeks. The Youden and Steiner statistical manual has been reprinted "as is" with a minor revision, as sales have continued at a steady rate. Wernimont's book, *Use of Statistics to Develop and Evaluate Analytical Methods,* has been very successful and will be reprinted. The book won the 1987 Youden Award of the American Statistical Association. A new AOAC statistical manual is being prepared under the direction of the AOAC Committee on Statistics and is slated for review jointly with the Editorial Board next spring.

Irwin Pomerantz presented a draft report of the Journal Review and Evaluation Committee, to be finalized in the next 2 months. *Journal* readers, authors, and reviewers and AOAC members responded to a questionnaire about the *Journal,* expressing satisfaction with the present contents and format. However, based on this survey and on an opinion poll of the *Journal* editors, the journal committee is expected to recommend that the *Journal* become a monthly publication as soon as possible. The *Journal* staff will prepare an "issues and options" paper concerning increased publication frequency. If it is decided to publish the *Journal* monthly, the page limit will probably be increased.

A policy for recruiting and appointing new *Journal* section editors, as vacancies occur, was approved. Also approved was a description of duties and responsibilities of *Journal* section editors. The description is to be shared with a new editor and his/her supervisor at the time of appointment. All future *Journal* section editors will be appointed for terms of 6 years (renewable). Annual performance appraisals will continue and present editors will continue their open-ended tenures.

Page charges were instituted for the *Journal* in late 1986. Early returns on this initiative are mixed; more time is needed for an assessment of the effects on costs and revenues. Page charges were confirmed for methods development papers leading to collaborative studies; collaborative study reports which are denied official status, but are deemed suitable for publication through peer review are exempt.

Evaluation of a proposal from the American Chemical Society to place the *Journal* full text on-line within the Scientific Technical Network (STN) led to a recommendation that the Board of Directors approve the proposal and contract. Readership and prestige of the *Journal* are expected to increase by its association, within STN, with many other leading ACS and international chemistry journals. Extension to the AOAC *Official Methods of Analysis* will be considered.

Increasing *Journal* publication frequency and placing the *Journal* online have strong implications of increased revenue through increased advertising income and increased readership. Publishing separate sections of *Official Methods of Analysis* would also increase its usefulness; however, appropriate printing and marketing strategies need to be developed.

Other topics covered were acquisition and review of special material for the *Journal,* such as review papers, editorials, and technical commentaries (advice to be sought from the editors); minor changes to the wording of the Editorial Board Terms of Reference; desktop publishing; and possibilities of publication exchanges with libraries and publishers outside the United States. In connection with desktop publishing, a proposal that AOAC consider publishing a "Laboratory Bulletin Board" will be studied.

Archives Committee

CHARLOTTE A. BRUNNER, *Chairman*
Food and Drug Administration,
Division of Cardio-Renal Drug Products,
Rockville, MD 20857

Other Members: W. V. Eisenberg; W. Landgraf; R. Pierce; H. L. Reynolds; E. Sarnoff; H. M. Stahr

Recommendations for the Board of Directors

A. The committee recommends that chairmen of AOAC committees and regional sections be requested to furnish the Archives Committee with copies of all materials that might be of historical interest and do not get published in the *Journal of the AOAC.*

B. The committee recommends that the Board of Directors approve funding to cover the cost of taping and transcribing oral histories, copying old documents and photocopies, shipping costs, conference calls, and a booth at the 1988 annual international meeting.

Discussion

The committee met on Wednesday morning, September 16, 1987, at the Cathedral Hill Hotel, San Francisco. Committee members gave informal progress reports on their activities during the past year. Dr. Stahr held discussions with Salsbury Laboratories, one of the earliest industry laboratories to develop and validate methods. They are willing to cooperate. He also arranged to obtain AOAC materials from Dr. Gehrke, and he and Wynne Landgraf will sort and prepare the material for delivery to the archives at Iowa State. He has also held discussions with the state experiment station and the state department of agriculture but neither organization could identify AOAC materials. Dr. Stahr talked to some former Wiley Award winners and asked for their cooperation. It was suggested that a search be made of the AOAC files on Wiley Award winners and AOAC Fellows for historical materials. Wynne Landgraf contacted the Society of Feed Microscopists and obtained a few items. Their historian has promised to look for AOAC-related materials. It was suggested that Dr. Walls of the University of Maryland would be a good source.

Dr. Eisenberg has been in contact with Wallace Janssen, the FDA historian, but most of that material is oriented to FDA rather than to AOAC. He plans to get in touch with retired FDA employees who were formerly active in AOAC. He will also approach the Food and Drug Law Institute.

Charlotte Brunner and Helen Reynolds have continued their examination of AOAC files for archives materials. They have been examining the AOAC Centennial files; when that is completed, they will look at the administrative files, such as financial reports, minutes of Board of Directors and Editorial Board meetings, and formation of committees and regional sections.

The proposed questions for use in obtaining oral histories were reviewed. One question was added, namely, "What do you consider your chief contribution to AOAC?" Names were suggested as candidates for oral histories, and committee members were assigned the task of obtaining the oral histories.

The Archives Committee booth at the 1987 meeting was discussed. The committee felt that the effort had been worthwhile. A number of members have been made aware of the existence and purpose of the committee, and several people agreed to furnish materials or to get in touch with persons who might have materials to contribute. Ideas for a booth at the 1988 meeting were discussed.

To achieve our goals, in addition to the various suggestions listed above, we will try to obtain more volunteers and members in the Washington, DC, area who will help with the time-consuming task of going through AOAC files. We are also asking that the various standing committees and regional sections be requested to regularly furnish the Archives Committee with copies of their documents for possible inclusion in the archives. It is hoped that these various efforts will eventually produce the kind of archival materials we need.

Committee on the Constitution

AUDREY V. GARDNER, *Chairman*
New York State Agricultural Experiment Station, Geneva, NY 14456

Other Members: W. R. Bontoyan; R. H. Bowers; A. Conetta; E. J. DeVries; C. A. Geisler; J. B. Kottemann; J. P. Minyard; R. J. Ronk; A. W. Tiedemann; B. Woodward

Recommendation to the Board of Directors

The Committee on the Constitution recommends that the Board of Directors consider amending the AOAC bylaws to: (*1*) incorporate all references to the Official Methods Board in a separate article; also to be considered are the proposed changes which have been suggested by the Official Methods Board; and (*2*) place all references to voting in a separate article.

Basis for Recommendations

The Official Methods Board has requested that the Board of Directors consider several amendments, including that the Official Methods Board determine the duties of Associate Referees, General Referees, and statistical consultants; that the size of each methods committee be increased to a minimum of 7; that the right of the Board of Directors to determine the number and tenure of General Referees be removed; and that the methods appeal procedure be formalized. It would also be advantageous to have all sections of the bylaws that relate to the Official Methods Board placed in one article for clarification and accessibility.

The various references to voting in the association are scattered throughout the current bylaws. It would be advantageous to have all these references placed in one article for clarity and easy reference. These would include voting on amendments to the bylaws, adoption of methods, acceptance of committee reports, election of officers, setting of fees, and other substantive matters by mail ballot, by proxy, or at the annual business meeting.

Discussion

The committee met on September 14, 1987, during the AOAC Annual International Meeting in San Francisco, CA. The 5 proposed bylaw amendments and their related housekeeping amendments, as approved by the Board of Directors,

were reviewed and discussed at length. The text of the proposed amendments is appended.

Members of the committee would be available during the meeting to inform association members of the amendments to be voted on at the business meeting on September 17.

The procedures to be followed for accepting and validating proxy votes, presenting the amendments for vote at the business meeting, and tabulating the voting results were reviewed.

The chairman wishes to thank the committee members for the extra effort necessary to complete the many tasks of the past year. Special thanks to Margaret Ridgell of the AOAC office for her assistance and encouragement in meeting deadlines, and to Virginia Schlottshauer, AOAC parliamentarian, for her expertise and guidance.

BYLAWS

Revised September 17, 1987

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.

[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 and Standing Committees, of Liaison Officers, and of General Referees
–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
Referees and Committees

Section 1. 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 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. 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.
B. *Committee Assignments.* Each Committee shall consist of at least four (4) 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 4. 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 5. 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 6. 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 7. Long-Range Planning Committee

The Committee shall make recommendations to the Board of Directors on improving the functions of the Association.

ARTICLE IX
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. A business meeting shall be held during the annual meeting, for voting on matters relating to the analytical methods and other business transactions of the Association.

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.

Section 3. Proxies

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.

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. 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

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 XI

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 XII

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 resident and working within the geographical boundaries of the section. Other individuals interested in the purpose of the regional section and resident 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 XIII

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 XIV

Amendments to the Bylaws

All proposed amendments of these bylaws shall be presented in writing to the Board of Directors at least ninety (90) days in advance of a meeting of the Association. The Board shall present the proposals to the Association membership, with recommendations, not later than the second day of a meeting of the Association, and submit them to the membership as the first order of business at the business meeting. A three-fourths vote of the Members voting by mail ballot is required for adoption.

AMENDMENT I

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.

Committee on Instrumental Methods and Data Handling

KENNETH R. HILL, *Chairman*
U.S. Department of Agriculture,
Agriculture Research Service,
Beltsville, MD 20705

Other Members: P. F. Kane, Vice Chairman; R. L. Beine; J. V. Bruno; W. Furman; T. Gale; L. Gelber; H. G. Lento; G. MacEachern; M. Margosis; H. M. McNair; W. J. Morris; W. A. Trujillo; P. Volk; M. Walters; R. S. Wayne

Recommendations for the Board of Directors

A. It is recommended that the Subcommittee on Instrumental Specifications be continued as the Subcommittee on Instrumental Test Methods, reporting to and directly responsible to the Official Methods Board. Support for this motion is contained in the attached status report by W. Furman.

B. It is moved that the former Subcommittee on Automated Methods and Equipment be replaced by the new Method Advisory Subcommittee on Instrumental Performance reporting to and directly responsible to the Official Methods Board. The proposed objectives of this group are contained in a document prepared by P. Kane. The exact composition and terms of reference for this subcommittee will be developed through future discussions with the Official Methods Board.

Discussion

The final meeting of the Instrumental Methods and Data Handling Committee was held on September 14, 1987, in San Francisco, CA, to review the activities of the subcommittees for the preceding year.

The Subcommittee on Instrumental Specifications reported that draft manuscripts on "AOAC Instrument Specifications" and "Proposed AOAC Instrument Specifications: Ultraviolet-Visible Spectrophotometers" are ready for submission to the *Journal*. Other test methods under development for the following instruments are expected to be ready for publication in the indicated time spans: differential pulse voltammeters (ca 6 months), analog instrument output (ca 6 months), pH meters (ca 6 months), analytical balances, single pan (ca 1 year), and infrared spectrophotometers (ca 2 years). The subcommittee recommended that its name be changed to Committee or Subcommittee on Instrument Test Methods to reflect more accurately the nature of its work and that it continue to function as a unit of the Official Methods Board. Guidance from the Official Methods Board would be sought on plans to (*1*) publish new instrument test methods in the *Journal* in the form of contributed papers, corresponding in function to Associate Referee reports, with recommendation for adoption by AOAC; (*2*) receive approval by vote of the association at the annual business meeting as is now done for new methods; and (*3*) publish approved instrument test methods in the *Journal* as "Changes in Official Methods" and then accumulate them as a new chapter in *Official Methods of Analysis*.

The Subcommittee on Gas and Liquid Chromatographic column Specifications reported on progress on the classification of C-18 LC columns through the publication (*J. Assoc. Off. Anal. Chem.* [1987] **70,** 465–469) of the paper entitled "Classification of Octadecyl-Bonded Liquid Chromatography Columns" by Milda J. Walters. The following proposals were submitted for consideration: (*1*) Submit column test procedures for review by the Official Methods Board with the goal of including them in the proposed new chapter for the *Official Methods of Analysis*. (*2*) Recommend that method developers (Associate Referees) test the C-18 columns suitable for their methods by the proposed scheme and submit the data to the Official Methods Board for inclusion in the classification table. (*3*) Recommend that the format for column specifications in a method take the following general form: *LC column.*—(Approximate dimensions) packed with octadecyl-bonded silica microparticle stationary phase having an HP Index of 4.0–4.5 and an SiOH Index < 0.9. *System Suitability.*—The LC system is suitable if the analyte peak elutes in 8–10 min, the %RSD for the peak responses of 5 injections of standard solution is $<1.5\%$, and the analyte-internal standard R is >2.0 (or $T < 1.5$ and $n > 3000$ for single ingredient without internal standard). (The numerical values are used only as examples and those appropriate to the particular method would be included by the author.) (*4*) Accumulate data on more brands of C-18 columns and make this information available through publication in the *Journal.*

Members of the Subcommittee on Laboratory Computers and Robotics reaffirmed their previous recommendations for a new approach to specifying weights and volumes and for a policy for dealing with robotic modifications to existing official methods. R. Beine agreed to continue work directly with the Official Methods Board on clarifying any issues arising from these recommendations.

The liaison member for the International Organization of Legal Metrology (OIML) attended meetings of the U.S. national working group PS17 and task group RS4 on March 13, 1987, May 20, 1987, and June 25, 1987. Draft recommendations for performance specifications for gas chromatographs and gas chromatograph/mass spectrometers were approved by the international working group for PS17 and were ready for a vote by the international committee of OIML on August 31, 1987. The initial draft recommendations for atomic absorption spectrophotometers, high performance liquid chromatographs, and portable instruments for airborne pollutants were reviewed by the international working group last September and new drafts should be available for comment soon. A meeting of the task group on liquid chromatographic systems was held on May 20, 1987, at AOAC headquarters and several significant changes were made on the second draft in response to questions arising from the international working group on PS17 which was held in Paris. Included were proposed test chemicals, concentrations, and acceptable detection limits for UV, fluorescence, electrochemical, and conductivity detectors. At the June 25th meeting of the PS17 in Washington, DC, it was determined that there would be no copyright restriction on the use of OIML specifications by AOAC; the AOAC liaison member suggested that PS17 consider the preparation of an article explaining the work of OIML and the U.S. national working group on PS17 for *The Referee*.

Committee on Interlaboratory Studies

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; T. Dols; R. L. Ellis; R. L. Epstein; D. W. Fink; J. Gallagher; M. Ihnat; S. E. Katz; P. C. Kelly; C. J. Kirschmer; T. P. Layloff; M. Margosis; K. A. McCully; A. Munson; J. O'Rangers; H. S. Rageb; R. C. Rund; S. Sherken; E. Smith; J. Springer; W. Stellar; L. Stoloff; B. K. Thompson; M. Thompson; J. S. Winbush; J. Winter; L. R. Williams; E. S. Windham; R. Wood

Recommendations for the Board of Directors

A. The committee recommends that the Board approve the document "Guidelines for Collaborative Study Procedure to Validate Characteristics of a Method of Analysis" attached as an Appendix.

B. The committee recommends that the Board of Directors endorse the continued participation of the committee in the additional efforts of the International Union for Pure and Applied Chemistry (IUPAC) to obtain harmonization of quality assurance systems in chemical analysis and harmonization of the presentation of the results obtained by collaborative studies and by actual analysis.

C. The committee recommends that the board approve the participation of AOAC as a cosponsor of a symposium on "Harmonization of Quality Assurance Systems" and a workshop on "Adoption and Presentation of the Results Obtained by Methods Standardized by Collaborative Study" for 1989.

Discussion

A. As a result of almost 5 years of work which began in 1980, this committee produced a guideline for the design, conduct, and analysis of AOAC collaborative studies that was approved by the Board of Directors. Based upon this document, a discussion paper of the issues requiring harmonization was prepared by the chairman with his recommendations for specific actions. These recommendations were the basis for a discussion by interested organizations at a workshop held in Geneva, Switzerland, May 4–5, 1987, and a final report containing modified recommendations was produced. This report was circulated to the participants and the revised recommendations were used to further modify the original AOAC guidelines. These guidelines have been reviewed by the committee at meetings held in Washington (September 1, 1987) and in San Francisco (September 13, 1987) and by the Statistics Committee on September 14, 1987, and further revised, primarily editorially. If approved, these AOAC guidelines may be designated as in accord with the "IUPAC-1987 recommendations."

The most important changes from the original guidelines are the greater emphasis on preliminary work by the Associate Referee prior to the initiation of the collaborative study; increasing the size of the usual study to 5 materials and 8 laboratories, using blind duplicates or split levels (Youden pairs); and the application first of the Cochran outlier test for extreme range of replicates within a laboratory, followed by the Grubbs tests for extreme laboratory averages. Outliers are removed only if they exceed the 1% critical value.

B. The IUPAC organizing committee considers the work so important that it wishes to continue the harmonization effort in 2 directions: developing quality assurance/quality control protocols and expressing the results of collaborative studies.

Review of the reports of the AOAC Committee on Laboratory Quality Assurance indicates that AOAC does not have such a protocol. The closest document to this is Garfield's *Quality Assurance Principles for Analytical Laboratories.*

The participants are aware that the collaborative study develops one set of precision parameters (and sometimes bias parameters) but that a different set of parameters must be used when these methods are applied in actual practice. ISO uses their repeatability and reproducibility critical values for this purpose (i.e., how close 2 values by the same analyst [repeatability] or by different laboratories [reproducibility] should check each other 19 times out of 20). AOAC uses ranges of means and standard deviations derived from the collaborative study, which have little applicability to actual practice.

C. The symposium conducted under the cosponsorship of AOAC during its centennial celebration at the National Academy of Sciences was considered to be extremely successful, so much so that the IUPAC organizing committee naturally turned to the AOAC again for continued participation. AOAC has the experience and competence to conduct such a meeting.

Benefits to AOAC

Other organizations, particularly those that are just beginning to use the collaborative study procedure, look to AOAC as a model for instructions as to the proper design, conduct, and performance of collaborative studies. The promulgation of this document, which has had the benefit initially of comment from scientists from all over the world as well as from within AOAC, has now been fine-tuned to correspond with the consensus of numerous other organizations with experience in collaborative studies. If AOAC approves this document at this meeting, it can claim the distinction of being the first organization formally to approve a protocol that can state that it is in conformity with the IUPAC-1987 recommendations. It will then have a document that can be distributed to all Associate Referees, new and old, and to methods committee statisticians and reviewers as the statement of AOAC policy on the design, conduct, and performance of collaborative studies.

By utilizing the efforts of other organizations in the area of quality assurance/quality control and expression of results, AOAC can concentrate on areas where it has had the most experience. Some of these areas might be further application to microbiological assays and to qualitative tests (yes-no decisions) where a number of collaborative studies have been published in recent years.

Cosponsorship of the proposed symposium will further enhance recognition of AOAC as one of the leaders in the area of collaborative studies of methods and will extend it into the even more important areas of quality assurance and quality control, which have become matters of societal concern in recent years.

Guidelines for Collaborative Study Procedure to Validate Characteristics of a Method of Analysis

Fourth (Final) Draft

(Incorporates symbols, terminology, and recommendations accepted by consensus by the participants at the IUPAC Workshop on Harmonization of Collaborative Analytical Studies, Geneva, Switzerland, May 4–5, 1987.)

This document has been prepared from the point of view of AOAC administrative arrangements. In AOAC, the Associate Referee is the individual scientist responsible for choosing the method, conducting intra- and interlaboratory tests, evaluating the results, and recommending approval of a method of analysis. Other organizations who may utilize this document may wish to substitute their own terminology. Although the directions were developed for chemical studies, some parts may be applicable to all types of collaborative studies.

Summary Statement of AOAC Recommendation for the Design of a Collaborative Study:

1. *Minimum number of materials:* 5 (only when a single level specification is involved for a single matrix may this minimum be reduced to 3).
2. *Minimum number of laboratories:* 8 reporting valid data for each material (only in special cases involving very expensive equipment or specialized laboratories may the study be conducted with an absolute minimum of 5 laboratories, with the resulting expansion in the confidence interval for the statistical estimates of the method characteristics).
3. *Minimum number of replicates:* 1, if within-laboratory repeatability parameters are not desired; 2, if these parameters are required. Replication should ordinarily be attained by blind replicates or split levels (Youden pairs).

Guide to Collaborative Study Procedure

(Section numbers correspond to Outline; not all section numbers are included in guide.)

1. Preliminary Work
   - 1.1 Determine purpose of method
   - 1.2 Choose method
   - 1.3 Optimize method
   - 1.5 Prepare description of method
   - 1.6 Invite participation
2. Design of Collaborative Study
   - 2.1 General principles
   - 2.2 Laboratories
   - 2.3 Materials
   - 2.4 Replications
3. Preparation of Materials
4. Submission of Materials
5. Obligations of Collaborators
6. Statistical Analysis
   - 6.1 Initial review of the data
   - 6.3 Outliers
   - 6.4 Bias (systematic error)
   - 6.5 Precision (random error)
     - 6.5.1 Reproducibility
     - 6.5.2 Repeatability
   - 6.6 False positive and false negative values
7. Final Report
8. References

1. Preliminary Work (Within One Laboratory)

1.1 Determine Purpose and Scope of the Study and Method

Determine purpose of the study (e.g., to determine attributes of a method, proficiency of analysts, reference values of a material, or to compare methods), the type of method (empirical, screening, practical, reference, definitive), and the probable use of the method (enforcement, surveillance, monitoring, acceptance testing, quality control, research). Also, on the basis of the relative importance of the various method attributes (bias, precision, specificity, limit of determination), select the design of the interlaboratory study. The directions in this document pertain primarily to determining the precision characteristics of a method, although many sections are also appropriate for other types of studies.

1.2 Alternatives for Method Selection

1.2.1 Sometimes obvious (only method available)
1.2.2 Critical literature review (reported within-laboratory attributes are often optimistic)
1.2.3 Survey of laboratories to obtain candidate methods; comparison of within-laboratory attributes of candidate methods (sometimes choice may still not be objective)
1.2.4 Selection by expert (AOAC-preferred procedure—selection by Associate Referee with concurrence of General Referee)
1.2.5 Selection by Committee (ISO-preferred procedure—often time-consuming)
1.2.6 Development of new method or modification of existing method when an appropriate method is not available. (Proceed as a research project.) (This alternative is time-consuming and resource-intensive; use only as a last resort.)

1.3 Optimize Either New or Available Method

1.3.1 Practical principles
(a) Do not conduct collaborative study with an unoptimized method. An unsuccessful study wastes a tremendous

amount of collaborators' time and creates ill will. This applies especially to methods that are formulated by committees and have not been tried in practice.

(b) Conduct as much experimentation within a single laboratory as possible with respect to optimization, ruggedness, and interferences. Analysis of the same material on different days provides considerable information on variability that may be expected in practice.

1.3.2 Alternative approaches to optimization

(a) Conduct formal ruggedness testing for identification and control of critical variables. See Youden and Steiner (8.1, pp. 33–36, 50–55). The actual procedure is even simpler than it appears. (This is an extremely efficient way for optimizing a method.)

(b) Use Deming simplex optimization to identify critical steps. See Dols and Armbrecht (8.3).

(c) Conduct trials by changing one variable at a time.

1.4 Develop Within-Laboratory Attributes of Optimized Method

(Some items can be omitted; others can be combined.)

1.4.1 Determine calibration function (response vs concentration in pure or defined solvent) to determine useful measurement range of method. For some techniques, e.g., radioimmunoassay, linearity is not a prerequisite. Indicate any mathematical transformations needed.

1.4.2 Determine analytical function (response vs concentration in matrix, including blank) to determine applicability to commodity(ies) of interest.

1.4.3 Test for interferences (Specificity):

(a) Test effects of impurities, ubiquitous contaminants, flavors, additives, and other components expected to be present and at usual concentrations.

(b) Test nonspecific effects of matrices.

(c) Test effects of transformation products, if method is to indicate stability, and metabolic products, if tissue residues are involved.

1.4.4 Conduct bias (systematic error) testing by measuring recoveries of analyte added to matrices of interest and to extracts, digests, or other treated solutions thereof. (Not necessary when method itself defines the property or component.)

1.4.5 Develop performance specifications for instruments and suitability tests for systems (which utilize columns or adsorbents) to ensure satisfactory performance of critical steps (columns, instruments, etc.) in method.

1.4.6 Conduct precision testing at the concentration levels of interest, including variation in experimental conditions expected in routine analysis (ruggedness).

In addition to estimating the "classical" repeatability standard deviation, s_r, the initiating laboratory may estimate the total within-laboratory variability expected by determining the variability at different days and with different calibration curves, by the same or different analysts within a single laboratory. This total within-laboratory estimate includes both between-run (between-batch) and within-run (within-batch) variability.

1.4.7 Delineate the range of applicability to the matrices or commodities of interest.

1.4.8 Compare the results of the application of the method with existing tested methods intended for the same purposes, if other methods are available.

1.4.9 If any of the preliminary estimates of the relevant performance of these characteristics are unacceptable, revise the method to improve them, and retest as necessary.

1.4.10 Have method tried by analyst not involved in its development.

1.4.11 Revise method to handle questions raised and problems encountered.

1.5 Prepare Description of Method

Note: A collaborative study of a method involves practical testing of the written version of the method, in its specific style and format, by a number of laboratories on identical materials.

1.5.1 Prepare method in format and style given in the *Handbook for AOAC Members* (1982), or other recognized manual, e.g., *ISO Guide 18* (8.4).

1.5.2 Clearly specify requirements for chromatographic materials, enzymes, antibodies, and other performance-related reagents.

1.5.3 Clearly describe and explain every step in the analytical method so as to discourage deviations. Use imperative directions; avoid subjunctive and conditional expressions as options as far as possible.

1.5.4 Edit method for completeness, credibility (e.g., buffer pH consistent with specified chemicals, volumes not greater than capacity of container), continuity, and clarity.

1.5.5 Check for inclusion of performance specifications and systems suitability tests (1.4.5), defined critical points, and convenient stopping points. Incorporate physical or chemical constants of working standard solutions, e.g., absorptivities, half-scale deflections, recoveries, etc., or properties of operating solutions and chromatographic materials, e.g., pH, volumes, resolution, etc., and any other indicators (e.g., sum equals 100%) that suggest analysis is proceeding properly.

1.5.6 If time and resources are available, conduct pilot study involving 3 laboratories.

1.6 Invite Participation

1.6.1 *Selection of candidate laboratories.* Laboratories invited to participate should have personnel experienced in the basic techniques employed; experience with the method itself is not a prerequisite for selection. Lists of possible participants can be developed through personal contacts, technical societies, trade associations, or literature search, and advertisements in the AOAC's news publication "The Referee."

1.6.2 *Letter of invitation.* Address a formal letter to the individual responsible for assignment of laboratory effort. State reason for selecting that laboratory (e.g., as a volunteer or has responsibility or familiarity with the problem or method), estimated number of person-hours required for performance, number of materials to be sent, number of analyses to be required, expected date for material distribution, and target date for completion of the study. *Emphasize the importance of management support in assigning the necessary time for the project.* Enclose a copy of the method and a return form or card (with postage affixed, if domestic), requiring only a check mark for acceptance or refusal of the invitation, a signature, and space for address corrections, telephone number, and date.

With large studies, involving several analysts per laboratory, several familiarization materials, receipt of items at different times, or similar recurrent situations, acceptance of the invitation should be followed by a letter suggesting that a study coordinator be appointed. The study coordinator should be responsible for receiving and storing the materials, assigning the work, dispensing materials and information related to the study, seeing that the method is followed as written, accumulating the data, assuring that the data are correctly reported, and submitting the report within the deadline.

(A file of letters previously used for these purposes is available.)

1.7 Instructions and Report Forms

1.7.1 Carefully design and prepare instructions and forms, and scrutinize them before distribution. A pilot study (1.5.6) is also useful for uncovering problems in these documents.

1.7.2 Send instructions and report forms immediately on receipt of acceptance, independent of materials, if selection of laboratories is not to be based on performance in pilot or training studies. The instructions should include in bold face or capital letters a statement: "THIS IS A STUDY OF THE METHOD, NOT OF THE LABORATORY. THE METHOD MUST BE FOLLOWED AS CLOSELY AS PRACTICABLE, AND ANY DEVIATIONS FROM THE METHOD AS DESCRIBED, NO MATTER HOW TRIVIAL THEY MAY SEEM, MUST BE NOTED ON THE REPORT FORM."

1.7.3 Include instructions on storage and handling, markings, and identifications to be noted, any special preparation for analysis, and criteria for use of practice or familiarization materials, if included (*see* 1.8). Precode the form for each laboratory and provide sufficient space for as much sequential data as may be required for proper evaluation of the results, including a check of the calculations.

Indicate the number of significant figures to be reported.

When recorder tracing reproductions are required to evaluate method performance, request their submission both in the instructions and as a check item on the form. Provide instructions with regard to labeling of recorder tracings, such as identification with respect to item analyzed, axes, date, submitter, experimental conditions, and instrument settings.

Include in the report form a signature line for the analyst and lines for a printed or typed version of the name and address for correct acknowledgment. Provide for a review by the laboratory supervisor. An example of a completed form is helpful. A questionnaire may be included or sent after completion of the analyses in which the questions can be designed to reveal if modifications have been made at critical steps in the method.

1.7.4 Request a copy of the calibration curve or other relationship between response and concentration or amount of analyte so that if discrepancies become apparent after examining all of the data, it can be determined whether the problem is in the calibration or in the analysis.

1.8 Familiarization or Practice Materials

If deemed necessary, supply as far ahead as practicable, familiarization materials, with instructions, before actual materials are sent. When familiarization materials have been submitted, supply forms for reporting progress toward satisfactory performance.

2. Design of the Collaborative Study

2.1 General Principles

2.1.1 The purpose of the collaborative study is to provide a realistic estimate of the attributes of a method, particularly the systematic and random deviations, to be expected when the method is used in actual practice. A collaborative study usually provides information on the best performance to be expected.

2.1.2 The design should attempt to identify and to include the possible sources of significant variability that may occur in actual practice, including between days, between runs, and between calibration curves, if these are significant factors. (Within-laboratory performance, 1.4.6, particularly on different days and with different calibration curves may provide a clue with respect to between-laboratory performance and is required infor-

mation for quality control.) The best measure of within-laboratory variability is obtained by using blind replicates or split levels (Youden pairs). The design must take into account how the data will be analyzed statistically.

2.1.3 Present materials sent for analysis as unknowns (blind) and coded in a random pattern. If necessary to conserve analyst time, an indication of the potential range of concentration or amount of analyte may be provided. If spiking solutions are used, provide one coded solution for each material. All spiking solutions should be identical in appearance and volume. Do not provide a single solution from which aliquots are to be removed for spiking. Any information with regard to concentration (e.g., utilizing factorial aliquots or serial dilutions of the same spiking solutions) or known replication is likely to lead to an underestimate of the variability.

2.1.4 The study must be extensive enough to assure sufficient data surviving in the face of possible loss of materials during shipment, inability of collaborators to participate after acceptance, and a maximum outlier rate of 2/9 (2 laboratory failures in a 9-laboratory study, when a minimum design is used).

2.1.5 Improper preparation of reference standards and standard solutions can cause a significant portion of the analytical error. A decision must be made whether such error is to be considered separately or as part of the method, i.e., will the analysts procure their own standards and prepare their own standard solutions or will standards be provided by the Associate Referee. The decision depends primarily on the availability of the standard. If the standard is readily available, the analysts should prepare their own. If the standard is not readily available, the standard may be supplied, but physical constants, e.g., absorptivity, of working standard solutions should be incorporated into the description as a check on proper preparation of the solution.

2.1.6 Obtain the necessary administrative and operational approvals. Review by potential users of the method is also desirable.

2.2 Laboratories

Laboratories must realize the importance of the study. A large investment is being made in testing the method and this probably will be the only collaborative study of the method that will be performed. Therefore, it is important to have a fair and thorough evaluation of the method.

2.2.1 *Type.* The most appropriate laboratory is one with a responsibility related to the analytical problem. Laboratory types may be representative (selection of laboratories that will be using the method in practice), reference (assumed to be "best"), or the entire population of laboratories (usually certified or accredited) that will be using the method. Final selection of participants should be based on a review with the General Referee and other Associate Referees of each laboratory's capabilities and past performance in collaborative studies, followed up, if possible, by telephone conversations or by personal visits. Selection may also be based on performance with familiarization materials. Sometimes only laboratories with dedicated or very specialized instruments must be used. If the study is intended for international consideration, laboratories from different countries should be invited to participate.

2.2.2 *Number of laboratories:* Minimum of 8 laboratories submitting valid data (to avoid unduly large confidence bands about the estimated parameters). Only in special cases of very expensive equipment or specialized laboratories may the study be conducted with an absolute minimum of 5 laboratories. Fewer laboratories widen the confidence limits of the mean and of the variance components (*see* design considerations 2.4.1 and 2.4.2). The optimum number of laboratories, balancing logistics and costs against information obtained, often is 8–10. However, larger studies are not discouraged.*

2.2.3 *Analysts:* Most designs require only 1 analyst per laboratory. If analyst-within-laboratory variability is a desired variance component, multiple analysts should be requested from all participating laboratories. Ordinarily 2 analysts from the same laboratory cannot be substituted for different laboratories, unless standard solutions, reagents, chromatographic columns and/or materials, instrument calibrations, standard curves, etc., are prepared independently, and no consultation is permitted during the work. Different laboratories from the same organization may be used as separate laboratories if they operate independently with their own instruments, standards, reagents, and supervision.

2.3 Test Materials

2.3.1 *Materials must be homogeneous. This is critical.* Establish homogeneity by testing a representative number of laboratory samples taken at random before shipment. (A collaborator who reports an outlying value will frequently claim receipt of a defective laboratory sample.) The penalty for inhomogeneity is an increased variance in the analytical results that is not due to the intrinsic method variability.

2.3.2 *Code laboratory samples* at random so that there is no preselection from order of presentation.

2.3.3 *Concentration range:* Choose analyte levels to cover concentration range of interest. If concentration range of interest is a tolerance limit or a specification level, bracket it and include it with materials of appropriate concentration. If design includes the determination of absence of analyte, include blank (not detectable) materials as part of range of interest.

2.3.4 *Number of materials:* Minimum number of materials is 5. However, when a single-level specification is involved, this may be reduced to an absolute minimum of 3.

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* In some cases Associate Referees are unable to obtain more than 5 participants. In such cases a study may be conducted with 5 laboratories, but it must be realized that the reliability of the resulting estimates of performance parameters is reduced considerably. Furthermore, in such cases the number of test materials in the study should be increased to provide some additional assurance. but the analysis of more test materials is not a substitute for fewer laboratories. Methods adopted on the basis of such a design may not be acceptable to other organizations that have adopted the IUPAC-87 recommendations.

2.3.5 *Nature of materials:* Materials should be representative of commodities usually analyzed, with customary and extreme values for the analyte.

2.3.6 *Size of laboratory samples:* Furnish only enough material to provide the number of test portions specified in the instructions. If additional test portions are required, the collaborator must request them, with an explanation.

2.3.7 *Interferences:* If pertinent, some materials, but not all, should contain contaminants and interferences in concentrations likely to be encountered, unless they have been shown to be unimportant through within-laboratory testing (*see* 1.4.4). The success of the method in handling interference on an intralaboratory basis will be demonstrated by passing systems suitability tests.

2.3.8 *Familiarization materials:* With new, complex, or unfamiliar techniques, provide material(s) of stated composition for practice, on different days, if possible (*see* 1.8). The valuable collaborative materials should not be used until the analyst can reproduce the stated value of the practice samples within a given range. However, it should be pointed out that one of the assumptions of analysis of variance is that the underlying distribution of results is independent of time (i.e., there is no drift). The Associate Referee must be satisfied that this assumption is met.

2.4 Replication

When within-laboratory variability is also of interest, as is usually the case, independent replication can be ensured by applying at least one of the following procedures (listed in suggested order of desirability; the nature of the design should not be announced beforehand):

2.4.1 *Split levels (Youden pairs):* A pair of materials of slightly different composition obtained either naturally or by diluting (or by fortifying) one portion of the material with a small amount of diluent (or of analyte). Both portions are supplied to the participating laboratories under a random code number and each portion should be analyzed only once; replication defeats the purpose of the design.

2.4.2 *Split levels for some materials and blind duplicates for other materials* in the same study (obtain only single values from each portion supplied).

2.4.3 *Blind duplicate laboratory samples, randomly coded. Note:* Triplicate and higher replication are relatively inefficient when compared with duplicate test samples because replication provides additional information only on individual within-laboratory variability, which is usually the less important component of error. It is more effective to utilize resources for the analysis of more levels and/or materials rather than for increasing the number of replicates for the individual materials.
PRACTICAL PRINCIPLE: With respect to replication, the greatest net marginal gain is always obtained in going from 2 to 3 as compared to going from 3 to 4, 4 to 5, etc.

2.4.4 *Independent materials.* (*Note:* Unrelated independent materials may be used as a split level in the calculations of the precision parameters or for plotting, but the more they differ, particularly with respect to concentration, the less reliable the information they provide on within-laboratory variability.)

2.4.5 *Use of known replicates* is a common practice. It is much preferable to use the same resources on blind replicates or split levels. (*See Note* of 2.4.3.)

2.4.6 Instead of obtaining repeatability parameters through the collaborative study, *use of quality control materials in each laboratory individually,* for its own use, independent of the collaborative study, for a separate calculation of s_r, using 2 (or more) replicates from each quality control test, according to the pattern developed for each product.

2.5 Other Design Considerations

2.5.1 The design can be reduced in the direction of less work and less cost, but at the sacrifice of reduced confidence in the reliability of the developed information.

2.5.2 More work (values) is required if more confidence is needed, e.g., greater confidence is required to enforce a tolerance at 1.00 ppm than at 1.0 ppm. (The distinction is a precision requirement of the order of 1% rather than 10%.)

2.5.3 The estimate of the standard deviation and of the corresponding relative standard deviation is a random variable. For example, 30 data points from a single population will permit estimation of the standard deviation *of an individual reading* only to within ±25% with about 95% confidence; 200 data points are required to estimate that standard deviation to within about 10%, with about 95% confidence. The distribution of standard deviations is actually asymmetrical for small numbers of values, e.g., the relative distribution about 1.00 for 30 values (about 95% confidence limits) is 0.78–1.30; for 200, 0.91–1.10.

2.5.4 The validity of extrapolating the use of a method beyond concentrations and components tested can be estimated only on the basis of the slope of the calibration curve (sensitivity) observed as a function of the nature and concentration of the matrix and contaminant components. If the signal is more or less independent of these variables, a reasonable amount of extrapolation may be utilized. The extrapolator assumes the burden of proof as to what is reasonable.

3. Preparation of Materials for Collaborative Studies

3.1 General Principles

3.1.1 Heterogeneity between portions from a single test material must be negligible compared to analytical variability, as measured within the Associate Referee's laboratory. *See also* 2.3.1.

3.1.2 The containers must not contribute extraneous analytes to the contents, and they must not adsorb or absorb analytes or other components from the matrix, e.g., water.

3.1.3 If necessary, the materials may be stabilized, preferably by physical means (freezing, dehydrating), or by chemical means (preservatives, antioxidants) which do not affect the performance of the method.

3.1.4 Compositional changes must be avoided, where necessary, by the use of vapor-tight containers, refrigeration, flushing with an inert gas, or other protective packaging.

3.2 Materials Suitable for Collaborative Studies

3.2.1 *A single batch of homogeneous, stable product* such as milk powder, peanut butter, vegetable oil, starch, etc., is the best type of material.

3.2.2 *Reference materials* supplied by standards organizations such as the National Bureau of Standards are excellent, unless they have easily recognizable characteristics (e.g., odor and color of NBS Orchard Leaves). However, they are of limited availability, composition, and analyte level. If available, they are expensive. Sometimes the certification organization may be interested in making reference materials available for the analyte under study, in which case it may assist in providing the material for the study.

3.2.3 *Synthetic materials* may be especially formulated with known amounts of analytes by actual preparation for the study. This procedure is best used for macroconstituents such as drugs or pesticide formulations.

3.2.4 *Spiked materials* consisting of normal or blank materials to which a known amount of analyte has been added may be used. The amount of analyte added should not be excessive in relation to the amount present (e.g., about 2×), and the analyte added should be in the same chemical form as present in the commodities to be analyzed subsequently.

In drug and pesticide residue-type problems, it is often necessary to use spiked materials in order to assess recovery. However, because incurred residues are likely to present different problems from those of spiked residues, collaborative studies should include some incurred test samples to ensure that the method is applicable under these conditions as well.

(a) *Preparation in bulk:* This requires thorough and uniform incorporation of the analyte, often by serial dilution of solids. The danger of segregation due to differences in densities always exists. Fluid materials susceptible to segregation should be prepared under constant agitation. Uniformity should be checked by direct analysis, with an internal standard, or by a marker compound (dye or radioactive label).

(b) *Laboratory samples, individually prepared:* A known amount of analyte is either weighed directly or added as an aliquot of a prepared solution to premeasured portions of the matrix in individual containers. The collaborator is instructed to use each entire portion for the analysis, transferring the contents of the container quantitatively or a substantial weighed fraction of the portion. (This is the preferred alternative to spiked solid materials at trace [ppm] levels, at the expense of considerably more work.)

(c) *Concentrated unknown solutions for direct addition by collaborators to their own commodities:* Should be used only as a last resort when instability of the analyte precludes distribution from a central point. To preclude direct analysis of the spiking solution, supply individual coded solutions to be added in their entirety to portions of the matrix for single analyses by each laboratory. All solutions should have the same volume and appearance. This type of material is analogous to that of 3.2.4(b) except for the source of matrix. This case should be used only for perishable commodities that are altered by all available preservation techniques.

3.2.5 *Materials analyzed by another, presumably accurate, method,* if available, in the Associate Referee's laboratory or by some or all the collaborators.

3.2.6 *Only as an absolutely last resort (usually with unstable materials and preparation of material studies) should the collaborators be permitted to prepare their own materials* for analysis. Since it is impossible to avoid the personal bias introduced by knowledge of the composition of the material, the materials should be prepared in each laboratory by an individual who will not be involved in the analyses.

3.3 Blanks

When the absence of a component is as important as its presence, when determinations must be corrected for the amount of the component or the presence of background in the matrix, or when recovery data are required, provision must be made for the inclusion of blank materials containing "none" (not detected) of the analyte. It is also important to know the variability of the blank and the tendency of the method to produce false positives. There are 2 types of blanks: matrix blanks and reagent blanks. Since laboratories often will utilize reagents from different sources, each laboratory should perform reagent blanks. Matrix blanks, when required, are an intrinsic part of the method, and the number of blanks needed depends on the combined variance of the material (s_M) and of the blank (s_B). The total variability of a blank corrected value will be $s = (s_M^2 + s_B^2)^{1/2}$.

3.4 Limit of Determination

If the limit of determination is important, it is necessary to provide a design which gives special attention to the number of blanks, and to the necessity for interpreting false positives and false negatives. In all cases, the definition of limit of determination used in the study must be given by the Associate Referee.

3.5 Controls

When separation from interferences is critical to the analysis, appropriate materials incorporating these interferences must be included.

3.6 Practical Principle

Always allow for contingencies and prepare more sets (e.g., 25% more) of laboratory samples than there are collaborators. Some packages may never arrive, some materials may spoil, and some may be lost or the container broken. New laboratories may have to be substituted for those which are unable to complete the promised work. Some sets may have to be analyzed at a later time for different purposes, such as to verify stability on storage.

4. Submission of Laboratory Samples

4.1 *Label laboratory samples legibly and without ambiguity.*

4.2 *Pack shipping cartons well and label properly to avoid transportation delays.* If the containers are breakable, pack well to minimize possibility of breakage. If material is perishable, ship frozen with solid CO_2, sufficient to last several days longer than anticipated travel time. Notify collaborators of shipping arrangements, including waybill numbers, arrival time, and required storage conditions. Use special transportation services, if necessary. For international delivery, mark as "Laboratory samples—no commercial value" or other designation as required by customs regulations of the country to which the package is being sent. Hazardous materials must be packed and labeled as required by transportation regulations. Animal and plant products sent across international borders may require special certification from health authorities.

4.3 *Include a return slip, to confirm safe receipt, with each package.* If not sent previously, include copy of method, instructions, and report forms.

4.4 *Provide instructions for proper storage* of laboratory samples between unpacking and analysis. Do not use thawed or decomposed test samples without consulting the Associate Referee.

4.5 *When it is important to have instruments calibrated with the same reference material,* supply reference material to collaborators. Provision for supplying reference standards is particularly important when commercial sources of standards have not yet been developed. The inclusion of a working standard solution as an unknown is useful to establish a consensus value for standardization of quality control parameters, such as absorptivity, retention time, and sensitivity (change in signal intensity divided by the change in concentration).

5. Obligations of Collaborators

5.1 *Analyze materials at times indicated, according to submitted protocol.* With unstable materials (e.g., with microbial or decomposition problems) analyses must be started at specified times.

5.2 *FOLLOW METHOD EXACTLY (this is critical).* Any deviation, such as the necessity to substitute reagents, columns, apparatus, or instruments, must be recorded at the time and reported. If the collaborator has no intention of following the submitted method, he or she should not participate in the study. If the collaborator wishes to check another method on the same materials, additional laboratory samples should be requested for that purpose, to be analyzed separately.

5.3 *Conduct exactly the number of determinations stated in the instructions.* Any other number complicates the statistical analysis. Too few determinations may require discarding the results from that laboratory for that material or inserting "missing values"; too many values may require discarding the contribution of that laboratory or at least some of the values. If a laboratory cannot follow instructions as to number of analyses to perform, it raises a question as to its ability to follow the method.

5.4 *Report individual values, including blanks.* Do not average or do other data manipulations unless required by the instructions. Undisclosed averaging distorts statistical measures. If blank is larger than determination, report the negative value; do not equate negative values to zero. Follow or request instructions with regard to reporting "traces" or "less than." Descriptive (i.e., nonquantitative) terms are not amenable to statistical analysis and should be avoided. When results are below the limit of determination, report actual calculated result, regardless of its value.

5.5 *Supply raw data, graphs, recorder tracings, photographs, or other documentation* as requested in the instructions.

5.6 *If analytical results appear unreasonable, investigate possible cause immediately,* first by checking for transcription and calculation mistakes, and then by reanalysis, if permitted by the protocol. Call Associate Referee to discuss suspicious values. If Associate Referee indicates a value may be an outlier, review the determination promptly to the extent possible, by recalculation, reanalysis, or preparation of new standards. If time and materials are available, obtain new laboratory samples for repeat analysis.

Since collaborators may have no basis for judging whether a value is an outlier, the results should be communicated to the Associate Referee as soon as the protocol is complete and before time and equipment are reassigned, so that repeat assays may be performed at once, if necessary.

Note: The sooner an apparent outlier is investigated, the greater the likelihood of finding a reason for its occurrence. The most frequent causes of correctable outliers are:

5.6.1 Incorrect calculations and arithmetic errors.

5.6.2 Errors in reporting, such as transposition of numbers, misplacement of the decimal point, or use of the wrong units.

5.6.3 Incorrect standards due to weighing or volumetric errors (check physical constants or compare against freshly prepared standard solutions).

5.6.4 Contamination of reagents, equipment, or test materials.

6. Statistical Analysis

6.1 Initial Review of Data (Data Audit)

The Associate Referee should first plot the collaborative data material by material (or one value against the other for a split level [Youden pair]), values vs laboratory, preferably in ascending or descending order of reported average concentration. Usually major discrepancies will be apparent: displaced means, unduly spread replicates, outlying values, differences between methods, consistently high or low laboratory rankings, etc.

Only valid data should be included in the statistical analysis. Valid data are values that the collaborator has no reason to suspect as being wrong. Invalid data may result when: (a) the method is not followed; (b) a nonlinear calibration curve is found although a linear curve is expected; (c) system suitability specifications were not met; (d) resolution is inadequate; (e) distorted absorption curves arise; (f) unexpected reactions occur; or (g) other atypical phenomena materialize. Other potential causes of invalid data are included in 5.6.1–5.6.4.

6.2 Statistical Approach

Perform calculations on each material individually or as split levels, as appropriate. Only if the variances are not significantly different from each other should the results across materials be pooled for analysis of variance. If the relative standard deviations of the results from different materials are not significantly different, it may be convenient to average them over the range tested and to thereby report just a single relative standard deviation. Consultation with a statistician is always desirable.

6.3 Outliers

Collaborative studies seem to have an inherent level of outliers, the number depending on the definition of outliers and the basis for calculation (analytes, materials, laboratories, or determinations). Rejection of more than 2/9 of the data from each material in a study, without an explanation (e.g., failure to follow the method), is ordinarily considered excessive. This corresponds to rejection of more than 1 laboratory from a 5–6 laboratory study or 2 from a 9 laboratory study. For larger studies, a smaller acceptable percentage of rejections may be more appropriate. Determine the probability that the apparent aberrant value(s) is part of the main group of values considered as a normal population by applying the following tests in order:

6.3.1 *Cochran test* for removal of laboratories (or indirectly for removal of extreme individual values from a set of laboratory values) showing significantly greater variability among replicate (within-laboratory) analyses than the other laboratories for a given material. Apply as a 1-tail test at a probability value of 0.01.
To calculate the Cochran test statistic: Compute the within-laboratory variance for each laboratory and divide the largest of these by the sum of all of these variances. The resulting quotient is the Cochran statistic which indicates the presence of a removable outlier if this quotient exceeds the critical value listed in the Cochran table for $P = 0.01$ (1-tail) and L (number of laboratories), Appendix A-1.

6.3.2 *Grubbs tests* for removal of laboratories with extreme averages. Apply in the following order: single value test (2-tail; $P = 0.01$); then if no outlier is found, apply pair value test (2 values at the highest end, 2 values at the lowest end, and 2 values, one at each end, at an overall $P = 0.01$).
To calculate the single Grubbs test statistic: Compute the average for each laboratory and then calculate the standard deviation (SD) of these L averages (designate as the original s). Calculate the SD of the set of averages with the highest average removed (s_H); calculate the SD of the set of averages with the lowest average removed (s_L). Then calculate the percentage decrease in SD as follows:

$$100 \times [1 - (s_L/s)] \quad \text{and} \quad 100 \times [1 - (s_H/s)]$$

The higher of these 2 percentage decreases is the single Grubbs statistic, which signals the presence of an outlier to be omitted if it *exceeds* the critical value listed in the single Grubbs tables at the $P = 0.01$ level, 2-tail, for L laboratories, Appendix A-2.
To calculate the Grubbs pair statistic, proceed in an analogous fashion, except calculate the standard deviations s_{2L}, s_{2H}, and s_{HL}, following removal of the 2 lowest, the 2 highest, and the highest and the lowest averages, respectively, from the original set of averages. Take the smallest of these 3 SD values and calculate the corresponding percentage decrease in SD from the original s. A Grubbs outlier pair is present if the selected value for the percentage decrease from the original s *exceeds* the critical value listed in the Grubbs pair value table at the $P = 0.01$ level, for L laboratories, Appendix A-2.

6.3.3 If the single value Grubbs test signals the need for outlier removal, remove the single Grubbs outlier and recycle back to the Cochran test as shown in the flowchart, Appendix A-3.
If the single value Grubbs test is negative, check for masking by performing the pair value Grubbs test. If this second test is positive, remove the 2 values responsible for activating the test and recyle back to the Cochran test as shown in the flowchart, Appendix A-3, and repeat the sequence of Cochran, single value Grubbs, and pair value Grubbs. Note, however, that no outliers should be removed if such removal results in an overall reduction of more than 2/9 in the number of laboratories whose values are removed.

6.3.4 If no outliers are removed for a given cycle (Cochran, single Grubbs, pair Grubbs), outlier removal is complete. Also, stop outlier removal whenever more than 2/9 of the laboratories are flagged for removal. With a higher removal rate, either the precision parameters must be taken without removal of all outliers or the method must be considered as suspect.

Note: The decision as to whether a value(s) should be removed as an outlier ultimately is not statistical in nature. The decision must be made by the Associate Referee on the basis of the indicated probability given by the outlier test and any other information that is pertinent. (However, for consistency with other organizations adhering to the harmonized outlier removal procedure, the estimate resulting from rigid adherence to the prescribed procedure should be reported.)

6.4 Bias (Systematic Deviation) of Individual Results

6.4.1 (Estimated) Bias = mean amount found − amount added (or known or assigned value)

Note: Error of a single value = the single value − amount added (true value)

% Recovery = (measured concentration in fortified material − measured concentration in unfortified material) × 100/(known increment in concentration)

The amount added should be a substantial fraction of, or more than, the amount present in the unfortified material.

6.4.2 A true or assigned value is known only in cases of spiked or fortified materials, certified reference materials, or by analysis by another (presumably unbiased) method. Concentration in the unfortified material is obtained by direct analysis or by the method of additions. In other cases, there is no direct measure of bias, and consensus values derived from the collaborative study itself often must be used for the reference point.

6.4.3 Notes:

(a) [Note for Youden Manual] Youden equates "true" or "pure" between-laboratory variability (not including the within-laboratory variability) to the variability in bias (or variability in systematic error) of the individual laboratories. Technically, this definition refers to the average squared difference between individual laboratory biases and the mean bias of the assay.

(b) The presence of random error limits the ability to estimate the systematic error. To detect the systematic error of a single laboratory when the magnitude of such error is comparable to that laboratory's random error, at least 15 values are needed, under reasonable confidence limit assumptions.

6.5 Precision (Random Error)

The relative basis (i.e., relative standard deviation, RSD) is the most useful measure of precision in chemical analytical work because the RSDs are usually independent of concentration or amount of analyte over a reasonable range of concentrations. Therefore, the use of RSD facilitates comparison of variabilities at different concentrations. When the RSD increases rapidly with a decrease in concentration or amount, the rise delineates the limit of usefulness of the method (limit of reliable measurement). The most important types of precision are:

6.5.1 *Reproducibility*—among-laboratories (including within-laboratories) precision, designated as s_R.
Note: This component is not obtained merely by calculating the standard deviation of all the data (except when there are no replicates) since this term must be corrected by a replication term (Youden, 8.1, p. 19). The correction term must be extracted by an analysis of variance technique (Steiner, 8.1, p. 78–81). However, this crude, overall calculation of the standard deviation of all the data can serve as a check on the arithmetic, since the 2 values are usually fairly close.

6.5.2 *Repeatability*—within-laboratory precision, designated as s_r.

6.5.3 *Among-laboratories (not including within-laboratory, variability).* Designated as s_L and used only for calculating s_R.

6.5.4 *Relationship among precision components.*

(a) The relationship among the 3 precision parameters is:

$$s_R^2 = s_L^2 + s_r^2$$

The parameters, s_R^2, s_L^2, and s_r^2, must be nonnegative, by definition. The *estimate* of s_L^2, however, can be negative. This frequently occurs in practice when s_r^2 is so large (poor repeatability) that it swamps out s_L^2. A negative estimate of the s_L^2 term arises from the fact that s_L^2 is calculated from the difference of 2 terms, each of which is calculated independently. If the second term is larger than the first, the difference is negative. When this occurs, s_L^2 is set equal to zero, which does result in a biased estimate of s_R^2. If this occurs with a number of the materials in the collaborative study, the method is probably unsatisfactory due to poor replication. Otherwise, such an occasional aberration can be tolerated.

(b) When only single determinations are performed on each material (except in the case of the split level design), there is no rigorous basis for calculating s_r^2, and within-laboratory variability cannot be estimated directly.

(c) The ISO definitions for repeatability value (r) and reproducibility value (R) (see 8.5) are simple multiples of the above measures of precision expressed as standard deviations. They are shown to be expressible in terms of the corresponding standard deviations below. The ISO definitions use a prediction interval statement: the value below which the absolute difference between 2, and only 2, single test results of identical test material may be expected to lie with a specified probability (usually 95%); in other words, assuming normal distribution, when duplicate measurements are performed, the absolute difference between the results of each of these duplicate measurements is expected to be below r or R in 95% of the cases. The relationship between the 2 definitions is:

$$\text{Repeatability value (r)} = 2 \cdot 2^{1/2} \cdot s_r = 2.8 \cdot \bar{X} \cdot RSD_r/100$$

$$\text{Reproducibility value (R)} = 2 \cdot 2^{1/2} \cdot s_R = 2.8 \cdot \bar{X} \cdot RSD_R/100$$

The coefficient 2.8 is derived from assumptions about the distribution of the sample populations, and s_r and s_R are repeatability and reproducibility standard deviations, respectively; RSD_r and RSD_R are the corresponding relative standard deviations, and $\bar{X}$ is the mean of the laboratory means.

6.5.5 *Confidence limits for precision terms.* Standard deviations and relative standard deviations from actual collaborative studies are merely estimates of "true values." The "confidence interval" (bounded by the confidence limits) is the range within which the true value is expected to lie with a stated degree of confidence (customarily 95%). The confidence intervals of precision terms are rarely given because about 200 values are required to estimate the standard deviation to within approximately 10%; for a small study of about 30 values, the standard deviation can be estimated to only about 25%.

6.6 Incorrect, Improper, or Illusory Values (False Positive and False Negative Values)

These results are not necessarily outliers (no a priori basis for a decision), since there is a basis for determining their incorrectness (a positive value on a blank material, or a zero (not found) or negative value on a spiked material). There is a statistical basis for the presence of false negative values: In a series of materials with decreasing analyte concentration, as the RSD increases, the percent false negatives increases from an expected 2% at an RSD = 50% to 17% at a RSD = 100%, merely from normal distribution statistics alone.

When false positives and/or false negatives exceed about 10% of all values, analyses become uninterpretable from lack of confidence in the presence or absence of the analyte, unless all positive laboratory samples are reanalyzed by a more reliable (confirmatory) method with a lower limit of determination than the method under study. When the proportion of zeros (not necessarily false negatives) becomes greater than approximately 30%, the distribution can become bimodal and even more uninterpretable (is the analyte present or absent?).

7. Final Report

7.1 The final report should contain a description of the materials used, their preparation, any unusual features in their distribution, and a table of all *valid* data, including outliers. When replication is performed, the individual values, not just averages, must be given, unless the method requires averages (e.g., microbiological methods). Values not used for specified reasons, such as decomposition, failure to follow method, or contamination, should not be included in the table since they may be included erroneously in subsequent recalculations. The report should include the statistical parameters calculated with and without specified outliers. Report the standard deviations, means, and the corresponding RSDs. Proofread tables very carefully since many errors are of typographical origin. Give the names of the participants and their organizations, if agreement has been obtained for their acknowledgment.

7.2 The final report should be published in a generally accessible publication, or availability of the report from the organization sponsoring the method should be indicated in the published method. Without public documentation, the significance of the study is very limited.

7.3 The report should be sent to all participants, preferably at the manuscript stage, so that clerical and typographical errors may be corrected before publication. If changes in values from the original submission are offered, they must be accompanied by an explanation.

7.4 Example of Table of Statistical Parameters: See Table 1.

8. References

8.1 W. J. Youden & E. H. Steiner (1975) *Statistical Manual of the AOAC.* Association of Official Analytical Chemists, 1111 North 19th Street, Suite 210, Arlington, VA 22209 USA. The fifth printing (1987) contains several explanatory footnotes.

8.2 *Handbook for AOAC Members* (1982). Availability as in 8.1.

8.3 T. Dols & B. Armbrecht (1976) *J. Assoc. Off. Anal. Chem.* **59**, 1204–1207.

8.4 International Organization for Standardization Guide 18, Geneva Switzerland. Available from American National Standards Institute, 1430 Broadway, New York, NY 10018 USA and other national standards organizations.

8.5 Ibid, ISO 5725-1986.

TABLE 1

[x] collaborative tests carried out at the international level in [year(s)] by [organization(s)] in which [y and z] laboratories participated, each performing [k] replicates, gave the following statistical results:

Results expressed in [units]

Material [Description and listed across the top in increasing order of magnitude of means]
Number of laboratories retained after eliminating outliers
Number of outlying laboratories removed
Mean
True or accepted value, if known
Repeatability standard deviation (s_r)
Repeatability relative standard deviation (RSD_r)
Repeatability *value*, r (2.8 × s_r)
Reproducibility standard deviation (s_R)
Reproducibility relative standard deviation (RSD_R)
Reproducibility *value*, R (2.8 × s_R)

* * *

The repeatability and reproducibility *values* may also be expressed as a relative value (as a percentage of the determined mean value), when the results so suggest.

If the recovery and precision values are more or less constant for all materials or for groups of materials, an overall average value may be presented. Although such averaging may not have statistical validity, it does have practical value.

Appendix A-1

Critical Values for the Cochran Test
(Abbreviated from ISO 5725-1986, $P = 0.01$)

L = number of laboratories at a given level (concentration)
r = number of replicates per laboratory

L	r = 2	r = 3	r = 4	r = 5	r = 6
3	0.993	0.842	0.883	0.834	0.793
4	0.968	0.864	0.781	0.721	0.676
5	0.928	0.788	0.696	0.633	0.588
6	0.883	0.722	0.626	0.564	0.520
7	0.838	0.664	0.568	0.508	0.466
8	0.794	0.615	0.521	0.463	0.423
9	0.754	0.573	0.481	0.425	0.387
10	0.718	0.536	0.447	0.393	0.357
11	0.684	0.504	0.418	0.366	0.332
12	0.653	0.475	0.392	0.343	0.310
13	0.624	0.450	0.369	0.322	0.291
14	0.599	0.427	0.349	0.304	0.274
15	0.575	0.407	0.332	0.288	0.259
16	0.553	0.388	0.316	0.274	0.246
17	0.532	0.372	0.301	0.261	0.234
18	0.514	0.356	0.288	0.249	0.223
19	0.496	0.343	0.276	0.238	0.214
20	0.480	0.330	0.265	0.229	0.205
21	0.465	0.318	0.255	0.220	0.197
22	0.450	0.307	0.246	0.212	0.189
23	0.437	0.297	0.238	0.204	0.182
24	0.425	0.287	0.230	0.197	0.176
25	0.413	0.278	0.222	0.190	0.170
26	0.402	0.270	0.215	0.184	0.164
27	0.391	0.262	0.209	0.179	0.159
28	0.382	0.255	0.202	0.173	0.154
29	0.372	0.248	0.196	0.168	0.150
30	0.363	0.241	0.191	0.164	0.145
35	0.325	0.213	0.168	0.144	0.127
40	0.294	0.192	0.151	0.128	0.114

Cochran statistic = (largest individual within-laboratory variance)/(sum of all the within-laboratory variances).

Appendix A-2

Critical Values for the Grubbs Single Value and Pair Value Tests Expressed as the Percent Reduction in the Standard Deviation Caused by Removal of the Suspect Value(s) (*See* 6.3.2 for calculating the Grubbs statistics.)

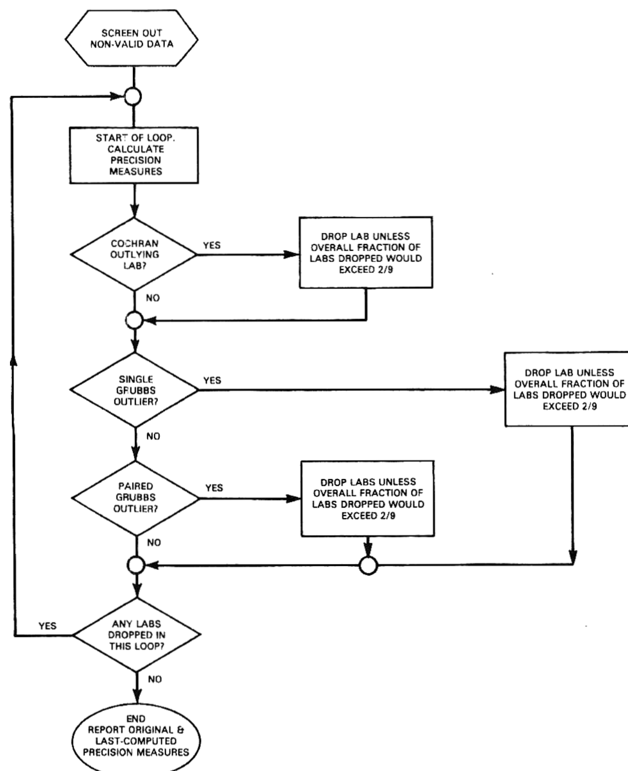
L = number of laboratories at a given level (concentration)

L	Single value test	Pair value test
4	91.3	99.7
5	80.7	95.4
6	71.3	88.3
7	63.6	81.4
8	57.4	75.0
9	52.3	69.4
10	48.1	64.6
11	44.5	60.5
12	41.5	56.8
13	38.9	53.6
14	36.6	50.8
15	34.6	48.3
16	32.8	46.0
17	31.2	44.0
18	29.8	42.1
19	28.5	40.4
20	27.3	38.9
21	26.2	37.4
22	25.2	36.1
23	24.3	34.9
24	23.4	33.7
25	22.7	32.7
26	21.9	31.7
27	21.2	30.8
28	20.6	29.9
29	20.0	29.1
30	19.5	28.3
35	17.1	25.0
40	15.3	22.5

Source: Patrick Kelly, Canada Packers, Toronto, Ontario, Canada. Single critical values calculated from available formulas; pair critical values from simulation and fitting and should be accurate to 0.1% absolute. (Submitted for publication to *Technometrics*.)

Appendix A-3

Flowchart
Complies with IUPAC-1987 Recommendations



Committee on International Coordination

BEN BORSJE, *Chairman*
P. H. Van Rynstraat 44,
3904 HJ Veenendaal,
The Netherlands

Other Members: K. Naguib, Vice Chairman; C. Y. Ang; W. R. Bontoyan; L.-H Chen; R. F. Coleman; L. F. Corominas; E. Hopkin; W. Horwitz; M. Ihnat; J. R. Iturbe; P. Martin; G. G. Moy; D. L. Park; H. Povlsen; M. Rogers; R. C. Rund; I. Santich; R. W. Weik; R. Wood

Recommendations for the Board of Directors

A. The Committee on International Coordination recommends that the Official Methods Board's criteria for acceptance of methods originating from other organizations be distributed to organizations with which AOAC has cooperative agreements and others with which AOAC has regular contacts.

B. The committee recommends that the AOAC staff develop a menu of services (short courses and symposia) and specific projects and describe conditions including funding (sponsors) that persons desiring such services must meet for AOAC to initiate action.

C. The committee recommends that the methods committees, the General Referees, the Associate Referees, and other relevant persons be informed of the contents, consequences, and their responsibilities under various AOAC cooperative agreements with other organizations.

D. The committee recommends that the Board of Directors consider the following: The committee is prepared to develop a strategic plan, in cooperation with the European and U.S. representatives, to advance the methods development, collaborative studies, and interactions of scientists in North America to scientists in Europe; in other words, a planned effort to advance the tenets of AOAC in all aspects to the scientific community in Europe. Does the Board of Directors want the International Coordination Committee, eventually in cooperation with the Long Range Planning Committee, to develop such a strategic plan? We are prepared to do so!

Basis for Recommendations

A. The committee noted with pleasure last year in Scottsdale that the Official Methods Board took a positive action in accepting internationally available methods of analysis. The committee is now requesting the Board of Directors to implement that action for the benefit of the international scientific community.

B. Following last year's request by our African member, the committee is studying the possibility of placing training courses in developing countries and other less-industrialized countries. We need to see what kind of funds can be raised and what type of volunteer input is required to organize training courses on a cost-recovery basis.

C. The committee has the impression that the implemen-

tation of cooperative agreements with AOAC needs more attention.

D. During the meeting of the AOAC Europe Steering Committee in San Francisco, 2 motions were adopted concerning the clarification of the position of AOAC Europe and the need for a permanent representation and strategy for AOAC Europe. After discussion of these motions, the International Coordination Committee adopted a motion covering both.

Other Discussion

Reports of the status of joint actions with other bodies indicated once again a high level of cooperation. These international organizations included: International Standards Organization, Analytical Division of the Royal Society of Chemistry (UK), International Union of Pure and Applied Chemistry, Food and Agriculture Organization of the United Nations, World Health Organization, International Dairy Federation, Nordic Committee on Food Analysis, European Economic Community, American Association of Cereal Chemists, American Association of Brewing Chemists, Association of Public Analysts (UK), and Federation of European Chemical Societies.

The committee was pleased to note that the AOAC/AMC agreement has been signed.

The committee was pleased to learn that the European Representative achieved observer status for AOAC at EEC expert committee meetings, and considers this a major step forward.

The portions of the strategic plan pertaining to the committee were discussed and will be reported on later.

The committee was pleased to receive guests from Brazil, Belgium, United Kingdom, Sweden, and Finland. Members from Mexico, Egypt, Denmark, United States, and The Netherlands were also present.

Intersociety Committee on Methods of Air Sampling and Analysis

BERNARD E. SALTZMAN, *Representative*
University of Cincinnati,
Kettering Laboratory,
Cincinnati, OH 45267-0056

The committee held one meeting during 1987 on June 23 in New York, NY. The executive secretary, George Kupchik, reported that 1100 copies of the second edition of the manual remained of the original 5900 printed. The third edition will be issued in 1988; therefore, a proposal by the American Public Health Association to offer these remaining copies to its non-U.S. distributors at 60% less than the list price was discussed. Because of the lack of a quorum, a mail ballot vote was taken and the proposal was accepted.

The editor, James P. Lodge, reported that the revised manuscript for the third edition of the manual was almost complete for specific methods, but some work on general techniques remained. He expects the manual to be ready for publication in early 1988.

The subcommittee chairmen reported on the status of remaining work in preparing sections of the manuscript.

Two proposals for publication of the third edition of the manual were discussed. The APHA proposal to handle distribution, storage, and accounting of an already published manual was not favored. A proposal by Lewis Publishers, Inc. (Chelsea, MI), for complete publication and handling was subsequently approved by mail ballot. A copy of the proposal will be sent to all participating societies. They will also distribute the manual as was done with previous editions.

Under the terms of the Articles of Agreement, the chairmanship was rotated to a representative of another society. Robert S. Saltzman (Dupont) representing the Instrument Society of America was elected chairman by a mail ballot for a 3 year term beginning in 1988.

The future program of the Intersociety Committee after publication of the third edition of the manual will be discussed at the next meeting. Sampling and analysis of indoor air pollutants, hazardous wastes, and toxic substances have been suggested.

The 9 societies currently participating in the Intersociety Committee are: Air Pollution Control Association; American Chemical Society; American Institute of Chemical Engineers; American Public Works Association; American Society of Civil Engineers; American Society of Mechanical Engineers; Association of Official Analytical Chemists; Health Physics Society; Instrument Society of America.

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 (AOCS); D. L. Park (AOAC); A. E. Pohland (IUPAC); O. L. Shotwell (AACC); R. D. Stubblefield (*Secretary*) (AOCS); A. E. Waltking (AOCS)

The annual meeting of the Joint Mycotoxin Committee was held on September 16, 1987, in San Francisco, CA.

(A.) The minutes of the Joint Mycotoxin Committee of September 16, 1986, were approved.

(B.) The report of the IUPAC Commission on Food Chemistry was given by Doug Park for Al Pohland. The commission has several projects on mycotoxins that are completed, under way, or initiated: (a) collaborative study on patulin in apple juice (S. Kubacki, completed); (b) collaborative study on ochratoxin A (P. Krogh, to be completed in 1988); (c) collaborative studies on aflatoxin methods (see AOAC report, completed); (d) limits and regulations for mycotoxins in foods and feeds (H. P. van Egmond, ongoing project); (e) guidelines for immunoassay methods (J.-M. Fremy, new project); (f) spectroanalytical parameters of *Fusarium* toxins (P. Thiel, ongoing project); (g) check sample program (M. Friessen, ongoing project); (h) decontamination (D. Park, new project).

(C.) Peter Scott highlighted the AOAC General Referee report on mycotoxins. (a) The ELISA screening method for aflatoxin B_1 in cottonseed products and mixed feeds was recommended for adoption to the Committee on Foods I; however, they have not yet made a decision. (b) Final reports are being prepared on the LC method for determining aflatoxins, the solvent-efficient TLC method, and a rapid-screening ELISA card method. These were collaboratively studied this year. (c) Numerous official first action methods in chapter 26 were recommended for official final action status. Three methods (aflatoxin in green coffee, **26.061-26.066**; identification of aflatoxin B_1 by TLC, **26.076-26.082**; and confirmation of aflatoxin M_1 in liver, **26.110**) were declared surplus. (d) An associate refereeship is being established for Immunochemical Methods for Mycotoxins.

(D.) Art Waltking gave an overview of the AOCS mycotoxin meeting of May 17, 1987, in New Orleans, LA. (a) The report of the Smalley Committee on Aflatoxin was reviewed. (b) The CB method for aflatoxins in corn and corn products was written in AOCS style for inclusion in *Official and Tentative Methods*. It has been forwarded to the AOCS Uniform Methods Committee.

(E.) The report of the AACC Mycotoxin Committee was given by Odette Shotwell. (a) The method for determining zearalenone by liquid chromatography, approved by both the AOAC and AOCS, was approved by the AACC. (b) The presumptive test for aflatoxin in corn, "the black light test," was written up for the AACC book of methods by Odette Shotwell. This will provide, "for information only," a proper procedure for doing this test. (c) A new representative to the Joint Mycotoxin Committee to replace Raymond Bowers is being sought.

(F.) The report of the International Dairy Federation Group E33-Mycotoxins was summarized by Peter Scott. (a) The method for the determination of aflatoxin M_1 in milk and milk products-IDF Standard 111:1982 (AOAC method **26.095-26.100**) will incorporate minor suggestions and be submitted for publication as a Provisional IDF Standard. (b) Sterigmatocystin in certain types of Dutch hard cheese is a topic of concern. A method presented by Dr. Battaglia appears to be promising for study. (c) The occurrence of α-cyclopiazonic acid in mold-ripened soft cheese was discussed.

(G.) Art Waltking led a discussion of Proposition 65 in California. This proposition has been passed and becomes effective in March 1988. Attorneys for the American Peanut Product Manufacturers Incorporated (APPMI) have presented their views on how the industry would be affected to the scientific advisory panel for Proposition 65, in attempts to get it to defer the initial listing of aflatoxin on their list of carcinogens. The APPMI noted that aflatoxin is a naturally occurring carcinogen, but listing of aflatoxin at this time will lead to lowered public confidence in federal standards and put a burden upon the peanut butter manufacturers.

(H.) There was a lengthy discussion of the current status of ELISA and affinity column chromatography kits. Some committee members have evaluated some of the kits, with generally good results. However, it was noted that mixed feed samples can produce background color in their extracts, which could lead to false positives. Other attendees commented upon the excellent cleanup of extracts by the affinity columns and the effects of temperature variation on the enzyme in ELISA tests. The discussion shifted to the problems encountered when an immunoassay method is written up. It is necessary to specify the criteria and specifications so that any manufacturer's kit would be acceptable without a new collaborative study, as long as it meets the specified criteria. This is no easy task and other Associate Referees are struggling with the same problem. It was recommended by the committee members that all AOAC Associate Referees involved in immunoassay methodology should collaborate to address the write-up problems.

(I.) Douglas Park stated that the FDA requested an in-depth review on the decontamination of aflatoxin-contaminated commodities (corn, peanuts, and cottonseed) by the use of ammonia. This review was written by Douglas Park (University of Arizona), Louise Lee (SRRC), Al Pohland (FDA), and Ralph Price (University of Arizona) and will be used to reach a decision in the clearance process.

(J.) Recent findings of trichothecenes in agricultural commodities were noted, including HT-2 toxin in wheat, diacetoxyscirpenol and DON in soybeans, and 3- and 15-acetyl DON in corn.

(K.) Meetings on mycotoxins that will take place in 1988 are the FASEB Summer Research Conference on Trichothecene, Blue-green Algal, and Marine Toxins: Mechanisms, Detection, and Therapy, at Copper Mountain, CO, July 25–28; 7th IUPAC International Symposium on Mycotoxins and Phycotoxins, Tokyo, Japan, August 16–19; and 5th International Congress of Plant Pathology, Kyoto, Japan, August 20–27. A conference and workshop on immunoassay of mycotoxins is planned for the 103rd AOAC Annual International Meeting in St. Louis, MO, September 25–28, 1989.

Committee on Laboratory Quality Assurance

JON E. McNEAL, *Chairman*
U.S. Department of Agriculture,
Food Safety and Inspection Service,
Washington, DC 20250

Other Members: R. Alvarez; J. B. Ault; R. A. Baldwin; C. S. Brannon; M. H. Brodsky; A. S. Y. Chau; D. Curley-Arndt; J. P. Dux; J. M. Guira; G. Hirsh; E. Klasta; J. Lanier; E. Meier; T. Mekaru; T. Stijve

Recommendations for the Board of Directors

A. The committee recommends that the Board of Directors direct the staff to proceed with the implementation of an AOAC analyst recognition program, with concomitant recognition for the organization to which the analyst belongs.

B. The committee recommends that the Board of Directors not pursue an AOAC certified reference materials program, but continue to direct the Laboratory Quality Assurance Committee to investigate the possibility of the association acting as a marketing agent for analytical standards.

Basis for Recommendations

A. A proposal is before the Board of Directors to implement an AOAC analyst accreditation program during 1988; accreditation would be based on successful participation in AOAC collaborative studies and/or the conducting of studies leading to official status for specific methods. Such a program would be intended to foster incentive for analysts and Associate Referees to participate vigorously in AOAC activities.

The committee believes that the accreditation of analysts should not be a part of the collaborative process, because the primary objective of the process is to evaluate the method, not the analyst. An inherent principle in the collaborative process is that analysts are expected to be competent before commencing their portion of the study.

Rather than helping to increase analyst participation, it is believed that such a program would lead to nonparticipation for fear of failure. If a study was successful but an analyst was not allowed accreditation because his/her findings were considered outliers, the analyst would have this stigma to live with, and it could lead to adverse effects on careers. Outliers can be characteristic of the method and not the analyst.

Not all participation in collaborative studies is voluntary; companies and supervisors often assign specific analysts for this type of work. Questions arise as to whether such a program might foster an elitist group within AOAC. What about the person who has many years of experience in the specific area but, because of present position, cannot participate in a collaborative study and therefore is not eligible for accreditation? What happens in companies that *must* use official methodology but have no accredited analysts or their one analyst has changed companies? Would salary differentials be created?

AOAC can specifically recognize analysts who have volunteered for or have been assigned to a collaborative study. Such recognition could be through the use of certificates of participation that one could justifiably and proudly display. A small fee could be charged to cover the implementation of the program, but it should not be considered a revenue-gathering exercise. Because an organization has contributed dollars to a study, in terms of people, time, and material, it too should have the opportunity for recognition.

B. The association does not have the resources to implement a standard reference materials program. Large initial outlays of funds are required for manufacturing and testing facilities, and there is a limited market. Such a program would create an unacceptable financial burden for AOAC.

An opportunity does exist for the association to become a marketing agent for standard reference materials if we act immediately. The U.S. Office of Management and Budget has asked the Environmental Protection Agency to externalize its analytical standards programs; this will allow outside organizations to contract for the preparation, testing, storage, etc., of these materials and to market them. The need for these standard materials is huge. EPA would still operate the bulk of the program; AOAC could do the marketing and share in the revenues. It appears that clients who have been receiving these materials free of charge will have to begin purchasing them regardless of whether groups outside EPA market them.

Other Discussion

AOAC Quality Assurance Short Courses: Full 2-day courses were held in Arlington, VA, on April 7–8, 1987, July 28–29, 1987, and in San Francisco, CA, on September 12–13, 1987, with 35, 54, and 45 attendees, respectively. On July 16, 1987, AOAC, under contract with EPA, presented a one-day modified course on Quality Assurance for Analytical Laboratories at the "EPA Solid Waste Testing and Quality Assurance Symposium." Because of restrictions in the contract, only 35 participants were allowed. On-site, however, over 200 people wished to take the course. Nine additional persons from the EPA Pesticide and Toxic Substances Office were added. There is still a large market for these courses and more will be scheduled during the coming year. Also the short course for microbiology laboratories should be offered again.

QA Committee Participation in AOAC Regional Sectional Meetings: Committee members participated in the Midwest and Southeast Regional Section meetings during 1987, giving presentations and conducting question/answer sessions concerning quality control and quality assurance for bench level analysts. Both presentations were received with great enthusiasm. Committee members are encouraged to continue their contact with AOAC regional sections and to participate in programs on quality assurance/quality control wherever practical. Presentations should be geared toward the specific needs of those attending these meetings and are not meant to be in competition with or as all-inclusive as the AOAC short courses.

Sampling: The symposium on sampling that was part of last year's annual meeting will not be published as a separate entity. Authors are encouraged to submit manuscripts of their papers for publication in the *Journal of the AOAC.* Frederick Garfield is preparing a publication entitled "A Guide to Sampling Food, Drugs, and Agricultural Commodities."

Future Activities

(*1*) *AOAC Check Sample Programs:* Proficiency check sample programs are not easy to administer, but they are possible. The committee believes that AOAC may have a role to play in this area. A subcommittee, chaired by Eugene Klesta, has been formed to determine in what areas AOAC could service its membership by providing check samples for needs not currently covered by existing programs. The

subcommittee will also look at areas where redundant programs now exist, with efforts directed towards consolidation.

(*2*) Members of the committee, through their affiliations with other professional societies and organizations, will be investigating the possibility that those groups with continuing education unit (CEU) programs would accept AOAC collaborative studies for CEU credit. If this can be done, it will encourage more participation in AOAC activities.

(*3*) The committee will look into the possibility of videotaping sections of the short course for sale to interested parties. There also appears to be an interest from laboratories and companies for on-site courses to which they could send more of their people. Preliminary discussions reveal that they are willing to pay for this service. These activities could be of great service to our membership and could more than pay for themselves.

Long Range Planning Committee

RICHARD L. ELLIS, *Chairman*
U.S. Department of Agriculture,
Food Safety and Inspection Service,
300 Twelfth St, SW, Washington, DC 20250

Other Members: R. A. Baldwin; W. R. Bontoyan; B. Borsje; W. P. Cochrane; F. J. Farrell; C. W. Gehrke; R. Matulis; J. P. Minyard; A. Munson; R. C. Rund; A. Shroff; W. Spain; G. R. Tichelaar; L. Whalen

Recommendations for the Board of Directors

A. The Long Range Planning Committee recommends that the Board of Directors approve the revised Terms of Reference for the committee.

B. The committee recommends that the Board of Directors transmit to the Official Methods Board the collection of recommendations concerning scientific activities of AOAC, dated 1987, and including scientific areas to be emphasized and expanded and new areas to be explored for development of methods for collaborative study.

Discussion

The proposed revised Terms of Reference specify an expanded committee membership which will allow broader and more representative participation in the long range planning activities of the association. Principally, this will bring into the committee increased representation from the private sector and additional representation from outside North America.

As part of its ongoing activities, the Long Range Planning Committee assesses the current status of topics and areas being addressed by AOAC and evaluates trends and events both within those areas and in potential new areas that may affect future directions of AOAC. Recommendations were developed in 11 areas, as follows:

(*1*) AOAC should nurture existing cooperative agreements with other methods development organizations in the *food, feeds, and pesticides areas,* such as those in existence with Nordic Committee on Food Analysis, International Dairy Federation, Association of Public Analysts, and others. AOAC should capitalize on its recognition in southeast Asia, for example, and expand its involvement in the Pacific Basin in fertilizers, pesticide formulations, and other commodity areas.

(*2*) AOAC should encourage industry involvement in the effort to harmonize methodology for *pharmaceuticals,* particularly in broadening traditional AOAC procedures to include validation of methods for new dosage forms.

(*3*) AOAC should encourage increased participation by *microbiologists,* by being more visible at symposia, meetings, and workshops of interest, and by offering appropriate and relevant microbiological progams within AOAC.

(*4*) Associate Referees should be appointed in *drug and device related microbiology* to develop methods in microbial detection, sterilization, and product evaluation, particularly of diagnostic test kits.

(*5*) AOAC should refocus attention on microbiological methods for *cosmetics.*

(*6*) AOAC should actively recruit and encourage federal, state, and local enforcement agencies to participate in rapid development and sharing of analytical methodology for characterization of *scheduled drugs, forensic evidence, and consumer products* allegedly tampered with.

(*7*) AOAC should consider giving official status to the appropriate EPA 600 series methods for contaminants in *ground water,* assuming the collaborative data support adoption.

(*8*) AOAC should pursue cooperation with the Environmental Protection Agency on collaborative study of proposed methods for *disinfectants*; at the same time, interests of other countries in validating such methods should be explored.

(*9*) AOAC should delete further consideration of methods for *hazardous substances* in nonfood products; we do not have the membership to support interest and study of such methods.

(*10*) Each methods committee and General Referee needs to adopt a strategy for developing methodology for *diagnostics and kits.* AOAC will need to be flexible in seeking innovative procedures, processes, reference standards, and related techniques to assess and incorporate these into *Official Methods of Analysis.* AOAC needs a policy to address inclusion of proprietary reagents in kits and possible implications of AOAC adoption of test kit methodology.

(*11*) AOAC should not expend any additional effort in the area of *human and veterinary biologics.*

As part of its specific responsibilities assigned under the association's long range plan, the committee will be developing a strategy for making better use of the liaison officers as information resources, both as transmitters of information about AOAC, and as contacts to facilitate cooperation between AOAC and organizations with mutual interests and activities. Part of this project will be a formal description of the roles and responsibilities for liaison officers.

The committee will be developing summary evaluations of new methods areas for expansion of AOAC and will recommend priorities for the expenditure of limited resources into these new areas. The committee expects to work with the Official Methods Board to suggest protocols for methods development in commodity or consumer product areas that do not fit the traditional collaborative study evaluation process.

Two specific topics that the committee will be studying will be evaluation of new venture areas, for possible development by other committees, and a review of the status and future of AOAC funding, to be done in cooperation with the Ways and Means Committee.

Committee on Meetings, Symposia, and Educational Programs

NICOLE F. HARDIN, *Chairman*
Food and Drug Administration,
4298 Elysian Fields Ave,
New Orleans, LA 70122

Other Members: R. Bianchi; P. D. Bland; M. H. Brodsky; J. P. Cherry; S. M. Glad-Anderson; T. L. Jensen; J. J. Karr; E. Meir; H. F. Morris; P. F. Ross; R. D. Stubblefield; R. Wood

The committee held 2 meetings during 1987: April 16, at AOAC's offices in Arlington, VA, and September 15, at the Annual International Meeting in San Francisco, CA.

At its April meeting, the committee reviewed its responsibilities under the association's long range plan (LRP). LRP project sheets were developed and submitted to the Board of Directors. Committee LRP obligations are being studied and prioritized for implementation.

Also at the April meeting, Fred Garfield reported to the committee on the association's short course program. The highly successful Quality Assurance Short Course had, at that time, been presented 21 times and the Microbiological Quality Assurance Short Course had been presented once. Mr. Garfield proposed a short course on sampling food, drugs, and agricultural commodities. The committee approved Mr. Garfield's initial general proposal and his proceeding to develop a final specific plan for proposal to the Board of Directors.

This year the committee completed establishment of symposium liaisons. After each symposium proposal is accepted, a committee member is assigned as a liaison between the symposium chairman and the committee. This liaison oversees symposium development and provides the symposium chairman ready access to the committee and assistance, if needed.

The committee has a first draft of guidelines for cooperative projects between AOAC and other organizations. These will be reviewed during the coming year and finalized for presentation to the Board of Directors.

Also this year, to comply with its revised Terms of Reference, the committee established 2 permanent subcommittees—one for the Annual International Meeting and one for educational programs. Robert Bianchi and Michael Brodsky, respectively, accepted chairmanship of these subcommittees.

At the committee's September meeting, William Horwitz presented a proposal for AOAC to cosponsor, with IUPAC, a symposium, "Harmonization of Protocols for Quality Assurance of Laboratory Studies." It would be accompanied by a one-day workshop on the expression and reporting of the results of collaborative studies. The committee enthusiastically received the presentation and supports this proposal.

Five symposia were presented at the Annual International Meeting in San Francisco. The lead symposium, "Robotics," chaired by Allen S. Carman and George Tichelaar, was very popular and well received. The symposium "Mycotoxins," chaired by Peter Scott, was the first in a series of "state of the art" symposia. Also presented were the following: the third symposium of Dennis Hinton's biotechnology series, "Biotechnology: Impact on Present and Future Methods of Analysis"; "Industrial Analytical Chemistry," chaired by James J. Karr; and "Cholesterol Oxidation," chaired by Gerhard Maerker and Jane Robens. Kenneth Helrich moderated this year's Regulatory Roundtable, "Seafood Toxins." The committee commends these chairmen on their excellent symposia.

The focus for the 1988 meeting is biotechnology, and 5 symposia proposals have been accepted by the committee: review of biotechnology methods and applications; pesticide residues; methods for detecting drug residues in food products of animal origin; laboratory information systems; and methods for phosphorus and nitrogen in fertilizers and fertilizer-related materials. Laboratory safety will be the subject of the 1988 Regulatory Roundtable.

The committee gratefully acknowledges the continuing support of its staff liaison, Administrative Manager Margaret R. Ridgell. Whatever the committee has accomplished would have been impossible without her assistance.

Committee on Membership

WARREN BONTOYAN, *Chairman*
Environmental Protection Agency,
Office of Pesticide Programs,
Beltsville, MD 20705

Other Members: W. F. Carey; W. P. Cochrane; G. Gentry; A. R. Hanks; K. R. Hill; D. Kassera; B. Larsen; A. J. Malanoski; D. L. Park; V. A. Thorpe; J. Williams; R. Young

The Membership Committee's recommendations regarding changes in the AOAC bylaws relating to membership, increased number of Directors, and mail ballots were incorporated into the Constitution Committee's recommendations and approved by the Board of Directors for a membership vote at the 1987 Annual International Meeting. Adoption of these recommendations will be one of the most significant bylaw changes in the history of the AOAC. It will be a clear signal that AOAC is a body of independent scientists whose primary goal is the development of reliable and scientifically defensible methods of analysis for use by regulatory agencies and regulated industry.

The next goal for the Membership Committee is to perform an in-depth review of membership benefits for the 3 classes of members (individual, sustaining, and honorary). After review, the committee will then recommend revisions or additions to the membership package. All proposed revisions and additions will be viewed from the standpoint of strengthening the association by addressing bench scientists' concerns and perceptions of the parent body relating to increased laboratory outputs, data quality, and professional development.

Because the spring workshops have been eliminated, it will be imperative that AOAC place a high priority on strengthening and expanding the role of members of the regional

sections by the development and implementation of specific plans to provide a close relationship with the parent body.

The committee will discuss the following in order to strengthen AOAC membership throughout the country:

(*1*) A standing committee should be created with the same level of responsibility and stature as the Long Range Planning Committee. The initial function would be to develop and maintain mandatory protocols, oversight, and review of the working relationship between the regional sections and headquarters staff and to furnish semi-annual assessments and recommendations to the Board of Directors to ascertain that bench scientists (who are the main beneficiaries of the regional sections) are an active and integral part of the association.

(*2*) Specific responsibilities (as determined by the Board of Directors) should be assigned to 2 or more directors to maintain close personal contact with the committee.

(*3*) Membership in the regional sections will require a continuous effort to make it possible for those scientists who cannot attend the Annual International Meeting to feel personally that they are an integral part of the association. Therefore, the committee would comprise 2 or more members from each of the regions (and appointed by the region), one of whom who would attend either the Annual International Meeting or one other meeting before the scheduled Board meetings.

(*4*) AOAC would need to establish a budget item for those members who could not obtain travel funds. The AOAC staff would be assigned the responsibility for proposing and implementing a creative approach to obtaining funds for this travel.

The committee is aware of the need for lower registration fees and lower AOAC costs in general to increase and maintain membership; however, how effective these measures have been or will be is questionable for active participation of bench scientists who cannot attend the Annual International Meetings. Therefore, the Membership Committee will solicit from members and nonmembers new and creative approaches for attracting new members, maintaining present membership, and improving esprit de corps. The solicitation may be done via *The Referee,* flyers enclosed with publications, letters to state, federal, and industry managers, and etc.

Consideration of AOAC's international membership benefits will depend on the initial success of recommendations (as approved by the Board of Directors) for North America.

An implementation plan was developed to "study and evaluate the costs, benefits, and potential liabilities of instituting an employment service for AOAC members." The committee will gather information from other organizations similar to AOAC. The AOAC membership will also be surveyed. The committee will evaluate all data and make appropriate recommendations to the Board of Directors regarding implementation of the plan.

The Chairman thanks and congratulates the members of the committee for developing the proposals as recommended by the Constitution Committee. The committee can take pride in the knowledge that it played a crucial part in taking the first step in assuring the AOAC will continue as the leading independent scientific organization for its second 100 years.

Committee on Regional Sections

RAYMOND H. BOWERS, *Chairman*
General Mills, Inc.,
9000 Plymouth Ave N,
Minneapolis, MN 55427

Other Members: G. H. Boone; A. V. Jain; K. A. McCully; L. Murray; D. Osheim; R. L. Polli; J. Wiskerchen

Recommendation for the Board of Directors

The committee recommends that the Board of Directors approve, for inclusion in the AOAC Regional Section operations guidelines, the Regional Section Committee's fundraising guidelines for regional sections.

Discussion

In response to the directive of the Board of Directors requiring submittal of regional section budgets prior to the commencement of the fiscal year, the committee has requested that the AOAC comptroller develop information on budget reporting requirements for regional sections, including appropriate forms for review by the committee and inclusion in the AOAC Regional Section operations guidelines.

Committee on Safety

EUGENE C. COLE, *Chairman*
University of North Carolina,
Department of Medicine, Division of Infectious Diseases,
Chapel Hill, NC 27599

Other Members: R. Bianchi; R. J. Everson; N. W. Henry; G. Markakis; R. Nelson; G. Roach; D. Root; M. G. Torchia

Recommendations for the Board of Directors

A. The committee recommends that the "Safety Checklist" for methods authors as now finalized by the committee after previous review by the Official Methods Board be adopted for use in submitting new methods for collaborative studies and for manuscript review prior to publication.

B. Regarding the revision of safety statements in *Official Methods of Analysis,* the committee recommends that the associate chapter editors be responsible for ensuring that each method is either updated with appropriate references to Chapter 51, "Laboratory Safety," or edited so that references to Chapter 51 are deleted and appropriate cautionary statements are placed at the beginning of the method.

Other Committee Activities

The Terms of Reference were reviewed and finalized.

The audio/visual safety training display at the Annual International Meeting where attendees could view commercially available safety videos was very successful. The committee recommends that the program be included again at the 1988 meeting with a larger room and more chairs and that half a day at a time be allocated to the display instead of hourly alternation which proved too disruptive. A list of commercially available laboratory safety audio/visual programs will be finalized by January 1, 1988.

The committee agreed to support a Regulatory Roundtable for 1988. The topic would be laboratory safety and would focus on recently enacted or proposed legislation affecting that area. The program will be finalized by January 19, 1988. Six tentative speakers have already been identified.

The committee agreed to submit to *The Referee* an article on laboratory fume hood operation and safety, based on Dave Root's submitted critique of the recent ACS program on fume hood safety.

The committee agreed to interpret new laboratory safety legislation for the association by publishing appropriate material in *The Referee* as indicated.

The committee recommended that preliminary revised drafts of the sections in the *AOAC Handbook for Members* pertaining to safety be drawn up for consideration by the Editorial Board. This includes the description of the Committee on Safety, Section XVI (p. 80), and Section 13, Chapter VIII (p. 49).

The committee will prepare a list of recommended liaison appointments of committee members to other professional associations; such appointments would be for the professional exchange of safety information.

The committee requests that the association subscribe to the *Laboratory Hazards Bulletin* (LHB) published by the Royal Society of Chemistry in England. Annual cost is $158.00. Each month the document would be routed throughout the committee membership. LHB contains laboratory safety abstracts from 100 or more publications worldwide, and includes information on safety legislation and training worldwide. This would be a valuable resource for committee activities.

Committee on Ways and Means

JOHN B. BOURKE, *Chairman*
U.S. Department of Agriculture,
CSRS, Washington, DC 20251

Other Members: A. Caputi; W. P. Cochrane; J. Goleb; L. Perlman; W. Phillips; M. R. Ready

Recommendations for the Board of Directors

A. The Committee on Ways and Means restates its recommendation that professional fundraisers be contacted when attempts are made to raise large sums for endowments or capital projects.

B. The committee recommends that the staff explore the possibility of forming a consortium of associations to construct and manage a common building.

C. The committee recommends that the Board of Directors study and the staff undertake to develop a short course, using the format of the quality assurance short course, on the design, implementation, and operation of Good Laboratory Practices (GLP) to be mandated by the U.S. Environmental Protection Agency in 1988.

Discussion

The committee discussed the potential for raising sufficient funds to construct an AOAC building as requested by the long range plan. It was felt that the size of the association limited its ability to raise the funds necessary for such an undertaking. However, the careful selection of related associations such as the Institute of Chemists, Society of Environmental Toxicology and Chemistry, and the American Horticultural Society and the pooling of resources could lead to a solution of the space and housing problems. Such an arrangement would also allow the sharing of common facilities such as conference room, educational facilities, and publishing, computer, and duplicating equipment. The committee recommends that the board appoint a special building committee to review in detail the needs of the association and work with the staff to undertake a feasibility study, including discussions with potential professional fundraisers.

A course on Good Laboratory Practices (GLP) should assist both public and private laboratories in meeting the EPA requirements and should assist in the conduct of in-house audits. The development, publication, and sales of supporting manuals, logbooks, check sheets, and continuing education supplies would also bring additional income to AOAC.

The entire area of continuing education offers many more possibilities to develop potentially profitable workshops in such areas as safety, laboratory waste disposal, electronics, automated data handling, chemical familiarization for the legal profession, to name a few. The committee recommends that interest centers be developed around a planned symposium held at the annual meeting. These centers should evaluate the potential for developing profitable workshops and training courses. In this case, the program committee

should keep the end product development in mind when planning symposia.

The great growth in the number of contract laboratories and the implementation of the proposed GLPs will lead many manufacturers of regulated chemicals and drugs to contract more of their research to specialists at this type of laboratory. The association, with its many contacts in the area of drugs and agricultural chemicals, should develop an advertising strategy to capitalize on these developments. The sale of advertising space in and the distribution of a catalog of food, drug, toxicological and agricultural chemical laboratories would be expected to serve a useful function as well as to generate significant income.

In the same vein, a directory of consultants and experts in these same areas would be a valuable resource not only to the private and public sectors of our disciplines but also to the legal profession. The committee recommends that consideration be given to the expansion of the current talent file of members into a file of scientists (both members and nonmembers) with descriptions of their particular expertise. Those wishing to locate a specific talent could be charged for successful queries.

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Committee on Pesticide Formulations and Disinfectants

Thomas L. Jensen (State Dept of Agriculture, 3703 S 14th St, Lincoln, NE 68502), *Chairman;* James J. Karr (Pennwalt Technical Center, 900 First Ave, King of Prussia, PA 19405); James P. Minyard (Mississippi State Chemical Laboratory, PO Box CR, Mississippi State, MS 39762); Richard S. Wayne (American Cyanamid Co., Agriculture Div., Box 400, Princeton, NJ 08540); H. Hedegaard Povlsen (Kemikaliekontrollen, Morkhoj Bygade 26, DK-2860 Soborg, Denmark); Stephen C. Slahck (Mobay Corp., Box 4913, Kansas City, MO 64120); Fran Porter (Florida Dept of Agriculture, 3125 Conner Blvd, Tallahassee, FL 32301); Richard H. Collier (Purdue University, Dept of Entomology, West Lafayette, IN 47907), *Secretary;* James Hansen (Union Carbide Corp, Agricultural Products, PO Box 8361, South Charleston, WV 25303), *Statistical Consultant*

PESTICIDE FORMULATIONS: CARBAMATE AND SUBSTITUTED UREA INSECTICIDES

Referee: Marshall Gentry, Florida Dept of Agriculture and Consumer Services, Division of Chemistry, Mayo Bldg, Tallahassee, FL 32301

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: 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
Benjamin A. Belkind, Sandoz Crop Protection Corp., 341 E Ohio St, Chicago, IL 60611

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
Benjamin A. Belkind, Sandoz Crop Protection Corp., 341 E Ohio St, Chicago, IL 60611

Methoxychlor
Juan F. Muniz, Oregon Dept of Agriculture, Laboratory Services, 635 Capitol St NE, Salem, OR 97310

Methyl Bromide
David McAllister, Great Lakes Chemical Corp., Box 2200, West Lafayette, IN 47906

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: 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

Cyhexatin
———

Cypermethrin
Stephen J. Eitelman, ICI Americas, Inc., PO Box 208, Goldsboro, NC 27530

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
———

Nicotine
Rodney J. Bushway, University of Maine, Dept of Food Science, Orono, ME 04469

Permethrin
Hershel F. Morris, State Dept of Agriculture, Box 16390-A, University Station, Baton Rouge, LA 70893

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

Rotenone and Other Rotenoids
Rodney J. Bushway, University of Maine, Dept of Food Science, Orono, ME 04469

PESTICIDE FORMULATIONS: HERBICIDES I

Referee: Peter D. Bland, ICI Americas, Inc., PO Box 208, Goldsboro, NC 27530

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

Bromoxynil
Laurence J. Helfant, Rhone Poulenc Ag Co., PO Box 12014, Research Triangle Park, NC 27709

Cacodylic Acid, MSMA, and DSMA
———

Cyanazine
Ronald D. Collins, Shell Development Co., PO Box 4248, Modesto, CA 95353

Dichlobenyl
A. Dereijke, Duphar BV, Analytical Dept, CJ Van Houtenlaan 36, 1381 CP Weesp, The Netherlands

Fomesafen
Stephen J. Eitelman, ICI Americas, Inc., PO Box 208, Goldsboro, NC 27530

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
William Y. Ja, Stauffer Chemical Co., 1200 S 47th St, Richmond, CA 94804

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

Chlorosulfuron
Glenn A. Sherwood, E. I. du Pont de Nemours & Co., Biochemicals Dept, Wilmington, DE 19898

Dicamba
Benjamin A. Belkind, Sandoz Crop Protection Corp., 341 E Ohio St, Chicago, IL 60611

Dimethyl Tetrachloroterephthalate
Brian Korsch, Ricerca, Inc., PO Box 1000, Painesville, OH 44079

Dinoseb
———

Fluometuron
Arthur H. Hofberg, Ciba-Geigy Corp., Analytical Section, PO Box 18300, Greensboro, NC 27419

Metasulfuron-methyl
Glenn A. Sherwood, Jr, E. I. du Pont de Nemours & Co., Biochemicals Dept, Wilmington, DE 19898

Methazole
Benjamin A. Belkind, Sandoz Crop Protection Corp., 341 E Ohio St, Chicago, IL 60611

Naptalam (Alanap)
Milton Parkins, Uniroyal Chemical Co., Crop Protection Analytical Div., Naugatuck, CT 06770

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: OTHER ORGANOPHOSPHORUS INSECTICIDES

Referee: Marshall Gentry, State Dept of Agriculture and Consumer Services, Division of Chemistry, Tallahassee, FL 32301

Crotoxyphos
Wendy King, State Dept of Agriculture and Consumer Services, Tallahassee, FL 32301

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: 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. Beikind, Sandoz Crop Protection Corp., 341 E Ohio St, Chicago, IL 60611

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)
Thomas T. Gale, Ciba-Geigy Corp., Box 18300, Greensboro, NC 27419

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, ICI Americas, Inc., PO Box 208, Goldsboro, NC 27530

Temephos
———

***S,S,S*-Tributyl Phosphorotrithioate**
William Betker, Mobay Corp., Box 4913, Kansas City, MO 64120

PESTICIDE FORMULATIONS: FUNGICIDES AND DISINFECTANTS

Referee: Peter D. Bland, ICI Americas, Inc., PO Box 208, Goldsboro, NC 27530

Anilazine (Dyrene)
Stephen C. Slahck, Mobay Corp., Box 4913, Kansas City, MO 64120

Benomyl
———

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
———

Dinocap
———

Dioxins in Pentachlorophenol
———

Dithiocarbamate Fungicides
Warren R. Bontoyan, Environmental Protection Agency, Office of Pesticide Programs, Beltsville, MD 20705

Oxythioquinox (Morestan)
Stephen C. Slahck, Mobay Corp., Box 4913, Kansas City, MO 64120

o-Phenylphenol
———

Quaternary Ammonium Compounds
———

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: RODENTICIDES AND MISCELLANEOUS PESTICIDES

Referee: Marshall Gentry, State Dept of Agriculture and Consumer Services, Division of Chemistry, Tallahassee, FL 32301

Brodifacoum
Richard S. Freedlander, ICI Americas Inc., PO Box 208, Goldsboro, NC 27530

Chlorophacinone
———

Diphacinone
———

α-Naphthylthiourea
———

Sampling
———

Strychnine
———

Warfarin
———

CIPAC STUDIES

Referee: James Launer, 1260 Acacia Dr S, Salem, OR 97302

MICROBIAL PESTICIDES

Referee: ———

DISINFECTANTS

Referee: Aram Beloian, Environmental Protection Agency, Benefits and Use Division, Washington, DC 20460

Antimicrobial Agents in Laundry Products
Luther B. Arnold and Jean Vollrath-Vaughn, Vikon Chemical Co., PO Box 1520, Burlington, NC 27215
Jamie McGee, Dow Corning Corp., Midland, MI 48640

Sporicidal Tests
Ted Wendt, Surgikos, Inc., 2500 Arbrook Dr, Arlington, TX 76010

Textile Antibacterial Preservatives
Luther B. Arnold and Jean Vollrath-Vaughn, Vikon Chemical Co., PO Box 1520, Burlington, NC 27215

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
Charles R. McDuff, Economics Laboratory, Inc., St. Paul, MN 55102

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 Chief Medical Examiner, 701 Jefferson Rd, South Charleston, WV 25309); 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); Robert C. Livingston (Food and Drug Administration, 5600 Fishers Ln, Rockville, MD 20857); Kaiser Aziz (Food and Drug Administration, Bureau of Medical Devices, 8751 Georgia Ave, Silver Spring, MD 20910); William W. Wright (U.S. Pharmacopeial Convention, 12601 Twinbrook Pkwy, Rockville, MD 20852), *Secretary*

DRUGS I

Referee: James W. Fitzgerald, Food and Drug Administration, Winchester Engineering and Analytical Center, 109 Holton St, Winchester, MA 01890

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

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

Pilocarpine

Irving Wainer, St. Jude's Children's Research Hospital, PO Box 318, Memphis, TN 38101

Rauwolfia Alkaloids

———

Rauwolfia serpentina

Ugo R. Cieri, Food and Drug Administration, 2nd and Chestnut Sts, Philadelphia, PA 19106

DRUGS III

Referee: Martin Finkelson, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Coumarin Anticoagulants

Ella Moore, 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 Moore, 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

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, West Point, PA 19484

D- and L-Amphetamines
Irving Wainer, St. Jude's Children's Research Hospital, 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, National Center for Drugs and Biologics, Washington, DC 20204

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

Beta-Methasone
David J. Krieger, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Penta-Erythritol Tetramitrate
Marvin Carlson, Food and Drug Administration, 240 Hennepin Ave, Minneapolis, MN 55401

Prednisolone
James F. Brower, Food and Drug Administration, Division of Drug Analysis, 1114 Market St, St. Louis, MO 63101

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: Richard A. Baldwin, 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

Heparin by Non-RIA Procedures
———

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

Ipronidazole in Turkey and Swine
Raymond B. Ashworth, U.S. Dept of Agriculture, Food Safety and Inspection Service, Washington, DC 20250

Screening Methods
Michael Thomas, Food and Drug Administration, Agricultural Research Center, Beltsville, MD 20705

COSMETICS

Referee: Ronald L. Yates, Food and Drug Administration, Division of Cosmetics Technology, Washington, DC 20204

Aloe
Alexis Eberendu, Carrington Laboratories, 9200 Carpelter Freeway, Dallas, TX 75247

Essential Oils and Fragrance Materials, Components
Harris H. Wisneski, Food and Drug Administration, Division of Cosmetics Technology, Washington, DC 20204

Nitrosamines
Hardy J. Chou, Food and Drug Administration, Division of Cosmetics Technology, Washington, DC 20204

Preservatives
Ann R. Stack, Food and Drug Administration, Division of Cosmetics Technology, Washington, DC 20204

Water and Alcohol
Mohinder Singh, Blistex, Inc., 1800 Swift Dr, Oak Brook, IL 60521

FORENSIC SCIENCES

Referee: ———

Blood
Ralph Plankenhorn, Pennsylvania State Police, Box P, Greensburg, PA 15601

Grouping Tests for Blood and Other Body Fluids
Henry C. Lee, State Police Forensic Science Laboratory, 294 Colony St, Meriden, CT 06450

Electrophoretic Methods
———

Immunoelectrophoresis of Biological Fluids
James D. Hauncher, Michigan State Police, 42145 W Seven Mile Rd, Northville, MI 48167

Explosives and Explosives Residues
———

Fingerprints
Charles M. Connor, 1025 Fashion Ave, Cincinnati, OH 45238

Gunshot Residue
———

Isoelectric Focusing Methods for Body Fluid Stains
———

Paints and Other Polymeric Materials
———

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;* Arthur E. Waltking (CPC International, Inc., 1120 Commerce Ave, Union, NJ 07083); John D. McKinney (Ranchers Cotton Oil, 2691 S Cedar, Fresno, CA 93725); Elmer George (State Dept of Agriculture and Markets, 1220 Washington Ave, Albany, NY 12235); H. B. S. Conacher (Health and Welfare Canada, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario K1A 0L2, Canada); Michael E. Knowles (Ministry of Agriculture, Fisheries and Food, Horseferry Rd, London SW1P 2AE, UK); Douglas Engebretson (Land O' Lakes, Inc., PO Box 116, Minneapolis, MN 55440); 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, General Foods Corp, Analytical Chemistry Dept, Prospect Plains Rd, Cranbury, NJ 08512

Methyl Xanthenes
John M. Newton, Food and Drug Administration, 50 Fulton St, San Francisco, CA 94102

Moisture
William P. Clinton, General Foods Corp., White Plains, NY 10625

Solvent Residues in Decaffeinated Coffee and Tea
B. Denis Page, Health and Welfare Canada, Health Protection Branch, Ottawa, Ontario K1A 0L2, Canada

Water Extract in Tea
———

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, Ottawa, Ontario K1A 0C5, Canada

Babcock Test and Babcock Glassware
Robert L. Bradley, University of Wisconsin-Madison, Food and Science Dept, Madison, WI 53706

Babcock, Mojonnier, and Kjeldahl Tests
———

Calcium, Phosphorus, and Magnesium in Cheese
Roger Pollman, New York State Dept of Agriculture and Markets, Albany, NY 12235

Chloride Meter
Bruce Vines, Kraft, Inc., 1 Kraft Ct, Glenview, IL 60025

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

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, University of Guelph, Dept of Food Science, Guelph, Ontario N1G 2W1, Canada

Moisture in Cheese
Raymond Matulis, Kraft Foods, Kraft Ct, Glenview, IL 60025

Mojonnier Method (Robotic)
Robert Bradley, University of Wisconsin, Food and Science Dept, 1605 Linden Dr, Madison, WI 53706

Nonfat Solids
John W. Sherbon, Cornell University, Dept of Dairy and Food Science, Ithaca, NY 14853

Nitrates in Cheese
James E. Hamilton, Food and Drug Administration, Division of Drug Labeling—Compliance, 5600 Fishers Ln, Rockville, MD 20857

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, Spain

Whey Proteins in Nonfat Dry Milk
C. Olieman, Netherlands Institute for Dairy Research, 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, Food Research Div., Tunney's Pasture, Ottawa, Ontario K1A 0L2, Canada

Brominated Oils
James F. Lawrence, Health and Welfare Canada, Food Research Div., 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
———

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, Food Directorate, 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

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 in Foods
———

MEAT, POULTRY, AND MEAT AND POULTRY PRODUCTS

Referee: Jon E. McNeal, U.S. Dept of Agriculture, Food Safety and Inspection Service, Washington, DC 20250

Automated Methods
———

Chemical Antibiotic Methods
William Moats, U.S. Dept of Agriculture, Meat Science Research Laboratory, Beltsville, MD 20705

Fat in Meat Products
Jon E. McNeal, U.S. Dept of Agriculture, Food Safety and Inspection Service, Washington, DC 20250

Identification of Meats, Serological Tests
Arthur P. Marin, State Dept of Agriculture and Markets, State Campus, Albany, NY 12235

Immunochemical Identification of Additives
Ron Berger, U.S. Dept of Agriculture, Food Safety and Inspection Service, Beltsville, MD 20705

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
———

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
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

Sample Preparation Techniques
Sylvan Eisenberg, Anresco, Inc., 1370 Van Dyke Ave, San Francisco, CA 94124

Species Identification Methods
———

Specific Ion Electrode Applications
Randy Simpson, U.S. Dept of Agriculture, Food Safety and Inspection Service, PO Box 6085, Athens, GA 30604

Temperature, Minimum Processing
Grover Pickel, U.S. Dept of Agriculture, Food Safety and Inspection Service, PO Box 6085, Athens, GA 30604

Total Fat
Max 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 and Moisture by Microwave Drying
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
Douglas L. Park, University of Arizona, Dept of Nutrition and Food Science, 309 Schantz Bldg, Tucson, AZ 85721

Alternaria Toxins
Edgar E. Stinson, U.S. Dept of Agriculture, Eastern Regional Research Center, 600 E Mermaid Ln, Philadelphia, PA 19118

Citrinin
David Wilson, University of Georgia, Dept of Plant Pathology, Tifton, GA 31794

Cyclopiazonic Acids
John A. Landsen, U.S. Dept of Agriculture, National Peanut Research Laboratory, Dawson, GA 31742

Emodin and Related Anthoquinones
———

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

***Penicillium islandicum* Toxins**
———

Secalonic Acid
———

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, 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 Colompo 79, 20133 Milano, Italy

Oxidized Fats
Michael Blumenthal, Libra Laboratories, Inc., 44 Stelton Rd, Piscataway, NJ 08854

Pork Fat in Other Fats
Laila El-Sayed Abdel Fattah, Centre of Science and Medical Sciences for Women, PO Box 22452, Riyadh 11495, Saudi Arabia

Sterols and Tocopherols
Robert J. Reina, Food and Drug Administration, 585 Commercial St, Boston, MA 02109

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
———

Hypoglycine in Ackee
———

Phytoestrogens
———

Pyrrolizidine Alkaloids
———

Steroidal Alkaloids
Allen S. Carman, Food and Drug Administration, 4298 Elysian Fields Ave, New Orleans, LA 70122

SEAFOOD PRODUCTS

Referee: Louis M. Gershman, 151 Collidge Ave, Watertown, MA 02172

Amines by TLC
Thomas R. Weber, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Ammonia in Seafood
Beverly A. Hunter, National Marine Fisheries Service, PO Drawer 1207, Pascagoula, MS 39567

Coprostanol
James F. Stewart, Food and Drug Administration, 3032 Bryan St, Dallas, TX 75204

Decomposition of Crabmeat
Kurt Steinbrecher, Food and Drug Administration, 909 First Ave, Seattle, WA 98174

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

Ethanol in Seafoods
Harold R. Throm, Food and Drug Administration, 909 First Ave, Seattle, WA 98174

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
———

Volatile Amines—TMA and DMA by GC
Ronald C. Lundstrom, National Marine Fisheries Service, Northeast Fisheries Center, Gloucester, MA 01930

Shellfish Decomposition
———

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 (Immunoassay Method)
Patrick Guire, BioMetric Systems, Inc., 9932 W 74th St, Eden Prairie, MN 55344

Paralytic Shellfish Poisons (LC Methods)
John J. Sullivan, Food and Drug Administration, 909 First Ave, Seattle, WA 98174

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

Laura L. Zaika (U.S. Dept of Agriculture, Eastern Regional Research Center, 600 E Mermaid Ln, Philadelphia, PA 19118), *Chairman;* Harry G. Lento (Campbell Soup Co., Campbell Pl, Camden, NJ 08151); 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); Robert A. Martin (Hershey Foods Corp., Hershey Technical Center, 1025 Reese Ave, Hershey, PA 17033), *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

Alcohol Content of High Solids Distilled Spirits
———

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

Color Intensity for Distilled Alcoholic Products
———

Ethanol in Wine by GC
Arthur Caputi, Jr, E & J Gallo Winery, PO Box 1130, Modesto, CA 95353

Ethyl Carbamate in Wine
Benjamin Canas, Food and Drug Administration, Division of Food Chemistry and Physics, Washington, DC 20204

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 (Ripper Method)
Barry Gump, California State University, Dept of Food Science and Enology, Fresno, CA 93740
Guy Baldwin, Paul Masson Vinyards, PO Box 97, Saratoga, CA 95070

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 Food, Nutrition, and Institute Management, University, AL 35486

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
———

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

Arsenic, Barium, and Heavy Metals
William J. McShane, Kraft, Inc., Research and Development Dept, 801 Waukegan Rd, Glenview, IL 60025

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

Intermediates, Uncombined, in Certifiable Water-Soluble Azo Colors
———

Intermediates in Other Certifiable Colors
Alan Scher, Food and Drug Administration, Division of Color Technology, Washington, DC 20204

Liquid Chromatography
Elizabeth A. Cox, Food and Drug Administration, Division of Color Technology, Washington, DC 20204

Subsidiary Colors in Certifiable Color Additives
John Bailey, 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: Kurt Schoen, David Michael & Co., 10801 Decatur Rd, Philadelphia, PA 19154

Additives in Vanilla Flavorings
Sidney Kahan, Kahansultants, Inc., 66 Peachtree Ln, Roslyn Heights, NY 11577

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 Technology, Washington, DC 20204

Adulteration of Apple Juice
Edgar R. Elkins, National Food Processors Association, 1401 New York Ave, 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

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

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
———

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: James Nelson, Food and Drug Administration, Import District, 850 Third Ave, Brooklyn, NY 11232

Aspartame, Benzoates, Saccharin, and Caffeine, Liquid Chromatography
———

Organic Preservatives (Thin Layer Chromatography)
Rosella Bigornia, General Foods Corp., White Plains, NY 10591

Sulfites
Walter Holak, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232
Martha Hudak-Roos, National Marine Fisheries Service, 3209 Frederick St, Pascagoula, MS 39568-1207
John J. Sullivan, Food and Drug Administration, 909 First Ave, 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

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
Thomas E. Haney, Durkee Foods, 16651 Sprague Rd, Strongsville, OH 44136

Ethylene Oxide and Ethylene Chlorohydrin Residues
Lynn Theiss, R. T. French Co., PO Box 23450, Rochester, NY 14692

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

Chromatographic Methods
Michael Gray, Bio-Rad Laboratories, 32nd & Griffin Ave, Richmond, CA 94804

Color, Turbidity, and Reflectance-Visual Appearance
Margaret A. Clarke, Sugar Processing Research, Inc., Box 19687, New Orleans, LA 70179

Corn Syrup and Corn Sugar
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
Maria Franca Morselli, University of Vermont, Botany Dept, Burlington, VT 05405

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 Sugar Methods of Analysis
Mary A. Godshall, Sugar Processing Research, Inc., Box 19687, New Orleans, LA 70179

Sugar in Cereal
L. Zygmunt, Quaker Oats Co., 617 W Main St, Barrington, IL 60010

Sugar 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
———

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

Dietary Fiber
Leon Prosky, Food and Drug Administration, Division of Nutrition, Washington, DC 20204

Fat in Food by Chloroform-Methanol Extraction
Chester E. Daugherty, Campbell Soups, Campbell Pl, Camden, NJ 08101

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
Raymond Cooke, Laboratory of the Government Chemist, Food Composition and Nutrition, Cornwall House, Stamford St, London, UK SE1 9NQ

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-LaRoche, 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); William G. 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); Richard Frank (Ministry of Agriculture, Food Technology, and Field Services, 801 Bay St, Toronto, Ontario M7A 1A5, Canada); Edgar R. Elkins (National Food Processors Assoc., 1401 New York Ave NW, Washington, DC 20005); 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 Chemical Technology, Washington, DC 20204

Atomic Absorption Spectrophotometry
Milan Ihnat, Agriculture Canada, Chemistry and Biology Research Institute, 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 Chemical Technology, Washington, DC 20204

Lead in Calcium Supplements
Patricia Maroney Benassi, Food and Drug Administration, 10 W 35th St, Chicago, IL 60616

Methyl Mercury in Fish and Shellfish
Susan Hight, Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Neutron Activation Analysis
William C. Cunningham, Food and Drug Administration, Division of Contaminants Chemistry, Washington, DC 20204

Organotin Compounds
Allen D. Uhler, Food and Drug Administration, Division of Contaminants Chemistry, Washington, DC 20204

Organometallics in Fish
Walter Holak, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Polarography
Susan Hight, Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

MULTIRESIDUE METHODS

Referee: Leon D. Sawyer, Food and Drug Administration, 200 C St SW, Washington, DC 20204

Comprehensive Multiresidue Methodology
Leon Sawyer, Food and Drug Administration, Washington, DC 20204
Darryl E. Johnson, Food and Drug Administration, 240 Hennepin Ave, Minneapolis, MN 55401

Fumigants
James L. Daft, Food and Drug Administration, 1009 Cherry St., Kansas City, MO 64106

Extraction of Low Moisture-High Fat Samples
Leon D. Sawyer, Food and Drug Administration, 200 C St SW, Washington, DC 20204

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

ORGANOHALOGEN PESTICIDES

Referee: Bernadette McMahon, Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Chlordane
Wilbur Saxton, Food and Drug Administration, 5003 Federal Office Building, Seattle, WA 98174

Chlorinated Dioxins
David Firestone, Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Chlorophenoxy Alkyl Acids

———

Ethylene Oxide and Its Chlorohydrin

———

Gel Permeation Chromatographic Cleanup
Timothy Spurgeon, ABC Laboratories, 7200 E ABC Ln, Box 1097, Columbia, MO 65205

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

Pentachlorophenol
George Yip, Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Pentachlorophenol in Blood
Virlyn Burse, Center for Environmental Health, Clinical Chemistry Division, 1600 Clifton Rd, Atlanta, GA 30333

Pentachlorophenol in Meat and Poultry Tissue
Douglas Gillard, Huntington Analytical Services, PO Box 250, Middleport, NY 14105

Polychlorinated Biphenyls (PCBs)
Leon D. Sawyer, Food and Drug Administration, 200 C St SW, Washington, DC 20204

Polychlorinated Biphenyls in Blood

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ORGANONITROGEN PESTICIDES

Referee: W. Harvey Newsome, Health and Welfare Canada, Food Research Division, Tunney's Pasture, Ottawa, Ontario, K1A 0L2, Canada

Anilazine

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Benzimidazole-Type Fungicides
Mikio Chiba, Agriculture Canada, Vineland Station, Ontario L0R 2E0, Canada

Captan and Related Fungicides
Dalia Gilvydis, Food and 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
Hafez Abdel-Kader, and 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**

———

Trifluralin

———

ORGANOPHOSPHORUS PESTICIDES

Referee: Gail Abbott Parker, Florida Dept of Agriculture and Consumer Services, 3125 Conner Blvd, Tallahassee, FL 32301

Disulfoton
Sunny Y. Szeto, Agriculture Canada, 6660 NW Marine Dr, Vancouver, British Columbia V6T 1X2, Canada

Extraction Procedures

———

Gel Permeation Chromatography Cleanup
Ronald Scharfe, Agriculture Canada, Pesticide Laboratory, Ottawa, Ontario K1A 0L2, Canada

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
Richard E. Needham, Food and Drug Administration, Winchester Engineering and Analytical Center, Winchester, MA 01890
Alfred V. Robinson, United States Testing Co., Inc., Richland Division, 2800 George Washington Way, Richland, WA 99352

Radium-228
Jacqueline Michel, Research Planning Institute, 925 Gervais St, Columbia, SC 29201

Strontium-89 and -90
Joseph A. Hutchinson, State Dept of Health, Radiological Sciences Lab., Albany, NY 12201

Tritium

———

Committee on Microbiology

Michael H. Brodsky (Ministry of Health, Laboratory Services Branch, Toronto, Ontario M5W 1R5, Canada), *Chairman;* John S. Gecan (Food and Drug Administration, Division of Microbiology, Washington, DC 20204); 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, 1090 Tusculum Ave, Cincinnati, OH 45226); Donald E. Lake (American Can Co., 433 N NW Highway, Barrington, IL 60010); Phillip Alioto (State Dept of Agriculture, 4702 University Ave, Madison, WI 53707); Donald A. Mastrorocco, Jr (Hershey Foods Corp., 19 E Chocolate Ave, Hershey, PA 17033), *Secretary;* Foster D. McClure (Food and Drug Administration, Division of Mathematics, Washington, DC 20204), *Statistical Consultant;* Richard E. Young (36 Drexel Ave, Florence, KY 41042), *Statistical Consultant*

ANALYTICAL MYCOLOGY AND MICROSCOPY

Referee: Stanley M. Cichowicz, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Chemical Methods for Detecting Mold
Ruth Bandler, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Geotrichum Mold in Canned and Frozen Fruits and Vegetables
Stanley M. Cichowicz, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Standardization of Plant Tissue Concentrations for Mold Counting
Stanley M. Cichowicz, 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

Bactoscan Methods

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β-Lactam Residues in Milk—Delvo-test
Wesley N. Kelley, State Dairy Laboratory, SDSU-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 Foods, Inc., Kraft Ct, Glenview, IL 60025

Listeria-DNA Probe

———

Listeria-Culture Methods

Joseph Lovett, Food and Drug Administration, 1090 Tusculum Ave, Cincinnati, OH 45226

Penicillins in Milk—Affinity Quantitation

Stanley E. Charm, Penicillin Assays, Inc., 36 Franklin St, Malden, MA 02148

Raw Milk in Cheese

———

Somatic Cells, Automated Optical Counting Method

Wesley N. Kelley, State Dairy Laboratory, SDSU-Dairymicro Bldg, Brookings, SD 57007

Somatic Cells, Fossomatic Counting Method

R. D. Mochrie, North Carolina State University, Animal Science Dept, Raleigh, NC 27695

FOOD MICROBIOLOGY

Referee: Wallace H. Andrews, 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
Gayle Lancette, Food and Drug Administration, Minneapolis Center for Microbiological Investigations, 240 Hennepin Ave, Minneapolis, MN 55401

***Bacillus cereus* Enterotoxin**

Reginald W. Bennett, 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

———

Enterobacteriaceae

Russell S. Flowers, Silliker Laboratories, Inc., 1304 Halsted St, Chicago Heights, IL 60411

***Escherichia coli,* Enzymatic Methods**

Lloyd J. Moberg, General Mills, Inc., 9000 Plymouth Ave N, Minneapolis, MN 55427

Genetic Methods for Detection of Bacterial Pathogens

Walter Hill, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Hydrophobic Grid Membrane Filter Methods

Phyllis Entis, QA Laboratories, Ltd, 135 The West Mall, Toronto, Ontario M9C 1C2, Canada

Iron Milk Test for *Clostridium perfringens*

Carlos Abeyta, Food and Drug Administration, 909 First Ave, Seattle, WA 98174

Petrifilm Methods

Roy E. Ginn, Dairy Quality Control Institute, 2353 Rice St, St. Paul, MN 55133
Vernal S. Packard, University of Minnesota, Dept of Food Science and Nutrition, St. Paul, MN 55108

Redigel Media

Jonathan Roth, RCR Scientific, Inc., PO Box 340, Goshen, IN 46526

***Salmonella* in Chocolate**

O. Bindschedler, Nestec, Ave Nestle 55, CH 1800 Vevey, Switzerland

Shellfish

William Watkins, Food and Drug Administration, Construction Batallion Center, North Kingstown, RI 02852

Sugars

———

***Vibrio cholerae* and Detection of its Toxins**

Angelo De Paolo, 2511 SW 35th Pl, 39 Arbor Apt, Gainesville, FL 32608

Yeasts, Molds, and Actinomycetes

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, 240 Hennepin Ave, Minneapolis, MN 55401
George Kapperud, National Institute of Public Health, Norwegian Defense Microbiology Laboratory, Geitmyrsvein 75, N-0462, Oslo 4, Norway

DRUG AND DEVICE RELATED MICROBIOLOGY

Referee: Gordon Oxborrow, Food and Drug Administration, Minneapolis Center for Microbiological Investigations, 240 Hennepin Ave, Minneapolis, MN 55401

Biological Sterility Indicators

Gordon Oxborrow, 3M, 3M Center, Bldg 270-5N-01, St. Paul, MN 55144

Chemical Indicators

Marvin L. Hart, 3M Co., Medical Systems and Microbial Products, St. Paul, MN 55082

Limulus Amebocyte Lysate Tests for Endotoxins

Christine W. Twohy, Food and Drug Administration, Minneapolis Center for Microbiological Investigations, 240 Hennepin Ave, Minneapolis, MN 55401

Packaging Integrity for Medical Devices

Ana M. Placencia, Food and Drug Administration, Sterility Research Center, 100 Union St, Minneapolis, MN 55455

Sterility Testing of Medical Devices

Michael J. Palmieri, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Testing and Standardization of Biological Indicators

Robert Berube, 3M Co., Medical Surgical Division, St. Paul, MN 55144
Gordon Oxborrow, Food and Drug Administration, 240 Hennepin Ave, Minneapolis, MN 55401

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

Joseph K. Nagy, Food and Drug Administration, Customhouse, Philadelphia, PA 19106

Botanical Drugs, Adulteration by Foreign Plant Materials

Frank D'Amelio, Bio Botanica, 75 Commerce Dr, Hauppauge, NY 11788

Botanicals

Marvin Nakashima, Food and Drug Administration, Division of Microbiology, Washington, DC 20204
Joseph A. McDonnell, Food and Drug Administration, 60 Eighth St NE, Atlanta, GA 30309

Canned and Dried Soups

Richard Klein, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Canned Crabmeat Shrimp, and Tuna

James D. Barnett, Food and Drug Administration, 4298 Elysian Fields Ave, New Orleans, LA 70122

Canned Fish and Fish Products

Wilfred A. Sumner, Food and Drug Administration, 50 United Nations Plaza, San Francisco, CA 94102

Capsicum Products

Susan Schena, Cal Compack Foods, PO Box 265, Santa Ana, CA 92702

Cheese

Mary-Ann Gardiner, Health and Welfare Canada, Microbial Research Division, Ottawa, Ontario K1A 0L2, Canada

Chocolate Products

———

Cocoa Powder and Press Cake

C. Robert Graham, Lancaster Laboratories, Inc., 2425 New Holland Pike, Lancaster, PA 17601

Dehydrated Vegetable Products

Francis J. Farrell, Thomas J. Lipton, Inc., 800 Sylvan Ave, Englewood Cliffs, NJ 07632

Grains, Whole, Cracking Flotation Methods

Richard Trauba, Food and Drug Administration, 240 E Hennepin Ave, Minneapolis, MN 55401

Insect Excreta in Flour

———

Meats, Processed
Phillip Alioto, State Dept of Agriculture, 4702 University Ave, Madison, WI 53707

Mite Contamination Profiles and Characterization of Damage to Foods
Diane Peace and Mary Ann Gardiner, Health and Welfare Canada, Bureau of Microbiological Hazards, Tunney's Pasture, Ottawa, Ontario K1A 0L2, Canada

Mites in Stored Foods
Jack L. Boese, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Mushroom Products, Dried
Jack L. Boese, Food and Drug Administration, Division of Microbiology, Washington, DC 20204
Alan R. Olsen, Food and Drug Administration, 1521 W Pico Blvd, Los Angeles, CA 90015

Mushrooms, Canned

———

Performance Evaluation of Methods for Filth
Jack L. Boese, Food and Drug Administration, Division of Microbiology, Washington, DC 20204
James Karpus and Alan Whiteman, Food and Drug Administration, 433 W Van Buren St, Chicago, IL 60607

Rye Bread
Richard R. Haynos, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Shrimp

———

Soluble Insect and Other Animal Filth
George P. Hoskin and Harriet R. Gerber, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Soybean Curd
Marvin Nakashima, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Spirulina
Marvin Nakashima, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Tomatoes and Mushrooms
Bernice Beavin, Food and Drug Administration, 900 Madison Ave, Baltimore, MD 21201

Urine Detection
Robert S. Ferrera, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Vertebrate Excreta, Chemical Identification Tests
Harriet R. Gerber, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Committee on Feeds, Fertilizers, and Related Materials

Rodney J. Noel (Office of the Indiana State Chemist, Purdue University, West Lafayette, IN 47907), *Chairman;* Louis W. Ferarra (Ferrara's Analytical and Technical Service, 82 Heritage Dr, Terre Haute, IN 47803); Alexander MacDonald (Hoffmann-La Roche Inc., 340 Kingsland St, Nutley, NJ 07110); Howard Casper (North Dakota State University, Veterinary Diagnostic Laboratory, Fargo, ND 58102); Stanley E. Katz (Rutgers University, Dept of Biochemistry and Microbiology, New Brunswick, NJ 08903); Thomas M. Parham (Arcadian Corp., PO Box 307, Geismar, LA 70734); Lori L. Rhodig (Smith Kline Animal Health Products, 4444 S 76th St, Omaha, NE 68127); Carclyn Geisler (Food and Drug Administration, U.S. Customhouse, Denver, CO 80202); Billy M. Colvin (University of Georgia, College of Veterinary Medicine, Box 1386, 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

Antibiotic Screening Methods
Gerald L. Stahl, Upjohn Co., Downtown Complex, Kalamazoo, MI 49001

Bacitracin
Linda L. Knotts, International Minerals & Chemicals Corp., PO Box 207, Terre Haute, IN 47808

Bacitracin (Chemical Method)
John B. Gallagher, International Minerals & Chemicals Corp., PO Box 207, Terre Haute, IN 47808

Bambermycins
Jean Olsen, Hoechst Pharmaceuticals, Inc., Rte 202-206 N, Somerville, NJ 08876

Chloramphenicol in Animal Tissues
Edward H. Allen, Food and Drug Administration, Bureau of Veterinary Mecicine, Beltsville, MD 20705

Chloramphenicol in Milk
Dieter Arnold, Robert Koch Institute, Nordufer 20, 1000 Berlin 65, West Germany

Chlortetracycline

———

Coban
Robert E. Scroggs, Elanco Products Co., Box 1750, Indianapolis, IN 46206

Cup Plate System for Antibiotic Analysis
Virginia A. Thorpe, State Dept of Agriculture, 1615 S. Harrison Rd, East Lansing, MI 48823

Design and Computerization of Microbiological Tests

———

Enzyme Immunoassay for Antimicrobial Compounds
Nitin Thaker, U.S. Dept of Agriculture, Agricultural Research Service, Beltsville, MD 20705

Erythromycins

———

Lasalocid (LC Method)
Edward Waysek, Hoffmann-La Roche, Inc., 340 Kingsland St, Nutley, NJ 07110

Lasalocid (Microbiological Assay)

———

Lincomycin
A. William Neff, The Upjohn Co., Agricultural Div., Kalamazoo, MI 49001

Microbiological Assays
Marietta S. Brady and Peter Kahn, Rutgers University, Dept. of Biochemistry and Microbiology, New Brunswick, NJ 08903

Monensin

———

Narasin
Robert E. Scroggs, Elanco Products Co., Box 1750, Indianapolis, IN 46206

Neomycin

———

Oxytetracycline
Dorothy M. Brennecke, 3981 Dover Pl, St. Louis, MO 63116

Rumensin
Robert E. Scroggs, Elanco Products Co., Box 1750, Indianapolis, IN 46206

Sampling
John Gallagher, International Minerals and Chemicals Corp., PO Box 207, Terre Haute, IN 47808

Statistics of Microbiological Assay

———

Tetracyclines in Tissues (Chromatographic Assay)
Raymond B. Ashworth, U.S. Dept of Agriculture, Food Safety and Inspection Service, Washington, DC 20250

Tetracyclines in Tissues (Microbiological Assay)
Stanley E. Katz, Rutgers University, Dept of Biochemistry and Microbiology, New Brunswick, NJ 08903

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)
Dorothy M. Brennecke, 3981 Dover Pl, St. Louis, MO 63116
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
———

Carbadox
Virginia A. Thorpe, State Dept of Agriculture, 1615 Harrison Rd, East Lansing, MI 48823

Ethopabate
Joseph Hildebrandt, Agway, Inc., 777 Warren Rd, Ithaca, NY 14850

Furazolidone and Nitrofurazone
Robert E. Smallidge, Purdue University, Dept of Biochemistry, West Lafayette, IN 47907

Lasalocid (Microbiological Assay)
Jacob M. Scheiner, Hoffmann-La Roche, 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

Phenothiazine
———

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
Robert K. Munns, Food and Drug Administration, 20th and California Sts, Denver, CO 80202

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, Box 27647, Raleigh, NC 27611

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
Paul Guenther, Colorado Dept of Agriculture, 1525 Sherman St, Denver, CO 80203

Fiber, Crude
David O. Holst, University of Missouri, Food Science and Nutrition, Columbia, MO 65211

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
Stuart Meridian, Marion Labs, 10236 Bunker Ridge Rd, Kansas City, MO 64137

Microscopy
Patricia Ramsey, CDFA Chemical Laboratories, 3292 Meadowview Rd, Sacramento, CA 95832

Minerals
———

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
———

Water by Karl Fischer Method
Raffaele Bernetti, CPC International, Inc., PO Box 345, Argo, IL 60501

FERTILIZERS

Referee: Frank J. Johnson, Tennessee Valley Authority, National Fertilizer Development Center, Muscle Shoals, AL 35660

Biuret
Lus 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

Free and Total Water
Russell D. Duncan, Tennessee Valley Authority, National Fertilizer Development Center, Muscle Shoals, AL 35660

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
———

Phosphorus
Joe R. Trimm, Tennessee Valley Authority, National Fertilizer Development Center, Muscle Shoals, AL 35660

Potash
Peter F. Kane, Purdue University, Dept of Biochemistry, West Lafayette, IN 47907

Sampling
Douglas Caine, Vigoro Industries, Inc., PO Box 4139, Fairview Heights, IL 62208

Sample Preparation
Rose A. Sweeney, University of Missouri, Experiment Station, Columbia, MO 65211

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, Soil Testing and Plant Laboratory, Athens, GA 30602

Ashing Methods
J. Benton Jones, University of Georgia, Dept of Horticulture, Athens, GA 30602

Atomic Absorption Methods
Robert A. Isaac, University of Georgia, Soil Testing and Plant Analysis Laboratory, Athens, GA 30602

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, Soil Testing and Plant Analysis Laboratory, Athens, GA 30602

Fluorine
Jay S. Jacobson, Boyce Thompson Institute, Tower Rd, Ithaca, NY 14853

Nitrate and Nitrite
———

Selenium
Oscar E. Olson and 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

———

Polyphenols

———

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

Chlorinated Phenols in Animal Tissues

———

Cholinesterase
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

Fluoride in Animal Tissue
David Osheim, U.S. Dept of Agriculture, National Veterinary Services Laboratory, Ames, IA 50010

Lead in Animal Tissue
Robert J. Everson, Purdue University, School of Veterinary Medicine, West Lafayette, IN 47907

Lipid-Soluble Vitamins
Karen S. Harlin, University of Illinois, Dept of Veterinary Bioscience, Urbana, IL 61801

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

Zinc in Animal Tissues
Dana Perry, Veterinary Diagnostic Laboratory, Dept of Veterinary Science, Tucson, AZ 85721

Committee on Hazardous Substances in Water and the Environment

Erika Hargesheimer (City of Calgary, Glenmore Waterworks Lab., Calgary, Alberta T2P 2M5, Canada), *Chairman;* Nile Frawley (Dow Chemical Co., 574 Building, Midland, MI 48640); Douglas J. Dube (State Laboratory of Hygiene, 465 Henry Mall, Madison, WI 53706); 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 St. Clair, Cincinnati, OH 45268); Mark F. Marcus (Chemical Waste Management, Inc., 150 W 137th St, Riverdale, IL 60627), *Secretary;* Forest C. Garner (Lockheed, 3877 Platis Circle, Las Vegas, NV 89103), *Statistical Consultant*

AIR

Referee: ———

BIOMONITORING

Referee: ———

BIOTA

Referee: ———

EFFLUENTS

Referee: ———

HAZARDOUS SUBSTANCES

Referee: ———

Ammonia As a Product Ingredient

———

Benzene in Consumer Products
Wayne G. Wamer, Food and Drug Administration, Division of Toxicology, Washington, DC 20204

Carbolic Acid (Phenolic) Compounds

———

Chlorinated Hydrocarbons

———

Diethylene Glycol and Ethylene Glycol

———

Flammable Substances in Pressurized Containers

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Flash Point of Solids and Semisolids

———

Formaldehyde

———

Hazardous Components in Resin Systems

———

Pentachlorophenol in Toy Paints
Hans E. A. M. Van Langeveld, Food Inspection Services, Florijnruwe 111, 6218 CA Maastricht, The Netherlands

Petroleum Distillates in Mixtures

———

Selenium

———

Toxic Metals in Paints

Warren K. Porter, Jr, Consumer Product Safety Commission, 200 C St SW, Washington, DC 20204

Turpentine

———

Viscosity of Liquids

———

SOILS AND SEDIMENTS

Referee: James Dragun, Stalwart Environmental Science and Services, 1700 N Opdike Rd, Auburn Hills, MI 48057

Adsorption Isotherms for Volatile Organics

Leverett R. Smith, 622 Clayton Ave, El Cerrito, CA 94530

Distribution Coefficients—Nonvolatile Organics

Danny R. Jackson, Battelle-Columbus Labs, 505 King Ave, Columbus, OH 43201

Distribution Coefficients—Volatile Organics

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Soil Column Leaching

———

WASTE MATERIALS

Referee: ———

Bioassays

Llewellyn R. Williams, Environmental Protection Agency, Environmental Monitoring Systems Lab., Box 15027, Las Vegas, NV 89114

Inorganic Analytes

Gerald McKee, Environmental Protection Agency, Environmental Monitoring Systems Lab., 26 W St. Clair St, Cincinnati, OH 45268

Organic Analytes

Paul Friedman, Environmental Protection Agency, Office of Solid Waste, Washington, DC 20460

Physical/Chemical Properties

Florence Richardson, Environmental Protection Agency, Office of Solid Waste, Washington, DC 20460

Prescreening

———

Sampling

———

INORGANICS IN DRINKING AND GROUND WATER

Referee: Kenneth P. Stoub, Waste Management, Inc., 3003 Butterfield Rd, Oakbrook, IL 60521

Arsenic and Selenium

———

Inductively Coupled Plasma

———

Ion Chromatography

———

ORGANICS IN DRINKING AND GROUND WATER

Referee: Andrew D. Sauter, 2356 Aqua Vista, Henderson, NV 89015

Environmental Protection Agency Methods

———

Phenols

———

Polychlorinated Biphenyls

Deborah Lazoski, Chemical Waste Management, 150 W 137th St, Riverdale, IL 60627

Screening for Polynuclear Aromatic Compounds

———

Total Organic Halogen

———

Volatile Organics–Capillary Analysis

———

ORGANICS IN SURFACE AND WASTE WATER

Referee: ———

Chemical Pollutants in Water and Wastewater

Larry B. Lobring, Environmental Protection Agency, 26 W St. Clair St, Cincinnati, OH 45268

Chlorinated Solvents in Water

Douglas J. Dube, State Laboratory of Hygiene, 465 Henry Mall, Madison, WI 53706

Herbicides in Water and Sediment

———

Major Ions and Nutrients in Water

———

Munitions in Wastewater

Peter Rissell, U.S. Army Toxic and Hazardous Materials Agency, Aberdeen Proving Ground, MD 21010

Thomas Jenkins, Cold Regions Research and Engineering Lab., Hanover, NH 03755-1290

Organohalogen Pesticides in Water

Marie Siewierski, Rutgers University, Cook College, New Brunswick, NJ 08903

Organophosphorus Pesticides in Water

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Phenols

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Salt

———

Triazine Herbicides in Water

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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), 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 .D01. Subsequent sections are numbered .D02, .D03, .D04, et seq. The D signifies that the method was adopted at the 1987 Annual International Meeting, and was published and became official in 1988 in the fourth 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

The following interim liquid chromatographic method for triamino-*s*-triazine in fertilizer mixes was adopted first action:

Triamino-s-Triazine in Fertilizer Mixes
Liquid Chromatographic Method
First Action

2.D01 ***Principle***

Ground sample is extd with H_2O and filtered. Triamino-*s*-triazine is detd by liq. chromatgy using external std and UV detection at 254 nm.

2.D02 ***Apparatus***

(**a**) *Liquid chromatograph.*—With UV detection at 254 nm. Operating conditions: flow rate 1.0 mL/min (1200 psi); column temp. ambient; chart speed 0.5 cm/min; injection vol. 20 μL; sample injector with fixed sample loop preferred. Pump LC mobile phase thru column until system is equilibrated. Allow 10 min run time for each injection. Retention time for triamino-*s*-triazine is 4–5 min. Re-equilibrate baseline before each injection.

(**b**) *LC column.*—LiChrosorb RP-18, 25 cm × 4.5 mm. (Use this type column; chemistry of triamino-*s*-triazine requires use of polar solv. system.)

(**c**) *Strip chart recorder.*—To match output of detector.

(**d**) *pH meter.*—Sensitivity 0.01. Stdze with pH 4 buffer soln.

(**e**) *Filters.*—2.4 cm glass fiber (Whatman 934-H or equiv.).

2.D03 ***Reagents***

(**a**) *Sodium phosphate.*—Anhyd., dibasic. Na_2HPO_4, reagent grade or equiv.

(**b**) *Diethylamine.*—Reagent grade or equiv.

(**c**) *Phosphoric acid.*—Reagent grade or equiv.

(**d**) *Water.*—Deionized or distd.

(**e**) *Buffer soln.*—pH 4.0.

(**f**) *Mobile phase.*—Deionized H_2O contg 1% (w/v) anhyd. Na_2HPO_4 and 1 mL diethylamine/L. Adjust to pH 4 with H_3PO_4.

(**g**) *Triamino-s-triazine std solns.*—(*1*) *Stock std soln.*—500 mg/L (ppm). Accurately weigh 50.0 mg triamino-*s*-triazine ref. std (Melamine Chemicals, Inc., PO Box 748, Donaldsonville, LA 70346) into 100 mL vol. flask. Dissolve in and dil. to vol. with deionized H_2O. (*2*) *Working std solns.*—50, 125, and 250 mg/L. Pipet 10, 25, and 50 mL stock std soln into sep. 100 mL vol. flasks and dil. to vol. with deionized H_2O. Use as calibration stds.

2.D04 ***Preparation of Sample***

Grind ≥225 g sample (triamino-*s*-triazine granules or dry-mix blends with other fertilizers) to pass No. 40 sieve, mix thoroly, and store in tightly stoppered bottle.

Accurately weigh 5–8 g well mixed, ground sample and transfer to 2 L vol. flask. Dil. to vol. with deionized H_2O and stir 2 h using stir bar and mag. stirrer. Filter portion for analysis thru 1 μm glass fiber filter. Pipet 1 mL filtrate into 100 mL vol. flask and dil. to vol. with deionized H_2O.

2.D05 ***Determination***

Equilibrate column with mobile phase for 30–60 min. Inject 20 μL std soln until peak hts agree ±2%. Inject 20 μL sample with attenuation set to give largest possible on-scale peaks. Reinject std after every 10th sample to verify calibration and ensure accurate quantitation.

2.D06 ***Calculations***

Calc. amt triamino-*s*-triazine as follows:

$$\text{Triamino-}s\text{-triazine, \%} = (PH/PH') \times [C/(5 \times W)] \times 100$$

where PH and PH' = peak hts for sample and std, resp.; C = concn of std, ppm; and W = sample wt, g.

Ref.: JAOAC **71**, May issue (1988).

CAS-108-78-1 (1,3,5-triazine-2,4,6-triamine; melamine)

3. PLANTS

The following first action methods were adopted final action:

(a) Metals in plants, emission spectrographic methods, **3.006–3.010**.

(b) Metals in plants, atomic absorption spectrophotometric method, **3.013–3.016**.

(c) Fluoride in plants, potentiometric method, **3.075–3.080**.

(d) Metals and other elements in plants, inductively coupled plasma spectroscopic method, **3.A01–3.A04.**

4. DISINFECTANTS

The following final action methods were repealed to first action:

(a) Use-dilution methods: testing disinfectants against *Salmonella choleraesuis*, **4.007–4.009**; testing disinfectants against *Staphylococcus aureus*, **4.010.**

(b) Tuberculocidal activity of disinfectants: presumptive in vitro screening test using *Mycobacterium smegmatis*, **4.036–4.038**; 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) Diazinon in formulations, gas chromatographic method, **6.425**.

(b) Thiocarbamates in herbicide formulations, gas chromatographic method, **6.581–6.585**.

(c) Metolachlor in formulations, AOAC–CIPAC gas chromatographic method, **6.A14–6.A18**.

(d) Permethrin in formulations, CIPAC–AOAC gas chromatographic method, **6.B06–6.B11**.

(e) Butachlor in formulations, AOAC–CIPAC gas chromatographic method, **6.B12–6.B16**.

(f) Propachlor in formulations, AOAC–CIPAC gas chromatographic method, **6.B17–6.B21**.

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(2) The following interim CIPAC–AOAC liquid chromatographic method for cyhexatin (tricyclohexylhydroxystannane) technical and formulations was adopted first action:

Cyhexatin Technical and in Pesticide Formulations
Liquid Chromatographic Method
First Action
CIPAC-AOAC Method

6.D01 ***Principle***

Sample is extd with *n*-decylbenzene internal std soln contg HOAc, MeOH, and H_2O. Cyhexatin is detd by liq. chromatgy using peak ht for quantitation.

6.D02 ***Apparatus***

(a) *Liquid chromatograph.*—With peak ht integrator or recorder, 10 μL sample loop, and detector at 214 nm. Operating conditions: column ambient; flow rate 2.0 mL/min; *A* range 1.0 AUFS; injection vol. 10 μL; retention times, cyhexatin ca 7 min and internal std ca 10 min. Adjust parameters to give peak ht for cyhexatin ca 75% full scale.

(b) *Chromatographic column.*—ODS bonded silica, 10 μm particle size, stainless steel, 25 cm × 4.6 mm id (E. Merck, available from Curtin Matheson Scientific, Inc., or VWR Scientific), or equiv.

6.D03 ***Reagents***

(a) *Methanol.*—LC grade,

(b) *Acetic acid.*—Glacial.

(c) *HCl soln.*—1M.

(d) *Sodium chloride.*—Analytical reagent grade.

(e) *Mobile phase.*—MeOH-H_2O-HCl-NaCl (93 + 7 + 0.001M + 0.005M). In 1 L g-s flask, combine 1 mL 1M HCl, 69 mL H_2O, and 0.29 g NaCl. Add 930 mL MeOH, mix, and degas.

(f) *n-Decylbenzene.*—Eastman Laboratory Chemicals No. 9195 (Eastman Kodak Co.), or equiv.

(g) *Cyhexatin reference std.*—Available from Dow Chemical Co.

6.D04 ***Preparation of Standards***

(a) *n-Decylbenzene internal std soln.*—Weigh 1.0 g *n*-decylbenzene into 1 L vol. flask. Add 49 mL H_2O and 1 mL HOAc. Dil. to vol. with MeOH and sonicate until dissolved.

(b) *Cyhexatin std soln.*—Accurately weigh ca 110 mg pure cyhexatin ref. std into 150 mL g-s flask. Add by pipet 100 mL internal std soln, shake well, sonicate for 10 min, and cool to ambient temp.

6.D05 ***Preparation of Sample***

(a) *Technical material.*—Accurately weigh ca 120 mg sample into 150 mL g-s flask. Add by pipet 100 mL internal std soln, shake well, and sonicate for 10 min. Cool to ambient temp. and centrifuge at 2000 rpm.

(b) *Wettable powder.*—Accurately weigh sample contg 110 mg cyhexatin into 150 mL g-s flask. Proceed as for *Technical material* beginning, "Add by pipet . . ."

(c) *Suspension concentrate.*—Accurately weigh sample contg ca 120 mg cyhexatin into 150 mL g-s flask. Add 10 mL H_2O and swirl until completely homogeneous. Proceed as for *Technical material* beginning, "Add by pipet . . ."

6.D06 ***Determination***

Inject 10 μL std solns until response ratio (cyhexatin peak ht/internal std peak ht) varies <2%. Make 2 sample injections followed by 1 std injection. Average peak ht ratios of stds immediately preceding and following sample injections, and average peak ht ratios of the 2 samples. Calc. cyhexatin as follows:

$$\text{Cyhexatin, \%} = (R/R') \times (W'/W) \times P$$

where R and R' = av. peak ht ratios for sample and std, resp.; W' = g cyhexatin in std soln; W = g sample extd for analysis; and P = % purity of std.

Ref.: JAOAC **71**, January issue (1988).

CAS-13121-70-5 (cyhexatin)

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(3) The following interim AOAC–CIPAC gas chromatographic method for alachlor (2-chloro-*N*-(2,6-diethylphenyl)-*N*-(methoxymethyl)acetamide) in microencapsulated formulations was adopted first action:

Alachlor in Microencapsulated Pesticide Formulations
Gas Chromatographic Method
First Action
AOAC-CIPAC Method

6.D07 ***Principle***

Sample is dissolved in acetone contg di-*n*-pentyl phthalate as internal std, analyzed by gas chromatgy with flame ionization detection, and quantitated by comparison of integrated peak areas.

6.D08 ***Safety***

LD_{50} of alachlor has been found to be 930 mg/kg in rat acute oral studies (Monsanto Co., 1985, MSDS No. 015972608). Alachlor has been detd to produce tumors in laboratory animals. Wear protective clothing to avoid excessive exposure.

6.D09 ***Apparatus***

(a) *Gas chromatograph.*—With flame ionization detector and on-column injection ports. Operating conditions: temps—column oven 230°, injection port 250°, detector 260°; gas flows (mL/min)—He carrier gas 35, H 30, air 250; sample size 1.0 μL; run time 15 min.

(b) *Column.*—Glass, 6 ft × 2 mm id (on-column configuration), packed with 10% SP-2250 on 100–120 mesh Supelcoport (Supelco Inc.), or equiv. SP-2250 is methyl-phenyl silicone (50 + 50).

6.D10 ***Reagents***

(**a**) *Acetone*.—Pesticide grade (Fisher or equiv.).
(**b**) *Di-n-pentyl phthalate internal std soln*.—Weigh 5.3 g di-*n*-pentyl phthalate (CTC Organics, PO Box 6933, Atlanta, GA 30315) into 1 L vol. flask. Dissolve in acetone and dil. to vol. with acetone.
(**c**) *Alachlor std soln*.—Recrystallize alachlor (Monsanto Co., PO Box 473, Muscatine, IA 52761) from MeOH. Accurately weigh 0.2 g recrystd alachlor into small flask. Add by pipet 30.0 mL internal std soln and shake mixt. to dissolve.

6.D11 ***Instrument Setup and Calibration***

Condition chromatgc column overnight at 250° with He flow at 35 mL/min. Suggested conditions represent best compromise for sepn and quantitation of cmpds of interest. Some minor adjustments may be required in other instruments and columns. Column, when working properly, should generate 4000–5000 plates calcd as follows: $N = 16(x/y)^2$, where N = no. of theoretical plates, x = distance from point of injection to peak max., and y = distance along baseline between intercept points of lines drawn tangent to slope of peak, with x and y measured in same units. Typical retention times for alachlor and internal std are ca 6 and 11.5 min, resp. Impurity in internal std (peak C), which elutes at ca 9.9 min, should be completely resolved from internal std peak at ca 11.5 min (Fig. **6:D1**). Internal std contains another impurity that elutes slightly after internal std causing slight tail on that peak. Careful control of integrator conditions is required to integrate internal std peak.

After instrument equilibration, make ≥3 injections of std soln before calibration.

6.D12 ***Determination***

Accurately weigh, to nearest 0.1 mg, ca 0.45 g alachlor microencapsulated formulation into 2 oz sample bottle. Avoid spilling sample on inside wall or neck of bottle; entire sample should be on bottom of bottle.

Pipet 30.0 mL internal std soln (**b**) into sample bottle. To reduce stirring time, use liq. stream from pipet to remove most of alachlor sample from bottom of bottle. Add mag. stirring bar (13 × 15 mm) and cap bottle with polyethylene-lined cap.

Mag. stir mixt. until sample is completely removed from inside wall and bottom of bottle. During stirring, aggregated sample turns fluffy and easily floats in acetone. For most samples, this requires ca 2–3 min moderately fast stirring. Then place bottles on shaker and shake 10 min at high speed.

Let solids settle and pipet off clear acetone soln.

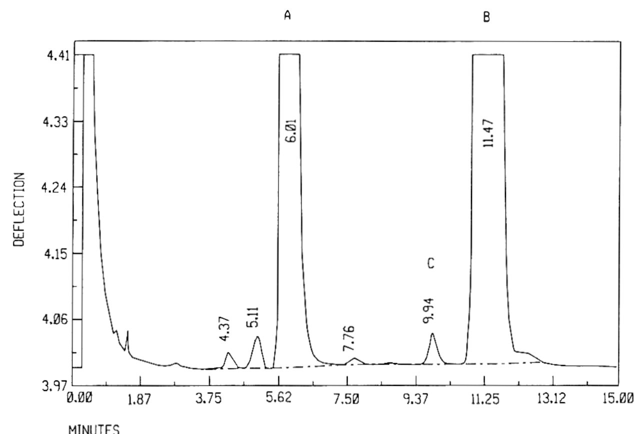

FIG. 6:D1—GC chromatogram of alachlor standard (A), internal standard di-*n*-pentyl phthalate (B), and unknown from internal standard (C)

Make replicate 1 μL injections of alachlor std soln and measure response ratio, R (area alachlor peak/area internal std peak) for each injection. Repeat until consecutive response ratios agree within 0.5%.

Make duplicate injections of acetone sample soln and det. av. R. Follow with injection of alachlor std soln. Det. av. R for std before and after sample injection.

$$\text{Alachlor, \%} = (R/R') \times (W'/W) \times P$$

where R and R' = av. response ratios for sample and std, resp.; W and W' = wt (g) of sample and std, resp.; P = % purity of std.

Ref.: JAOAC **70**, 1056 (1987).

CAS-15972-60-8 (alachlor)

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(4) The following interim AOAC–CIPAC liquid chromatographic method for anilazine (4,6-dichloro-*N*-(2-chlorophenyl)-1,3,5-triazin-2-amine) in formulations was adopted first action:

Anilazine in Pesticide Formulations
Liquid Chromatographic Method
First Action
AOAC-CIPAC Method

(Method is suitable for formulations with anilazine as only active ingredient.)

6.D13 ***Principle***

Anilazine is detd by liq. chromatgy using octanophenone as internal std. Adequate resolution is controlled by monitoring sepn of bis-compound (major impurity of anilazine) from anilazine and internal std peaks.

6.D14 ***Apparatus***

(**a**) *Liquid chromatograph*.—Able to generate >7 MPa (>1000 psi) and measure A at 250 nm. Operating conditions: column temp. ambient; mobile phase flow rate ca 1.7 mL/min (ca 800 psi); chart speed 0.5 cm/min; injection vol. 20 μL; A range 0.32 AUFS. Retention times: anilazine ca 2.5 min, bis-compound ca 4.0 min, octanophenone ca 6.6 min. Pump LC mobile phase thru column until system is equilibrated (flat baseline). Allow 1 min after elution of internal std before next injection.
(**b**) *Chromatographic column*.—250 × 4.6 mm id packed with ≤10 μm C_{18} bonded silica gel capable of resolving bis-compound from anilazine and internal std peaks (Du Pont ODS, or equiv.).
(**c**) *Chart recorder*.—Min. 250 mm span, 10 mV range, 30 cm/h speed.
(**d**) *Bath*.—Ultrasonic.
(**e**) *Filters*.—0.45 μm porosity (Gelman Acrodisc-CR, or equiv.).

6.D15 ***Reagents***

(**a**) *Acetonitrile*.—LC grade or distd in glass (Burdick & Jackson Laboratories, Inc., or equiv.).
(**b**) *Octanophenone internal std soln*.—Dil. 4 mL octanophenone (Aldrich Chemical Co., Inc., or equiv.) to 250 mL with CH_3CN.
(**c**) *Water*.—LC grade or distd in glass (Burdick & Jackson Laboratories, Inc., or equiv.).
(**d**) *LC mobile phase*.—CH_3CN–H_2O (80 + 20).
(**e**) *Anilazine std soln*.—Accurately weigh ca 230 mg anilazine ref. std (Mobay Corp., Agricultural Chemicals Div., PO Box 4913, Hawthorne Rd, Kansas City, MO 64120-0013) into 100 mL vol.

flask. Pipet 10 mL internal std soln into flask, dil. to vol. with CH_3CN, and mix well. Pipet 5 mL of this soln into 100 mL vol. flask, dil. to vol. with CH_3CN, and mix well. Filter portion of final soln for LC analysis.

6.D16 ***Preparation of Sample***

(**a**) *Formulations excluding flowable.*—Accurately weigh sample contg ca 230 mg anilazine into 100 mL vol. flask. Pipet 10 mL internal std soln into flask, dil. to vol. with CH_3CN, and sonicate 1 min. Mix well. Pipet 5 mL of this soln into 100 mL vol. flask, dil. to vol. with CH_3CN, and mix well. Filter portion of final soln for LC analysis.

(**b**) *Flowable.*—Accurately weigh sample contg ca 230 mg anilazine into 100 mL vol. flask. Add 5 mL LC grade or distd in glass H_2O and swirl until sample is thoroughly dispersed. Pipet 10 mL internal std soln into flask, dil. to vol. with CH_3CN, and sonicate 1 min. Mix well. Pipet 5 mL of this soln into 100 mL vol. flask, dil. to vol. with CH_3CN, and mix well. Filter portion of final soln for LC analysis.

6.D17 ***Determination***

Inject anilazine std soln and adjust operating parameters so that anilazine elutes in 2.5–3.0 min. Adjust injection vol. and attenuation to give largest possible on-scale peaks. Bis-compound must be resolved from anilazine and octanophenone peaks. If not, change or repack column.

Using same injection vol. for all sample and std injections, make repetitive injections of ref. std soln and calc. response ratios by dividing anilazine peak ht by internal std peak ht. Response ratios must agree within $\pm 1\%$. Average duplicate response ratios obtained with std solns.

Inject duplicate aliquots of each sample soln. Average duplicate response ratios for each sample soln. Response ratios must agree within $\pm 1\%$. If not, repeat detn, starting with std injections.

Re-inject ref. std soln twice. Average response ratios of stds immediately preceding and following sample injection. These must agree within $\pm 1\%$. If not, repeat detn.

6.D18 ***Calculation***

$$\text{Anilazine, wt } \% = (R/R') \times (W'/W) \times P$$

where R and R' = av. response ratios for sample and std solns, resp., W' and W = wt (mg) of anilazine in std and sample solns, resp., and P = % purity of anilazine std.

Ref.: JAOAC **71**, January issue (1988).

CAS-101-05-3 (anilazine)

7. ANIMAL FEED

(1) The following interim $CuSO_4/TiO_2$ mixed catalyst Kjeldahl method for crude protein in animal feed was adopted first action:

Protein (Crude) in Animal Feed
$CuSO_4/TiO_2$ Mixed Catalyst Kjeldahl Method
First Action

(Caution: See 51.030, 51.037.)

7.D01 ***Principle***

Sample is digested in H_2SO_4, using $CuSO_4/TiO_2$ as catalysts, converting N to NH_3 which is distd and titrd.

7.D02 ***Reagents***

(**a**) *Sodium hydroxide soln.*—Dissolve ca 450 g NaOH pellets or flakes (low N) in H_2O, cool, and dil. to 1 L; or use soln with sp. gr. $\geq$1.36.

(**b**) *Boiling stones.*—Alundum, 8–14 mesh (No. 1590-D18; Thomas Scientific Co., 99 High Hill Rd at I-295, PO Box 99, Swedesboro, NJ 08085-0099).

(**c**) *Methyl red indicator.*—Dissolve 1 g Me red (Na salt) in 100 mL MeOH.

(**d**) *Hydrochloric or sulfuric acid std soln.*—0.5N. Prep. as in **50.011–50.017** or **50.039–50.040.**

(**e**) *Sodium hydroxide std soln.*—0.1N. Prep. as in **50.032–50.035.**

After stdzg both acid and base by methods suggested in (**d**) and (**e**), also check one against the other. In addn, check entire method by analyzing NBS Std Ref. material No. 194, $NH_4H_2PO_4$, certified 12.15% N, and a high purity lysine·HCl.

7.D03 ***Apparatus***

(**a**) *Digestion.*—Kjeldahl flasks with capacity of 500–800 mL.

(**b**) *Distillation.*—Digestion flask (e.g., Corning No. 2020) connected to distn trap by rubber stopper. Distn trap is connected to condenser with low-S tubing. Outlet of condenser tube should be $<$4 mm diam.

7.D04 ***Determination***

Weigh 0.250–1.000 g sample into digestion flask. Add 16.7 g K_2SO_4, 0.01 g anhyd. $CuSO_4$, 0.6 g TiO_2, 0.3 g pumice, 0.5–1.0 g Alundum granules, and 20 mL H_2SO_4. (Add addnl 1.0 mL H_2SO_4 for each 0.1 g fat or 0.2 g other org. matter if sample wt is $>$1 g.)

Include at least 1 sample of high purity lysine·HCl in each day's run as check of correctness of digestion parameters. If recovery is not complete, make appropriate adjustments.

To digest sample, first adjust heat to bring 250 mL H_2O at 25° to rolling boil in 5 min. Add a few boiling chips to prevent superheating. Then heat samples at this 5-min boil rate until dense white fumes clear bulb of flask (ca 10 min), swirl gently, and continue heating addnl 40 min. (Note: Reagent proportions, heat input, and digestion time are critical factors—do not change.) Cool, cautiously add about 250 mL H_2O, and cool to room temp. (Note: Add H_2O as soon as possible to reduce amt of caking. If excessive bumping occurs during distn, increase diln H_2O from 250 mL to ca 300 mL.)

Prep. titrn beaker by adding appropriate vol. of acid std soln to amt of H_2O such that condenser tip will be sufficiently immersed to trap all NH_3 evolved. Add 3–4 drops of indicator soln (**c**).

Add addnl 0.5–1.0 g Alundum granules to cooled digestion flask. Optionally, 2–3 drops of tributyl citrate may also be added to reduce foaming. Slowly down side of flask, add sufficient NaOH soln (**a**) such that mixt. will be strongly alk. Immediately connect flask to distn app., mix completely, and distill at ca 7.5-min boil rate until $\geq$150 mL distillate is collected in titrn beaker.

Titr. excess std acid in distillate with NaOH std soln (**e**). Correct for blank detn on reagents. Calc. % nitrogen:

$$\% \text{ N} = \{[(N_{acid})(mL_{acid}) - (mL_{bk})(N_{NaOH}) - (mL_{NaOH})(N_{NaOH})] \times 1400.67\}/\text{mg sample}$$

where mL_{NaOH} = mL std base needed to titr. sample; mL_{acid} = mL std acid used for that sample; mL_{bk} = mL std base needed to titr. 1 mL std acid minus mL std base needed to titr. reagent blank carried thru method and distd into 1 mL std acid; N_{acid} = normality of std acid; N_{NaOH} = normality of std base. Calc. % crude protein, defined as 6.25 × % nitrogen, or 5.7 × % nitrogen for wheat grains.

Ref.: JAOAC **70**, 907(1987).

(2) Make an editorial change in the final action photometric method for phosphorus in animal feed, **7.125–7.128**. Add the following applicability statement:

(Not applicable to mineral-mix feeds)

Ref.: JAOAC **59**, 937(1976).

8. BAKING POWDERS AND BAKING CHEMICALS

No additions, deletions, or other changes.

9. BEVERAGES: DISTILLED LIQUORS

The following first action method was adopted final action: Total acidity in cordials and liqueurs, **9.135**.

10. BEVERAGES: MALT BEVERAGES AND BREWING MATERIALS

(1) The following first action methods were adopted final action:

(a) Ethanol in beer, ASBC–AOAC gas chromatographic method, **10.028–10.033**.

(b) *N*-Nitrosodimethylamine in beer, ASBC-AOAC gas chromatographic method I, **10.098–10.106**.

(c) Extract of barley for malting, ASBC-AOAC method, **10.120–10.125**.

(d) Aphids in hops, flotation method, **10.182**.

(e) Total solids in liquid and pressed yeast, 16 hour drying method, **10.222–10.224**.

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(2) The following interim ASBC-AOAC digital density meter method for specific gravity of beer and wort was adopted first action:

Specific Gravity of Beer and Wort
Digital Density Meter Method
First Action
ASBC–AOAC Method

10.D01 ***Principle***

Natural frequency of hollow oscillator varies with density of material that fills tube. Electronic measurement of a time period is converted to digital display of density.

10.D02 ***Apparatus and Reagents***

(a) *Digital density meter.*—Mettler DMA-46 (Mettler Instrument Corp., Box 100, Princeton, NJ 08540), or equiv.

(b) *Hypodermic syringe.*—As recommended in density meter instructions.

10.D03 ***Calibration of Density Meter***

Calibrate digital density meter at 20.0 ± 0.1° with air and H_2O. Use hypodermic syringe to inject liqs into sample tube. Fill tube completely so no gas or solids are entrapped.

10.D04 ***Preparation of Sample***

Decarbonate beer samples as in **10.001**. Samples must be brilliantly clear and free from any particulate matter, and must be at 20° test temp. Filter if required.

10.D05 ***Determination***

Inject clear, decarbonated sample into filling tube. Be sure no gas is entrapped in tube. Let thermostated sample tube reach thermal equilibrium (0.5–4 min). Obtain sp gr measurement.

Refs.: J. Am. Soc. Brew. Chem. **36**, 118(1978). ASBC: Beer-2,A.

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(3) Make an editorial change in the final action titrimetric method for alpha-amylase in malt, **10.155–10.158.**

In **10.155(a):** Soluble starch is available from American Society of Brewing Chemists, 3340 Pilot Knob Rd, St. Paul, MN 55121.

In **10.155(b):** beta-Amylase powder is no longer available from Wallerstein Co. Henley Chemical, Inc., 50 Chestnut Ridge Rd, Mont Vale, NJ 07654 is a distributor of Sterge beta-amylase.

In **10.156:** Hellige apparatus is now available from Orbeco Analytical Systems, Inc., 185 Marine St, Farmingdale, NY 11735.

11. BEVERAGES: WINES

(1) The following first action methods were adopted final action:

(a) Alcohol in wines, dichromate oxidation method, **11.008–11.011.** Alternative use of an electric distillation unit ("Changes in Official Methods" (1985) JAOAC **68,** 374).

(b) Alcohol in wines, gas chromatographic method, **11.012–11.014.**

(c) Caloric content of wines, **11.022.**

(d) Volatile acidity of wines exclusive of SO_2, by barium hydroxide treatment, **11.046.**

(e) Citric and malic acids in wines, **11.052.**

(f) Carbohydrate content in wine, **11.A05.**

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(2) The following interim titrimetric method for determination of carbon dioxide in wine was adopted first action:

Carbon Dioxide in Wines
Titrimetric Method
First Action

(Applicable to wines contg ≤400 mg CO_2/mL)

11.D01 ***Principle***

Sample is made alk. with NaOH and carbonate formed is titrd with stdzd H_2SO_4. Degassed sample is titrd as blank.

11.D02 ***Apparatus and Reagents***

(a) *pH meter.*—Stdzd at pH 4 and 10 with appropriate buffers.

(b) *Buret.*—25 mL with automatic zero.

(c) *Magnetic stirrer.*

(d) *Sulfuric acid.*—0.0455N H_2SO_4. At this normality, 1 mL = 20 mg CO_2/100 mL.

(e) *Carbonic anhydrase soln.*—0.1 mg/mL H_2O. Keep refrigerated; soln is stable for 1 week. (Use carbonic anhydrase from bovine erythrocytes. Available from Sigma Chemical Co., Cat. No. C-7500.)

(f) *Sodium hydroxide soln.*—50% soln of reagent grade NaOH.

11.D03 ***Determination***

Cool sample to ca 5°, open bottle, and add equiv. of 5 mL NaOH soln per 375 mL sample. Immediately recap bottle, mix contents, pipet 10 mL portion of sample into 40 mL H_2O, add 3 drops carbonic anhydrase soln, and titr. to pH 8.6 with 0.0455N H_2SO_4. Refill buret and continue titrn to pH 4.0. Record titer between pH 8.6 and 4.0.

Obtain blank as follows: Degas 25 mL duplicate of sample with agitation ca 1 min under ≥28 in. vac. in 500 mL filter flask contg 3 drops carbonic anhydrase soln. Add 0.33 mL NaOH soln. Pipet 10 mL degassed sample into 150 mL beaker contg 40 mL H_2O. Stir and titr. as for sample detn.

Calc. as follows:

$$\text{mg } CO_2/100 \text{ mL} = (\text{mL sample} - \text{mL blank}) \times 20 \times 1.013$$

Ref.: JAOAC **70**, 1060(1987).

CAS-124-38-9 (carbon dioxide)

12. BEVERAGES: NONALCOHOLIC AND CONCENTRATES

No additions, deletions, or other changes.

13. CACAO BEAN AND ITS PRODUCTS

No additions, deletions, or other changes.

14. CEREAL FOODS

(1) The following first action method was adopted final action:

Phytate in foods, anion-exchange method, **14.B01–14.B05.**

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(2) Make a nonsubstantive change in the final action titrimetric method for fat acidity of grains, **14.069–14.071,** to use toluene in place of benzene in the titration medium, **14.069(a).**

15. COFFEE AND TEA

No additions, deletions, or other changes.

16. DAIRY PRODUCTS

(1) Make a nonsubstantive change in the final action Babcock method for fat in cream, **16.177–16.178:**

Change **16.178(a)** to read:

. . . centrf. 5 min. Fill bottle to neck with hot H_2O and centrf. 2 min. Add hot H_2O until liq. column. . . .

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(2) Make a nonsubstantive change in the first action infrared spectroscopic method for determination of fat, lactose, and protein in milk, **16.083–16.092:**

Add the following sentence in **16.087:**

Use A, B, and A + B filters for measurement.

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(3) The interim microbial receptor assay for rapid detection and identification of 7 families of antimicrobial drugs in milk was adopted first action:

Antimicrobial Drugs in Milk

Microbial Receptor Assay

First Action

(Min. detectable concns (ng/mL) are penicillin G, 3; tetracycline, 600; erythromycin, 20; streptomycin, 10; novobiocin, 10; sulfamethazine, 5; chloramphenicol, 80.)

16.D01 ***Principle***

Assay is based on binding reaction between drug functional group and receptor site on added microbial cells. ^{14}C or ^{3}H binding is measured by scintillation counter and compared with zero std milk to detect antimicrobials. The greater the amt of antibiotic present in the sample, the lower the counts. Method does not detect metabolites, only active drugs.

16.D02 ***Apparatus***

App. is available from Penicillin Assays Inc., 36 Franklin St, Malden, MA 02148.

(a) *Heaters.*—Two dry well incubators of anodized Al, each with 6 holes, 5.23 cm deep, 1.35 cm diam., controlled at 45 and 85 ± 1°, for 13 × 100 mm glass test tube.

(b) *Mixer.*—Maxi Mix II (Thermolyne Corp., 2555 Kerper Blvd, Dubuque, IA 52001), or equiv.

(c) *Bench centrifuge.*—12-place, 1300 ×*g*. Whisperfuge (Damon Scientific, 300 Second Ave, Needham Heights, MA 02194), or equiv.

(d) *Cold plate.*—To accommodate 7 mL plastic scintillation vials holding at −3 to −5°.

(e) *Scintillation counter.*—To accommodate 13 × 100 mm glass tube. Window settings such that <10% of ^{3}H crosses into ^{14}C channel and <30% of ^{14}C crosses into ^{3}H. Counting time is 1 min. Efficiency is >80%. Replication in counting same tube at ca 1000 cpm is ca 10%.

(f) *Scintillation fluid dispenser.*—(Oxford [Monoject Scientific, 1831 Olive St, St. Louis, MO 63103]; or equiv.).

(g) *Labware.*—Cotton swabs. Wash bottle. 13 × 100 mm test tubes, wall thickness 0.084 ± 0.005 cm. Plastic stoppers for 13 × 100 mm tubes. Plastic scintillation vials, 7 mL. Pipets capable of delivering 200 µL, 300 µL, and 5 mL.

16.D03 ***Reagents***

Reagents are available from Penicillin Assays Inc.

(a) *Scintillation fluid.*—Optifluor (Packard Instrument Co., 2200 Warrenville Rd, Downers Grove, IL 60515), or equiv.

(b) *Microbial binders.*—(*1*) *Reagent D:* Freeze-dried suspension of *Bacillus stearothermophilus.* Reagent is stable 1 year at −20°. When reconstituted with Diluent D, it is stable 1 week at −20° or 24 h at −5°. About 1 × 10^8 cells are added per assay or 200 µL, used for binding beta-lactams, tetracyclines, streptomycin, macrolides, and novobiocin.

(*2*) *Reagent R:* Freeze-dried irradiated, nonviable suspension of microorganism, with receptor sites for sulfonamides and chloramphenicol (microorganisms inhibited by these drugs possess required receptor sites). Stable 1 year at −20° freeze-dried, and 6 h at −5° reconstituted. About 1 × 10^9 cells are used per 200 µL for binding sulfonamides and chloramphenicol.

(c) *Antibiotic tracer reagents.*—(*1*) Penicillin ^{14}C 125 µCi/µmol, 0.0024 µCi/test. (*2*) Tetracycline ^{3}H 0.5 µCi/µmol, 0.052 µCi/test. (*3*) Erythromycin ^{14}C 38 µCi/µmol, 0.0031 µCi/test. (*4*) Streptomycin ^{3}H 26 µCi/mmol, 0.0185 µCi/test. (*5*) Novobiocin ^{3}H 55 µCi/mmol, 0.0148 µCi/test. (*6*) Sulfamethazine ^{3}H 59 µCi/mmol, 0.0311 µCi/test. (*7*) Chloramphenicol ^{14}C 35 µCi/mmol, 0.0126 µCi/test.

Antibiotic tracer reagents have stabilizers added to preserve their biological activity. Freeze-dried tracer reagents are stable ≥12

months at −20°; reconstituted tracer reagents are stable 2 weeks at −20°.

In certain cases ^{14}C and ^{3}H tracers are combined to facilitate screening for more than 1 family in each tube. This is done with penicillin ^{14}C and tetracycline ^{3}H as well as erythromycin ^{14}C and streptomycin ^{3}H.

(d) *Diluent C.*—70% soln of propylene glycol for reconstituting radiolabeled tracer antibiotics.

(e) *Diluent D.*—Soln contg 5% sucrose, 10% dimethyl sulfoxide (DMSO), 1.2% NaCl for reconstitution of binding microorganisms for Reagents D and R.

(f) *Reagent NH.*—12% $(NH_4)_2SO_4$.

16.D04 ***Reconstitution of Reagents***

(a) *Tracer reagents.*—Add room temp. diluent to freeze-dried tracer. Dissolution occurs immediately. Remove amt reagent needed for 1 day into plastic screw-cap vial. Place vial in cold plate at −3 to −5° for up to 24 h. Store remaining tracer as liq. at −20° for up to 2 weeks. Small plastic vials may be refilled as needed from storage vial held at −20°.

(b) *Microbial binding reagents.*—Reconstitute *Reagent D* by adding vol. of cold Diluent D (4°) indicated on label, usually 12 mL. Break up clumps of cells by using large glass test tube and vortex mixer. Mix well. Let reconstituted Reagent D warm to room temp. (25°) and hold for ca 5 min. Dispense into vials and place in cold plate at −5° for use or store at −20°. Reagent D may be stored at −20° for 1 week or 12 h at −5°. Thaw frozen Reagent D by placing in tap H_2O (ca 20°) for 5 min and cool in cold plate ≥5 min before use.

Reconstitute *Reagent R* by adding amt of Diluent D indicated on bottle. Mix well. Reagent R may be stored in cold plate 6 h at −5°.

16.D05 ***General Instructions***

(a) *Exempt quantities of ^{14}C and ^{3}H.*—Amts of radioactivity used in this method are sufficiently low as to be exempt from U.S. Nuclear Regulatory Commission (NRC) and agreement state regulations. This applies to laboratories unlicensed by NRC.

Check appropriate national and local regulations for use of exempt amts of ^{14}C and ^{3}H.

(b) *Disposal of scintillation fluid.*—Liq. scintillation counting solns which are readily soluble or dispersible in H_2O (Optifluor, or equiv.) and which contain <0.05 μCi ^{3}H or ^{14}C per g soln can be dispersed in large vol. of H_2O and disposed thru sanitary sewerage system (in this method, 2.8 g Optifluor/assay is used). It is recommended that ratio of running H_2O to scintillation fluid exceed 10:1. Dispersion may be cloudy due to presence of high bp hydrocarbon. No special fire prevention precautions are necessary. Note that local regulations must be observed if these differ from federal regulations.

16.D06 ***Performance Check***

Check reagents and equipment by testing zero std milk. Compare with previous zero milk results or with manufacturer's typical count sheet that accompanies reagents. If comparable results are obtained, proceed.

Combine tracers as follows: penicillin and tetracycline (P&T), erythromycin and streptomycin (E&St), novobiocin (N), sulfonamides and other PABA analogs (Sm), chloramphenicol (C).

(*1*) Mark 5 tubes: (P&T), (E&St), (N), (Sm), (C).

(*2*) Add 200 μL tracer reagent to corresponding tube; wipe pipet before each addn. Pipet reagent directly to bottom of tube.

(*3*) *For chloramphenicol only*, add 300 μL Reagent NH.

(*4*) Add 5 mL milk sample to each test tube.

(*5*) Gently add 200 μL *Reagent D* to (P&T), (E&St), and (N) tubes. Add *Reagent R* to (Sm) and (C) tubes.

(*6*) Mix by letting milk swirl and settle ca 10 times to ensure thoro distribution of added reagents. This should take ca 10 s.

(*7*) Incubate (P&T) and (E&St) 3 min at 85°; incubate (N), (Sm), and (C) 3 min at 45°.

(*8*) Centrf. tubes 3 min and then decant milk completely.

(*9*) Hold tubes at 45° tilt and rinse by filling tube once with H_2O from wash bottle; do not disturb ppt at bottom of tube. Tilt tubes down to drain. Wipe dry with 2 or more swabs while wiping out fat ring.

(*10*) Add 300 μL H_2O and resuspend pellet completely, using mixer.

(*11*) Dispense 3 mL scintillation fluid into tube, cap, and shake tube gently.

(*12*) Measure activity in analyzer ^{3}H channel and ^{14}C channel. Read (T), (St), (N), and (Sm) on ^{3}H channel and (P), (E), and (C) on ^{14}C channel.

Note whether number is above control point for neg. sample or below for pos. Control point is number about 3 std devs from av. of 6 zero samples. Reassay pos. samples.

16.D07 ***Double Positive***

To det. whether double pos. in 1 tube is true double pos. or is result of single pos. and "cross talk" between ^{14}C and ^{3}H channels, retest sample with single tracers in duplicate and compare with 2 zero detns carried out with single tracers. If single tracer result compares with zero single tracer, then that "pos." is due to cross talk.

16.D08 ***Control Point Determination***

Set control point ≥3 std devs from av. zero. For convenience, use percentage to est. 3 std devs.

For penicillin, use ca 2.5 std devs because <0.008 IU/mL must be detected to accommodate *B. stearothermophilus* disc assay std where required by U.S. Food and Drug Administration Pasteurized Milk Ordinance (1983; revision).

To det. control points, subtract following percentages from av. of 6 zero detns.

Antibiotic	*% subtracted from average zero*
Beta-lactam	15
Tetracyclines	20
Macrolides	20
Streptomycin	25
Novobiocin	20
Sulfonamides	20
Chloramphenicol	20

Ref.: JAOAC **71**, March issue (1988).

17. EGGS AND EGG PRODUCTS

No additions, deletions, or other changes.

18. FISH AND OTHER MARINE PRODUCTS

(1) Make an editorial change in the final action biological method for paralytic shellfish poison, **18.086–18.092.**

In **18.086(a):** The standard is now available from Food and Drug Administration, Division of Contaminants Chemistry, Natural Products and Instrumentation Branch, 200 C St, SW, Washington, DC 20204.

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(2) The following interim physical separation method for determination of minced fish flesh in mixed fillet–minced cod blocks was adopted first action:

Minced Fish Flesh in Mixed Fillet–Minced Cod Blocks
Physical Separation Method
First Action

18.D01 ***Principle***

Mixed fillet–minced cod block is air-thawed, drained, weighed, and immersed in cold H_2O bath. Fillets are sepd from mince by hand and placed on perforated tray to drain. H_2O from bath is poured thru sieve, and any fillet pieces on sieve are removed and added to drain tray. Fillets are drained 15 min and weighed. Amt of minced fish is calcd from drained wt of block and drained wt of fillets.

18.D02 ***Apparatus***

(a) *Trays*.—Al or galvanized. (*1*) Shallow; large enough to hold 1 fish block for thawing. (*2*) Perforated, with holes ⅜ in. diam. drilled to cover entire bottom of tray, or made from U.S. Standard No. 8 metal wire cloth.

(b) *Container*.—Tub of sufficient size to hold fish block plus H_2O.

(c) *Scale*.—Sensitive to 0.25 oz (7 g).

(d) *Sieve*.—U.S. No. 8.

18.D03 ***Determination***

Thaw sample (entire fish block) on preweighed tray at ambient (room) temp. (usually takes overnight). Perform detn within 8 h after block is completely thawed.

Drain any exuded fluid (thaw-drip) and det. wt of drained fish block (wt = W_B).

Immerse drained fish flesh in tub of cold, 10–21° (50–70°F), H_2O. Use ratio of 2–3 parts H_2O to 1 part fish by wt. Sep. fillets by hand and wash minced fish flesh from fillets in tub. Place washed fillets on upper section of preweighed perforated tray. Incline tray at angle of 20–40° to facilitate draining.

Pour H_2O contg minced fish and any small pieces of fillet from tub thru sieve. Remove any fillet pieces from sieve, place them on drain tray with fillets, and let fillets drain 15 min. Remove any excess H_2O from lower section of tray and weigh drained fillets (wt = W_F).

18.D04 ***Calculation***

$$\text{Minced fish, } \% = [(W_B - W_F)/W_B] \times 100$$

where W_B = wt of thawed, drained fish block, and W_F = wt of drained fillets.

Ref.: JAOAC **71**, January issue (1988).

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

In the method for titratable acidity of fruit products, **22.058–22.059**, add the following as an introductory paragraph:

Titratable acidity can be expressed conventionally in g acid per 100 g or per 100 mL product, as appropriate, by using the factor appropriate to the acid: for malic acid use 0.067 as factor; oxalic acid, 0.045; citric acid monohydrate, 0.070; tartaric acid, 0.075; sulfuric acid, 0.049; acetic acid, 0.060; lactic acid, 0.090.

23. GELATIN, DESSERT PREPARATIONS, AND MIXES

No additions, deletions, or other changes.

24. MEAT AND MEAT PRODUCTS

The following interim enzyme-linked immunosorbent assay for determination of soy protein in meat products was adopted first action:

Soy Protein in Raw and Heat-Processed Meat Products
Enzyme-Linked Immunosorbent Assay
First Action

(Method is semiquant.; may be quant. when nature of added soy protein is known, and especially if calibration sample is available.)

24.D01 ***Principle***

Enzyme-linked Immunosorbent assay (ELISA).—Inhibition mode of ELISA is applied (Fig. **24:D1**) in which soy protein analyte (antigen) is reacted with fixed vol. of appropriate antiserum (antibody-pos.) in excess, and unreacted antibody is detd after isolation on immunosorbent, in this case inside surface of sensitized plastic well onto which antigen has been passively immobilized. Antiserum contains specific rabbit serum globulins; captured antibody is detd after addn of another immunoreagent (conjugate) consisting of second antibody (e.g., raised in goat to isolated rabbit serum globulins) to which enzyme has been covalently attached. Captured enzyme is detd by adding chromogenic substrate. Washing steps are incorporated after each interaction stage to remove any non-immobilized species, and each sample is generally assayed at several serial dilns.

Determination of soy protein.—Acetone powder of sample is prepd and solubilized in hot aq. urea soln. After diln, renatured proteins are analyzed by ELISA using com. available antibodies. Response is compared to that observed with std com. soy protein and with suitable blanks.

24.D02 ***Apparatus***

App. specified here (as guide) has been tested and used for collaborative study but does not imply comprehensive survey or recommendation over alternatives; equiv. app. may be used. Several com. semi-automated aids to ELISA are available at various levels of convenience and expense; these can be worthwhile, since systematic washing of ELISA plates is of great importance, and the final step involves measurement of optical density of 250 μL portion of soln.

(a) *ELISA washing system*.—Automatic system: Dynawasher II (AM 55, Dynatech Laboratories, Inc., 14340 Sullyfield Circle, Chantilly, VA 22021) or Nunc-Immuno Wash (Nos. 447430 or 448909, Gibco Ltd, PO Box 35, Paisley PA3 4EF, Scotland, or Thomas Scientific, 99 High Hill Rd at I-295, PO Box 99, Swedesboro, NJ 08085-0099). Multichannel manual washing system may be used.

(b) *ELISA reader*.—Automatic ELISA reader (Dynatech AM 133), Minireader II (Dynatech AM 118), or equiv. app.

(c) *Pipetting systems*.—Variable 8- or 12-channel pipetter, 50–200 μL (Nos. 77-857-00 or 77-887-00, Flow Labs Ltd, Second Ave, Industrial Estates, Irvine, Ayrshire KA12 8NB, Scotland, or Flow Labs Inc., 7655 Old Springhouse Rd, McLean, VA 22102); reagent reservoirs, separate reservoir needed for each soln (77-824-01, Flow

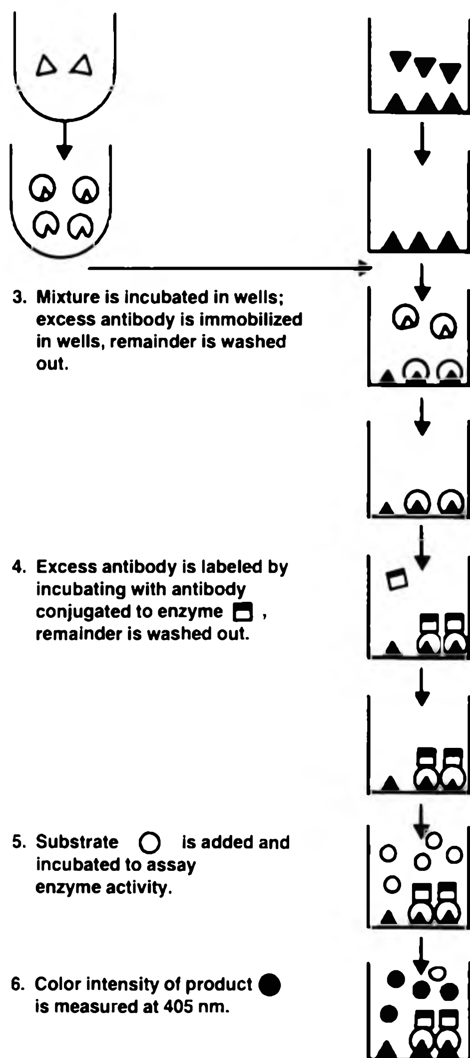

FIG. 24:D1—Determination of soy protein by ELISA procedure: scheme illustrating individual steps of procedure

Stages 1 and 5 require addition of solutions by pipet and incubation of mixture; stages 2, 3, and 4 also involve subjecting wells to standardized draining/filling procedure which can be done in automatic or manual washer. Quantitative interactions on which assay is based occur in monomolecular layer on inner surface of well; normally, 96 wells are used simultaneously for blanks, standards, and samples at different serial dilutions. Optical density of final solution (250 µL) is measured at stage 6; this can be done in situ using special reader.

Labs Ltd); pipet tips and tip carrier. Pipets (5–50 µL; 50–200 µL; 100–1000 µL; 1000–5000 µL) with tips (Anachem, Charles St, Luton, Bedfordshire LU2 0EB, England). Graduated glass pipet (200 µL), with filler, for 2-mercaptoethanol.

(d) *Labware for sample extraction.*—Homogenizer. Measuring cylinders. Conical flasks. Macerator, e.g., IKA Ultra-Turrax Disperser T 18/10 with 18N shaft (Sartorius Instruments Ltd, 18 Avenue Rd, Belmont, Surrey SM2 6JD, England). Filter funnel, 7 cm, 3-piece Whatman (1950 017, FSA Laboratory Supplies, Bishop Meadow Rd, Loughborough Leicestershire LE11 0RG, England) and Whatman No. 541 filter paper, with Buchner flask and vac. system. Funnels. Mortar and pestle. Graduated Pyrex test tubes, 10 mL, with *plastic* stoppers (1628 10, FSA Laboratory Supplies; or equiv.) and holding tray. Boiling H_2O bath.

(e) *Labware for ELISA.*—Immunoassay plates, 96-well (Microtiter, Dynatech; or Nunc-Immuno Plate I, Gibco [No. 2-39454] or Thomas Scientific) with lids (e.g., No. 76-405-04, Flow Labs Ltd). Sealable plastic bags to fit ELISA plates. Plastic storage tubes (Micronic PPN tubes, Flow Labs Ltd No. 61-226-C2) for serial dilution and pre-incubation, with 8-cap strips and tube holder.

(f) *General labware.*—Vol. flasks (10, 25, 50 mL) with plastic stoppers. Balance (top pan). Balance (weighing to 100 µg). pH meter (reading to 0.02 pH unit). Incubator (37 ± 0.5°). Whirlimixer. Clock (timing to 1 s). Magnetic stirrer. Semilogarithmic graph paper (linear/4 log cycles). Flexi-curves.

24.D03 *Reagents*

Use anal. grade chemicals and distd H_2O. Chemicals used as received include acetone, urea, 2-mercaptoethanol (**Caution**: toxic and volatile), and sodium azide (**Caution**: toxic material: prevent contact with skin and eyes, and avoid breathing dust). Store concd antibody-pos. and conjugate solns at 2–8°, antibody-neg. soln at −20°, and substrate tablets at <0°.

(a) *Chloroform–methanol.*—2 + 1 v/v.

(b) *Ethanol–water (acidified).*—80 + 20 v/v acidified with 2 drops HCl/L.

(c) *Tris-HCl buffer.*—0.25M tris(hydroxymethyl)methylamine (30.3 g/L), adjusted to pH 8.60 with HCl.

(d) *Sensitizing soln.*—Unico 75 (Unimills B.V., Zwijndiecht, The Netherlands) has been used as arbitrary std.

(*1*) Freshly prep. 50-fold concd sensitizing soln by mixing 4.5 mL 0.2M Na_2CO_3, 8.0 mL 0.2M $NaHCO_3$, A µL soy protein std antigen soln, and H_2O to 50 mL. A is calcd to correspond to 2.5 mg soy protein in the acetone powder; soln described in **24.D05(b)** contains 100 mg/25 mL, in which case A = 625. Store remaining std soln overnight at 2–8°.

(*2*) Prep. working diln by mixing 4.5 mL 0.2M Na_2CO_3, 8.0 mL 0.2M $NaHCO_3$, and H_2O to 50 mL, and then removing 1.0 mL of soln and replacing it with 1.0 mL 50-fold concd sensitizing soln.

(e) *Phosphate buffer saline pH 7.1–Tween 20–azide (PBSTA).*—Prep. 10-fold concd soln without Tween (PBSA) by mixing 85.0 g NaCl, 10.7 g Na_2HPO_4, 3.9 g $NaH_2PO_4 \cdot 2H_2O$, 5.0 g NaN_3, and H_2O to 1000 mL. Prep. working PBSTA soln by mixing 100 mL PBSA, 1.5 mL Tween 20, and 900 mL H_2O.

(f) *Immunoreagents.*—Amts of antibody and conjugate added depend on properties of immunoreagents recd. Stdze each batch and calc. B and C as described in **24.D04**.

(*1*) *Antibody-pos. soln:* Rabbit antiserum to soy protein (Hoechst U.K. Ltd, OTNG05, manufactured and purified by Behringwerke from rabbits immunized with mixt. of com. untreated and heat-denatured antigens), B µL dild with PBSTA to 50 mL.

(*2*) *Antibody-neg. soln:* Normal rabbit serum (e.g., Flow Labs Ltd No. 29-411-46), B µL dild with PBSTA to 50 mL.

(*3*) *Conjugate soln:* Goat anti-rabbit IgG (H + L)–alkaline phosphatase conjugate (Lq) (Miles Laboratories Research Products No. 61-275-1), C µL dild with PBSTA to 25 mL.

(g) *Substrate soln.*—*p*-Nitrophenyl phosphate in buffer. Prep. by mixing 2.25 mL 0.2M Na_2CO_3, 4.00 mL 0.2M $NaHCO_3$, 25 µL 0.5M $MgCl_2 \cdot 6H_2O$, and H_2O to 25 mL; add 25 mg *p*-nitrophenyl phosphate

as 5 tablets (5 mg each) of Sigma 104 phosphatase substrate (Sigma Chemical Co. No. 104-105).

24.D04 *Standardization*

(a) *Antibody-positive and antibody-negative.*—Test new batches on an ELISA plate (prepd as described in **24.D05(c)** and **(d)**). Make up series of dilns (e.g., in range 100–100 000) of antibody pos. (rows A–D) and antibody neg. (rows E–H) in PBSTA, pre-incubate without soy protein, and then proceed as described in **24.D05(h)–(k)**. Use antiserum diln curve to obtain titer of antibody pos. Calc. value of B, e.g.:

$$\text{Titer} = 1 \text{ in } 3000;\ B = 2 \times (1/3000) \times 50\,000\ \mu L = \text{ca } 30\ \mu L$$

Also test antibody on std meat products contg known soy protein concn and on potential cross-reacting materials.

(b) *Conjugate.*—Test new batches of conjugate on an ELISA plate (prepd as described in **24.D05(c)** and **(d)**). Prep. antibody pos. as for BP (**24.D05(f)**) and pre-incubate this soln with PBSTA separately (**24.D05(g)**). Incubate in plate (**24.D05(h)**) in rows A–D (antibody pos.) and rows E–H (PBSTA). Use conjugate at various dilns (e.g., in range 500–5000) in **24.D05(i)** and then follow **24.D05(j)** and **(k)**. Calc. value of C (e.g., C = 15) such that conjugate pos. is >1.000 and conjugate neg. is <0.050.

(c) *ELISA plate.*—Test plate batches for variability across plate. Plate pattern in protocol uses only wells (B–G) (2–11) for quantitation because of some variation in outer wells. Majority of samples contg soy protein would then be on linear portion of std graph, eliminating necessity for reassay.

24.D05 *Determination*

Perform analyses in duplicate.

(a) *Acetone powder preparation.*—Weigh *representative* sample (20 g) of meat product and macerate as follows (e.g., by Ultra-Turrax) in sequence of org. solvs, filtering off residue at each stage for re-extn. Ext. with 200 mL $CHCl_3$–MeOH (3 times), then with 200 mL acidified EtOH (3 times), and finally with 200 mL acetone (3 times). Air-dry residue overnight and grind it in mortar. Take sep. samples for detn of total protein by Kjeldahl procedure (**24.027, 24.028–24.036, 24.037,** or **24.038–24.040**): use observed level of total protein (N × 6.25) in acetone powder to calc. appropriate wt of sample to be taken in (**b**) for ELISA of soy protein.

(b) *Solubilization of samples and std.*—Weigh into sep. 10 mL graduated test tubes std soy acetone powder (97–103 mg protein) and each sample of acetone powder (95–105 mg protein). To each tube add in order: 2 mL Tris-HCl buffer, 6.0 g urea, 2 mL H_2O, 200 µL 2-mercaptoethanol, and H_2O to 10 mL. Stopper and mix; adjust vols if necessary and heat in steam or boiling H_2O bath for 60 ± 1 min, immersing up to 10 mL level. Cool for 3–5 min and transfer quant. to 25 mL vol. flask. Dil. to vol. with H_2O used in transfer, ensuring resolubilization of any crystd urea. Store sample solns overnight at 2–8°.

(c) *ELISA plate sensitization.*—Fill reagent reservoir with sensitizing soln. Using multichannel pipetter, transfer 200 µL to each well in ELISA plate. Place lidded plate in plastic bag and seal; incubate 16 ± 1 h at 37°.

(d) *ELISA plate washing and storage.*—Always wash ELISA plates by using stdzd washing procedure. With automatic washing systems, "one 5-fold wash" is cycle in which each row of wells is successively drained to waste and refilled with PBSTA; this is repeated 4 more times and finally wells are drained without being refilled. With manual washing systems, 1 row of wells is drained and refilled with PBSTA 5 times before being finally drained; sequence is repeated with successive rows. "One 10-fold wash" includes first a 5-fold wash followed by refilling all wells with PBSTA, and then a second 5-fold wash.

If sensitized ELISA plate is not to be used immediately, remove it from incubator at end of 16 h and wash it 5-fold as above. Efficient and consistent washing is essential part of assay. Washed plate may be blotted and stored. Use absorbent tissue to dry sides of plate; invert plate to remove any remaining liq. from wells, and then dry top of plate. Ideally, inside surface of wells including base should remain untouched throughout washing and blotting steps. Place lidded plate in plastic bag and seal; store upside-down in deep freeze (−20°) until required.

(e) *Serial dilution of sample and std solns.*—Ensure that sensitized ELISA plate to be used in (**h**) has sufficient time (45–60 min) to warm from −20° or cool from 37°, and that sample and std solns are also at room temp. Transfer 750 µL aliquots of each of these solns to 10 mL vol. flasks; dil. to vol. with PBSTA and mix by repeated inversion. Fill Micronic tube holder with tubes and pipet 600 µL of dild sample and std solns each into appropriate tubes as defined in Table **24:D1**: std S1 in triplicate and samples T1–Z1 in duplicate. Table **24:D1** illustrates std pattern of tubes which corresponds to wells in ELISA plate, designed to make least use of outermost wells and to facilitate multichannel pipetting. All other tubes must now receive 400 µL PBSTA; make this transfer from reagent reservoir using multichannel pipetter (two 200 µL portions). Carry out serial diln of each sample in duplicate by using multichannel pipet to mix 600 µL aliquots (by withdrawing 200 µL and replacing it 3 times), and then to transfer 200 µL of each to an adjacent tube that already contains 400 µL PBSTA. Mix as before and repeat diln, discarding 200 µL aliquot from final tube. This results in a number of 1:3 serial dilns of which only 3 are analyzed in duplicate; normally, use undild, 3-fold dild, and 9-fold dild sample solns for assay when suspected level of soy protein in sample is unknown or low (0–11 g soy protein/100 g total protein). Choose higher dilns if levels are higher (3-, 9-, 27-fold of 11–33 g/100 g; 9-, 27-, 81-fold for 33–100 g/100 g), or if ELISA has already demonstrated that sample solns are too concd. Dil. std soln similarly, but prep. 7 serial dilns for assay (undild soln to 729-fold diln each in triplicate).

(f) *Blanks.*—Four types of blank are used, each in triplicate: substrate (BS), conjugate (BC), antibody-pos. (BP), and antibody-neg. (BN) blanks. Relevant Micronic tubes already contain 400 µL PBSTA; add 2 addnl 200 µL portions of PBSTA to BS and BC tubes, antibody-pos. soln (2 × 200 µL) to BP tubes, and antibody-neg. soln (2 × 200 µL) to BN tubes. Ideal, final optical density readings (**k**) corresponding to these blanks should be as follows: BS <0.010; BC <0.050; BP >1.000; BN <0.200.

(g) *Pre-incubation with antiserum against soy protein.*—Fill reagent reservoir with antibody-pos. soln. Use multichannel pipet to

Table 24:D1 Recommended Standard Pattern of Micronic Tubes in Their Holder and Corresponding Wells in ELISA Plate for Assay of 7 Samples in Duplicate[a]

	Column											
Row	1	2	3	4	5	6	7	8	9	10	11	12
A	BS	S1	S1	S1	00	00	00	00	00	00	00	00
B	BS	S2	S2	S2	T1	U1	V1	W1	X1	Y1	Z1	00
C	BS	S3	S3	S3	T2	U2	V2	W2	X2	Y2	Z2	00
D	BC	S4	S4	S4	T3	U3	V3	W3	X3	Y3	Z3	00
E	BC	S5	S5	S5	T1	U1	V1	W1	X1	Y1	Z1	00
F	BC	S6	S6	S6	T2	U2	V2	W2	X2	Y2	Z2	00
G	BP	S7	S7	S7	T3	U3	V3	W3	X3	Y3	Z3	00
H	BP	BP	BN	BN	BN	00	00	00	00	00	00	00

[a] The 96 positions are arranged in 12 columns (1–12) and 8 rows (A–H). Samples are each analyzed at 3 dilutions, standard (in triplicate) at 7 dilutions, and 4 blanks in triplicate.

Key: BS, substrate blank; BC, conjugate blank; BP, antibody-positive blank; BN, antibody-negative blank; 00, unused; S1–S7, standard (1–7 indicates 7 serial dilutions); T–Z, 7 samples (1–3 indicates 3 serial dilutions).

add antibody-pos. soln (2 × 200 μL) to each of sample and std tubes. Place PBSTA (2 × 200 μL) in remaining outer tubes (00 in Table **24:D1**). Carefully affix cap strips to tubes. Invert tube holder ≥3 times to mix, and place in 37° incubator for 30 ± 1 min, minimizing untimed interval since addn of immunoreagents.

(h) *Antibody step*.—If freshly prepd sensitized ELISA plates **(c)** are to be used immediately, remove from incubator at end of 16 h, and let cool to room temp. (ca 30 min). If stored, sensitized ELISA plates **(d)** are to be used, remove from freezer, let warm to room temp. (45–60 min), and fill wells with PBSTA by using ELISA plate-washing system. In last 10 min of pre-incubation step **(g)**, wash freshly prepd plate 10-fold or stored plate 5-fold (using stdzd washing and blotting procedure **(d)**). Complete these operations by end of 30 min pre-incubation of Micronic tubes **(g)**.

Remove tube holder from incubator at stipulated time and invert 3 times to mix. Carefully remove caps. Using multichannel pipetter, transfer 200 μL aliquot of each soln to wells in corresponding positions in ELISA plate (Table **24:D1**). Before each transfer, ensure homogeneity by removing 200 μL aliquot and pipetting it back into remaining 600 μL of soln. Then place lidded plate in plastic bag and seal; incubate 120 ± 2 min at 37°, minimizing untimed interval since previous incubation.

(i) *Conjugate step*.—Remove ELISA plate from incubator at end of 120 min; wash 10-fold and blot as in **(d)**. Fill a reagent reservoir with conjugate soln. Add 200 μL PBSTA to "substrate blank" BS wells. Use multichannel pipetter to add conjugate soln to all other wells (apart form BS wells) in same order as in antibody step **(h)**. Then place lidded plate in plastic bag and seal; incubate 120 ± 2 min at 37°, minimizing untimed interval since previous incubation.

(j) *Substrate step*.—Remove ELISA plate from incubator at end of 120 min; wash 10-fold and blot as in **(d)**. Fill reagent reservoir with substrate soln, and use multichannel pipetter to add this soln (200 μL) to each well in plate in same order as in antibody step **(h)**. Then place lidded plate in plastic bag and seal; incubate 30 ± 0.25 min at 37°, minimizing untimed interval since previous incubation.

(k) *Termination of enzymic reaction and color measurement*.—Fill reagent reservoir with 0.2M NaOH soln (**Caution:** corrosive). Prep. multichannel pipet for addn of 50 μL aliquots. Remove ELISA plate from incubator at end of 30 min and add 50 μL 0.2M NaOH soln to each well in plate in same order as in antibody step. Critical timing of 30 min incubation is important because it facilitates some comparison between different plates; however, it is essential to ensure *equal* enzymic reaction times (within 15 s) in each individual well of single plate by means of systematic timing. Measure and record optical density at 405–410 nm of soln in each well, using ELISA reader or equiv.; these data should be observed between 5 and 60 min after addn of NaOH.

24.D06 ***Calculation***

For each plate, construct calibration curve (Fig. **24:D2**) of log concn of soy protein as acetone powder (N × 6.25) in the 7 std serial dilns vs corresponding optical density (mean of 3 observations). Calc. concn of total protein (N × 6.25) in each diln of each sample soln. From each mean observed optical density read from curve corresponding concn of soy protein in each diln of each sample soln. Calc. wt soy protein/100 g total protein for 3 dilns corresponding to each sample of acetone powder. If all 3 values correspond to central portion (i.e., dilns 2–6) of calibration curve (Fig. **24:D2**), take mean as final result. Optical densities that are too low imply that soln assayed was too concd; higher serial dilns should be chosen for a second ELISA. Result can also be calcd in terms of wt soy protein/100 g sample as received or in terms of wt soy ingredient (e.g., flour contg 45% protein) per 100 g sample as received. Note that the 3 ways of defining soy levels in sample are not identical and must be distinguished carefully.

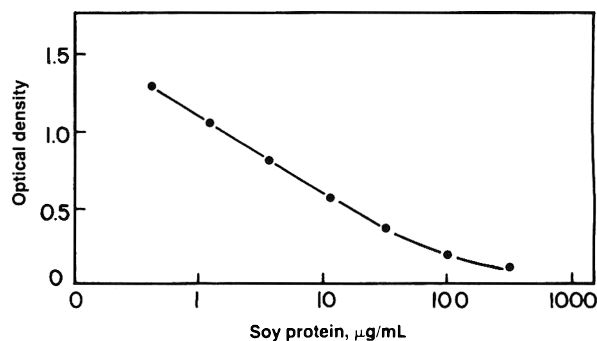

FIG. 24:D2—Determination of soy protein by ELISA procedure: calibration curve showing relationship between concentration of soy protein in standard solution and observed optical density

24.D07 ***Suggested Timetable***

ELISA procedure itself (Fig. **24:D1**), excluding prepn of all solns and calcn of results, should be carried out in a few hours during single work day. Complete analysis of 7 samples in duplicate on single plate (Table **24:D1**) requires 1 week from receipt of samples and involves 2–3 days actual labor. Realistic timetable might be as follows; however, it is possible to use the intervals to achieve higher throughput or to dovetail other jobs as necessary.

Day one: secs **24.D03(a)** and **(b)**; **24.D05(a)**
Day two: secs **24.D03(c)** and **(e)**; **24.D05(a)**
Day three: secs **24.D03(d)** and **(e)**; **24.D05(b)** and **(c)**
Day four (ELISA): secs **24.D03(f)** and **(g)**; **24.D05(d)–(k)**
Day five: sec. **24.D06**

Refs.: J. Sci. Food Agric. **36**, 499(1985). J. Assoc. Publ. Analysts **22**, 59(1984). JAOAC **71**, March issue (1988).

25. METALS AND OTHER ELEMENTS

(1) The following first action methods were adopted final action:

(a) Arsenic, cadmium, lead, selenium, and zinc in food, multielement method, **25.001–25.007**.

(b) Cadmium and lead in food, anodic stripping voltammetric method, **25.008–25.015**.

(c) Methyl mercury in fish and shellfish, gas chromatographic method, **25.146–25.152**.

(d) Tin in canned foods, atomic absorption spectrophotometric method, **25.A01–25.A04**.

---

(2) Make a nonsubstantive change in the final action AOAC-ASTM atomic absorption spectrophotometric method for determination of cadmium and lead in earthenware, **25.024–25.027**.

Change **25.025(c)**(*2*) to read:

Working solns.—Dil. 0.0, 1.0, 3.0, 5.0, 10.0, and 15.0 mL stock soln to 1 L with 4% HOAc (0, 1, 3, 5, 10, and 15 μg/mL).

---

(3) The following interim rapid gas chromatographic method for determination of methyl mercury in fish and shellfish was adopted first action:

Mercury (Methyl) in Fish and Shellfish
Rapid Gas Chromatographic Method
First Action

25.D01 *Principle*

Org. interferences are removed from homogenized seafood by acetone wash followed by toluene wash. Protein-bound Me Hg is released by addn of HCl and extd into toluene. Toluene ext is analyzed for CH_3HgCl by electron capture GC.

25.D02 *Reagents*

Equiv. reagents may be used.

(a) *Solvents.*—Acetone, toluene, and isopropanol, all distd in glass (Burdick and Jackson Laboratories, Inc., or MC/B Manufacturing Chemists, Norwood, OH 45212). **Caution**: Toluene is harmful if inhaled and is flammable; conduct all operations with toluene in laboratory hood.

(b) *Hydrochloric acid soln (1 + 1).*—Add concd HCl to equal vol. distd or deionized H_2O and mix. Use 2 vols toluene to ext potential interferences from 1 vol. HCl soln by vigorously shaking mixt. 15 s in separator. Discard toluene ext. Repeat extn step 4 times. Soln may be mixed in advance. However, extn must be performed immediately before HCl soln is used to avoid formation of electron-capturing compds which produce extraneous peaks in chromatograms.

Before beginning analysis, check quality of reagents by chromatographing blank taken thru method. Do not use HCl and solvs which produce extraneous peaks at retention time of Me Hg.

(c) *Carrier gas.*—GC quality Ar–CH_4 (95 + 5).

(d) *Sodium sulfate.*—Anhyd. reagent grade. Heat overnight in 600° furnace, let cool, and store in capped bottle. Line cap with acetone-washed Al foil to prevent contamination from cap. Peaks appearing at 14–15 min may be eliminated by refiring Na_2SO_4 (600° overnight).

(e) *Methyl mercuric chloride std solns.*—Keep tightly stoppered. Seal stopper with Teflon tape. *(1) Stock std soln.*—1000 μg Hg/mL. Weigh 0.1252 g CH_3HgCl (ICN-K&K Laboratories, Inc.) into 100 mL vol. flask. Dil to vol. with toluene. *(2) High level intermediate std soln.*—40 μg Hg/mL. Dil. 10.0 mL stock std soln to 250.0 mL with toluene. *(3) Low level intermediate std soln.*—2.0 μg Hg/mL. Dil. 10.0 mL high level intermediate std soln to 200.0 mL with toluene. *(4) Working std solns.*—0.005–0.10 μg Hg/mL. Prep. monthly by dilg with toluene in vol. flasks as follows: Dil. 10.0 mL of 2.0 μg Hg/mL soln to 200.0 mL for 0.10 μg Hg/mL. Dil. 20.0 mL of 0.10 μg Hg/mL soln to 25.0 mL, 15.0 mL to 25.0 mL, 10.0 mL to 25.0 mL, 10.0 mL to 50.0 mL, 10.0 mL to 100.0 mL, and 10.0 mL to 200.0 mL for 0.080, 0.060, 0.040, 0.020, 0.010, and 0.005 μg Hg/mL, resp.

(f) *Mercuric chloride column treatment soln.*—1000 ppm $HgCl_2$. Dissolve 0.1 g $HgCl_2$ in 100 mL toluene.

(g) *Fortification solns.*—*(1) Stock soln.*—1000 μg Hg/mL. Weigh 0.1252 g CH_3HgCl into 100 mL vol. flask. Dil to vol. with H_2O. *(2) Working fortification soln.*—15 μg Hg/mL. Dil. 1500 μL stock fortification soln to 100.0 mL with H_2O.

25.D03 *Apparatus*

Wash all glassware with detergent (Micro Laboratory Cleaner, International Products, Trenton, NJ 08601) and rinse thoroly with hot tap H_2O followed by distd or deionized H_2O. Then rinse 3 times with acetone and 3 times with toluene. Dry in hood.

Equiv. app. may be used except use packed column specified.

(a) *Centrifuge.*—Model IEC CRU-5000 (International Equipment Co., Needham Heights, MA 02194).

(b) *Centrifuge tubes.*—Glass, 50 mL capacity with Teflon-lined screw caps (Cat. No. 9212-K78, Thomas Scientific, 99 High Hill Rd at I-295, PO Box 99, Swedesboro, NJ 08085-0099).

(c) *Graduated cylinders.*—Glass, class A, 50 mL capacity, with ground-glass stoppers (Kimble 20036).

(d) *Transfer pipets.*—Disposable glass, Pasteur-type.

(e) *Dropping pipets.*—Glass, 5 mL capacity (No. 13-710B, Fisher Scientific Co.).

(f) *Mechanical shaker.*—Model S-500 shaker-in-the-round, with Model PT-0 timer (Kraft Apparatus, Inc., Mineola, NY 11501).

(g) *Gas chromatograph.*—Hewlett-Packard Model 5710A equipped with linear ^{63}Ni electron capture detector, Model 7131A recorder, and 6 ft × 2 mm id silanized glass column packed with 5% DEGS-PS on 100–120 mesh Supelcoport (Supelco, Inc.). Pack column *no closer* than 2.0 cm from injection and detector port nuts and hold packing in place with 2 cm high quality, silanized glass wool at both ends. Install oxygen scrubber and molecular sieve dryer (No. HGC-145, Analabs) between carrier gas supply and column. Condition column according to manufacturer's instructions as follows: Flush column 0.5 h with carrier gas flowing at 30 mL/min at room temp. Then heat 1 h at 50°. Next, heat column to 200° at 4°/min and hold at 200° overnight. Do not connect column to detector during this conditioning process. Maintain 30 mL/min carrier gas flow at all times during conditioning, treatment, and use. Operating conditions: column 155°, injector 200°, detector 300°; carrier gas flow 30 mL/min; recorder chart speed 0.5–1.0 cm/min. Under these conditions and with $HgCl_2$ column treatment procedure described below, CH_3HgCl peak appears 2–3 min after injection of ext.

25.D04 *Mercuric Chloride Column Treatment*

Column of 5% DEGS-PS, conditioned according to manufacturer's instructions, can be used to det. CH_3HgCl only after treatment by $HgCl_2$ soln, (f). Because column performance degrades with time, also treat column periodically during use. Perform appropriate $HgCl_2$ treatment procedures described below.

(a) *Following 200° column conditioning and after every 2–3 days of analyses.*—If column has just been conditioned according to manufacturer's instructions or has been used 2–3 days to analyze exts, proceed as follows: Adjust column temp. to 200° and inject 20 μL $HgCl_2$ treatment soln 5 times at 5–10 min intervals. Maintain 200° temp. overnight. Chromatogram will contain large, broad peaks. Adjust column temp. to 155° next morning and inject 20 μL $HgCl_2$ treatment soln 2 more times. Large, broad chromatgc peaks appearing at ca 1–2 h signal completion of treatment process and that column is ready for use.

(b) *On day preceding analyses.*—If column has been treated by procedure (a) or used 1 day at 155° to analyze exts, column may be treated at end of working day for next day's use as follows: Lower column temp. to 115° and inject 20 μL $HgCl_2$ treatment soln 1 time. After large, broad peaks appear in chromatogram (11–20 h), treatment process is complete. Next working day, increase column temp. to 155° operating temp. When baseline is steady, column is ready for use.

(c) *During extract analyses at 155°.*—If column has been used at 155° to analyze exts or if column performance and peak ht have degraded enough to require $HgCl_2$ treatment, inject two 20 μL aliquots of $HgCl_2$ treatment soln. Large, broad peaks will appear in chromatogram 1–2 h after $HgCl_2$ injection, signaling completion of treatment process. Wait for steady baseline; then column is ready for use.

25.D05 *Extraction of Methyl Mercuric Chloride*

Perform all operations except weighing in laboratory hood. Take empty centrf. tube thru all steps for method blank detn. Accurately weigh 1 g homogenized test sample into 50 mL centrf. tube. Add 25 mL acetone; tightly cap and *vigorously* shake tube by hand 15 s.

Loosen cap and centrf. 5 min at 2000 rpm. Carefully decant and discard acetone. (Use dropping pipet to remove acetone, if necessary.) Repeat 25 mL acetone wash step 2 more times. Break up tissue with glass stirring rod before shaking tube, if necessary. Add 20 mL toluene; tightly cap and vigorously shake tube by hand 30 s. Loosen cap and centrf. 5 min at 2000 rpm. Carefully decant (or draw off with dropping pipet) and discard toluene. Extraneous peaks in final GC chromatogram may indicate that more vigorous shaking with acetone and toluene is required. In products for which Me Hg recoveries are to be detd, fortify tissue at this point by adding working fortification soln, (g), to centrf. tubes.

Add 2.5 mL HCl soln, (**b**), to centrf. tube contg acetone- and toluene-washed sample. Break up tissue with glass stirring rod, if necessary. Ext CH_3HgCl by adding 20 mL toluene and shaking tube *gently* but *thoroly* 5 min on mech. shaker at setting 5 (2 min by hand). Loosen cap and centrf. 5 min at 2000 rpm. If emulsion is present after centrifugation, add 1 mL isopropanol and gently stir into toluene layer with glass stirring rod to reduce emulsion. Do not mix isopropanol with aqueous phase. Add equal amts of isopropanol to blank and test solns. If emulsion is not present, do not add isopropanol to blank or test solns. Vigorous mixing of isopropanol with HCl may produce interfering peaks in chromatograms. Recentrifuge. With dropping pipet, carefully transfer toluene to graduated cylinder. Rinse walls of centrf. tube with 1-2 mL toluene and transfer rinse to graduated cylinder. Repeat extn step 1 more time. Combine both exts in graduated cylinder, dil. to 50 mL with toluene, stopper, and mix well. Add 10 g Na_2SO_4 and mix again. Tightly stoppered exts (sealed with Teflon tape) may be refrigerated and held overnight at this point. Analyze by GC.

25.D06 ***Gas Chromatography***

Verify that system is operating properly by injecting 5 μL std soln contg 0.005 μg Hg/mL into GC system. Diff. between CH_3HgCl peak hts for 2 injections should be ≤4%. Check detector linearity by chromatographing all working std solns.

Inject 5 μL std soln with concn approx. equal to or slightly greater than concn of ext. Immediately after CH_3HgCl peak appears, inject another 5 μL ext. Immediately after CH_3HgCl and background peaks for ext appear, inject another 5 μL aliquot of std soln. Because column performance and peak ht slowly decrease with time, calc. Hg concn in each test sample by comparing peak ht for each test ext to average peak ht for std solns injected immediately after test ext.

Correct ht of CH_3HgCl peak for test ext by subtracting ht of peak for method blank obtained at same attenuation and recorder sensitivity. Calc. Me-bound Hg content of test sample expressed as μg Hg/g (ppm Hg) by comparing ht of peak from injection of test ext to av. ht of peak from dup. injections of std soln as follows:

$$\mu g\ Hg/g\ fish = (R/R') \times (C'/C) \times 50$$

where R = corrected ht of CH_3HgCl peak from injection of test ext, R' = av. ht of CH_3HgCl peak from dup. injections of std soln, C = wt (g) of test portion, C' = concn (μg/mL) of Hg in std soln, and 50 = final vol. (mL).

Ref.: JAOAC **70**, 24(1987).

CAS-7439-97-6 (mercury)

26. NATURAL POISONS

(1) The following first action methods were adopted final action:

Aflatoxin M_1 standards for thin layer chromatography, **26.008–26.011**.

Aflatoxins in food, Romer minicolumn method, **26.014–26.019**.

Aflatoxins in corn and peanuts, Holaday–Velasco minicolumn method, **26.020–26.025**.

Aflatoxins in peanuts and peanut products, CB method, **26.026–26.031**.

Aflatoxins in peanuts and peanut products, BF method, **26.032–26.036**.

Aflatoxins in coconut, copra, and copra meal, thin layer chromatographic method, **26.044–26.048**.

Aflatoxins in corn, thin layer chromatographic method, **26.049–26.051**.

Aflatoxins in cottonseed products, thin layer and liquid chromatographic methods, **26.052–26.060** (includes sample preparation, **26.B01**).

Aflatoxins in green coffee, thin layer chromatographic method, **26.061–26.066**.

Aflatoxins in pistachio nuts, thin layer chromatographic method, **26.067–26.068**.

Aflatoxins in soybeans, thin layer chromatographic method, **26.069**.

Aflatoxins in eggs, thin layer chromatographic method, **26.070–26.075**.

Identification of aflatoxin, by derivative formation on TLC plate, **26.083**.

Aflatoxin M_1 in dairy products, thin layer chromatographic method, **26.090–26.094**.

Aflatoxins B_1 and M_1 in liver, thin layer chromatographic method, **26.101–26.106**.

Aflatoxins B_1 and M_1 in liver, confirmation method, **26.107–26.109**.

Ochratoxins in barley, thin layer chromatographic method, **26.111–26.118**.

Ochratoxin A in green coffee, thin layer chromatographic method, **26.119–26.125**.

Sterigmatocystin in barley and wheat, thin layer chromatographic method, **26.132–26.138**.

Zearalenone in corn, thin layer chromatographic method, **26.139–26.147**.

Aflatoxin B_1 identification, confirmation method, **26.A01–26.A08**.

α-Zearalenol and zearalenone in corn, liquid chromatographic method, **26.A09–26.A16**.

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(2) Make a nonsubstantive change in the final action thin layer chromatographic–spectrometric method for preparation of aflatoxin standards, **26.004–26.007**.

Add as final sentences in **26.007:**

Do not store stock solns of aflatoxin stds in MeOH. To store, evap. MeOH soln to dry film, or evap. and then redissolve residue in benzene–CH_3CN.

---

(3) Make nonsubstantive changes in the first action (final 1988) CB method for aflatoxins in peanuts and peanut products, **26.026–26.031**.

(a) In **26.031(b)**, 1st par., change line 9 to read:

. . . spot 2, 5, and 10 μL aflatoxin stds, **26.011**, or resolution ref. std, **26.012**. Spot 5 μL std. . .

(b) In **26.031(c)**, 2nd par., change line 3 to read:

. . . spot 3.5, 5.0, and 6.5 μL aflatoxin stds, **26.011**, corresponding to aflatoxins observed on preliminary plate, or use resolution ref. std, **26.012**. Spot 5.0 μL. . .

---

(4) The following first action methods were declared surplus:

(a) Aflatoxins in green coffee, **26.061–26.066** (final 1988).

(b) Identification of aflatoxin B_1, thin layer chromatographic method, **26.076–26.082**.

(c) Aflatoxin M_1 in liver, confirmatory method, **26.110**.

27. NUTS AND NUT PRODUCTS

No additions, deletions, or other changes.

28. OILS AND FATS

No additions, deletions, or other changes.

29. PESTICIDE RESIDUES

No additions, deletions, or other changes.

30. SPICES AND OTHER CONDIMENTS

(1) The following first action method was adopted final action:

Moisture in spices, distillation method, **30.B01–30.B03**.

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(2) Make an editorial correction in method **30.083–30.084**. The title should read:

Permanganate Oxidation Number (6), Titrimetric Method, Final Action

31. SUGARS AND SUGAR PRODUCTS

(1) The following first action methods were adopted final action:

(a) Purity of lactose, liquid chromatographic method, **31.064–31.071**.

(b) Corn syrup and cane sugar in maple syrup, carbon ratio mass spectrometric method, **31.185–31.190**.

---

(2) Make nonsubstantive changes in the final action thin layer chromatographic method for high fructose corn sirup in honey, **31.156–31.157**.

(a) In **31.157(a)**: (*1*) Change the last sentence of the first paragraph to read, "Evap. eluate in 50 mL beaker on steam bath in current of air or N, or by other appropriate means."

(*2*) Change the last sentence of the second paragraph to read, "Dissolve residue in 0.1–0.2 mL H_2O."

(b) Change **31.157(c)** to read:

Interpretation.—Pure honey will show 1 or 2 large blue-gray or blue-brown spots at R_f >0.35. Any blue streaks or series of spots extending from origin provide presumptive evidence of presence of corn sirup, high fructose corn sirup (HFCS), including high fructose sirup derived from plant source other than corn. In this event, addnl test must be. . .

---

(3) Make a nonsubstantive change in the final action carbon ratio mass spectrometric method for corn sirup in honey, **31.158–31.161**.

In **31.161**, change the last paragraph to read:

. . . from TLC method **31.156**. Corresponding $\delta^{13}C$ values for predominantly citrus honey are $-21.9‰$ and $-20.0‰$. Samples with $\delta^{13}C$ value less negative than $-21.5‰$ relative to PDB ($-20.0‰$ for citrus honey) is considered adulterated.

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(4) The following final action methods were repealed to first action:

(a) Canadian lead number of maple products, Fowler modification, **31.191**.

(b) Winton lead number of maple products, **31.192–31.194**.

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(5) The following interim Roberts copper method for dextran in raw cane sugar was adopted first action:

Dextran in Raw Cane Sugar
Roberts Copper Method
First Action

31.D01 ***Principle***

All high MW material (>10,000 daltons) is sepd from sugar by pptn in 80% ethyl alcohol. Pptd material is filtered off, washed, and then redissolved. Alk. Cu(II) reagent is added which selectively ppts dextran from other high MW material in raw cane sugar. This ppt is filtered off, and dextran content is detd colorimetrically as carbohydrate by phenol–H_2SO_4 reaction, which breaks down dextran complex to glucose units and gives color with intensity proportional to total amt dextran in sample. Samples and reaction mixts must be kept free of contamination by sugar dust and other dust that may contain carbohydrate and react positively in phenol-H_2SO_4 color test.

31.D02 ***Apparatus***

(a) *Colorimeter or spectrophotometer*.—To read *A* or *T* at 485 nm.

(b) *Sintered glass filters*.—Coarse, pore size C, 15 mL.

(c) *Nessler tubes*.—35 mL, or flat-bottom test tubes short enough to fit inside filter flask.

31.D03 ***Reagents***

Use reagent grade chems unless otherwise specified. Use distd or deionized H_2O.

(a) *Absolute alcohol*.—Use absolute alcohol; do not use 95% alcohol.

(b) *Ethyl alcohol, 80%*.—Dil. 80 mL absolute alcohol (a) with 20 mL H_2O.

(c) *Filter aid*.—Anal. grade, acid-washed.

(d) *NaOH reagent soln*.—2.5N NaOH, satd with Na_2SO_4. Dissolve 100 g NaOH in H_2O and dil. to 1 L. Sat. with anhyd. or hydrated Na_2SO_4, adding crystals until some are left undissolved. Store in reagent bottle that has stopper of material other than glass. Soln may be stored 1 month.

(e) *Cu stock soln*.—Dissolve 3.0 g $CuSO_4 \cdot 5H_2O$ in 50 mL H_2O. Dissolve 30.0 g Na citrate in 50 mL H_2O. Mix these 2 solns and dil. to 1 L with H_2O. Soln may be stored 2 weeks.

(f) *Cu reagent soln*.—Dil. 50 mL Cu stock soln with 50 mL H_2O. Dissolve 12.5 g anhyd. Na_2SO_4 in this soln. Reagent must be freshly prepd on each day of use and must not be stored.

(g) *Wash soln*.—To 50 mL H_2O, add 10 mL Cu reagent soln and 10 mL 2.5N NaOH reagent soln.

(h) *Phenol soln, 5%*.—Dissolve 5.0 g pure phenol in H_2O and dil. to 100 mL. Soln may be stored 2 weeks.

(i) *Dextran*.—(Pharmacia Co., Inc., 800 Centennial Ave, Piscataway, NJ 08854). Any MW range may be used.

(j) *Sulfuric acid, 2N*.—Dissolve 98 g H_2SO_4 in H_2O and dil. to 1 L. Soln may be stored 1 month. **(Caution: See 51.030.)**

31.D04 ***Preparation of Standard Curve***

Det. moisture content of dextran to allow moisture correction when dextran is weighed for std curve. Weigh 500 mg std dextran in weighing dish and dry 4 h at 105°. Cool in desiccator over anhyd. $CaCl_2$. Weigh and calc. moisture content as follows:

$$\text{Moisture, \%} = [(\text{original wt} - \text{dry wt})/\text{original wt}] \times 100$$

Weigh 500 mg dextran (corrected for calcd moisture content), dissolve in H_2O, and dil. to 500 mL. Do not use predried dextran which may not dissolve properly. Soln contains 1.0 mg dextran/mL. Use freshly prepd soln for each std curve detn.

Dil. 100 mL 1.0 mg dextran/mL std soln to 1 L (0.1 mg dextran/mL). Use this soln in aliquots of 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 mL, each dild to 100 mL, to prep. std curve. Each soln contains, resp., 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, and 0.1 mg dextran/mL.

Use A (or T) data to construct calibration curve with mg dextran/mL as x-axis.

31.D05 ***Phenol-H_2SO_4 Test***

In series of 10 test tubes, 20 × 150 mm, place 2 mL of each dextran std soln. Place 2 mL H_2O in an 11th tube as blank. Add 1 mL 5% phenol soln to each tube. Swirl tubes gently to mix phenol and carbohydrate solns.

To each tube add 10 mL concd H_2SO_4, preferably using plastic automatic pipet, releasing acid rapidly to ensure that soln is thoroly mixed. Do not let pipet touch soln, and do not let acid remain on top of soln. Mix on Vortex mixer.

Place tubes (in rack) in boiling H_2O bath for 2 min. Let cool 30 min.

Read color as A or T, preferably A, on colorimeter or spectrophtr at 485 nm against blank soln. Set A to zero on blank and then read A of solns, using same cell (or matched cells) for blank and test solns. Make duplicate colorimetric readings and calc. av. value.

For best accuracy, A should be between 0.1 and 0.6 (T should be >25%). If A is >0.6, diln step is required. This step should not be necessary for prepn of std curve, but may be required for sugar samples.

To prep. dild soln from soln from which 2 mL aliquot was taken (final 25 mL soln in sugar sample detn), pipet 5 mL into 25 mL vol. flask and dil. to vol. Use 2 mL of this dild soln in phenol–H_2SO_4 detn. In this case, diln factor of 5 must be included in calcn of ppm dextran.

31.D06 ***Determination***

Accurately weigh 40.0 g sugar in beaker and dissolve in small amt H_2O. Quant. transfer soln to 100 mL vol. flask and dil. to vol. with H_2O. Filter ≥50 mL of this soln thru qual. paper in funnel to remove coarse suspended material.

Pipet 10 mL filtrate into 100 mL beaker, add 0.3–0.4 g anal. filter aid, and stir; add 40 mL absolute alcohol and stir. Let stand 5 min to form ppt. Filter ppt off on sintered glass filter on rubber ring or rubber sleeve-topped vac. filter flask.

Wash ppt 5 times with 80% ethyl alcohol, each time filling filter with alcohol, and letting alcohol be drawn thru ppt. Do not let ppt go dry and do not let alcohol overflow filter. This step is important to remove sugar that might remain adsorbed to ppt and interfere in phenol–H_2SO_4 reaction.

When last portion of alcohol wash has been completely drawn thru ppt, quant. transfer ppt plus filter aid to 25 mL vol. flask. Use min. amt H_2O for transfer. First, place 50 or 60 mm long-stem funnel in empty 25 mL flask. Invert sintered glass funnel contg ppt into long-stem funnel; fill stem of sintered glass funnel with H_2O and blow ppt plus filter aid into long-stem funnel, using air hose or mouth. Use H_2O from wash bottle to wash remaining traces of solid from sintered glass funnel (2 times) into long-stem funnel, and wash all ppt (broken up with spatula) into 25 mL vol. flask. Amt H_2O used must not exceed vol. of 25 mL flask. Adjust to vol. with H_2O.

Filter soln thru fluted Whatman No. 42, 110 mm, paper in 60 or 80 mm funnel. Collect ≥10 mL filtrate for analysis.

Pipet 10 mL filtrate into 20 × 100 mm glass or plastic test tube. Pipet 2 mL 2.5N NaOH reagent soln into test tube. Pipet 2 mL Cu reagent soln into test tube and add 0.2 g anal. filter aid. Place test tubes (in rack) in boiling H_2O bath 5 min to ppt Cu-dextran complex on filter aid, and then let cool 20 min.

Filter ppt, which contains Cu–dextran complex, on sintered glass filter (coarse pore size, 15 mL). Rinse test tube 2 times with 10 mL portions of wash soln and pour these into sintered glass filter.

Discard filtrate.

Place filter, contg ppt, on vac. flask contg short (35 mL) Nessler tube (or flat-bottom vial) positioned so that stem of sintered glass funnel extends into Nessler tube.

Pour 2 mL 2N H_2SO_4 soln onto ppt in sintered glass funnel; turn on vac. and draw acid soln thru ppt. Repeat this procedure, then rinse ppt with 2 mL H_2O.

Quant. transfer filtrate, which contains solubilized dextran to 25 mL vol. flask and dil. to vol. with H_2O.

Pipet 2 mL of this soln into 20 × 150 mm test tube and follow procedure for *Phenol–H_2SO_4 Test*.

31.D07 ***Calculation***

Read dextran concn, mg/mL, from std calibration curve.

Dextran in sample, ppm = [(mg/mL dextran from std curve) × (mL final soln of Cu–dextran complex) × (mL soln of alcohol ppt) × 10^5]/[(mL aliquot taken for Cu pptn) × (mL aliquot taken for alcohol pptn) × (wt sample solids dild to 100 mL)]

If A = wt sample solids, g, dild to 100 mL; B = aliquot taken for alcohol pptn, mL; C = soln of alcohol ppt, mL; D = aliquot taken for Cu pptn, mL; E = final soln of Cu–dextran complex, mL; F = dextran (from std curve), mg/mL, then:

$$\text{Dextran, ppm} = (F) \times (E) \times (C/D) \times (1/B) \times (1/A) \times 10^5$$

Therefore, for aliquots specified in procedure: $A = 40$, $B = 10$, $C = 25$, $D = 10$, and $E = 25$.

Ref.: JAOAC 71, March issue (1988).

CAS-9004-54-0 (dextran)

32. VEGETABLE PRODUCTS, PROCESSED

No additions, deletions, or other changes.

33. WATERS; AND SALT

No additions, deletions, or other changes.

34. COLOR ADDITIVES

The following interim rapid cleanup for spectrophotometric and thin layer chromatographic identification of FD&C color additives in foods was adopted first action:

FD&C Color Additives in Foods
Rapid Cleanup for Spectrophotometric and Thin Layer Chromatographic Identification
First Action

(Applicable to sepn and identification of FD&C Red Nos. 3 and 40, Blue Nos. 1 and 2, Yellow Nos. 5 and 6, Green No. 3, and former FD&C Red No. 2)

34.D01 *Principle*

Color additives are sepd on reverse phase C_{18} cartridge and identified by spectrophotometry and thin layer chromatgy.

34.D02 *Apparatus*

(a) *C_{18} cartridges.*—Sep-Pak (Waters Associates, Inc.), or equiv. Cartridges are reusable when flushed with 3–4 mL 50% isopropanol to remove all residual coloring and stored in isopropanol until needed.

(b) *Syringe.*—10 mL with Luer tip.

(c) *Spectrophotometer.*—UV-visible, recording, capable of scanning wavelength range from 750 to 350 nm.

(d) *Spectrophotometric cells.*—3.5 mL, 1 cm path length.

(e) *TLC plates.*—Silica gel G, precoated. Uniplates (Alltech Associates, Inc., 2051 Waukegan Rd, Deerfield, IL 60015), or equiv.

(f) *Filter paper.*—Folded. Whatman No. 2V, 12.5 cm, or equiv.

(g) *Pipets.*—Disposable, 5 μL. Microcaps (Drummond Scientific Co., 500 Parkway, Broomall, PA 19008), or equiv.

34.D03 *Reagents*

(a) *Isopropanol solns.*—2.5, 5, 13, 20, and 50% LC grade isopropanol in H_2O.

(b) *Acetic acid soln.*—1% HOAc in H_2O.

(c) *Hydrochloric acid.*—Concd, anal. reagent grade.

(d) *NaOH soln.*—50% NaOH in H_2O w/v.

(e) *FD&C reference stds.*—(Available from Food and Drug Administration, Division of Color Technology, 200 C St, SW, Washington, DC 20204, and commercial sources). *Stock soln:* 100 mg ref. std/100 mL H_2O. *Working soln:* Dil. 1 mL stock soln to 100 mL with appropriate isopropanol soln to obtain spectra. Use 1 cm cells as described in *Identification*.

(f) *TLC solvent system.*—*n*-Butanol–methyl ethyl ketone–NH_4OH–H_2O (5 + 3 + 1 + 1).

34.D04 *Preparation of Samples*

Sample size depends on intensity of colorants present. Prior to C_{18} cartridge extn, filter samples thru prewetted paper, sintered glass filters, or disposable filters (Lid/X [Xydex Corp., 4 Alfred Cr, Bedford, MA 01730; or equiv.]). *Note:* Filter paper may absorb coloring.

If lakes of colors are present use alternative methodology.

Following are examples of prepns for commonly encountered samples.

(a) *Oriental noodles.*—Place 5 g product and 20 mL H_2O in beaker over low heat with gentle swirling until coloring is leached. Filter soln, and apply 2 mL filtrate onto cartridge.

(b) *Candy.*—Place in beaker 2 or more units depending on intensity of product coloring. Use sep. beaker for each different colored candy. Add 10 mL H_2O to each beaker and proceed as for noodles (a).

(c) *Carbonated soda and similar drinks.*—Use sample "as is." Apply 2 mL onto cartridge.

(d) *Gelatin dessert.*—Place 3 g product and 90 mL H_2O in beaker over low heat with gentle swirling until dissolved. Filter soln and apply 5 mL filtrate onto cartridge. Use cartridge only once and discard. Use new cartridge for repeat extn.

(e) *Powdered drink mix.*—Place 1 g product in beaker, add 200 mL H_2O, and mix by gentle swirling until sample is dissolved. Filter soln and apply 2 mL filtrate onto cartridge.

(f) *Dye mixtures.*—Apply 1–2 mL aq. soln of dye mixt. directly onto cartridge.

34.D05 *Separation*

Remove plunger from 10 mL syringe, and place long end of cartridge on Luer tip of syringe barrel. Add 3 mL isopropanol to syringe barrel and replace plunger. Pump soln thru cartridge, remove cartridge, and discard eluate. Repeat using 5 mL 1% HOAc followed by filtered sample and appropriate vol. of isopropanol soln according to scheme in Fig. **34:D1**. Collect only colored portion. Elute at moderate flow rate that results in droplets rather than streams of eluant. Monitor sepn closely and discard fractions that contain overlapping bands.

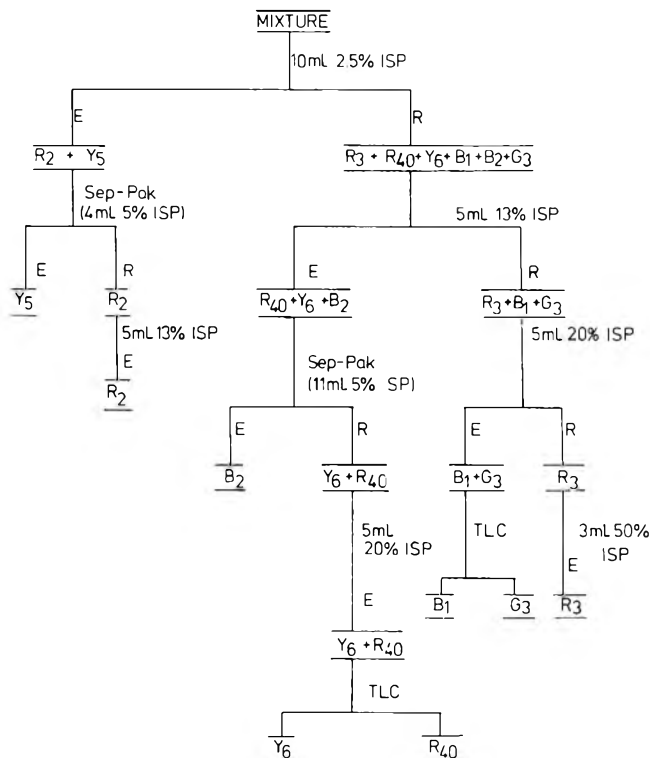

FIG. 34:D1—Scheme for identification of FD&C color additives: isopropanol (ISP); E = elutes and R = retains. R_2, Red No. 2; R_3, Red No. 3; R_{40}, Red No. 40; Y_5, Yellow No. 5; Y_6, Yellow No. 6; B_1, Blue No. 1; B_2, Blue No. 2; G_3, Green No. 3

34.D06 *Identification*

Ref. std solns must be prepd in same strength isopropanol used to elute test sample. Identify sepd color additives by visible spectrophotometry with further confirmation by thin layer chromatgy.

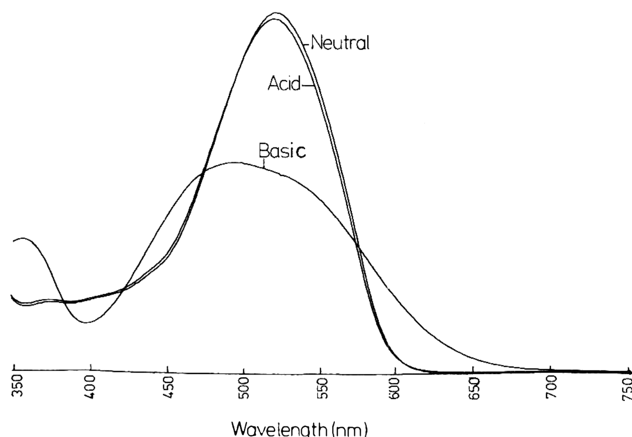

FIG. 34:D2—Spectra of former FD&C Red No. 2 in three 13% isopropanol solutions

(a) *Spectrophotometry.*—Use neut.–acid–basic combination spectra as shown in Fig. **34:D2**. Perform following steps in sequence on same soln to obtain spectra under 3 conditions: (*1*) *Neutral soln.*—Fill 3.5 mL 1 cm absorption cell ca ¾ full with sample eluate and scan visible spectrum from 750 to 350 nm.

(*2*) *Acid soln.*—To neut. soln in cell add 1 drop (Pasteur pipet equiv.) of HCl. Obtain visible spectrum as for neut. soln.

(*3*) *Basic soln.*—To acid soln in cell add 2 drops 50% NaOH soln. Obtain visible spectrum as for neut. soln.

(b) *Thin layer chromatography.*—Use silica gel G plates and TLC solv. system (**f**). Use sample eluate "as is" if color intensity is adequate for visualization, or conc. to small vol. if judged too dild. Streak 3 cm band of unknown sample along line 1 cm above base of TLC plate, and let dry. Apply 1 μL appropriate ref. stds to plate at spaced intervals atop dried sample streak, dry, and develop to 10 cm. One common band that develops at R_f of corresponding ref. std identifies color additive.

Ref.: JAOAC **71**, May issue (1988).

35. COSMETICS

No additions, deletions, or other changes.

36. DRUGS: GENERAL

(1) The following first action methods were adopted final action:

(a) Methocarbamol in drugs, liquid chromatographic method, **36.212–36.218**.

(b) Trimethobenzamide hydrochloride in drugs, ion-pair column chromatographic method, **36.A01–36.A06**.

(c) Phenothiazine drugs, microcrystalline identification, **36.A07–36.A10**.

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(2) The following interim liquid chromatographic method for determination of flucytosine in capsules was adopted first action:

Flucytosine in Drug Capsules
Liquid Chromatographic Method
First Action

36.D01 ***Principle***

Flucytosine content of capsules is detd by liq. chromatgy on C_{18} reverse phase column, using H_2O–MeOH–HOAc mobile phase contg 1-octanesulfonic acid Na salt, *p*-aminobenzoic acid as internal std, and UV detection at 285 nm.

36.D02 ***Apparatus***

(a) *Liquid chromatograph.*—Model 8800 solv. pump with variable wavelength detector capable of monitoring elution at 285 nm (Du Pont Instruments Div.), injection valve with 20 μL sample loop (Valco Instruments Co., Inc., PO Box 55603, Houston, TX 77055), and suitable strip chart recorder. Equiv. LC system may be used. Operating conditions: flow rate 1.5 mL/min; detector 285 nm, 0.32 AUFS; chart speed 0.5 cm/min; temp. ambient; injection vol. 20 μL. To detect fluorouracil, main degradation/precursor product of flucytosine, monitor sepn at 266 nm to maximize sensitivity.

(b) *Chromatographic column.*—Stainless steel, 300 mm × 3.9 mm id, packed with 10 μm μBondapak C_{18} (Waters Associates, Inc.), or equiv. column that meets LC system suitability requirements.

36.D03 ***Reagents***

(a) *1-Octanesulfonic acid NA salt.*—(Eastman Kodak Co.).

(b) *p-Aminobenzoic acid.*—Certified ACS Grade (Fisher Scientific Co.), or equiv.

(c) *Mobile phase.*—H_2O–MeOH–HOAc (785 + 200 + 15 v/v/v) contg 2 g/L of 1-octanesulfonic acid Na salt.

(d) *Internal std soln.*—Accurately weigh ca 160 mg *p*-aminobenzoic acid and transfer to 200 mL vol. flask. Add 100 mL mobile phase, sonicate 5 min, shake mech. 25 min, dil. to vol. with mobile phase, and mix well.

(e) *Flucytosine std soln.*—Accurately weigh ca 30 mg USP Flucytosine Ref. Std and transfer to 50 mL vol. flask. Add 25 mL mobile phase, sonicate 5 min with gentle swirling, shake mech. 25 min, dil. to vol. with mobile phase, and mix. Transfer 10.0 mL of this soln to 100 mL vol. flask, add 5.0 mL internal std soln, dil. to vol. with mobile phase, and mix. Prep. this soln fresh daily.

36.D04 ***Sample Preparation***

Accurately weigh contents of ≥20 flucytosine capsules and det. av. wt/capsule. Accurately weigh portion of powder equiv. to ca 100 mg flucytosine and transfer to 100 mL vol. flask. Add 50 mL mobile phase, sonicate 5 min with gentle swirling, shake mech. 25 min, dil. to vol. with mobile phase, and mix well. Filter portion of soln thru suitable paper or 0.45 μm membrane filter, discarding first 10 mL filtrate. Transfer 6.0 mL filtrate to 100 mL vol. flask, add 5.0 mL internal std soln, dil. to vol. with mobile phase, and mix well. From this point, complete detn on same day.

36.D05 ***Suitability Test and Determination***

Inject 20 μL each of sample and std solns into LC system by using sampling valve or high pressure microsyringe. Operate system as described in *Apparatus*. Adjust detector sensitivity so peak response for flucytosine is between 40 and 75% full scale. In suitable chromatogram, CV of peak ht (or area) ratios for 6 replicate injections of std soln should be ≤2.0%, and resolution factor, *R*, for flucytosine peak and internal std peak should be ≥2.

Calc. resolution factor as follows:

$$R = [2(t_2 - t_1)]/(W_2 + W_1)$$

where t_2 and t_1 = retention times of 2 components, and W_2 and W_1 = corresponding widths of peaks, measured by extrapolating sides of peaks to baseline.

36.D06 ***Calculation***

Calc. amt flucytosine in dosage form, using response ratios based on either peak hts or peak areas, according to following equation:

$$\text{Flucytosine, mg/capsule} = 1.667C \times (R/R') \times (T/W)$$

where C = concn, μg/mL, of flucytosine in std soln; R and R' = response ratios for flucytosine peak to internal std peak for sample and std, resp ; T = av. capsule wt, mg; W = wt sample taken for assay, mg.

Ref.: JAOAC **71**, January issue (1988).

CAS-2022-85-7 (flucytosine)

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(3) The following interim liquid chromatographic method for determination of levodopa and levodopa–carbidopa in solid dosage forms was adopted first action:

Levodopa and Levodopa-Carbidopa in Solid Dosage Forms
Liquid Chromatographic Method
First Action

36.D07 ***Principle***

Levodopa in tablets or capsules and levodopa-carbidopa in tablets are detd by reverse phase liq. chromatgy on C_{18} column with 3% HOAc as mobile phase, and UV detection at 280 nm. Methyldopa is internal std for levodopa tablets or capsules; acetaminophen is internal std for levodopa-carbidopa tablets.

36.D08 ***Apparatus***

(a) *Liquid chromatograph.*—Isocratic system equipped with detector capable of monitoring *A* at 280 nm. suitable strip chart recorder, and injection valve with 20 μL sample loop.

(b) *Chromatographic column.*—300 × 3.9 mm id, μBondapak C_{18}, 10 μm particle size (Waters Associates, Inc), or equiv. column that meets suitability requirements.

(c) *Membrane filters.*—0.45 μm porosity (Millipore, or equiv.).

36.D09 ***Reagents***

(a) *Mobile phase.*—3% aq. HOAc.

(b) *Methyldopa internal std soln.*—2 mg/mL. Accurately weigh ca 200 mg USP Methyldopa Ref. Std into 100 mL vol. flask, add 50 mL 0.1N HCl, and sonicate to dissolve std. Dil. to vol. with mobile phase, and mix.

(c) *Acetaminophen internal std soln.*—0.5 mg/mL. Accurately weigh ca 125 mg USP Acetaminophen Ref. Std into 250 mL vol. flask, add 75 mL MeOH, and sonicate to dissolve std. Dil. to vol. with mobile phase, and mix.

(d) *Levodopa std soln.*—Just prior to use, dry USP Levodopa Ref. Std 4 h at 105°. Store in tightly covered, light-resistant container. Accurately weigh ca 100 mg dried std into 50 mL vol. flask. Add 30 mL 0.1N HCl, and sonicate to dissolve. Dil. to vol. with 0.1N HCl, and mix. Filter soln thru 0.45 μm membrane filter, discarding first 5 mL filtrate. Pipet 5 mL filtrate and 10 mL methyldopa internal std soln into 100 mL vol. flask, dil. to vol. with mobile phase, and mix.

(e) *Levodopa-carbidopa std soln.*—Dry USP Carbidopa Ref. Std to const wt at 100° under reduced pressure not exceeding 5 mm Hg. Store in tightly covered, light-resistant container. Accurately weigh ca 100 mg dried USP Levodopa Ref. Std (**d**) into 50 mL vol. flask. Add accurately weighed amt of dried carbidopa std so that carbidopa-to-levodopa ratio corresponds to that found in com. levodopa-carbidopa tablet. Add 30 mL 0.1N HCl, sonicate to dissolve, dil. to vol. with 0.1N HCl, and mix. Filter soln thru 0.45 μm membrane filter, discarding first 5 mL filtrate. Pipet 10 mL filtrate into 100 mL vol. flask, and add vol. of acetaminophen internal std soln so that acetaminophen concn is 1.25 times carbidopa concn. Dil. to vol. with mobile phase, and mix.

36.D10 ***Sample Preparation***

(a) *Levodopa tablets or capsules.*—Weigh and finely powder ≥20 tablets or composite contents of 20 capsules. Weigh portion of powder equiv. to ca 100 mg levodopa into 50 mL vol. flask, and proceed as directed under levodopa std soln (**d**), beginning "Add 30 mL 0.1N HCl. . ."

(b) *Levodopa-carbidopa tablets.*—Weigh and finely powder ≥20 tablets. Weigh portion of powder equiv. to ca 100 mg levodopa into 50 mL vol. flask, and proceed as directed under levodopa-carbidopa std soln (e), beginning "Add 30 mL 0.1N HCl. . ."

(c) *Levodopa-carbidopa tablets for content uniformity determination.*—Dissolve 1 tablet in sufficient 0.1N HCl to prep. soln contg 2 mg levodopa/mL. Filter soln thru 0.45 μm membrane filter, discarding first 5 mL filtrate. Pipet 10.0 mL filtrate into 100 mL vol. flask, add acetaminophen internal std soln (15 mL for levodopa-carbidopa 100/25 tablets, 5 mL for all other dosage levels), dil. to vol. with 0.1N HCl, and mix.

36.D11 ***System Suitability Test and Assay***

Equilibrate LC system with mobile phase at 1.5 mL/min. Inject 20 μL std soln. Approx. retention times are levodopa, 3 min; methyldopa, 4.5 min; carbidopa, 5 min; and acetaminophen, 9 min. Calc. resolution factor, *R*, as follows:

$$R = [2(t_2 - t_1)]/(W_2 + W_1)$$

where t_2 and t_1 = retention times of the 2 components, and W_2 and W_1 = corresponding widths of bases of peaks obtained by extrapolating relatively straight sides of peaks to baseline. *R* between levodopa and carbidopa and between carbidopa and acetaminophen should be >3.5. *R* between levodopa and methyldopa should be >2.

Change flow rate to improve resolution. For levodopa-carbidopa tablets, change detector sensitivity between levodopa peak (approx. 0.64 AUFS) and carbidopa peak (approx. 0.08 or 0.04 AUFS). Set detector sensitivity to 35–95% AUFS. If necessary, adjust vol. of internal std soln added to sample soln and std soln to obtain satisfactory detector response for std soln. Inject std soln 5 times and compare peak hts. Calc. CV as follows:

$$CV, \% = \frac{100}{\bar{X}} \left[\frac{\sum_{i=1}^{N} (X_i - \bar{X})^2}{N-1} \right]^{1/2}$$

where $\bar{X}$ = mean of set of *N* measurements, and X_i = an individual detn of ratio of peak ht of analyte to peak ht of internal std. In suitable system, CV = ≤2.0.%

Proceed with sample analysis by injecting 20 μL each of sample soln and corresponding std soln.

36.D12 ***Calculation***

Using peak ht ratios *R* and *R'* relative to internal std, calc. mg drug/tablet or capsule as follows:

$$\text{mg/tablet or capsule} = (R/R') \times C \times (D/W) \times T$$

where *R* and *R'* = peak ht ratios for sample and std solns, resp.; *C* = concn of std soln, mg/mL; *W* = wt of sample taken, mg; *D* = sample diln; and *T* = av. tablet or capsule wt, mg.

Ref.: JAOAC **70**, 987 (1987).

CAS-59-92-7 (levodopa)
CAS-38821-49-7 (carbidopa)
CAS-28860-95-9 (carbidopa anhydrous)

37. DRUGS: ACIDIC

(1) The following first action method was adopted final action: Primidone in drug tablets, liquid chromatographic method, **37.A17–37.A22.**

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(2) The following interim liquid chromatographic method for determination of diethylpropion hydrochloride in drug substance and tablets was adopted first action:

Diethylpropion Hydrochloride in Drug Substance and Tablets
Liquid Chromatographic Method
First Action

37.D01 ***Principle***

DEPH content of drug substance and tablets is detd by reverse phase liq. chromatgy using anthracene as internal std, UV detection

at 254 nm, and $MeOH-H_2O$–phosphate mobile phase. Purity is confirmed by UV, LC, and spot tests.

37.D02 ***Apparatus***

(a) *Liquid chromatograph.*—Equipped with 30 cm × 4 mm id stainless steel column packed with ODS bonded to microparticulate silica; UV detector; and suitable integrator. Operating conditions: column temp. ambient; mobile phase flow rate ca 1.5 mL/min; vol. injected 20 μL; detector wavelength 254 nm.

(b) *UV-visible scanning spectrophotometer.*

37.D03 ***Reagents***

Use deionized, purified H_2O and anal. reagent grade chemicals unless otherwise specified.

(a) *Mobile phase.*—Dissolve 0.40 g KH_2PO_4 and 2.26 g anhyd. Na_2HPO_4 in H_2O to make 1000 mL. Mix 1 part of this soln with 4 parts of MeOH, and filter thru suitable membrane of ≤ 1 μm porosity. pH of resulting soln should be ca 7.7. Degas soln by sparging with He. Make adjustments, if necessary, to obtain desired retention times and resolution.

(b) *H_3PO_4 soln.*—(1 in 2000). Dil. 1 mL H_3PO_4 (85%) to 2 L with H_2O.

(c) *Internal std soln.*—Dissolve 20 mg anthracene in 500 mL MeOH. Store in air-tight container.

(d) *DEPH std soln.*—Dry USP Diethylpropion Hydrochloride Ref. Std over silica gel ≥ 4 h. Accurately weigh ca 25 mg dried std and transfer to 200 mL vol. flask contg 40 mL H_3PO_4 soln. Pipet 40.0 mL internal std soln into flask, dil. to vol. with MeOH, and mix.

37.D04 ***Chromatographic System Suitability Test***

Let chromatgc system equilibrate and inject replicate 20 μL portions of DEPH std soln. Typical retention times for diethylpropion and anthracene are ca 6 and 9 min, resp., at flow rate between 1.0 and 2.0 mL/min. In suitable system, resolution, R, between these peaks is > 3.0 and relative std dev. (RSD) for ratios of peak responses, R_s, for 6 replicate injections of DEPH std soln is ≤ 1.0%.

37.D05 ***Sample Preparation***

(a) *Bulk drug assay soln.*—Dry DEPH bulk drug over silica gel ≥ 4 h. Accurately weigh ca 25 mg dried sample and transfer to 200 mL vol. flask contg 40 mL H_3PO_4 soln. Pipet 40.0 mL internal std soln into flask, dil. to vol. with MeOH, and mix.

(b) *Tablet assay soln.*—Weigh and finely powder ≥ 20 DEPH tablets. Into 200 mL vol. flask, transfer accurately weighed amt of powder equiv. to ca 25 mg DEPH. Add 40 mL H_3PO_4 soln and shake mech. ≥ 15 min. Transfer 40.0 mL internal std soln to flask, dil. to vol. with MeOH, and mix. Centrifuge portion of soln, and filter supernate thru suitable membrane filter ≤ 1 μm porosity.

37.D06 ***Determination***

(a) *Bulk drug.*—Sep. inject equal vols (ca 20 μL) of DEPH std soln and bulk drug assay soln in duplicate into LC system, record chromatograms, and measure response for major peaks. Rel. retention times are ca 0.7 for diethylpropion and 1.0 for anthracene. Calc. amt DEPH, mg, in portion of DEPH drug substance taken as follows:

$$\text{mg DEPH} = F \times C \times (R_u/R_s)$$

where F = vol. sample soln/1000 μg/mg = 200 mL/1000 μg/mg = 0.2; and where C = concn, μg/mL, of USP DEPH Ref. Std in DEPH std soln, and R_u and R_s are ratios of peak responses of diethylpropion to anthracene obtained from bulk drug assay soln and DEPH std soln, resp.

(b) *Tablets.*—Use tablet assay soln and follow procedure for bulk drug detn. Calc. amt DEPH, mg/tablet, as follows:

$$\text{DEPH, mg/tablet} = \{[0.2C \times (R_u/R_s)]/\text{mg sample}\} \times W$$

where W = av. tablet wt, mg.

37.D07 ***Identification Tests***

(a) *Bulk drug.*—UV spectrum of 1 in 100,000 soln in 0.1N HCl exhibits max. and min. at same wavelengths as that of similar prepn of USP DEPH Ref. Std concomitantly measured, and molar a values (anhyd. basis) at wavelength max. at ca 253 nm do not differ by > 3.0%.

(b) *Tablets.*—Chromatogram of tablet assay soln obtained as directed for tablet assay exhibits major peak for diethylpropion, with retention time that corresponds with that exhibited in chromatogram of DEPH std soln, both relative to internal std.

37.D08 ***Chromatographic Purity***

(a) *Test preparations.*—*Test prepn A.*—Dissolve 250 mg bulk drug in 20 mL H_3PO_4 soln in 100 mL vol. flask, dil. to vol. with MeOH, and mix. *Test prepn B.*—Transfer 1.0 mL test prepn A to 100 mL vol. flask, dil. to vol. with 4:1 mixt. of MeOH and H_3PO_4 soln, and mix.

(b) *LC system.*—Use system described in *Apparatus.*

(c) *Procedure.*—Inject 20 μL test prepn B into LC system, adjust detector output to obtain peak ht for diethylpropion ≥ 40% but < 100% full scale deflection on chart, adjust integrator sensitivity accordingly, and det. area of diethylpropion peak. Inject 20 μL test prepn A, chromatograph for total time equal to 3 times retention time of diethylpropion, and det. sum of areas of impurity peaks between solv. front peak and diethylpropion peak. Perform solv. blank detn by injecting 20 μL of 4:1 mixt. of MeOH and H_3PO_4 soln. Calc. % chromatgc impurities by formula:

$$\%\ \text{impurities} = 100\ (r_A - r_S)/[100r_B + (r_A - r_s)]$$

where r_A = sum of areas of impurity peaks in chromatogram obtained from test prepn A, r_S = sum of areas of peaks in chromatogram obtained from solv. blank, and r_B = area of diethylpropion peak in chromatogram obtained from test prepn B. Impurities found should be ≤ 0.5%.

37.D09 ***Secondary Amines***

(a) *Acetaldehyde test soln.*—Mix 4 mL acetaldehyde, 3 mL alcohol, and 1 mL H_2O. Prep. soln fresh.

(b) *Procedure.*—Dissolve 100 mg bulk drug in 2 mL CH_2Cl_2 in centrf. tube. Transfer to second tube 2 mL std soln of diethylamine·HCl (DEAH) (dried 2 h at 105° before using) in CH_2Cl_2, which has known concn of 250 μg/mL. Treat each soln as follows: Ext with 2 mL buffer soln contg 5.7 g Na_2CO_3 and 3.0 g $NaHCO_3$/100 mL H_2O. Centrifuge, if necessary, to clarify upper phase, and immediately transfer 0.5 mL of soln to spot plate. Immediately add 2 drops of acetaldehyde test soln, and then, in rapid succession, add 1 drop of Na nitroferricyanide soln (1 in 100) to each spot. Immediately and simultaneously, briefly stir both spots to mix reagents. Any blue color formed within 3 min by bulk drug soln is not visibly more intense than that of DEAH std soln (< 0.5% of secondary amines as DEAH). Note: Failure of DEPH std to form blue color has been shown to be due to decomposed acetaldehyde.

Ref.: Pharm. Forum Sept.–Oct. 1985, p. 791.

CAS-90-84-6 (diethylpropion)
CAS-134-80-5 (diethylpropion HCl)

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(3) The following interim liquid chromatographic method for determination of coumarin anticoagulants dicumarol, phenprocoumon, and warfarin sodium in tablets was adopted first action:

Dicumarol, Phenprocoumon, and Warfarin Sodium in Drug Tablets

Liquid Chromatographic Method

First Action

37.D10 ***Principle***

Coumarin anticoagulants dicumarol, phenprocoumon, and warfarin Na are identified and quant. detd in tablets by reverse phase LC on C_{18} column with tetrahydrofuran–MeOH–H_2O–HOAc mobile phase, and photometric detection at 311 nm.

37.D11 ***Apparatus***

(a) *Liquid chromatograph.*—Equipped with Du Pont Model 8800 solv. pump, variable wavelength detector, and strip chart recorder (E.I. du Pont de Nemours & Co.), or equiv.; and Rheodyne Model 7125 injection valve with 20 μL sample loop (Rheodyne Inc., PO Box 996, Cotati, CA 94928), or equiv. Operating conditions: column temp. ambient; solv. flow rate 1.5 mL/min; detector wavelength 311 nm; detector attenuation 16 AUFS; recorder range 1 mV; chart speed 0.5 cm/min.

(b) *Chromatographic column.*—Stainless steel. 30 cm × 3.9 mm id, packed with 10 μm μBondapak C_{18} (Waters Associates, Inc.), or equiv.

37.D12 ***Reagents***

(a) *Solvents.*—LC grade MeOH and reagent grade glacial HOAc (Fisher Scientific Co.); tetrahydrofuran (Mallinckrodt, Inc.); and H_2O double distd in glass.

(b) *Mobile phase.*—Tetrahydrofuran–MeOH–H_2O–HOAc (35 + 10 + 65 + 0.1 v/v/v/v). Filter thru 0.45 μm membrane and degas under vac.

(c) *Dicumarol std soln.*—0.05 mg/mL. Accurately weigh ca 25 mg USP Ref. Std Dicumarol into 100 mL vol. flask, dissolve in and dil. to vol. with 0.01N NaOH, and mix. Pipet 5 mL of this soln into 25 mL vol. flask, dil. to vol. with mobile phase, and mix.

(d) *Warfarin Na std soln.*—0.2 mg/mL. Accurately weigh ca 10 mg USP Ref. Std Warfarin Na into 50 mL vol. flask, and dissolve in mobile phase. Dil. to vol. with mobile phase, and mix.

(e) *Phenprocoumon std soln.*—0.12 mg/mL. Accurately weigh ca 3 mg USP Ref. Std Phenprocoumon into 25 mL vol. flask, and dissolve in mobile phase. Dil. to vol. with mobile phase, and mix.

37.D13 ***Preparation of Sample***

(a) *Tablet composites.*—Weigh and finely powder ≥20 tablets. Transfer accurately weighed amt of powder to suitable vol. flask and quant. dissolve in mobile phase with aid of ultrasonic bath. Dil. to vol. with mobile phase to prep. soln contg ca 0.12 mg/mL of phenprocoumon or 0.2 mg/mL of warfarin Na. For dicumarol samples, first dissolve powder in 0.01N NaOH with aid of ultrasonic bath to obtain soln contg 0.25 mg/mL; then quant. dil. 5.0 mL aliquot of soln with mobile phase to final dicumarol concn of ca 0.05 mg/mL. Filter all sample prepns prior to injection into LC system.

(b) *Single tablets.*—Place 1 powdered tablet in suitable vol. flask, and proceed as described for tablet composites.

37.D14 ***Determination***

Equilibrate system with mobile phase at 1.5 mL/min until baseline is steady. Use sampling valve to inject measured vol. of std soln into LC system. Adjust injection vol. and operating parameters so std soln gives peak ht ca 60% full scale. Under these conditions, 3 replicate injections of a std soln should give RSD ≤3% and tailing factor ≤2.0. Make alternate injections of equal vols of std and sample solns. Measure peak responses in sample and std solns.

37.D15 ***Calculations***

Calc. amt coumarin anticoagulant in sample as follows:
Tablet composite sample:

$$\text{mg/tablet} = (H/H') \times (W'/D') \times (D/W) \times A$$

Single tablet sample:

$$\text{mg/tablet} = (H/H') \times (W'D') \times D$$

where H and H' = peak responses of sample and std solns, resp.; W and W' = mg sample and std taken, resp.; D and D' = diln factors for sample and std solns, resp.; and A = av. tablet wt, mg. To calc. amt warfarin Na in either tablet composites or individual tablets, use 1.071 as multiplier in above equations (1.071 = ratio of MW of warfarin Na/MW of warfarin).

Ref.: JAOAC **70**, 834 (1987).

CAS-66-76-2 (dicumarol)
CAS-435-97-2 (phenprocoumon)
CAS-81-81-2 (warfarin)
CAS-129-06-6 (warfarin Na)

38. DRUGS; ALKALOIDS AND RELATED BASES

No additions, deletions, or other changes.

39. DRUGS: STEROIDS AND HORMONES

(1) The following first action method was adopted final action:

Hydrocortisone in drugs, liquid chromatographic, infrared spectroscopic, and thin layer chromatographic methods, **39.047–39.055.**

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(2) The following methods were declared surplus:

(a) Conjugated estrogens in drugs, final action spectrophotometric method, **39.001–39.005.**

(b) Dexamethasone phosphate in drugs, first action spectrophotometric method, **39.056–39.060.**

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(3) The following interim liquid chromatographic method for determination of cortisone acetate in bulk drug and dosage forms was adopted first action:

Cortisone Acetate in Bulk Drug and Dosage Forms

Liquid Chromatographic Method

First Action

39.D01 ***Principle***

Bulk drug or dosage form is dissolved in CH_3CN–0.025M acetate pH 4 buffer (1 + 1) and analyzed by external std method. Cortisone acetate is resolved from extraneous components by reverse phase liq. chromatgy and detected at 254 nm.

39.D02 ***Apparatus***

(a) *Liquid chromatograph.*—Equipped with isocratic pump system with UV detector (254 nm) and suitable recorder. Operate at ambient temp.

(b) *LC column.*—Reverse phase octadecylsilane, 10 μm.
(c) *Ultrasonic bath.*
(d) *Table top centrifuge.*

39.D03 ***Reagents***

Use LC grade reagents.

(a) *Sodium acetate pH 4 buffer.*—Mix 20 mL 1N HCl, 150 mL 0.5N KCl, and 50 mL 0.5M NaOAc in 1 L vol. flask. Dil. to vol. with H_2O (0.025M and 0.1 μ soln).
(b) *Diluent.*—Mix CH_3CN and pH 4 buffer (1 + 1). Let mixt. equilibrate to room temp.
(c) *Mobile phase.*—Degas mixt. of 450 mL CH_3CN and 550 mL H_2O. Adjust vol. of CH_3CN as needed to obtain suitable retention time.

39.D04 ***Preparation of Standard Solutions***

Accurately weigh ca 30 mg USP Ref. Std and transfer to 100 mL vol. flask. Add diluent, sonicate until std is dissolved, and dil. to vol. with diluent. Further dil. soln 10 mL to 50 mL, 10 mL to 25 mL, and 5 mL to 10 mL to prep. 3 std solns.

39.D05 ***Preparation of Samples***

Bulk drug.—Accurately weigh ca 25 mg bulk drug and transfer to 250 mL vol. flask. Dissolve in and dil. to vol. with diluent. Use an aliquot, concn ca 0.1 mg/mL, for LC analysis.

Tablets.—Weigh and finely powder 10 tablets. Accurately weigh equiv. of ca 2 tablets or amt necessary to prep. soln not to exceed 1.0 mg/mL concn. Transfer sample to vol. flask, dissolve in diluent, and sonicate ca 5 min. Dil. as needed to concn of ca 0.1 mg/mL. Centrf. aliquot of prepd soln, and use portion of supernate for LC analysis. Filter supernate thru 0.45 μm filter before analysis if necessary.

Suspension.—Measure sample vol. of 1 or more vials as follows: Shake vial vigorously until product is homogeneous (but ≥15 s). Remove sample immediately by successive use of clean, dry hypodermic syringes of appropriate size. Deliver samples into same stdzd cylinder graduated to contain. Read vol. Transfer contents of cylinder to vol. flask. Rinse all glassware twice with diluent, and add rinses to vol. flask. Concn of soln should not exceed 1.0 mg/mL. Dil. soln as needed to concn of ca 0.1 mg/mL for LC analysis. Filter soln thru 0.45 μm filter before analysis if necessary.

39.D06 ***System Suitability Tests***

Condition column with mobile phase until baseline is acceptable. Cortisone acetate peak should fulfill following performance specifications: column efficiency, ≥1500 theoretical plates; asymmetry or tailing factor (at 5% peak ht), ≤2; capacity factor, k′, ≥2; relative std deviation <1% for 5 replicate 20 μL injections.

39.D07 ***Determination***

Inject each of 3 std solns before and after all samples. Use peak area to calc. amt of each sample, μg/mL, with respect to stds. Curve-fit samples and calc. results mathematically or by calculator.

39.D08 ***Calculations***

(*1*) Calc. concn, μg/mL, of cortisone acetate in each std soln (C_{std}) as follows:

$$C_{std} = (W_{std}/V_{std}) \times (V_d V_{df}) \times (P/100) \times 1000$$

where W_{std} = wt of std, mg; V_{std} = vol. of std, mL; V_d = vol. of aliquot transferred for diln, mL; V_{df} = vol. of flask used for diln, mL; P = purity of std as %.

(*2*) Use linear regression procedure of PA_{std} vs C_{std} to prep. std curve mathematically or by computer. Correlation coefficient (r) should be ≥0.999 and intercept < ±3.0.

Substitute calcd values for consts m and c, and variable X in following equation to calc. individual Y values:

$$Y = \mathrm{m}X + \mathrm{c}$$

where Y = std concn, μg/mL; and X = av. of peak areas for stds injected before and after samples.

Enter PA_{sam} (av. peak areas for samples = X) on std curve and obtain value for C_{sam} (concn of sample, μg/mL = Y).

(*3*) Calc. cortisone acetate in bulk drug and dosage forms as follows:

Bulk drug:

$$\text{Cortisone acetate, mg} = (C_{sam} \times D)/1000$$

Suspension:

$$\text{Cortisone acetate, mg/mL} = [C_{sam} \times (D/N)]/1000$$

Tablets:

$$\text{Cortisone acetate, mg/tablet} = C_{sam} \times D \times [W_{tab}/(W_{sam} \times 1000)]$$

where D = diln factor; N = vol. for vials sampled, mL; W_{tab} = av. tablet wt, mg; W_{sam} = sample wt, mg.

Ref.: JAOAC **71**, May issue (1988).

CAS-50-04-4 (cortisone acetate)

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(4) The following interim liquid chromatographic method for determination of dexamethasone acetate in bulk drug and suspensions was adopted first action:

Dexamethasone Acetate in Bulk Drug and Suspensions

Liquid Chromatographic Method

First Action

39.D09 ***Principle***

Bulk drug or suspension is dissolved in CH_3CN–0.025M phosphate pH 6 buffer (1 + 1) and analyzed by external std method. Dexamethasone acetate is resolved from extraneous components by reverse phase liq. chromatgy and detected at 254 nm.

39.D10 ***Apparatus***

(a) *Liquid chromatograph*—Equipped with isocratic pump system with UV detector (254 nm) and suitable recorder. Operate at ambient temp.
(b) *Column.*—Reverse phase octadecylsilane, 10 μm.
(c) *Ultrasonic bath.*

39.D11 ***Reagents***

Use LC grade reagents.

(a) *Potassium phosphate pH 6 buffer*—Mix 3 mL 1N NaOH, 138 mL 0.5N KCl, and 50 mL 0.5M KH_2PO_4 in 1 L vol. flask. Dil. vol. with H_2O (0.025M and 0.1 μ soln).
(b) *Diluent.*—Mix CH_3CN and 0.025M phosphate pH 6 buffer (1 + 1). Let mixt. equilibrate to room temp.
(c) *Mobile phase.*—Degas mixt. of 450 mL CH_3CN and 550 mL H_2O. Adjust vol. of CH_3CN as needed to obtain suitable retention time.

39.D12 ***Preparation of Standard Solutions***

Dry USP Ref. Std 2 h in 105° oven. Accurately weigh ca 30 mg dried std and transfer to 100 mL vol. flask. Add diluent, sonicate

until std is dissolved, and dil. to vol. with diluent. Further dil. soln 10 mL to 50 mL, 10 mL to 25 mL, and 5 mL to 10 mL to prep. 3 std solns.

39.D13 *Preparation of Samples*

Bulk drug.—Accurately weigh ca 25 mg bulk drug that has been dried 2 h in 105° oven and transfer to 250 mL vol. flask. Dissolve in and dil. to vol. with diluent. Use an aliquot, concn ca 0.1 mg/mL, for LC analysis.

Suspension.—Measure sample vol. of 1 or more vials as follows: Shake vial vigorously until product is homogeneous (but ≥15 s). Remove sample immediately by successive use of clean, dry hypodermic syringes of appropriate size. Deliver samples into same stdzd cylinder graduated to contain. Read vol. Transfer contents of cylinder to vol. flask. Rinse all glassware twice with diluent, and add rinses to vol. flask. Concn of soln should not exceed 0.8 mg/mL. Dil. soln as needed to concn of ca 0.1 mg/mL. Filter soln thru 0.45 μm filter before LC analysis if necessary.

39.D14 *System Suitability Tests*

Condition column with mobile phase until baseline is acceptable. Dexamethasone acetate peak should fulfill following performance specifications: column efficiency, ≥1500 theoretical plates; asymmetry or tailing factor (at 5% peak height), ≤2; capacity factor, k' ≥2; relative std deviation <1% for 5 replicate 20 μL injections.

39.D15 *Determination*

Inject each of 3 std solns before and after all samples. Use peak area to calc. amt of each sample, μg/mL, with respect to stds. Curve-fit samples and calc. results mathematically or by calculator.

39.D16 *Calculations*

(*1*) Calc. concn, μg/mL, of dexamethasone acetate in each std soln (C_{std}) as follows:

$$C_{std} = (W_{std}/V_{std}) \times (V_d/V_{df}) \times (P/100) \times 1000$$

where W_{std} = wt of std, mg; V_{std} = vol. of std, mL; V_d = vol. of aliquot transferred for diln, mL; V_{df} = vol. of flask used for diln, mL; P = purity of std as %.

(*2*) Use linear regression procedure of PA_{std} vs C_{std} to prep. std curve mathematically or by computer. Correlation coefficient (*r*) should be ≥0.999 and intercept $< \pm 3.0$.

Substitute calcd values for consts m and c, and variable *X* in following equation to calc. individual *Y* values:

$$Y = mX + c$$

where *Y* = std concn, μg/mL; and *X* = av. of peak areas for stds injected before and after samples.

Enter PA_{sam} (av. peak areas for samples = *X*) on std curve and obtain value for C_{sam} (concn of sample, μg/mL = *Y*).

(*3*) Calc. dexamethasone acetate and dexamethasone equiv. in bulk drug and suspension, resp., as follows:

Bulk drug:

$$\text{Dexamethasone acetate, mg} = (C_{sam} \times D)/1000$$

Suspension:

$$\text{Dexamethasone equiv., mg/mL} = [C_{sam} \times (D/N) \times F]/1000$$

where *D* = diln factor; *N* = vol. for vials sampled, mL; *F* = factor to convert acetate to free base = 0.903.

Ref.: JAOAC **71**, May issue (1988).

CAS-55812-90-3 (dexamethasone acetate monohydrate)
CAS-50-02-2 (dexamethasone)

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(5) The following interim quantitative and identification methods for dexamethasone in drug substance and elixirs were adopted first action:

Dexamethasone in Drug Substance and Elixirs

Quantitative and Identification Methods

First Action

39.D17 *Principle*

Dexamethasone content in drug substance and elixir is detd by normal phase LC using quaternary mobile phase with controlled H_2O content, UV detection at 254 nm, and cortisone as internal std. Identity is confirmed in bulk drug substance and elixir by TLC and in drug substance by IR spectroscopy and relative LC retention time ratios. Alcohol content in elixir is detd by GC on porous polymer column using internal std and flame ionization detector.

Liquid Chromatographic Method

39.D18 *Apparatus*

(**a**) *Liquid chromatograph.*—Model 8100 (Spectra-Physics, 3333 N First St, San Jose, CA 95134-1995) equipped with Model 100-10 photometric detector (Hitachi/NSA, 460 E Middlefield Rd, Mountain View, CA 94043), 15–30 μL injection valve (Valco Instruments Co., Inc., PO Box 55603, Houston, TX 77255), and Model CR1A integrator (Shimadzu Scientific Instruments, Inc., 7102 Riverwood Rd, Columbia, MD 21046). Equiv. LC system, UV detector, autosampler, and strip chart recorder may be used. LC pumping system in which bubbles develop in mobile phase is unsuitable. 1 μg dexamethasone should produce 50% full scale response with appropriate detector and recorder or integration settings at 254 nm. Mobile phase flow rate 1.2 mL/min at ambient temp.

(**b**) *Chromatographic column.*—Stainless steel, 25 cm × 4.6 mm id, packed with 5 μm Zorbax-Sil (E.I. du Pont de Nemours and Co.), or equiv. meeting appropriate LC system suitability requirements. Stainless steel guard column, 3 cm × 4.6 mm id, packed with 10 μm silica particles may be used. If necessary, dry silica column by eluting with 20 mL CH_2Cl_2–HOAc–2,2-dimethoxypropane (90 + 2 + 2 v/v/v).

39.D19 *Reagents*

(**a**) *Solvents.*—Glacial HOAc (J.T. Baker or equiv.), UV grade MeOH and CH_2Cl_2 (Burdick and Jackson Laboratories, Inc., or equiv.), and distd-in-glass H_2O.

(**b**) *Methanol soln.*—Pipet 5.0 mL H_2O into 100 mL vol. flask and dil. to vol. with MeOH.

(**c**) *Mobile phase.*—Pipet 1.0 mL glacial HOAc and 45.0 mL MeOH soln into 1 L vol. flask, and dil. to vol. with CH_2Cl_2. Degas mixt. Adjust MeOH content to obtain retention times of approx. 6 and 9 min for cortisone and dexamethasone, resp. Cortisone retention time should be used for mobile phase composition adjustments; increased MeOH content decreases retention time.

(**d**) *Sodium bicarbonate soln.*—1M. Dissolve 8.4 g $NaHCO_3$ in 100 mL H_2O.

(**e**) *Internal std soln.*—Dissolve 30 mg cortisone (Sigma Chemical Co., or equiv.) in 4.0 mL MeOH and dil. to 100.0 mL with CH_2Cl_2.

(**f**) *Dexamethasone std soln.*—4.0 mg/100 mL. Transfer ca 25 mg accurately weighed USP Ref. Std Dexamethasone (previously dried 30 min at 105°) to 25 mL vol. flask, and dissolve in and dil. to vol. with MeOH. Transfer 2.0 mL aliquot of this soln to 50 mL vol. flask contg 6.0 mL internal std soln, and dil. to vol. with CH_2Cl_2. Do not filter thru membrane filter.

39.D20 ***Sample Preparation***

(**a**) *Drug substance*.—Prep. as directed for *Dexamethasone std soln*, using 25 mg dexamethasone. Do not filter thru membrane filter.

(**b**) *Elixir*.—Transfer accurately measured 10 mL portion of Dexamethasone Elixir, contg 1 mg dexamethasone, to 125 mL separatory funnel, add 5 mL 1M $NaHCO_3$ soln, and ext with four 20 mL portions of CH_2Cl_2. Collect exts in 250 mL separatory funnel contg 5 mL H_2O. Back-wash combined exts and filter thru cotton wet with CH_2Cl_2 into suitable beaker. Rinse H_2O back-wash and 125 mL separatory funnel consecutively with 10 mL CH_2Cl_2. Filter this rinse into beaker. Evap. filtrate on steam bath under jet of air to approx. 10 mL and quant. transfer with CH_2Cl_2 to 25 mL vol. flask contg 1.0 mL MeOH and 3.0 mL internal std soln. Dil. to vol. with CH_2Cl_2. Do not filter thru membrane filter.

39.D21 ***Determination***

Equilibrate column with mobile phase at 1.2 mL/min. Monitor response at 254 nm. Make 3 replicate injections of dexamethasone std soln. Using either peak area or peak ht measurements for each injection, calc. coefficient of variation (CV) of peak response ratios of dexamethasone to internal std. In suitable system, CV should be $\leq 2.5\%$ and resolution factor, R_s, for dexamethasone peak and internal std peak should be ≥ 3. Make duplicate injections of std and sample solns and det. response ratio for each. Relative retention ratios of dexamethasone to internal std should agree within $\pm 2.0\%$. If relative retention ratios differ by $> 2.0\%$, then dry silica column as described in *Apparatus* (**b**).

39.D22 ***Calculations***

Calc. content of dexamethasone as follows:

Drug substance:

$$\text{Dexamethasone, mg} = 625 \times C \times (RR/RR')$$

Elixirs:

$$\text{Dexamethasone, mg/5 mL} = 12.5 \times C \times (RR/RR')$$

where C = final concn of std soln (mg/mL), and RR and RR' = av. response ratio for peak ht or area of analyte to that of internal std for sample and std solns, resp.

Thin Layer Chromatographic Identification

39.D23 ***Apparatus, Reagents, and Test Solutions***

(**a**) *Thin layer plates*.—Glass, 20 × 20 cm, coated with 250 μm layer of silica gel G with fluorescent indicator (Analtech Cat. No. 02011 [Analtech Inc., 75 Blue Hen Dr, PO Box 7557, Newark, DE 19711], or equiv.).

(**b**) *Developing solns*.—(*1*) *Drug substance*.—$CHCl_3$–diethylamine (2 + 1). (*2*) *Elixir*.—$CHCl_3$–acetone–glacial HOAc (80 + 40 + 1).

(**c**) *TLC std test solns*.—(*1*) *Drug substance*.—Prep. 1 mg/mL soln of USP Ref. Std Dexamethasone in CH_2Cl_2–MeOH (1 + 1). (*2*) *Elixir*.—Evap. 10 mL of *Dexamethasone std soln* (**f**) just to dryness on steam bath. Dissolve residue in 1 mL CH_2Cl_2–MeOH (1 + 1). Prep. individual 400 μg solns of dexamethasone (Sigma Chemical Co., or equiv.) and cortisone in CH_2Cl_2–MeOH (1 + 1) to serve as chromatgc identification stds.

(**d**) *TLC sample test solns*.—(*1*) *Drug substance*.—Prep. as directed for *TLC std test soln*. (*2*) *Elixir*.—Evap. 10 mL of elixir sample prepn (**b**) just to dryness on steam bath. Dissolve residue in 1 mL CH_2Cl_2–MeOH (1 + 1).

39.D24 ***Chromatography***

Equilibrate suitable chromatgc tank with appropriate developing solv. Spot 5 μL of each test soln ca 2.5 cm from bottom of coated plate. Let spots dry and develop chromatogram until solv. front has moved 10 cm from origin. Remove plate, mark solv. front, air-dry plate, and locate spots under shortwave UV light. For drug substance, R_f of major spot in sample test soln corresponds to that for std test soln. For elixir, relative R_f of dexamethasone to cortisone for TLC sample test soln corresponds to that for TLC std test soln.

39.D25 ***Infrared Spectroscopic Identification***

Drug substance.—Prep. KBr dispersions from previously dried sample and std material. Scan spectra between 2.5 and 15.0 μm. Compare sample and std spectra. If difference appears, dissolve portions of both sample and std in CH_3CN, evap. solns to dryness, and repeat test on residues. Sample and std prepns exhibit maxima at same wavelength.

39.D26 ***Identification by Relative Retention Times***

Drug substance.—Compare retention ratios of main peak to internal std peak obtained for dexamethasone std soln and for assay sample prepn as directed in LC assay. Ratios that do not differ by $>2.0\%$ confirm identity.

Alcohol in Elixir

Gas Chromatographic Method

39.D27 ***Apparatus and Reagents***

(**a**) *Gas chromatograph*.—Model 5830A, with flame ionization detector and electronic integrator (Hewlett-Packard), or equiv. Operating conditions: column temp. 165° and N gas flow adjusted so that 2-propanol elutes in 3–5 min.

(**b**) *Chromatographic column*.—Glass, 6 ft × 4 mm id, packed with 80–100 mesh copolymer of ethylvinylbenzene and divinylbenzene that has nominal surface area of 500–600 sq m/g and av. pore diam. of 0.0075 μm. This material has been washed with org. solvs and acids and then preconditioned in bulk in O-free atm. Super-Q (Alltech Associates Cat. No. 2735 [Applied Science Labs, 2051 Waukegan Rd, Deerfield, IL 60015]) has been found to be suitable.

(**c**) *Internal std soln*.—Dil. 5.0 mL 2-propanol with H_2O to 250 mL.

(**d**) *Alcohol std soln*.—Dil. 5.0 mL absolute alcohol with H_2O to 250 mL. Pipet 10 mL of this soln and 10 mL internal std soln into 100 mL vol. flask and dil. to vol. with H_2O.

39.D28 ***Preparation of GC Column***

With small plug of silanized glass wool in end of column, apply vac. to exit of column and add packing in small amts to inlet end. With aid of gentle vibration, pack column firmly. Condition column overnight at 235° with slow N flow. Check column for voids after conditioning. Gently vibrate column to remove voids. Check column performance by injecting alcohol std soln and calcg following: resolution ≥ 3; RSD $< 1.5\%$ for alcohol peak area relative to 2-propanol peak area with 6 replicate injections; and tailing factor ≤ 2.0 for alcohol.

39.D29 ***Sample Preparation***

Pipet 4 mL elixir and 10 mL std soln into 100 mL vol. flask and dil. to vol. with H_2O.

39.D30 ***Determination and Calculation***

Inject ca 5 μL each of sample and std solns in duplicate. Calc. % alcohol in elixir as follows:

$$\%\ \text{alcohol (v/v)} = (RR/RR') \times C \times D$$

where RR and RR' = av. response ratio for peak area of analyte

to that of internal std for sample and std, resp.; C = % alcohol in std soln; and D = sample diln factor.

Ref.: JAOAC **70**, 967(1987).

CAS-50-02-2 (dexamethasone)

40. DRUGS: ILLICIT

(1) The following first action method was adopted final action:

Diazepam in drug tablets, liquid chromatographic method, **40.A01–40.A06**.

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(2) The following interim liquid chromatographic method for determination of enantiomers of amphetamine was adopted first action:

Enantiomers of Amphetamine
Liquid Chromatographic Method
First Action

(Applicable to bulk drug, and syrup and capsule dosage forms)

40.D01 *Principle*

Samples are dissolved in CH_2Cl_2, and 2-naphthoyl amide derivatives are formed by adding 2-naphthoyl chloride. Isomers are detd by liq. chromatgy on chiral stationary phase LC column, with hexane–isopropyl alcohol–CH_3CN (97 + 3 + 0.5) mobile phase and detection at 254 nm.

40.D02 *Apparatus*

(**a**) *Liquid chromatograph.*—Spectra-Physics Model 8000 equipped with Valco 7000 psi injection valve with 10 μL injection loop, temp.-controlled oven or column H_2O jacket, and printer plotter. Equiv. app may be used.

(**b**) *Detector.*—Spectra-Physics Model 770 UV-vis, or equiv., set at 254 nm, 0.04 AUFS, and time constant at 4 s.

(**c**) *Data integration system.*—Set peak width to peak threshold ratio at 1:60 for Spectra-Physics Model 8000 or at appropriate settings for equiv. chromatgc data system.

(**d**) *LC column.*—Pirkle covalent D-phenyl glycine analytical column (J.T. Baker Chemical Co. (Bakerbond Chiral Phase [DNBPG] column) or Regis Chemical Co., 8210 N Austin Ave, Morton Grove, IL 60053).

40.D03 *Reagents*

(**a**) *Solvents.*—Use UV quality, LC grade H_2O, hexane, isopropyl alcohol, CH_3CN, and CH_2Cl_2.

(**b**) *LC mobile phase.*—Hexane–isopropyl alcohol–CH_3CN (97 + 3 + 0.5 v/v/v).

(**c**) *2-Naphthoyl chloride.*—98% (Aldrich Chemical Co.).

(**d**) *Reagent solns.*—20% NaOH soln; 0.01M soln of 2-naphthoyl chloride in CH_2Cl_2; 0.01M H_2SO_4 soln.

40.D04 *Preparation of LC Standard and Sample Solutions*

(**a**) *Standard.*—Dissolve 10 mg USP Ref. Std in 5 mL CH_2Cl_2, and add 5 mL 20% NaOH soln. Continue with (**e**).

(**b**) *Bulk drug.*—Dissolve 10 mg bulk drug in 5 mL CH_2Cl_2, and add 5 mL 20% NaOH soln. Continue with (**e**).

(**c**) *Syrup.*—Mix 10 mL syrup with 5 mL CH_2Cl_2, and add 5 mL 20% NaOH soln. Continue with (**e**).

(**d**) *Capsules.*—Place 1 capsule in 5 mL 20% NaOH soln, ultrasonicate 45 min or until dissolved. Filter resulting soln. Add 5 mL CH_2Cl_2 to filtrate. Continue with (**e**).

(**e**) *Working solns.*—Transfer soln from (**a**), (**b**), (**c**), or (**d**) to 30 mL separatory funnel. Add 10 mL 0.01M soln of 2-naphthoyl chloride in CH_2Cl_2, and shake mixt. 1 min. Transfer org. phase to another 30 mL separatory funnel. Wash aq. phase with 5 mL CH_2Cl_2, and combine org. layers. Wash combined org. layers with 5 mL 0.01M H_2SO_4, and filter washed org. layer thru syringe (10 mL plastic syringe with plunger removed) contg glass cotton plug and anhyd. Na_2SO_4. Discard aq. layers.

40.D05 *Liquid Chromatography*

(**a**) *Column preparation.*—Equilibrate overnight chiral stationary phase LC column with mobile phase at flow rate of 0.25 mL/min with temp. controlled at 20 ± 1°. Circulate solv. during analysis without interruption. Use flow rate of 2 mL/min during analysis.

(**b**) *System suitability test.*—After derivatization, inject 10 μL aliquots of 50:50 mixt. of *d:l*-amphetamine (system suitability std; available from Sigma Chemical Co.) into chromatgc column. Fig. **40:D1** shows LC resolution of this mixt. Repeat analysis in triplicate. Calc. mean and coefficient of variation as follows:

$$\text{mean}, \bar{X} = (X_1 + X_2 + \ldots X_n)/n$$

$$\text{Coeff. of var., \%} = (\text{std dev./mean}) \times 100$$

Efficiency is optimum when resolution is ≥1.2 and max. relative std dev. is ≤2.0; however, suitable results can be obtained with resolution as low as 0.8.

(**c**) *Procedure.*—Inject 10 μL aliquots of working solns of std, bulk drug, syrup, or capsules. Make each injection in duplicate.

40.D06 *Calculations*

Calc. % area of *l*-amphetamine (percent *l*-amphetamine) and *d*-amphetamine (percent *d*-amphetamine) in std and samples as follows:

$$l\text{-Amphetamine, \%} = [PA_l/(PA_l + PA_d)] \times 100$$

$$d\text{-Amphetamine, \%} = [PA_d/(PA_l + PA_d)] \times 100$$

where PA_l and PA_d = peak areas for *l*- and d-amphetamines, resp.

Ref.: JAOAC **71**, May issue (1988).

CAS-156-34-3 (*l*-amphetamine)
CAS-51-64-9 (*d*-amphetamine)

41. DRUGS AND FEED ADDITIVES IN ANIMAL TISSUES

No additions, deletions, or other changes.

42. DRUGS IN FEEDS

(1) The following first action methods were adopted final action:

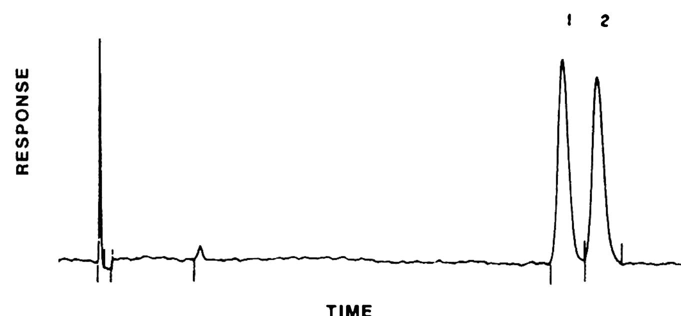

FIG 40:D1—Chromatogram of system suitability standard composed of 50:50 mixture of *d:l*-amphetamine: 1, *l*-amphetamine; 2, *d*-amphetamine.

(a) Dimetridazole in feeds, spectrophotometric method, **42.063–42.068**, including an editorial change to correct the dilution factors in **42.068**(a): Change the factors to read: "where diln factor = 66.67 for feeds contg 0.015%; 250.0, 0.06%; and 333.3, 0.1%."

(b) Furazolidone and zoalene in feeds, qualitative tests, **42.077–42.080**.

(c) Melengestrol acetate in feed supplements, gas chromatographic method, **42.088–42.096**.

(d) Sulfaquinoxaline in feeds, spectrophotometric method—method I, **42.179–42.183**.

(e) Furazolidone in feeds, liquid chromatographic method, **42.A01–42.A06**.

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(2) The following first action methods were declared surplus:

(a) Arprinocid in feeds, liquid chromatographic method, **42.021–42.026**.

(b) Arprinocid in premixes, spectrophotometric method, **42.027–42.032**.

(c) Diethylstilbestrol in feeds, spectrophotometric method, **42.059–42.062**.

43. VITAMINS AND OTHER NUTRIENTS

(1) The following first action methods were adopted final action:

(a) Nutrients in ready-to-feed milk-based infant formula, **43.A21–43.A40**: Compositing of sample, **43.A21**; sampling liquids for analysis, **43.A22**; riboflavin, **43.A23**; vitamin B_6 (pyridoxine, pyridoxal, pyridoxamine), **43.A24–43.A27**; vitamin C (reduced ascorbic acid), **43.A28–43.A31**; niacin and niacinamide (nicotinic acid and nicotinamide), **43.A32–43.A36**; minerals, **43.A37–43.A40**.

(b) Nutrients in ready-to-feed milk-based infant formula, **43.B01–43.B39**: Proximate analysis, **43.B01–43.B05**; chloride, **43.B06–43.B10**; cobalamin (vitamin B_{12} activity), **43.B11–43.B22**; phosphorus, **43.B23–43.B28**; thiamine (vitamin B_1), **43.B29–43.B39**.

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(2) The final action ion exchange chromatographic method for determination of sulfur amino acids in food and feed ingredients, **43.A08–43.A13**, was extended first action to include processed foods.

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(3) The following interim Food Chemicals Codex–U.S. Pharmacopeia–AOAC gas chromatographic method for mixed tocopherols concentrate was adopted first action:

Tocopherol Isomers in Mixed Tocopherols Concentrate
Gas Chromatographic Method
First Action
Food Chemicals Codex–U.S. Pharmacopeia–AOAC Method

43.D01 ***Principle***

Sample is refluxed with internal std soln of hexadecyl hexadecanoate in pyridine–propionic anhydride (2 + 1). Isomers are detd by gas chromatgy with flame ionization detection.

43.D02 ***Apparatus***

Use low-actinic glassware for all solns contg tocopherols.

(a) *Gas chromatograph*.—With flame ionization detector and glass-lined sample-introduction system or on-column injection. Operating conditions: column, isothermal between 240 and 260°; injection port, ca 290°; detector block, ca 300°. Adjust carrier gas flow rate to obtain hexadecyl hexadecanoate peak ca 18–20 min after sample introduction when 2% column is used, or 30–32 min for 5% column

(b) *Column*.—2 m × 4 mm borosilicate glass packed with 2–5% methylpolysiloxane gum on 80–100 mesh acid-base washed, silanized chromatgc diat. earth. *Note:* Cure and condition column as necessary; typically, condition 1 h at ca 300° with no carrier gas flow. Let column cool. Connect carrier gas to column, start flow, and maintain 4 h. Connect column to detector and perform system suitability check.

(c) *Flasks*.—Erlenmeyer, 50 mL, 19/38 ground-glass neck (Kimble 26510, or equiv.).

43.D03 ***Reagents***

(a) *Internal std soln*.—Accurately weigh ca 600 mg hexadecyl hexadecanoate (> 99%; Analabs No. LMS-067, or equiv.) and transfer to 200 mL vol. flask. Dissolve in and dil. to vol. with pyridine–propionic anhydride (2 + 1), and mix.

(b) *Tocopherol std solns*.—Accurately weigh 12, 25, 37, and 50 mg portions of USP Alpha Tocopherol Ref. Std and transfer to sep. 50 mL erlenmeyers. Pipet 25.0 mL internal std soln into each flask, mix, and reflux 10 min under H_2O-cooled condensers.

(c) *Carrier gas*.—N, dry.

43.D04 ***Assay Preparation***

Accurately weigh ca 60 mg sample and transfer to 50 mL erlenmeyer. Pipet 10.0 mL internal std soln into flask, mix, and reflux 10 min under H_2O-cooled condenser.

43.D05 ***System Suitability Test***

Chromatograph suitable number of injections of assay prepn, as directed in *Calibration*. Resolution factor, R, between major peaks occurring at retention times of ca 0.50 (delta-tocopheryl propionate) and 0.63 (beta- plus gamma-tocopheryl propionates), relative to hexadecyl hexadecanoate at 1.00, should be ≥2.5. Calc. R as follows:

$$R = [2(t_2 - t_1)]/(w_1 + w_2)$$

where t_2 and t_1 = retention times of 2 components, and w_2 and w_1 = corresponding widths of bases of peaks, obtained by extrapolating relatively straight sides of peaks to baseline.

43.D06 ***Calibration***

Chromatograph successive 2–5 µL portions of each tocopherol std soln until relative response factor, F, for each is const, i.e., within range of ca 2% for 3 consecutive injections. If graphic integration is used, adjust instrument to obtain ≥70% max. recorder response for hexadecyl hexadecanoate peak. Measure areas under first (alpha-tocopheryl propionate) and second (hexadecyl hexadecanoate) major peaks (excluding solv. peak), and record values as PA_s and PA_1, resp. Calc. factor, F, for each concn of tocopherol std soln as follows:

$$F = (PA_s/PA_1) \times (C_1 \times C_s)$$

where C_1 and C_s = exact concns, mg/mL, of hexadecyl hexadecanoate and USP Alpha Tocopherol Ref. Std in tocopherol std soln. Prep. curve by plotting peak area for alpha-tocopheryl propionate vs relative response factor.

43.D07 ***Determination***

Inject 2–5 μL assay prepn into chromatograph and record chromatogram. Measure areas under 4 major peaks occurring at relative retention times of 0.50, 0.63, and 0.76, and 1.00, and record values as PA_δ, $PA_{\beta+\gamma}$, PA_α, and PA_1, corresponding to delta-tocopheryl propionate, beta- plus gamma-tocopheryl propionates, alpha-tocopheryl propionate, and hexadecyl hexadecanoate, resp. Calc. wt, mg, of each tocopherol form as follows:

$$\text{delta-Tocopherol, mg} = (10C_1/F) \times (PA_\delta/PA_1)$$
$$\text{beta- plus gamma-Tocopherol, mg} = (10C_1/F) \times (PA_{\beta+\gamma} + /PA_1)$$
$$\text{alpha-Tocopherol, mg} = (10C_1/F) \times (PA_\alpha/PA_1)$$

where F = value from relative response factor curve (see *Calibration*) for each of corresponding peaks produced by assay prepn. *Note:* Relative response factor for delta-tocopheryl propionate and for beta- plus gamma-tocopheryl propionates has been detd empirically to be same as for alpha-tocopheryl propionate.

Ref.: Pharm. Forum (Jan.–Feb. 1987) p. 2155.

CAS-59-02-5 (α-tocopherol)
CAS-148-03-8 (β-tocopherol)
CAS-7616-22-0 (γ-tocopherol)
CAS-119-13-1 (δ-tocopherol)

(4) The interim ion exchange chromatographic method for determination of tryptophan in foods and food and feed ingredients was adopted first action:

Tryptophan in Foods and Food and Feed Ingredients
Ion Exchange Chromatographic Method
First Action

43.D08 ***Principle***

Protein is hydrolyzed under vac. with 4.2N NaOH. After pH adjustment and clarification, tryptophan is sepd by ion exchange chromatgy with measurement of ninhydrin chromophore or by reverse phase liq. chromatgy with UV detection.

43.D09 ***Apparatus***

(a) *Amino acid analyzer.*—Dionex D-500, or equiv., operated in accordance with manufacturer's instructions. Column, 50 cm × 1.75 mm id stainless steel, packed with DC5A cation exchange resin (Dionex Corp., PO Box 3603, Sunnyvale, CA 94088). Operating conditions: flow rate 8 mL/h. Column temp.: hold at 59° for 0 to 50:00 min, then increase to 65° for 50:00 to 90:00 min. Pump regenerant 0.2N NaOH for 0 to 5:35 min. Elution buffers: Na citrate elution buffer 1, pH 4.25, 0.2N, 5:35 to 50:00 min; then Na citrate elution buffer 2, pH 5.3, 0.14N with 4% 2-propanol, 50:00 to 90:00 min.

(b) *Modified micro-Kjeldahl flasks.*—25 mL micro-Kjeldahl flasks fitted with 12 mm id × 15 cm long neck. Constrict neck to ca 6 mm id 5 cm above bulb of flask (available as special order from Ace Glass, Inc.). These modifications can be made easily by experienced glass blower.

(c) *LC system.*—Waters 6000 instrument, or equiv., operated according to manufacturer's instructions. Column: 25 cm × 4.6 mm id stainless steel μBondapak C_{18} (Waters Chromatography Div., Millipore, Milford, MA 01757). Operating conditions: flow rate 1.5 mL/min; mobile phase 0.0085M NaOAc (adjusted to pH 4.0 with HOAc)–MeOH (95 + 5); UV at 280 nm, 0.01 AUFS.

(d) *Vacuum pump.*—Capable of 10 μm (Welch Duo-Seal Model No. 1400 equipped with diffusion pump head, or equiv.).

(e) *Membrane filter.*—0.45 μm.

43.D10 ***Reagents***

(a) *Water.*—Purified by Milli-Q system (Millipore Corp.), or equiv. Use thruout method.

(b) *Tryptophan std soln.*—*(1) Stock soln:* 1 mg/mL. Dissolve 250 mg L-tryptophan (Calbiochem, PO Box 12087, San Diego, CA 92112-4180) in 100 mL H_2O with 6 drops HCl. Add 3 drops "pHix" buffer preservative (Pierce Chemical Co.), and dil. to 250 mL with H_2O. *(2) Working soln I:* 0.1 mg/mL. Dil. 10 mL stock soln to 100 mL with H_2O. *(3) Working soln II:* 0.04 mg/mL. Dil. 4 mL stock soln to 100 mL with H_2O. Refrigerate std solns when not in use. Prep. solns fresh monthly.

(c) *Na citrate loading buffer.*—pH 4.25, 0.2N (Pierce Chemical Co.). Filter twice thru 0.45 μm filter.

(d) *Na citrate eluting buffer.*—pH 5.3, 0.14N, 4% 2-propanol. Dil. 262 mL Beckman pH 4.95 buffer (Beckman Instruments, Inc.) and 40 mL 2-propanol to 1 L with H_2O. Filter twice thru 0.45 μm filter.

(e) *Regenerant soln.*—0.2N NaOH with EDTA. Dissolve 32 g NaOH and 3 g Na_2EDTA in H_2O and dil. to 4 L with H_2O. Filter twice thru 0.45 μm filter.

43.D11 ***Preparation of Samples***

Grind test sample in centrifugal mill fitted with 1 mm screen, and mix thoroly. Weigh test portion contg 100 mg protein (≤300 mg) into modified micro-Kjeldahl flask.

(a) *Test samples with >5% lipids.*—Add 10 mL pet ether to weighed sample in flask. Swirl gently to mix and sonicate 20 min. Let settle. Centrf. if necessary. Siphon off as much pet ether as possible, taking care not to remove any solids. Evap. remaining pet ether under gentle stream of N. Continue as under *Preparation of Hydrolysates.*

(b) *Test samples with <5% lipids.*—Continue as under *Preparation of Hydrolysates.*

43.D12 ***Preparation of Hydrolysates***

Deaerate 4.2N NaOH by bubbling with N for 10 min. Add 10 mL deaerated 4.2N NaOH to each flask. Add 3 drops 1-octanol. Immediately freeze treated soln in dry ice–EtOH bath. Then remove flask from bath and evacuate to 10 μm. Turn off vac. and seal neck of flask at constriction with dual head O/natural gas flame. Place sealed flask in beaker contg H_2O at room temp. until sample soln is melted. Place flask in 110° oven for 20 h.

Let flasks cool to room temp. Etch neck of each flask with glass knife and break seal by touching white hot glass rod to etch mark. Tap neck of flask to break off tip into clean 50 mL beaker. Rinse neck of flask with 1 mL pH 4.25 Na citrate buffer soln, collecting rinse in beaker. Quant. transfer hydrolysate to same 50 mL beaker, rinsing flask with two 2 mL portions of pH 4.25 Na citrate buffer soln. Neutze soln with 3.5 mL HCl, and stir vigorously. Adjust pH to 4.25 ± 0.05.

Quant. transfer soln to 25 mL vol. flask, and dil. to vol. with H_2O. Pour into 40 mL centrf. tube and centrf. 20 min at 1150 $\times g$. Filter supernate thru glass fiber paper (Whatman GF/A). Transfer filtrate to centrf. tube and centrf. 10 min at 23 000 $\times g$.

43.D13 ***Determination***

Load 40 μL test or std soln into amino acid analyzer. Operate system according to manufacturer's instructions. Inject stds at beginning and end of run and after every 5 or 6 test solns.

For LC sepn, inject 15 μL test or std soln. Measure A at 280 nm.

43.D14 ***Calculations***

Measure peak areas for tryptophan in test samples and stds. Calc. % tryptophan as follows:

$$\text{Tryptophan, g/100 g} = [(PA/PA') \times C \times (V/W)] \times 100$$

where PA and PA' = peak areas of sample and std, resp.; C = g std/mL; V = vol. of final diln, mL; W = wt sample, g.

Ref.: JAOAC **71**, May issue (1988).

CAS-73-22-3 (L-tryptophan)

44. EXTRANEOUS MATERIALS: ISOLATION

(1) The following first action methods were adopted final action:

Filth in cocoa, chocolate, and press cake, flotation method, **44.006–44.007**.

Filth in ground coffee and coffee substitutes, sedimentation and flotation method, **44.008**.

Aphids in hops, AOAC–ASBC flotation method, **44.009–44.011**.

Light filth in tea, flotation method, **44.014–44.016**.

Sediment in dairy products, sediment test method, **44.021**.

Filth in shelled nuts, **44.028–44.029**.

Filth in pecans, **44.030–44.031**.

Filth in shredded coconut, **44.032**.

Filth and extraneous material in peanut butter, sedimentation/flotation methods, **44.033–44.038**.

Insect infestation (internal) of wheat, cracking flotation method, **44.040–44.042**.

Light filth (pre- and post-milling) in white flour, flotation method, **44.052**.

Insect excreta in flour, **44.054**.

Light filth in rice flours (powders), extruded rice products, and rice paper, flotation method, **44.055–44.057**.

Light filth in soy flour, flotation method, **44.060**.

Light filth in wheat gluten, flotation method, **44.061**.

Light filth in starch, sieving method, **44.062**.

Light filth in white breads and high-fat products, flotation method, **44.067**.

Light filth in breading of frozen food products, flotation method, **44.068**.

Light filth in alimentary pastes, flotation method, **44.069**.

Light filth in corn and rice cereals and corn chip products, flotation method, **44.070**.

Light filth in whole wheat cereals, flotation method, **44.071**.

Light filth in barley, oatmeal, and mixed dry infant cereal, flotation method, **44.072–44.073**.

Light filth in canned crabmeat, flotation method, **44.080**.

Light filth in fish (canned) and fish products, flotation method, **44.082**.

Light filth in canned shrimp, flotation method, **44.083**.

Light filth in pork sausage (uncooked) and ground beef or hamburger, enzyme digestion method, **44.084–44.085**.

Filth in apple butter, flotation method, **44.086**.

Thrips and other insects in frozen blackberries and frozen raspberries, flotation method, **44.089–44.090**.

Filth in jam and jelly, **44.094**.

Filth in citrus and pineapple juices (canned), **44.095–44.096**.

Light filth in raisins, microscopic examination method, **44.097**.

Filth in potato chips, flotation method, **44.101**.

Filth in corn chips, flotation method, **44.102**.

Filth in candy, flotation method, **44.103**.

Filth in sirups, molasses, and honey, filtration methods, **44.105**.

Filth in sugars, filtration method, **44.106**.

Foreign matter in canned corn, flotation and macroscopic methods, **44.109**.

Filth in green leafy vegetables, **44.110–44.111**.

Filth in pureed infant food, **44.113–44.114**.

Light filth in dehydrated potato products, flotation method, **44.117**.

Filth in sauerkraut, sieving method, **44.118**.

Filth in tomato products, **44.119–44.120**.

Light filth in spices and condiments, flotation method, **44.125–44.126**.

Filth in ground annatto, **44.129**.

Heavy filth in ground capsicums, sedimentation method, **44.130**.

Light filth in ground capsicums, flotation method, **44.131–44.132**.

Light filth in ground nutmeg, flotation method, **44.140–44.141**.

Light filth in reconditioned nutmeg, flotation method, **44.142**.

Filth in paprika, **44.145–44.147**.

Light filth in pepper, **44.148–44.149**.

Filth in prepared horseradish, flotation method, **44.155**.

Light filth in prepared mustard, flotation method, **44.156**.

Light filth in gums (plant, crude), flotation method, **44.158**.

Light filth in crude and refined papain, flotation method, **44.161**.

Insect penetration through packaging materials, microscopic examination method, **44.162**.

Mold in fruit nectars, purees, and pastes, Howard mold count, **44.201–44.203**.

Mold in dehydrated tomato powder, Howard mold count, **44.211**.

Mold in soft drinks, *Geotrichum* mold count, **44.217**.

Mold in citrus juices, *Geotrichum* mold count, **44.218**.

Mold in vegetables, fruits and juices (canned), *Geotrichum* mold count, **44.219**.

Mold in comminuted fruits and vegetables, *Geotrichum* mold count, **44.220–44.222**.

Mold in cream style corn, *Geotrichum* mold count, **44.223**.

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(2) The first action flotation method for light filth in whole peppermint leaves, **44.A06–44.A08**, was adopted first action for light filth in whole leaves of alfalfa, papaya, and spearmint.

Ref.: JAOAC **70**, 997 (1987).

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(3) The following interim thin layer chromatographic method for detection of coprostanol as an indicator of mammalian feces was adopted first action:

Mammalian Feces

Thin Layer Chromatographic Method for Coprostanol

First Action

(Applicability includes identification of feces in heat-processed materials.)

44.D01 ***Principle***

Suspected fecal material is extd with hexane. Coprostanol, characteristic sterol of mammalian feces, is resolved from other sterols in ext by TLC, and produces blue spot when heated with phosphomolybdic acid.

44.D02 ***Apparatus***

(**a**) *Thin layer plates.*—Glass, 20 × 20 cm, precoated with 250 μm layer of silica gel. Prechanneled, with preadsorbent zone. (Whatman, Supelco, or equiv. plates.)

(**b**) *Dipping tank and accessories.*—Glass (Kontes Cat. No. K416160, or equiv.).

(**c**) *Chromatographic tank with lid.*—Glass (Kontes Cat. No. K416180, or equiv.).

(**d**) *Spotting pipets.*—20 μL, glass (Drummond Scientific Co., PO Box 700, Broomall, PA 19008, or equiv.).

44.D03 ***Reagents***

(a) *Alcoholic phosphomolybdic acid (PMA).*—Dissolve 50 g PMA (Fisher Certified ACS, or equiv.) in 500 mL alcohol, filter soln, and dil. to 1 L with alcohol. Store in dark. Discard if greenish tinge appears.

(**b**) *Developing solvent.*—Ether–heptane (55 + 45).

(c) *Coprostanol std soln.*—5 μg coprostanol (Supelco std)/mL hexane.

(**d**) *Cholesterol std soln.*—5 μg cholesterol (Supelco std)/mL hexane.

44.D04 ***Preparation of Sample***

Weigh sample of suspected fecal material to nearest 0.1 mg. If origin of feces is unknown or if herbivore dung is suspected, use ≥5 mg sample to reduce possibility of false neg. conclusions. Analyses may be made on much smaller test portions of samples, such as those from reconditioning operations of rodent-contaminated products, where more is known about sample.

Transfer sample to glass vial (ca 1–2 mL). Gently crush sample with glass rod if particle is >3–4 mm diam. Add hexane at ratio of 10 μL/mg feces, but not less than 30 μL per sample. Cap vial; let stand 1 h.

44.D05 ***Preparation of TLC Plates***

Prep. plates ≥24 h before use to ensure evapn of alcohol. To prevent contamination, wear vinyl gloves when handling plates. Dip plates, top edge leading, into 5% alc. PMA until soln is 2–3 mm below preadsorbent-silica gel juncture. Do not let PMA diffuse into preadsorbent zone. Hold plate 10–15 s, then remove plate from soln and let it stand vertically ca ½ h, preadsorbent edge up, on paper toweling. Do not let dust settle on damp surface. Store plates in clean, dark container with gel surface free of any contact. Store plates ≤3 months.

44.D06 ***Determination***

Use No. 1 (soft) pencil to lightly draw solv. front line 10 cm from preadsorbent-silica gel line. Spot 20 μL vols of stds and samples and at least 1 hexane reagent blank individually onto preadsorbent regions of plates. After std or sample has been applied, number lane with soft pencil and record lane no. and sample. If all lanes are not required, avoid lanes nearest edges. Let hexane evap. 10 min.

Work in hood. Apply thin bead of silicone grease around chromatgc tank top to seal lid. Pour 55 mL ether and 45 mL heptane into 100 mL mixing cylinder. Invert cylinder 3–4 times to mix and pour contents into TLC tank. Immediately cover tank and let system equilibrate 10 min. Place TLC plate in tank and cover tank. Develop to solv. front line (ca 20 min). Remove plate and air-dry in hood ca 5 min. Place plate in 120° forced-draft oven 20 min. Remove plate and circle spots with soft pencil. Record R_f for coprostanol and cholesterol stds. Record R_f for any spots that appear in coprostanol and cholesterol regions. Cholesterol and coprostanol should be completely sepd.

Feces are indicated by presence of 2 spots: at R_f ca 0.30 (cholesterol std) and at R_f ca 0.40 (coprostanol std). For small samples (≤2 mg) all spots may be faint and effort should be made to detect indicator spots. For larger samples, spots will be distinct. In addn to distinct cholesterol spot, cockroach excreta may present trace reaction in coprostanol region. Therefore, coprostanol spot should be approx. same or even greater in intensity than cholesterol spot to conclude that material is mammalian or bird feces rather than insect excreta. Coprostanol may occur in feces of some birds; therefore, if there is no evidence to eliminate possibility that material could be bird feces, pos. results should be reported as "fecal material from mammal or bird."

Colors fade in light. Photocopy machine may be used to make permanent record of plate.

Ref.: JAOAC **70**, 499 (1987).

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(4) Make an editorial correction in the final action flotation and macroscopic methods for foreign matter in canned corn. **44.109.** Change line 7 to read:

. . . add to percolator, **44.002(h)**(*3*). Reserve . . .

45. FORENSIC SCIENCES

No additions, deletions, or other changes.

46. MICROBIOLOGICAL METHODS

(1) The following first action methods were adopted final action:

(a) Bacterial and coliform counts in milk, dry rehydratable film methods, **46.B05–46.B07**.

(b) *Salmonella* in foods, enzyme immunoassay screening method, **46.B21–46.B29**.

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(2) The following interim pectin gel method for aerobic plate count of foods was adopted first action as an alternative to use of agar-based medium (**46.005(g)**):

Aerobic Plate Count

Pectin Gel Method

First Action

46.D01 ***Principle***

Method uses pretreated petri plates contg thin "hardener" layer, and liq. medium contg nutrients with pectin as only gelling agent. Liq. medium, 12–15 mL, is poured into pretreated petri plate and undild or dild sample is added. Plate is rotated and rocked to mix sample and medium. Plates are then allowed to stand on level surface 30–40 min until medium solidifies. Total process is done at ambient temp. Plates are then incubated and counted.

46.D02 ***Materials***

Note: Before pectin base medium formulated from individual ingredients is used, comparability to commercially available medium must be demonstrated.

Pectin gel tubes and plates—Pectin gel is available as sterile liq. in individual tubes contg sufficient gel to pour 1 plate. Use tubes of Redigel and pretreated petri plates (RCR Scientific, Inc., 206 W Lincoln Ave, Goshen, IN 46526), or equiv. that meet specifications.

To prep. plate count pectin gel from individual ingredients, suspend 5.0 g pancreatic digest of casein, 2.5 g yeast ext, and 1.0 g glucose, 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 7.0 ± 0.1. 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.D03 ***Preparation of Samples***

Prep. all decimal dilns with 90 mL sterile diluent (Butterfield's phosphate buffer) plus 10 mL of previous diln unless otherwise specified. 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.

(a) *Dairy products.*—Measure (or weigh) 11 mL (or g) sample and dil. in 99 mL Butterfield's 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°.

(b) *Nondairy products.*—Weigh 50 g sample into 450 mL Butterfield's diluent and blend 2 min at 10 000 to 12 000 rpm. Prep. further dilns by dispensing 10 mL sample into 90 mL diluent so that total colonies/plate is in 30–300 range. Incubate plates 48 ± 2 h at 35 ± 1°.

46.D04 ***Determination***

(*1*) Lift lid of pretreated petri plate and pour liq. pectin gel from 1 tube (12–15 mL) into plate. Replace lid and swirl plate to cover bottom with pectin gel. Prep. number of plates needed for samples being run (duplicate plates for each diln). Plates *must* be used within 5 min after liq. pectin gel is poured.

(*2*) Add 1 mL 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. pectin gel) after dispensing sample to rest point in pipet tip. *Immediately* rotate and rock plate to mix sample thoroly with pectin gel. Do not spill pectin gel 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 (ca 30–40 min), and then incubate 48 ± 2 h at 35 ± 1° for nondairy products and 48 ± 3 h at 32 ± 1° for dairy products.

(*4*) Count duplicate plates in suitable range (30–300 colonies for nondairy products, 25–250 colonies for dairy products). If plates do not contain proper range of colonies, record diln counted and note number of colonies found. Average counts obtained and report as aerobic plate count/g or mL.

Ref.: JAOAC **71**, March issue (1988).

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(3) The following interim microbiological method for enumeration of *E. coli* in chilled and frozen foods, exclusive of chilled and frozen shellfish, was adopted first action:

E. coli in Chilled and Frozen Foods

Microbiological Method

First Action

(Not applicable to chilled and frozen shellfish)

46.D05 ***Principle***

Lauryl sulfate tryptose broth with added 4-methyl-umbelliferyl-β-D-glucuronide (MUG) is used as medium in 3-tube MPN method. Tubes are incubated 24 ± 2 h at 35°. Fluorescent-pos. tubes are streaked onto eosin methylene blue agar (Levine) plates, which are incubated 24 ± 2 h at 35°. Typical colonies are picked and confirmed as *E. coli*.

46.D06 ***Media and Reagents***

See introductory par. to **46.013**.

(a) *Plate count agar.*—See **46.005(g)**.

(b) *Eosin methylene blue agar (Levine).*—See **46.005(d)**.

(c) *Tryptophane broth.*—Dissolve by heating, with stirring, 10.0 g tryptone or trypticase in 1 L H_2O. Dispense in 10 mL portions into test tubes and autoclave 15 min at 121°. Final pH, 6.9 ± 0.2.

(d) *Buffered glucose broth (MR–VP medium).*—For Me red–Voges Proskauer (MR–VP) tests. Dissolve 7.0 g proteose peptone, 5.0 g glucose, and 5.0 g K_2HPO_4 in ca 800 mL H_2O w gentle heat and occasional stirring. Filter, cool to 20°, and dil. to 1 L. Dispense 10 mL portions into test tubes and autoclave 12–15 min at 121°. Max. exposure to heat should be ≤30 min. Final pH, 6.9 ± 0.2. BBL, Division of Bioquest, or Difco dehydrated medium may be used.

(e) *Koser's citrate broth.*—See **46.005(e)**.

(f) *Butterfield's buffered phosphate diluent.*—See **46.013(m)**.

(g) *Lauryl sulfate tryptose broth with MUG.*—Prep. lauryl sulfate tryptose broth, **46.013(b)**, and add 50 mg 4-methyl-umbelliferyl-β-D-glucuronide (MUG). Dissolve with gentle heat, if necessary. Dispense 10 mL portions into 20 × 150 mm test tubes. Autoclave 15 min at 121°. Final pH, 6.8 ± 0.1. Difco dehydrated medium, or equiv., may be used.

(h) *Peptone dilution water.*—Dissolve 1.0 g peptone in 1 L H_2O. Prep. diln blanks with this soln, dispensing enough to allow for losses during autoclaving. Adjust pH to 7.0 ± 0.1. Autoclave 15 min at 121°.

(i) *Lactose broth.*—Dissolve on H_2O bath, with stirring, 3.0 g beef ext and 5.0 g polypeptone or peptone in 1 L H_2O. Add 5.0 g lactose. Dispense 450 mL portions into 750 mL flasks and autoclave 15 min at 121°. Max. exposure to heat should be ≤30 min. Final pH, 6.7 ± 0.2.

46.D07 ***Preparation of Sample***

Prep. all decimal dilns with 90 mL sterile diluent, peptone diln H_2O (h) or **46.013(m)** (*2*), plus 10 mL previous diln unless otherwise specified. 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.

Frozen or chilled foods.—Use balance with capacity of ≥2 kg and sensitivity of 0.1 g to aseptically weigh 50 g unthawed (if frozen) sample into sterile high-speed blender jar. Add 450 mL diluent, **(i)** or **46.013(m)**(*2*), and blend 2 min at 10 000–12 000 rpm. (If necessary to temper frozen sample to remove 50 g portion, hold ≤18 h at 2–5°.) Not >15 min should elapse from time sample is blended until all dilns are made in appropriate media.

If entire sample consists of <50 g, weigh portion equiv. to ½ sample and add vol. of sterile diluent required to make 1:10 diln. Total vol. in blender jar must completely cover blades.

46.D08 ***Determination***

Notes: (*1*) Test tubes used in MPN method should be checked under UV light to be sure glass does not fluoresce. (*2*) To avoid false-pos. fluoresence, longwave UV light used in method should not exceed 6 watts (Blak-Ray, Model UVL-56, or equiv.).

Seed 3-tube most probable number (MPN) series into lauryl sulfate tryptose broth contg MUG (g), using 1 mL inocula of 1:10, 1:100, and 1:1000 dilns, with triplicate tubes at each diln. Incubate 24 ± 2 h at 35° for fluorescence as evidenced by fluorescence of medium when tube is held under longwave UV light (366 nm).

Streak fluorescent-pos. tubes on eosin methylene blue agar plates (b), and incubate plates 24 ± 2 h at 35°.

Pick 2 or more well isolated typical colonies from eosin methylene blue agar plates and transfer to agar slants prepd from agar medium (a). Incubate 18–24 h at 35°. If typical colonies are not present, pick 2 or more colonies most likely to be *E. coli*. Pick ≥2 from every plate.

Confirm *E. coli* as specified in **46.016**.

Ref.: JAOAC **71**, May issue (1988).

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(4) The following interim elevated temperature enrichment method for isolation of *Vibrio cholerae* in oysters was adopted first action:

Vibrio cholerae in Oysters
Elevated Temperature Enrichment Method
First Action

46.D09 ***Principle***

Recovery of *V. cholerae* is based on selection of typical colonies on isolation agar. Although *V. cholerae* grows well at 35°, many other species of competing bacteria also proliferate in enrichment broth when incubated at 35°. Some species of competing microflora mimic colonial appearance of *V. cholerae* on isolation medium. Subsequent selection of these mimicking colonies decreases probability of recovering any *V. cholerae* colonies that may be present, and increases labor and materials needed for analysis. Ability of almost all strains of *V. cholerae* to grow at 42° distinguishes them from many other bacterial species associated with oysters and results in higher confirmation rate of suspects as *V. cholerae*.

46.D10 ***Culture Media and Reagents***

(**a**) *AP broth.*—Suspend 10.0 g peptone and 10.0 g NaCl in 1 L H_2O and mix thoroly. Adjust pH so that value after sterilization is 8.5 ± 0.2. Dispense portions into 500 mL flasks so that final vol. after autoclaving 10 min at 121° is 225 mL.

(**b**) *TCBS agar.*—Suspend 5.0 g yeast extract, 10.0 g proteose peptone No. 3, 10.0 g Na citrate, 10.0 g $Na_2S_2O_3$, 8.0 g oxgall, 20.0 g sucrose, 10.0 g NaCl, 1.0 g ferric citrate, 0.04 g bromthymol blue, 0.04 g thymol blue, and 15 g agar in 1 L H_2O and mix thoroly. Heat with frequent agitation until medium just boils, 1–2 min. Cool in H_2O bath and pour 20 mL portions into 15 × 100 mm petri dishes. Let dry ca 2 h with covers partially removed; then close plates. Final pH, 8.6 ± 0.2. Do not autoclave. Do not use wet plates.

(**c**) *T_1N_1 agar.*—Suspend 10.0 g tryptone or trypticase, 10.0 g NaCl, and 20.0 g agar in 1 L H_2O and mix thoroly. Heat with frequent agitation until medium boils. Dispense into 16 × 125 mm screw-cap tubes (if tubed medium is required). Autoclave 15 min at 121°. Slant tubes until cool or let medium cool to 50° and pour into 15 × 100 mm petri dishes. Let dry 2 h with plates covered. Do not use wet plates. Final pH, 7.2 ± 0.2.

(**d**) *Tryptone broth.*—Suspend 10.0 g tryptone or trypticase in 1 L H_2O and mix thoroly. Dispense 5 mL portions into 16 × 125 mm or 16 × 150 mm test tubes. Autoclave 15 min at 121°. Final pH, 6.9 ± 0.2.

(**e**) *Kligler iron agar (KIA).*—Suspend 3.0 g beef extract, 3.0 g yeast extract, 15.0 g peptone, 5.0 g proteose peptone, 10.0 g lactose, 1.0 g dextrose, 0.2 g $FeSO_4$, 5.0 g NaCl, 0.3 g $Na_2S_2O_3$, 12.0 g agar, and 0.024 g phenol red in 1 L H_2O, mix thoroly, and heat with occasional agitation. Boil ca 1 min until ingredients dissolve. Fill 13 × 100 mm screw-cap tubes ⅓ full and cap to maintain aerobic conditions during use. Autoclave 15 min at 121°. Before medium solidifies, place tubes in slanted position so that deep butts (ca 3 cm) and adequate slants (ca 5 cm) are formed on solidification. Final pH, 7.4 ± 0.2.

(**f**) *Hugh-Leifson glucose broth (HLGB).*—Suspend 2.0 g peptone, 0.5 g yeast extract, 30.0 g NaCl, 10.0 g dextrose, 0.015 g bromcresol purple, and 3.0 g agar in 1 L H_2O, mix thoroly, and heat with agitation. Boil ca 1 min until ingredients are dissolved. Final pH, 7.4 ± 0.2. Fill 13 × 100 mm screw-cap test tubes ⅓ full and cap. Autoclave 15 min at 121°. After inoculation cover with ca 1 mL sterile mineral oil to test for fermentation of dextrose.

(**g**) *Purple carbohydrate broth.*—Suspend 10.0 g proteose peptone No. 3, 1.0 g beef extract, 5.0 g NaCL, and 0.015 g bromcresol purple in 1 L H_2O and heat with gentle agitation until dissolved. Dissolve 10.0 g inositol or 10.0 g mannitol in basal broth. Dispense 2.5 mL portions into 13 × 100 mm test tubes. Autoclave 10 min at 121°. Final pH, 6.8 ± 0.2.

(**h**) *Decarboxylase test media (Moeller).*—Suspend 5.0 g peptone, 5.0 g beef extract, 0.5 g dextrose, 0.01 g bromcresol purple, 0.005 g cresol red, and 0.005 g pyridoxal in 1 L H_2O and heat with gentle agitation until dissolved. Dissolve 10.0 g L-lysine·2HCl, 10.0 g L-arginine·HCl, or 10.0 g L-ornithine·2HCl in basal broth. Use 1 portion of basal medium, without adding any amino acid, as control. Dispense 3–4 mL portions into 13 × 100 mm screw-cap tubes. Cap loosely and autoclave 10 min at 121°. Screw caps on tightly for storage. After inoculation cover with ca 1 mL sterile mineral oil. Final pH, 6.0 ± 0.2.

46.D11 ***Diagnostic Reagents***

(**a**) *Oxidase test soln.*—Dissolve 1.0 g *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine·2HCl in 100 mL H_2O. Store ≤7 days in dark glass bottle in refrigerator. Do not autoclave.

(**b**) *String test soln.*—Dissolve 0.5 g Na desoxycholate in 100 mL H_2O. Store tightly capped in refrigerator. Do not autoclave.

(**c**) *V. cholerae polyvalent (O) antiserum.*—Contains agglutinins for Inaba and Ogawa (O) antigens (Difco, or equiv.). Rehydrate with 5.0 mL sterile physiological saline soln (**e**). Store refrigerated.

(**d**) *V. cholerae individual somatic (O) antisera.*—For Inaba and Ogawa (O) groups (Difco, or equiv.). Rehydrate and store as described in (**c**).

(**e**) *Sterile physiological saline soln.*—Dissolve 8.5 g NaCl in 1 L H_2O and autoclave 15 min at 121°.

(**f**) *NaOH soln, 1N.*—Dissolve 42.11 g 95% reagent grade NaOH in sterile H_2O and dil. to 1 L.

(**g**) *HCl soln, 1N.*—Dil. 89 mL HCl to 1 L with sterile H_2O.

(**h**) *Sterile mineral oil.*—Autoclave 500 mL mineral oil in 1 L flask for 30 min at 121°.

(**i**) *Bromcresol purple soln, 0.2%.*—Dissolve 0.2 g bromcresol purple in sterile H_2O and dil. to 100 mL.

46.D12 ***Apparatus***

(**a**) *Incubator.*—Air, 35 ± 2°.

(**b**) *H_2O bath.*—Covered, 42 ± 0.2°.

(**c**) *High-speed blender.*—2 speed, with high-speed operation at 18 000–21 000 rpm, and 1 L glass or metal blender jars with covers. Use 1 jar for each test sample.

(d) *Sterile equipment*.—(*1*) Flasks or jars, 500 mL capacity. (*2*) Knives and spoons for opening and manipulating oysters. (*3*) Petri dishes, 15 × 100 mm. (*4*) Pipets, 1.0 and 10.0 mL with 0.1 mL graduations. (*5*) Inoculating needles and loops, ca 3 mm. (*6*) Culture tubes, 13 × 100 mm, 16 × 125 mm, and tube racks. (*7*) Wooden applicator sticks.

(e) *Balance*.—2000 ± 0.1 g capacity.

V. cholerae Recovery

46.D13 **Preparation of Test Sample**

Aseptically remove oyster meats and liquor from ca 12 shell stock oysters or 12 shucked oysters from container. Aseptically weigh ca 200 g oyster meat and liquor into sterile empty blender jar. Blend at high speed 1 min. Aseptically weigh 25 g portions into 500 mL flasks contg 255 mL AP broth. Cover flask with sterile Al foil. Swirl mixt. 25 times clockwise and 25 times counterclockwise to suspend oyster homogenate. Incubate 6–8 h at 42 ± 0.2° in H_2O bath.

46.D14 **Isolation**

Gently remove flasks from H_2O bath. Streak 3 mm loopful of surface or pellicle growth from incubated AP broth on TCBS agar plate. Incubate plates 18–24 h at 35°. Typical *V. cholerae* colonies on TCBS agar appear large, smooth, yellow, and slightly flattened with opaque centers and translucent peripheries. Colonies of *V. mimicus*, which is closely related to *V. cholerae*, appear as smooth, green, slightly flattened colonies.

46.D15 **Treatment of Typical or Suspicious Colonies**

Inoculation of T_1N_1 agar.—Pick with needle 2–5 suspicious colonies from TCBS agar plate. Streak to T_1N_1 agar and incubate 18–24 h at 35°.

Initial screening reactions.—Scrape agar surface with sterile wooden applicator stick and touch to filter paper impregnated with oxidase reagent.

Oxidase test.—*V. cholerae* cultures are oxidase pos. and should produce dark purple spot within 1 min.

String test.—Emulsify oxidase pos. cultures in drop of 0.5% Na desoxycholate by stirring with same wooden applicator stick used previously. Within 1 min, *V. cholerae* cultures form mucoid mass, which strings (string test) when stick is lifted 2–3 cm from slide. Treat oxidase and string test pos. cultures as presumptive *V. cholerae* and submit them to further examination.

Inoculation of Kligler iron agar (KIA) and tryptone broth—Inoculate KIA slant with each suspect colony by streaking slant and stabbing butt with inoculating needle. After inoculating KIA with needle, do not obtain more inoculum from colony and do not heat needle, but directly inoculate tryptone broth. Incubate KIA and tryptone broth overnight at 35°. Cap tubes lightly to maintain aerobic conditions while incubating slants to prevent excessive H_2S production.

KIA.—*V. cholerae* cultures typically have alk. (red) slant and acid (yellow) butt, without H_2S (blackening of agar) or gas (cracking or lifting of agar). Do not eliminate KIA culture as *V. cholerae* solely on basis of acid slant.

Tryptone broth.—*V. cholerae* cultures typically produce growth in tryptone broth without added NaCl. Discard only apparent non-*V. cholerae* cultures. Test retained presumptive pos. KIA and tryptone cultures to det. if they are *V. cholerae*. Biochem. reactions characteristic of *V. cholerae* are summarized in Table **46:D1**.

Table 46:D1 Biochemical reactions of *V. cholerae*

Test or substrate	Positive (+)	Negative (−)	*V. cholerae* reaction
H_2S (KIA)	blackening	no blackening	−
Gas (KIA)	lifting or cracking	no lifting or cracking	−
Tryptone	visible growth	no visible growth	+
HLGB	yellow	purple	+
Mannitol	yellow	purple	+
Inositol	yellow	purple	−
Decarboxylase broth:			
Lysine	purple	yellow	+
Arginine	purple	yellow	−
Ornithine	purple	yellow	+

V. cholerae Identification

46.D16 **Identification Tests**

Pure 18–24 h T_1N_1 agar cultures are required for inoculation of biochem. media. Select isolated colony and transfer with needle to each biochem. medium without obtaining more inoculum or heating needle.

Dextrose fermentation.—After inoculation, cover with ca 1 mL sterile mineral oil and incubate overnight at 35°. *V. cholerae* gives pos. test, shown by acid reaction (yellow). Discard all cultures that give neg. test.

Acid production from mannitol and inositol.—Incubate at 35° and read daily up to 4 days. Pos. tests are shown by acid production (yellow). *V. cholerae* gives pos. mannitol and neg. inositol test. Do not eliminate culture as *V. cholerae* solely on neg. mannitol test.

Decarboxylase broth.—After inoculation cover with ca 1 mL sterile mineral oil and incubate at 35°. Read daily up to 4 days. Pos. test is shown by purple alk. reaction thruout broth (final color is slightly darker than original purple of medium). Sometimes tubes that become yellow after 8–12 h incubation change to purple later. Neg. test is permanently yellow thruout broth and is seen with decarboxylase control tube without added amino acid. If medium appears to be discolored (neither purple nor yellow), add several drops of 0.2% bromcresol dye. *V. cholerae* gives pos. (purple) reaction in lysine and ornithine and neg. (yellow) reaction in arginine.

Serological Tests for *V. cholerae*

Reconstitute antisera with 5.0 mL sterile 0.85% saline and refrigerate. Pretest all antisera with known test cultures to ensure reliability of results with unknown cultures. **Caution:** Handle viable cultures carefully to prevent contaminating environment. Use pure 18–24 h T_1N_1 cultures for all serological tests. Perform serological test only on cultures that give biochem. reactions typical of *V. cholerae*.

46.D17 **Polyvalent Somatic O Group 1 Slide or Plate Test**

Use wax pencil to mark off 2 sections ca 1 × 2 cm on inside of glass or plastic petri dish. Place 1 drop of 0.85% saline soln to one section and 1 drop of *V. cholerae* polyvalent somatic (O) antisera to other section. With sterile wooden applicator stick or inoculating loop or needle, emulsify culture in saline soln for one section and repeat for other section contg antiserum. Tilt mixt. in both sections back and forth 1 min and observe against dark background. Any degree of agglutination is pos. reaction.

Classify polyvalent somatic (O) group 1 test as:

Positive.—Agglutination in culture–saline–serum mixt.

Negative.—No agglutination in culture–saline–serum mixt.

Nonspecific.—Both mixts agglutinate.

46.D18 **Determination of Individual Somatic O Group 1 Serotypes**

Test only somatic O group 1 pos. cultures in individual O group 1 antisera. Perform serological somatic O group 1 test on culture as

above, by using Inaba and Ogawa antiserum instead of *V. cholerae* polyvalent somatic O group 1 antiserum.

Classify individual somatic O group 1 test as:

Inaba positive.—Agglutination in culture–saline–Inaba antiserum mixt. and no agglutination in culture–saline or in culture–saline–Ogawa antiserum mixt.

Ogawa positive.—Agglutination in culture–saline–Ogawa antiserum mixt. and no agglutination in culture–saline or in culture–saline–Inaba antiserum mixt.

Hikojima positive.—Agglutination in both culture–saline–Inaba antiserum mixt. and culture–saline–Ogawa antiserum mixt. but no agglutination in culture–saline mixt.

Negative.—No agglutination in culture–saline–Inaba antiserum mixt., culture–saline–Ogawa antiserum mixt., or in culture–saline mixt. This pattern indicates faulty individual somatic O group 1 antisera or presence of non-O group 1 antisera in polyvalent somatic O group 1 antiserum.

Nonspecific.—All mixts agglutinate.

Ref.: JAOAC **71**, May issue (1988).

The recommendation to adopt first action the elevated temperature enrichment method for *Vibrio cholerae* in oysters was inadvertently omitted at the AOAC Business Meeting, September 17, 1987, San Francisco, CA.

47. MICROCHEMICAL METHODS

No additions, deletions, or other changes.

48. RADIOACTIVITY

No additions, deletions, or other changes.

49. VETERINARY ANALYTICAL TOXICOLOGY

No additions, deletions, or other changes.

50. STANDARD SOLUTIONS AND CERTIFIED REFERENCE MATERIALS

No additions, deletions, or other changes.

51. LABORATORY SAFETY

No additions, deletions, or other changes.

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 of Analysis" 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 will be official 30 days after publication of this fourth supplement.

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).

Acephate, residues in fruits and vegetables, **29.A01*** (final 1986)
Acetaminophen, in drug tablets, **37.C01***
Acids, citric and malic in wines, **11.052** (final 1988)
in apple juice and cranberry juice cocktail, **22.B01***
titratable acidity of fruit products, **22.058** (rev. 1988)
total acidity in distilled liquors, **9.135** (final 1988)
volatile acidity of wines, **11.046** (final 1988)
Acids (volatile), in oils and fats, **28.039** (rev. 1985)
Acrylonitrile, in food, **21.A01***
Adulteration, detection in meat and poultry products, **24.C01***
of Florida orange juice, **22.B06*** (rev. 1987)
Aerobic plate count, frozen, chilled, precooked, or prepared foods, **46.013** (rev. 1987), **46.B01*** (final 1987), **46.D01***
Aflatoxins, B_1 and M_1 in liver, **26.101** (final 1988), **26.107** (final 1988)
BF method, **26.032** (final 1988)
CB method, **26.026** (rev. 1988; final 1988)
confirmation of B_1, **26.A01*** (final 1988)
Holaday-Velasco minicolumn method, **26.020** (final 1988)
identification by derivative formation, **26.083** (final 1988)
identification of B_1, **26.076** (surplus 1988)
in almonds, **26.014** (final 1988)
in coconut, copra, and copra meal, **26.044** (final 1988)
in corn, **26.014** (final 1988), **26.020** (final 1988), **26.049** (final 1988)
in cottonseed products, **26.014** (final 1988), **26.052** (rev. 1986; final 1988), **26.B01*** (final 1988)
in eggs, **26.070** (final 1988)
in green coffee, **26.061** (final 1988; surplus 1988)
in milk, **26.B02***
in mixed feeds, **26.014** (final 1988)
in peanuts and peanut products, **26.014** (final 1988), **26.020** (final 1988), **26.026** (final 1988), **26.032** (final 1988)
in pistachio nuts, **26.014** (final 1988), **26.067** (final 1988)
in soybeans, **26.069** (final 1988)
M_1 in dairy products, **26.090** (rev. 1987; final 1988)
M_1 in liver, **26.110** (surplus 1988)
Romer minicolumn method, **26.014** (final 1988)
standards, **26.004** (rev. 1988), **26.008** (final 1988)
toxicity of B_1, **26.084** (surplus 1987)
Alachlor, in formulations, **6.A05*** (final 1987), **6.D07***
Alcohol, in dexamethasone elixirs, **39.D27***
in distilled liquors, **9.020** (rev. 1987), **9.026** (final 1985), **9.119** (final 1985)
in wines, **11.008** (rev. 1985; rev. final 1988), **11.012** (final 1988)
Alcoholic beverages, see Distilled liquors; Malt beverages; Wines
Aldicarb, residues in fruits and vegetables, **29.A05*** (final 1986)
Alimentary pastes, filth in, **44.069** (final 1988)
Allergenic extracts, **38.205** (final 1985; amended 1986), **38.B01***
Allopurinol, in drug tablets, **37.A01*** (final 1987)
Almonds, aflatoxins in, **26.014** (final 1988)
Aluminum, in plants, **3.006** (final 1988)
American Spice Trade Association, ASTA-AOAC method for piperine in pepper preparations, **30.C01***
Amino acids, sulfur amino acids in food and feed ingredients, **43.A08*** (final 1986)
sulfur amino acids in processed foods, **43.A08** (1988*)
tryptophan, in foods and food and feed ingredients, **43.D08***
Aminocarb, technical and formulations, **6.A25*** (final 1987)
Amitriptyline, in drug tablets and injectables, **36.207** (final 1986)
Amphetamine, determination of enantiomers, **40.D01***
α-Amylase, in malt, **10.155** (rev. 1988)
Anilazine, in formulations, **6.D13***
Annatto, filth in ground product, **44.129** (final 1988)
Antibiotics, in feeds, **42.203** (corr. 1985)
in milk, **16.135** (rev. 1986), **16.152** (rev. 1987), **16.D01***
Aphids, in hops, **10.182** (final 1988), **44.009** (final 1988)
Apple butter, filth in, **44.086** (final 1988)
Apple juice, organic acids in, **22.B01***
Apricots, total sulfites in, **20.C01***
Arprinocid, in feeds, **42.021** (surplus 1988)
in premixes, **42.027** (surplus 1988)
Arsenic, in liver tissue, **49.B01***
trace levels in food, **25.001** (final 1988)
ASBC, ASBC-AOAC digital density meter method, specific gravity of beer and wort, **10.D01***
ASBC-AOAC method, ethanol in beer, **10.028** (final 1988)
extract of malting barley, **10.120** (final 1988)
N-nitrosodimethylamine in beer, **10.098** (final 1988)
potassium in beer, **10.C01***
sodium in beer, **10.C06***
Ascorbic acid, in foods, **43.076** (final 1985)
in milk-based infant formula, **43.A28*** (final 1988)
Ash, of milk-based infant formula, **43.B01*** (final 1988)
Autoradiography, detection of *E. coli* enterotoxins, **46.B08*** (rev. 1987; final 1987)
Babcock method, fat in cream, **16.177** (rev. 1988)
fat in milk, **16.065** (rev. 1986), **16.B02***
Bacteria, count in milk, **46.B05*** (final 1988)
in low acid canned foods, **46.071** (rev. 1987; final 1987)
Baked goods, filth in white bread and high-fat products, **44.067** (final 1988)
Barium, in plants, **3.006** (final 1988)
Barley, extract of malting barley, **10.120** (final 1988)
sterigmatocystin in, **26.132** (final 1988)
Bayleton (triadimefon), in formulations, **6.A31***
Beer, ethanol in, **10.028** (final 1988)
N-nitrosodimethylamine in, **10.098** (final 1988)
potassium in, **10.C01***
sodium in, **10.C06***
specific gravity, **10.D01***
Bendiocarb, in formulations, **6.B40***
Beta-lactam antibiotics, in milk, **16.135** (rev. 1986), **16.152** (rev. 1987)
BF method, **26.032** (final 1988)
BHC, in formulations, **6.221** (final 1986)
residues in fruits and vegetables, **29.A01*** (final 1986; rev. 1987)
Bioassays, chicken embryo bioassay for toxicity of aflatoxin B_1, **26.084** (surplus 1987)
mouse adrenal cell and suckling mouse assay for *E. coli* enterotoxins, **46.049** (final 1987)
Boron, in fertilizers, **2.135** (final 1985)
in plants, **3.006** (final 1988), **3.A01*** (final 1988)
Bread, filth in, **44.067** (final 1988)
total dietary fiber in, **43.A14*** (rev. 1986; final 1986; rev. 1987)
Bufencarb, residues in fruits and vegetables, **29.A05*** (final 1986)
Butachlor, in formulations, **6.B12*** (final 1988)
N*-(Butoxymethyl)-2-chloro-*N*-(2,6-diethylphenyl)acetamide (butachlor)**, **6.B12 (final 1988)
Butter, aflatoxin M_1 in, **26.090** (rev. 1987; final 1988)
Butylate, in formulations, **6.581** (final 1988)
Cacao bean and its products, *Salmonella* in, **46.A06*** (final 1986)
Cadmium, in cookware, **25.016** (final 1986)
in earthenware, **25.024** (rev. 1988)
trace levels in food, **25.001** (final 1988), **25.008** (rev. 1987; final 1988)

Calcium, in deboned poultry and beef, **24.062** (final 1985; rev. 1987)
in milk-based infant formula, **43.A37*** (final 1988)
in plants, **3.006** (final 1988), **3.013** (final 1988), **3.A01*** (final 1988)
Caloric content, of wines, **11.022** (final 1988)
Candy, filth in, **44.103** (final 1988)
Canned foods, ethanol in salmon, **18.B01***
filth in citrus and pineapple juices, **44.095** (final 1988)
filth in corn, **44.109** (final 1988; corr. 1988)
filth in crabmeat, **44.080** (final 1988)
filth in fish and fish products, **44.082** (final 1988)
filth in shrimp, **44.083** (final 1988)
mold count in cream style corn, **44.223** (final 1988)
sodium chloride in vegetables, **30.032** (rev. 1987)
sporeforming organisms in low-acid products, **46.A01***
tin in, **25.A01*** (final 1988)
total solids in tomato products, **32.A01***
Capsicums, filth in ground product, **44.130** (final 1988), **44.131** (final 1988)
Carbamate pesticides, aminocarb, **6.A25*** (final 1987)
bendiocarb, **6.B40***
carbofuran, **6.B45***
methiocarb, **6.546** (final 1987)
propoxur, **6.565** (final 1987)
residues, **29.A05*** (final 1986)
thiocarbamates, **6.581** (final 1988)
Carbaryl, residues in fruits and vegetables, **29.A05*** (final 1986)
Carbidopa, with levodopa in solid dosage forms, **36.D07***
Carbofuran, in formulations, **6.B45***
residues in fruits and vegetables, **29.A05*** (final 1986)
Carbohydrates, in milk-based infant formula, **43.B05***
in wines, **11.A05*** (final 1988)
Carbon dioxide, in wines, **11.D01***
Casein, residual phosphatase in, **16.127** (rev. 1986), **16.B03***
CB method, **26.026** (rev. 1988; final 1988)
Cereal foods, deoxynivalenol in wheat, **26.B07***, **26.B13***
ethylene dibromide residues in grains and grain products, **29.B01*** (final 1987)
fat acidity of grains, **14.069** (rev. 1988)
filth in corn and rice cereals and corn chip products, **44.070** (final 1988)
filth in infant cereals, **44.072** (final 1988)
filth in rice flours (powders), extruded rice products, and rice paper, **44.055** (final 1988)
filth in white flour, **44.052** (final 1988)
filth in whole wheat cereals, **44.071** (final 1988)
insect infestation of oats, **44.A01***
mammalian feces in corn meal, **44.B01***
phytate in dried foods, **14.B01*** (final 1988)
starch in flour, **14.031** (surplus 1987)
sugars in presweetened cereals, **14.075** (rev. 1985)
total dietary fiber in, **43.A14*** (rev. 1986; final 1986; rev. 1987)
zearalenone and α-zearalenol in corn, **26.A09***
Cesium, cesium-137 in foods, **48.B01*** (rev. 1987)
Cheese, acrylonitrile in, **21.A01***
aflatoxin M_1 in, **26.090** (rev. 1987; final 1988)
Salmonella in powders, **46.A06*** (final 1986)
sampling, **16.B01***
Chelated iron, in fertilizers, **2.157** (rev. 1986)
Chicken embryo bioassay, for toxicity of aflatoxin B_1, **26.084** (surplus 1987)
Chloramphenicol, in milk, **16.D01***
Chlordimeform, in formulations, **6.A09*** (final 1987)
Chloride, in milk-based infant formula, **43.B06***
4-Chloro-α-(4-chlorophenyl)-α-(trichloromethyl)benzene-methanol (dicofol), **6.B22***
2-Chloro-*N*-(2,6-diethylphenyl)-*N*-(methoxymethyl)acetamide (alachlor), **6.A05*** (final 1987), **6.D07***
2-Chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxyl-1-methyl-ethyl)acetamide (metolachlor), **6.A14*** (final 1988)
2-Chloro-*N*-(1-methylethyl)-*N*-phenylacetamide (propachlor), **6.B17*** (final 1988)
N'*-(4-Chloro-2-methylphenyl)-*N,N*-dimethylmethanimidamide (chlordimeform)**, **6.A09 (final 1987)
1-(4-Chlorophenoxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazoi-1-yl)-2-butanone (triadimefon), **6.A31*** (final 1987)
Chlorothiazides, in drug dosage forms, **37.A11*** (final 1987)
Chlorpropamide, in drug tablets, **37.B01***
Chlorpyrifos, residues in fruits and vegetables, **29.A01*** (final 1986; rev. 1987)
Chocolate and chocolate products, filth in, **44.006** (final 1988)
Salmonella in, **46.A06*** (final 1986)
CIPAC, AOAC–CIPAC method, alachlor in formulations, **6.D07***
AOAC–CIPAC method, anilazine in formulations, **6.D13***
butachlor in formulations, **6.B12*** (final 1988)
chlordimeform in formulations, **6.A09*** (final 1987)
isofenphos in formulations, **6.C01***
metolachlor in formulations, **6.A14*** (final 1988)
propachlor in formulations, **6.B17*** (final 1988)
CIPAC–AOAC method, aminocarb in formulations, **6.A25*** (final 1987)
cyhexatin in formulations, **6.D01***
cypermethrin in formulations, **6.B01***
permethrin in formulations, **6.B06*** (final 1988)
triadimefon in formulations, **6.A31*** (final 1987)
Citric acid, in fruit juices, **22.B01***
in wines, **11.052** (final 1988), **11.A06***
Cobalamin, in milk-based infant formula, **43.B11***
Cocoa, filth in, **44.006** (final 1988)
Coconut, aflatoxins in, **26.044** (final 1988)
filth in shredded product, **44.032** (final 1988)
Codex Alimentarius, Codex Alimentarius–AOAC method for total soluble solids in vinegar, **30.C06***
Coffee, aflatoxins in green coffee, **26.061** (final 1988; surplus 1988)
filth in ground coffee and coffee substitutes, **44.008** (final 1988)
ochratoxin A in green coffee, **26.119** (final 1988)
Colchicine, drug substance and dosage forms, **38.A01*** (final 1987)
Coliforms, count in foods, **46.030** (final 1985)
count in milk, **46.B05*** (final 1988)
Color additives, in foods, **34.D01***
Color classification, of honey, **31.A01*** (final 1986)
Color units, of whisky, **9.002** (final 1985)
Cookware, cadmium and lead in, **25.016** (final 1986)
Copper, in liver, **46.A01*** (final 1987)
in milk-based infant formula, **43.A37*** (final 1988)
in plants, **3.006** (final 1988), **3.013** (final 1988), **3.A01*** (final 1988)
in serum, **49.003** (final 1986)
Copra and copra meal, aflatoxins in, **26.044** (final 1988)
Coprostanol, as indicator of mammalian feces, **44.D01***
Corn, aflatoxins in, **26.014** (final 1988), **26.020** (final 1988), **26.049** (final 1988)
filth in canned product, **44.109** (final 1988; corr. 1988)
filth in cereal and corn chip products, **44.070** (final 1988)
mold count in cream style product, **44.223** (final 1988)
zearalenone and α-zearalenol in, **26.A09*** (final 1988)
zearalenone in, **26.139** (final 1988)
Corn chips, filth in, **44.102** (final 1988)
Corn meal, mammalian feces in, **44.B01***
Corn syrups, dry substance, **31.226** (rev. 1985*; rev. final 1986)
in honey, **31.156** (rev. 1988), **31.158** (rev. 1988)
in maple syrup, **31.185** (final 1988)
Corn syrups and sugars, saccharides in, **31.245** (corr. 1986)
Cortisone acetate, bulk drug and dosage forms, **39.D01***
Cosmetics, γ-BHC in lindane shampoo and lotions, **6.221** (final 1986)
Cottonseed products, aflatoxins in, **26.052** (rev. 1986; final 1988), **26.B01*** (final 1988)
aflatoxins in cottonseed meal, **26.014** (final 1988)
Crabmeat, filth in canned product, **44.080** (final 1988)
Cranberry juice cocktail, organic acids in, **22.B01***
Cream, fat in, **16.177** (rev. 1988)
filth in, **44.021** (final 1988)
Crude fat, in meat, **24.005** (rev. 1985)
Cycloate, in formulations, **6.581** (final 1988)
Cyhexatin, in formulations, **6.D01***
Cypermethrin, technical and formulations, **6.A01*** (final 1987), **6.B01***
Cysteic acid, in food and feed ingredients, **43.A08*** (final 1988)
in processed foods, **43.A08** (1988*)

Dairy products, acrylonitrile in cold pack cheese, **21.A01***
added water in milk, **16.101** (surplus 1987)
aflatoxin M_1 in butter, cheese, and milk, **26.090** (rev. 1987; final 1988)
aflatoxins in milk, **26.B02***
alcohol in alcoholic-type products, **9.119** (final 1985)
antibiotics in milk, **16.135** (rev. 1986), **16.152** (rev. 1987), **16.D01***
cheese sampling, **16.B01***
dry rehydratable film method for bacterial and coliform counts in milk, **46.B05***
fat in cream, **16.177** (rev. 1988)

Dairy products (*Contd*)
fat in milk, **16.065** (rev. 1986), **16.074** (surplus 1987), **16.083** (rev. 1988), **16.B02***
lactose in milk, **16.059** (final 1985), **16.083** (rev. 1988)
nutrients in infant formula, **43.A21*** (amended 1986), **43.B01***
protein in milk, **16.083** (rev. 1988)
residual phosphatase in casein, **16.127** (rev. 1986), **16.B03***
Salmonella in cheese powders and nonfat dry milk, **46.A06*** (final 1986)
sediment in, **44.021** (final 1988)
somatic cells in milk, **46.171** (rev. 1987)
sulfur amino acids in nonfat dry milk and β-lactoglobulin, **43.A08*** (final 1986)
tin in canned evaporated milk, **25.A01*** (final 1988)
Dalapon, in formulations, **6.296** (rev. 1987; final 1987)
DDT, residues in fruits and vegetables, **29.A01*** (final 1986)
Decomposition in food, ethanol in canned salmon, **18.B01***
Definitions of Terms and Explanatory Notes, pp. xxiii and xxiv (14th ed.) (rev. 1987)
Deoxynivalenol, in wheat, **26.B07***, **26.B13***
Dexamethasone, drug substance and elixirs, **39.D17***
Dexamethasone acetate, bulk drug and suspensions, **39.D09***
Dexamethasone phosphate, in dosage forms, **39.056** (surplus 1988)
Dextran, in raw cane sugar, **31.D01***
Diastatic activity, of honey, **31.162** (rev. 1987)
Diazepam, in drug tablets, **40.A01*** (final 1988)
Diazinon, in formulations, **6.425** (corr. 1985; final 1988)
4,6-Dichloro-*N*-(2-chlorophenyl)-1,3,5-triazin-2-amine (anilazine), **6.D13***
3-(2,2-Dichloroethenyl)-2,2-dimethylcyclopropanecarboxylic acid cyano(3-phenoxyphenyl)methyl ester (cypermethrin), **6.A01*** (final 1987), **6.B01***
3-(2,2-Dichloroethenyl)-2,2-dimethylcyclopropanecarboxylic acid (3-phenoxyphenyl)methyl ester (permethrin), **6.B06*** (final 1988)
Dicofol, in formulations, **6.B22***
Dicumarol, in tablets, **37.D10***
Dieldrin, residues in fruits and vegetables, **29.A01*** (final 1986; rev. 1987)
Diethylpropion, drug substance and tablets, **37.D01***
Diethylstilbestrol, in feeds, **42.059** (surplus 1988)
2,3-Dihydro-2,2-dimethyl-7-benzofuranol methylcarbamate (carbofuran), **6.B45***, **29.A05*** (final 1986)
2-(Dimethylamino)-*N*-[[(methylamino)carbonyl]oxy]-2-oxo-ethanimidothioic acid methyl ester (oxamyl), **29.A05*** (final 1986)
4-(Dimethylamino)-3-methylphenol methylcarbamate (ester) (aminocarb), **6.A25*** (final 1987)
2,2-Dimethyl-1-,3-benzodioxol-4-ol methylcarbamate (bendiocarb), **6.B40***
3,5-Dimethyl-4-(methylthio)phenol methylcarbamate (methiocarb), **29.A05*** (final 1986)
Dimetridazole, in feeds, **42.063** (rev. 1988; final 1988)
2,4-Dinitrotoluene (DNT), as waste in waters, **33.B01***
Disinfectants, sporicidal activity, **4.033** (rev. 1985)
tuberculocidal activity, **4.036** (rev. 1987; repealed 1988), **4.039** (rev. 1987; repealed 1988)
use-dilution methods, **4.007** (rev. 1987; repealed 1988), **4.010** (rev. 1987; repealed 1988), **4.011** (rev. 1987)
Distilled liquors, alcohol in, **9.020** (rev. 1987), **9.026** (final 1985), **9.119** (final 1985)
color units of whisky, **9.002** (final 1985)
total acidity in, **9.135** (final 1988)
DNA colony hybridization method, detection of *E. coli* enterotoxins, **46.035** (final 1987), **46.B08*** (rev. 1987; final 1987)
detection of *Salmonella*, **46.C07***
Drugs: acidic, acetaminophen, **37.C01***
allopurinol in tablets, **37.A01*** (final 1987)
chlorpropamide in tablets, **37.B01***
coumarin anticoagulants in tablets, **37.D10***
dicumarol in tablets, **37.D10***
diethylpropion HCl drug substance and tablets, **37.D01***
methyldopa and methydopa-chlorothiazide in tablets, **37.A11*** (final 1987)
phenprocoumon in tablets, **37.D10***
primidone in tablets, **37.A17** (interim 1985; 1986*; final 1988)
sulfamethoxazole, **37.A07*** (final 1987)
sulfisoxazole in dosage forms, **37.166** (final 1986)
warfarin sodium in tablets, **37.D10***
Drugs: alkaloids and related bases, colchicine drug substance and dosage forms, **38.A01*** (final 1987)
physostigmine in dosage forms, **38.074** (final 1987)
pilocarpine, isopilocarpine, and pilocarpic acid in drugs, **38.144** (final 1987)
Drugs: general, amitriptyline, in tablets and injectables, **36.207** (final 1986)
flucytosine in capsules, **36.D01***
fluoride in tablets and solutions, **36.063** (final 1986)
levodopa and levodopa–carbidopa in solid dosage forms, **36.D07***
mercury in dosage forms, **36.085** (final 1985)
methocarbamol in dosage forms, **36.212** (final 1988)
microchemical tests for phenothiazine drugs, **36.A07*** (final 1988)
phenethylamines, **36.176** (corr. 1985)
trimethobenzamide in dosage forms, **36.A01*** (final 1988)
Drugs: illicit, amphetamine, determination of enantiomers, **40.D01***
diazepam in tablets, **40.A01*** (final 1988)
Drugs: steroids and hormones, conjugated estrogens, **39.001** (surplus 1988)
cortisone acetate bulk drug and dosage forms, **39.D01***
dexamethasone acetate bulk drug and suspensions, **39.D09***
dexamethasone drug substance and elixirs, **39.D17***
dexamethasone phosphate in dosage forms, **39.056** (surplus 1988)
hydrocortisone drug substance and tablets, **39.047** (final 1988)
prednisolone in tablets and bulk, **39.B01***
Drugs in feeds, antibiotics, **42.203** (corr. 1985)
arprinocid, **42.021** (surplus 1988), **42.027** (surplus 1988)
diethylstilbestrol, **42.059** (surplus 1988)
dimetridazole, **42.063** (rev. 1988; final 1988)
furazolidone, **42.077** (final 1988), **42.A01*** (final 1988)
melengestrol acetate in feed supplements, **42.088** (final 1988)
roxarsone, **42.B01***
sulfaquinoxaline, **42.179** (final 1988)
zoalene, **42.077** (final 1988)
Dry substance, in corn syrups, **31.226** (rev. 1985*; rev. final 1986)

Earthenware, cadmium and lead in, **25.024** (rev. 1988)
Eggs and egg products, aflatoxins in eggs, **26.070** (final 1988)
Salmonella in, **46.B21***
Salmonella in powdered eggs, **46.A06*** (final 1986)
EPTC, in formulations, **6.581** (final 1988)
Erucic acid, in oils and fats, **28.A14***
Erythromycin, in milk, **16.D01***
Escherichia coli, detection of enterotoxins, **46.B08*** (rev. 1987; final 1987)
detection of heat-labile enterotoxins, **46.035** (final 1987)
enterotoxin detection by mouse adrenal cell and suckling mouse assay, **46.049** (final 1987)
in chilled and frozen foods, **46.D05***
in foods, **46.030** (final 1985)
invasiveness of mammalian cells, **46.020** (final 1987)
Estrogens, conjugated, in drugs, **39.001** (surplus 1988)
Ethanol, in beer, **10.028** (final 1988)
in canned salmon, **18.B01***
Ether extract, of meat, **24.005** (rev. 1985)
Ethion, in formulations, **6.436** (corr. 1985)
2-[[Ethoxy[(1-methylethyl)amino]phosphinothioyl]oxy]benzoic acid 1-methylethyl ester (isofenphos), **6.C01***
Ethylene dibromide, residues in grains and grain products, **29.B01*** (final 1987)
3-(1-Ethylpropyl)phenol methylcarbamate, mixture with 3-(1-methylbutyl)phenyl methylcarbamate (1:3) (bufencarb), **29.A05*** (final 1986)
Evaporated milk, tin in, **25.A01*** (final 1988)
Excrement, insect excreta in flour, **44.054** (final 1988)
mammalian feces, coprostanol as indicator, **44.D01***
mammalian feces in corn meal, **44.B01***
of birds and insects on foods and containers, **44.186** (rev. 1986), **44.B07***
urine stains on foods and containers, **44.163** (rev. 1987)
Extract, of malting barley, **10.120** (final 1988)
Extraneous materials: isolation, see also specific product
aphids in hops, **10.182** (final 1988), **44.009** (final 1988)
coprostanol as indicator of mammalian feces, **44.D01***
excrement (bird and insect) on foods and containers, **44.186** (rev. 1986), **44.B07***
filth, in alimentary pastes, **44.069** (final 1988)
in apple butter, **44.086** (final 1988)
in breading of frozen food products, **44.068** (final 1988)
in candy, **44.103** (final 1988)
in canned citrus and pineapple juices, **44.095** (final 1988)

in canned crabmeat, **44.080** (final 1988)
in canned fish and fish products, **44.082** (final 1988)
in canned shrimp, **44.083** (final 1988)
in cocoa, chocolate, and press cake, **44.006** (final 1988)
in corn and rice cereals and corn chip products, **44.070** (final 1988)
in corn chips, **44.102** (final 1988)
in crude and refined papain, **44.161** (final 1988)
in crude plant gums, **44.158** (final 1988)
in dehydrated potato products, **44.117** (final 1988)
in green leafy vegetables, **44.110** (final 1988)
in ground coffee and coffee substitutes, **44.008** (final 1988)
in infant cereals, **44.072** (final 1988)
in jam and jelly, **44.094** (final 1988)
in meat and meat products, **44.084** (final 1988)
in peanut butter, **44.033** (final 1988)
in pecans, **44.030** (final 1988)
in potato chips, **44.101** (final 1988)
in pureed infant food, **44.113** (final 1988)
in raisins, **44.097** (final 1988)
in rice flours (powders), extruded rice products, and rice paper, **44.055** (final 1988)
in sage, **44.A07***
in sauerkraut, **44.118** (final 1988)
in shelled nuts, **44.028** (final 1988)
in shredded coconut, **44.032** (final 1988)
in sirups, molasses, and honey, **44.105** (final 1988)
in soy flour, **44.060** (final 1988)
in spices and condiments, **44.125** (final 1988), **44.129** (final 1988), **44.130** (final 1988), **44.131** (final 1988), **44.140** (final 1988), **44.142** (final 1988), **44.145** (final 1988), **44.148** (final 1988), **44.155** (final 1988), **44.156** (final 1988)
in starch, **44.062** (final 1988)
in sugars, **44.106** (final 1988)
in tea, **44.014** (final 1988)
in tomato products, **44.119** (final 1988)
in unground marjoram, **44.A04***
in wheat gluten, **44.061** (final 1988)
in white breads and high-fat products, **44.067** (final 1988)
in white flour, **44.052** (final 1988)
in whole leaves of alfalfa, papaya, and spearmint, **44.A06** (1988*)
in whole spearmint leaves, **44.A06***
in whole wheat cereals, **44.071** (final 1988)
Geotrichum mold count, in canned vegetables, fruits, and juices, **44.219** (final 1988)
in citrus juices, **44.218** (final 1988)
in comminuted fruits and vegetables, **44.220** (final 1988)
in cream style corn, **44.223** (final 1988)
in soft drinks, **44.217** (final 1988)
Howard mold count in dehydrated tomato powder, **44.211** (final 1988)
in fruit nectars, purees, and pastes, **44.201** (final 1988)
in ground spices, **44.213** (corr. 1986)
in canned corn **44.109** (final 1988; corr. 1988)
insect excreta in flour, **44.054** (final 1988)
insect infestation (internal) of wheat, **44.040** (final 1988)
insect infestation of oats, **44.A01***
insect penetration through packaging materials, **44.162** (final 1988)
insects in frozen fruits, **44.089** (final 1988)
isolation techniques, **44.001** (rev. 1987)
mammalian feces in corn meal, **44.B01***
rot fragments in comminuted tomato products, **44.224** (rev. 1985)
sediment in dairy products, **44.021** (final 1988)
Table 44:01 (rev. 1987)
urine stains on foods and containers, **44.163** (rev. 1987)

Fat, crude fat in meat, **24.005** (rev. 1985)
crude fat in meat and poultry products, **24.A04***
in cream, **16.177** (rev. 1988)
in milk, **16.065** (rev. 1986), **16.074** (surplus 1987), **16.083** (rev. 1988), **16.B02***
in milk-based infant formula, **43.B02***
Fat acidity, of grains, **14.069** (rev. 1988)
Fats, see Oils and fats
Fatty acids, crude, in oils and fats, **28.033** (rev. 1985; final 1985)
melting point, **28.012** (rev. 1986)
FD&C colors, in foods, **34.D01***
Feces, mammalian, detection of coprostanol as indicator, **44.D01***
mammalian, in corn meal, **44.B01***
Feeds, see also Drugs in feeds
aflatoxins in, **26.014** (final 1988)
crude protein in animal feeds, **7.021** (rev. 1987), **7.D01***
nitrate in forages, **49.B05***
phosphorus in, **7.125** (rev. 1988)
sulfur amino acids in feed ingredients, **43.A08*** (final 1986)
Fenitrothion, technical and formulations, **6.A19***
Fensulfothion, in formulations, **6.B28***
Fertilizers, boron in, **2.135** (final 1985)
iron in, **2.157** (rev. 1986)
melamine in, **2.D01***
potassium in, **2.097** (final 1985); **2.108** (surplus 1986); **2.114** (surplus 1986)
sampling, **2.001** (rev. 1987)
sulfur in, **2.182** (final 1985)
triamino-*s*-triazine in, **2.D01***
Fiber, total dietary fiber in foods, **43.A14*** (rev. 1986; final 1986; rev. 1987)
Filth, see Extraneous materials: isolation
Fish and other marine products, ethanol in canned salmon, **18.B01***
filth in breading of frozen products, **44.068** (final 1988)
filth in canned crabmeat, **44.080** (final 1988)
filth in canned products, **44.082** (final 1988)
filth in canned shrimp, **44.083** (final 1988)
methyl mercury in, **25.146** (final 1988), **25.D01***
minced fish flesh in mixed fillet–minced cod blocks, **18.D01***
paralytic shellfish poison in, **18.086** (rev. 1988)
parasites in fish muscle, **18.A01** (procedure 1985)
pesticide and PCB residues in, **29.029** (final 1985)
poliovirus 1 in oysters, **46.A12***
total sulfites in shrimp, **20.C01***
V. cholerae in oysters, **46.D09***
Flour, filth in, **44.052** (final 1988), **44.055** (final 1988), **44.060** (final 1988)
insect excreta in, **44.054** (final 1988)
starch in, **14.031** (surplus 1987)
Fluazifop-butyl, technical and formulations, **6.353** (final 1985)
Flucytosine, in capsules, **36.D01***
Fluoride, in drug tablets and solutions, **36.063** (final 1986)
in plants, **3.075** (final 1988)
Food, see specific analyte or commodity
Food additives: direct, saccharin, **20.204** (corr. 1985)
total sulfites in foods, **20.C01***
Food additives: indirect, acrylonitrile in food, **21.A01***
nitrosamines in baby bottle rubber nipples, **21.C01***
nitrosamines in fried bacon, **24.054** (final 1987)
N-nitrosodimethylamine in beer, **10.098** (final 1988)
Food Chemicals Codex, –U.S. Pharmacopeia–AOAC method for mixed tocopherols concentrate, **43.D01***
Food packaging, excrement (bird and insect) on. **44.186** (rev. 1986), **44.B07***
insect penetration, **44.162** (final 1988)
urine stains on, **44,163** (rev. 1987)
Frozen foods, filth in breading of fish products, **44.068** (final 1988)
insects in blackberries and raspberries, **44.089** (final 1988)
Fructose, in wines, **11.A01***
Fruits and fruit products, acidity (titratable) of fruit products, **22.058** (rev. 1988)
acids in juices, **22.B01***
adulteration of Florida orange juice, **22.B06*** (rev. 1987)
filth in apple butter, **44.086** (final 1988)
filth in canned citrus and pineapple juices, **44.095** (final 1988)
filth in jam and jelly, **44.094** (final 1988)
filth in raisins, **44.097** (final 1988)
insects in frozen blackberries and raspberries, **44.089** (final 1988)
mold count in canned fruits and juices, **44.219** (final 1988)
mold count in citrus juices, **44.218** (final 1988)
mold count in comminuted products, **44.220** (final 1988)
mold count in nectars, purees, and pastes, **44.201** (final 1988)
pesticide multiresidue methods, **29.A01*** (final 1986), **29.A05*** (final 1986)
tin in canned pineapple juice, **25.A01***
total sulfites in apricots, **20.C01***
total sulfites in orange juice, **20.C01***
vitamin C in, **43.076** (final 1985)
Furazolidone, in feeds, **42.077** (final 1988), **42.A01*** (final 1988)

Gelatin, dessert preparations, and mixes, pentachlorophenol residues in gelatin, **29.A14***

Genetic probe method, detection of *E. coli* enterotoxins, 46.035 (final 1987), 46.B08* (rev. 1987; final 1987)
detection of *Salmonella*, 46.C07*
***Geotrichum* mold count**, 44.217 (final 1988), 44.218 (final 1988), 44.219 (final 1988), 44.220 (final 1988), 44.223 (final 1988)
Gercock bottle, 16.B02*
Glucose, in wines, 11.A01*
Glycerides, in oils and fats, 28.B01*
Glycerol and glycols, in tobacco, 3.155 (final 1985)
Grains, see also Cereal foods
fat acidity, 14.069 (rev. 1988)
insect infestation (internal) in wheat, 44.040 (final 1988)
ochratoxins in barley, 26.111 (final 1988)
sterigmatocystin in barley and wheat, 26.132 (final 1988)
zearalenone and α-zearalenol in corn, 26.A09* (final 1988)
zearalenone in corn, 26.139 (final 1988)
Grapes, carbamate insecticide residues in, 29.A05* (final 1986)
Green beans, tin in canned products, 25.A01* (final 1988)
Groundwater, 2,4-DNT, TNT, RDX, and HMX in, 33.B01*
Gums, filth in crude plant gums, 44.158 (final 1988)

Hazardous substances, nitrosamines in latex infant pacifiers, 5.B01*
Helium leak test, bacteria in canned low acid foods, 46.071 (rev. 1987; final 1987)
High fructose corn syrup, dry substance, 31.226 (rev. 1985*; rev. final 1986)
in honey, 31.156 (rev. 1988), 31.158 (rev. 1988)
HMX, as waste in waters, 33.B01*
Holaday–Velasco minicolumn method, 26.020 (final 1988)
Honey, acrylonitrile in honey butter, 21.A01*
color classification, 31.A01* (final 1986)
corn syrups in, 31.156 (rev. 1988), 31.158 (rev. 1988)
diastatic activity, 31.162 (rev. 1987)
filth in, 44.105 (final 1988)
sugars in, 31.128 (rev. 1987)
Hops, aphids in, 10.182 (final 1988), 44.009 (final 1988)
Horseradish, filth in prepared product, 44.155 (final 1988)
Howard mold count, 44.201 (final 1988), 44.211 (final 1988)
Humectants, in tobacco, 3.155 (final 1985)
Hybridization method, detection of *E. coli* enterotoxins, 46.035 (final 1987), 46.B08* (rev. 1987; final 1987)
detection of *Salmonella*, 46.C07*
Hydrocortisone, drug substance and tablets, 39.047 (final 1988)
Hydrophobic grid membrane filter method, aerobic plate count of foods, 46.B01* (final 1987)
coliforms and *E. coli* in foods, 46.030 (final 1985)
Salmonella in foods, 46.A06* (final 1986)

Immunoassays, detection of adulteration of meat and poultry products, 24.C01*
Salmonella in foods, 46.B21* (rev. 1987; final 1988), 46.C17*
soy protein in meat products, 24.D01*
Inductively coupled plasma emission spectroscopy method, elemental analysis of plants, 3.A01* (final 1988)
Infant foods, filth in cereals, 44.072 (final 1988)
filth in pureed food, 44.113 (final 1988)
nutrients in formula products, 43.292 (final 1986)
proximate analysis of milk-based formula, 43.B01* (final 1988)
vitamins and nutrients in milk-based formula, 43.A21* (amended 1986; final 1988), 43.B01* (final 1988)
Infant products, nitrosamines in baby bottle rubber nipples, 21.C01*
nitrosamines in pacifiers, 5.B01*
Insects, in frozen blackberries and raspberries, 44.089 (final 1988)
in wheat, 44.040 (final 1988)
penetration through packaging materials, 44.162 (final 1988)
Iodine, iodine-131 in foods, 48.B01* (rev. 1987)
Iron, in fertilizers, 2.157 (rev. 1986)
in milk-based infant formula, 43.A37* (final 1988)
in plants, 3.006 (final 1988), 3.013 (final 1988)
ISO, pycnometer method for apparent weight per unit volume and specific gravity of oils and fats, 28.A01*
Isofenphos, technical and formulations, 6.C01*
IUPAC, IUPAC-AOAC method for erucic acid in oils and fats, 28.A14*
IUPAC–AOAC method for triglycerides in oils and fats, 28.B01*

Kelthane (dicofol), in formulations, 6.B22*
Kjeldahl methods, automated method for crude protein in animal feeds, 7.021 (rev. 1987)
$CuSO_4/TiO_2$ mixed catalyst method for crude protein in animal feeds, 7.D01*

β-Lactam antibiotics, in milk, 16.152 (rev. 1987)
Lactoglobulin, sulfur amino acids in, 43.A08* (final 1986)
Lactose, in milk, 16.059 (final 1985), 16.083 (rev. 1988)
purity, 31.064 (final 1988)
Latex infant pacifiers, nitrosamines in, 5.B01*
Lead, in cookware, 25.016 (final 1986)
in earthenware, 25.024 (rev. 1988)
trace levels in food, 25.001 (final 1988), 25.008 (rev. 1987; final 1988)
Lead number, of maple products, 31.191 (repealed 1988), 31.192 (repealed 1988)
Lettuce, pesticide multiresidues, 29.A01* (final 1986; rev. 1987)
Levodopa, and levodopa–carbidopa in solid dosage forms, 36.D07*
Lexone (metribuzin), technical and formulations, 6.553 (final 1986)
Lindane, in formulations, 6.221 (final 1986)
Liver, aflatoxin M_1 in, 29.110 (surplus 1988)
aflatoxins B_1 and M_1 in, 26.101 (final 1988), 26.107 (final 1988)
arsenic in, 49.B01*
copper in, 49.A01* (final 1987)
Lotion, γ-BHC in, 6.221 (final 1986)

Magnesium, in milk-based infant formula, 43.A37* (final 1988)
in plants, 3.006 (final 1988), 3.013 (final 1988), 3.A01* (final 1988)
Malic acid, in fruit juices, 22.B01*
in wines, 11.052 (final 1988)
Malt beverages, α-amylase in malt, 10.155 (rev. 1988)
aphids in hops, 10.182 (final 1988), 44.009 (final 1988)
ethanol in beer, 10.028 (final 1988)
extract of malting barley, 10.120 (final 1988)
N-nitrosodimethylamine in beer, 10.098 (final 1988)
potassium in beer, 10.C01*
sodium in beer, 10.C06*
specific gravity of beer and malt, 10.D01*
total solids in liquid and pressed yeast, 10.222 (final 1988)
Manganese, in milk-based infant formula, 43.A37* (final 1988)
in plants, 3.006 (final 1988), 3.013 (final 1988), 3.A01* (final 1988)
Manufacturers and suppliers, xxiii and xxiv (14th ed.) (rev. 1987)
Maple products, Canadian lead number, 31.191 (repealed 1988)
corn syrup and cane sugar in maple syrup, 31.185 (final 1988)
Winton lead number, 31.192 (repealed 1988)
Margarine, acrylonitrile in, 21.A01*
total *trans* isomers in, 28.A06* (rev. 1986)
Marine toxins, paralytic shellfish poison in seafood, 16.086 (rev. 1988)
Marjoram, filth in unground product, 44.A04*
Meat and meat products, adulteration detection, 24.C01*
aflatoxin M_1 in liver, 26.110 (surplus 1988)
aflatoxins B_1 and M_1 in liver, 26.101 (final 1988), 26.107 (final 1988)
calcium in deboned poultry and beef, 24.062 (final 1985; rev. 1987)
crude fat in, 24.A04*
crude fat or ether extract, 24.005 (rev. 1985)
filth in pork sausage (uncooked) and ground beef or hamburger, 44.084 (final 1988)
moisture in, 24.A01*
nitrosamines in fried bacon, 24.054 (final 1987)
organochlorine residues in beef and swine fats, 29.037 (1985*; final 1986)
organochlorine residues in poultry fat, 29.037 (final 1985)
Salmonella in raw poultry, 46.A06* (final 1986)
salt in, 24.010 (rev. 1986; final 1987)
sample preparation, 24.001 (rev. 1985)
soy protein in meat products, 24.D01*
species identification, 24.C01*
tin in canned products, 25.A01*
Melamine, in fertilizers, 2.D01*
Melengestrol acetate, in feed supplements, 42.088 (final 1988)
Melting point, fats and fatty acids, 28.012 (rev. 1986)
Membrane filter method, aerobic plate count of foods, 46.B01* (final 1987)
coliforms and *E. coli* in foods, 46.030 (final 1985)
Salmonella in foods, 46.A06* (final 1986)
Mercury, in drug dosage forms, 36.085 (final 1985)
methyl mercury in fish and shellfish, 25.146 (final 1988), 25.D01*

Metals and other elements, see also specific analyte
arsenic, trace levels in food, **25.001** (final 1988)
arsenic in liver tissue, **49.B01***
as nutrients in milk-based infant formula, **43.A37***
cadmium, trace levels in food, **25.001** (final 1988)
cadmium and lead in cookware, **25.016** (final 1986)
cadmium and lead in earthenware **25.024** (rev. 1988)
cadmium and lead in food, **25.008** (rev. 1987; final 1988)
copper in liver, **49.A01***
copper in serum, **49.003** (final 1986)
elemental analysis of infant formula, **43.292** (final 1986)
fluoride in plants, **3.075** (final 1988)
in plants, **3.006** (final 1988), **3.013** (final 1988), **3.075** (final 1988), **3.A01*** (final 1988)
lead, trace levels in food, **25.001** (final 1988)
lead and cadmium in cookware, **25.016** (final 1986)
lead and cadmium in earthenware, **25.024** (rev. 1988)
lead and cadmium in food, **25.008** (rev. 1987; final 1988)
mercury in drugs, **36.085** (final 1985)
methyl mercury in fish and shellfish, **25.146** (final 1988), **25.D01***
multielement analysis in foods, **25.001** (final 1988)
selenium, trace levels in food, **25.001** (final 1988)
tin in canned foods, **25.A01*** (final 1988)
tin in food, **25.161** (surplus 1986)
zinc, trace levels in food, **25.001** (final 1988)
Methamidophos, residues in fruits and vegetables, **29.A01*** (final 1986)
Methiocarb, residues in fruits and vegetables, **29.A05*** (final 1986)
technical and formulations, **6.546** (final 1987)
Methionine, in food and feed ingredients, **43.A08*** (final 1988)
in processed foods, **43.A08** (1988*)
Methocarbamol, in dosage forms, **36.212** (final 1988)
Methomyl, residues in fruits and vegetables, **29.A05*** (final 1986)
N*-[[(Methylamino)carbonyl]oxy]ethanimidothioic acid methyl ester (methomyl)**, **29.A05 (final 1986)
6-Methyl-1,3-dithiolo[4,5,-b]quinoxalin-2-one (oxythioquinox), **6.B34***
Methyldopa, and methyldopa with chlorothiazide in drug tablets, **37.A11*** (final 1987)
Methyl mercury, in fish and shellfish, **26.146** (final 1988), **25.D01***
2-Methyl-2-(methylthio)propanal *O*-[(methylamino)carbonyl]-oxime (aldicarb), **29.A05*** (final 1986)
Metolachlor, in formulations, **6.A14*** (final 1988)
Metribuzin, technical and formulations, **6.553** (final 1986)
Microbiological methods, aerobic plate count of frozen, chilled, precooked, or prepared foods, **46.013** (rev. 1987), **46.B01*** (final 1987), **46.D01***
antibiotics in feeds, **42.203** (corr. 1985)
bacteria in low acid canned foods, **46.071** (rev. 1987; final 1987)
DNA colony hybridization method for detection of *E. coli* enterotoxins, **46.035** (final 1987), **46.B08*** (rev. 1987; final 1987)
DNA colony hybridization method for detection of *Salmonella*, **46.C07***
dry rehydratable film method for bacterial and coliform counts in milk, **46.B05*** (final 1988)
E. coli in chilled and frozen foods, **46.D05***
E. coli invasiveness of mammalian cells, **46.020** (final 1987)
enzyme immunoassay of *Salmonella* in foods, **46.B21*** (rev. 1987; final 1988), **46.C17***
helium leak test for bacteria in canned low acid foods, **46.071** (rev. 1987; final 1987)
hydrophobic grid membrane filter method, for aerobic plate count of foods, **46.B01*** (final 1987)
for coliforms and *E. coli* in foods, **46.030** (final 1985)
for *Salmonella* in foods, **46.A06*** (final 1986)
in vivo and in vitro detection of *E. coli* enterotoxins, **46.049** (final 1987)
most probable number of *S. aureus* in food ingredients and nonprocessed foods, **46.062** (repealed 1987), **46.C01***
niacin and niacinamide in milk-based infant formula, **43.A32***
optical somatic cell counting in milk, **46.171** (rev. 1987)
poliovirus 1 in oysters, **46.A12***
Salmonella in foods, **46.117** (rev. 1986), **46.A06*** (final 1986), **46.B21*** (rev. 1987; final 1988), **46.C17***
S. aureus in food ingredients and nonprocessed foods, **46.062** (repealed 1987), **46.C01***
somatic cells in milk, **46.171** (rev. 1987)
tissue culture method for invasiveness of mammalian cells by *E. coli*, **46.020** (final 1987)
V. cholerae in oysters, **46.D09***
vitamin B_6 in milk-based infant formula, **43.A24*** (amended 1986; final 1988)
Microchemical tests, phenothiazine drugs, **36.A07*** (final 1988)
Microwave oven methods, crude fat in meat and poultry products, **24.A04***
moisture in meat and poultry products, **24.A01***
total solids in canned tomato products, **32.A01***
Milk, added water in, **16.101** (surplus 1987)
aflatoxin M_1 in, **26.090** (rev. 1987; final 1988)
aflatoxins in fluid product, **26.B02***
antibiotics in, **16.135** (rev. 1986), **16.152** (rev. 1987), **16.D01***
dry rehydratable film method for bacterial and coliform counts in, **46.B05***
fat in, **16.065** (rev. 1986), **16.074** (surplus 1987), **16.083** (rev. 1988), **16.B02***
lactose in, **16.059** (final 1985), **16.083** (rev. 1988)
protein in, **16.083** (rev. 1988)
somatic cells in, **46.171** (rev. 1987)
Moisture, in meat and poultry products, **24.A01***
in oils and fats, **28.003** (final 1985)
in spices, **30.005** (repealed 1987)
in spices and other condiments, **30.B01*** (final 1988)
Molasses, filth in, **44.105** (final 1988)
Mold, in citrus juices, **44.218** (final 1988)
in comminuted fruits and vegetables, **44.220** (final 1988)
in cream style corn, **44.223** (final 1988)
in dehydrated tomato powder, **44.211** (final 1988)
in fruit nectars, purees, and pastes, **44.201** (final 1988)
in ground spices, **44.213** (corr. 1986)
in soft drinks, **44.217** (final 1988)
in vegetables, fruits, and juices (canned), **44.219** (final 1988)
Molinate, in formulations, **6.581** (final 1988)
Monocrotophos, residues in fruits and vegetables, **29.A01*** (final 1986; rev. 1987)
Morestan (oxythioquinox), in formulations, **6.B34***
Most probable number (MPN), of *S. aureus* in food ingredients and nonprocessed foods, **46.062** (repealed 1987), **46.C01***
Multiresidue methods, carbamate insecticides, **29.A05*** (final 1986)
organochlorine and organophosphorus pesticides, **29.A01*** (final 1986)
organochlorine pesticides in beef and swine fats, **29.037** (1985*; final 1986)
organochlorine pesticides in fish, **29.029** (final 1985)
organochlorine pesticides in poultry fat, **29.037** (final 1985)
PCBs in fish, **29.029** (final 1985)
Mustard, filth in prepared product, **44.156** (final 1988)
Mycotoxins, see also Aflatoxins
deoxynivalenol in wheat, **26.B07***, **26.B13***
general reagents, **26.002(a)** (corr. 1986)
ochratoxin A in green coffee, **26.119** (final 1988)
ochratoxins in barley, **26.111** (final 1988)
sterigmatocystin in barley and wheat, **26.132** (final 1988)
zearalenone and α-zearalenol in corn, **26.A09*** (final 1988)
zearalenone in corn, **26.139** (final 1988)

1-Naphthalenol methylcarbamate (carbaryl), **29.A05*** (final 1986)
Niacin and niacinamide, in milk-based infant formula, **43.A32*** (final 1988)
Nitrate, in forages, **49.B05***
Nitrogen, in allergenic drug extracts, **38.205** (final 1985; amended 1986), **38.B01***
in animal feeds, **7.021** (rev. 1987), **7.D01***
Nitrosamines, in baby bottle rubber nipples, **21.C01***
in beer, **10.098** (final 1988)
in fried bacon, **24.054** (final 1987)
N*-Nitrosodibutylamine**, in latex infant pacifiers, **5.B01
***N*-Nitrosodimethylamine**, in beer, **10.098** (final 1988)
Nonalcoholic beverages, mold count in soft drinks, **44.217** (final 1988)
Nonfat dry milk, *Salmonella* in, **46.117** (rev. 1986), **46.A06*** (final 1986)
sulfur amino acids in, **43.A08*** (final 1986)
Novobiocin, in milk, **16.D01***
Nutmeg, filth in ground product, **44.140** (final 1988)
filth in reconditioned product, **44.142** (final 1988)
Nuts and nut products, see also Peanuts and peanut products
aflatoxins in almonds, **26.014** (final 1988)
aflatoxins in pistachio nuts, **26.014** (final 1988), **26.067** (final 1988)
filth in pecans, **44.030** (final 1988)
filth in shelled nuts, **44.028** (final 1988)

Ochratoxins, in barley, **26.111** (fina 1988)
in green coffee, **26.119** (final 1988)
Oils and fats, see also Fat
acids (volatile) in, **28.039** (rev. 1985)
acrylonitrile in margarine, **21.A01***
erucic acid in, **28.A14***
fatty acids (crude) in, **28.033** (rev. 1985; final 1985)
melting point of fats and fatty acids, **28.012** (rev. 1986)
moisture in, **28.003** (final 1985)
specific gravity, **28.A01***
specific gravity of oils, **28.006** (repealed 1985), **28.008** (repealed 1985)
total *trans* isomers in margarines, **28.A06*** (rev. 1986)
triglycerides in, **28.B01***
weight (apparent) per unit volume, **28.A01***
Omethoate, residues in fruits and vegetables, **29.A01*** (final 1986; rev. 1987)
Optical somatic cell counting, in milk, **46.171** (rev. 1987)
Orange juice, adulteration, **22.B06*** (rev. 1987)
total sulfites in, **20.C01***
Organochlorine pesticide residues, BHC, **29.A01*** (final 1986; rev. 1987)
DDT, **29.A01*** (final 1986; rev. 1987)
dieldrin, **29.A01*** (final 1986; rev. 1987)
in beef and swine fats, **29.037** (1985*; final 1986)
in fish, **29.029** (final 1985)
in poultry fat, **29.037** (final 1985)
pentachlorophenol, **29.A14***
Organohalogen pesticides, alachlor, **6.A05*** (final 1987), **6.D07***
butachlor, **6.B12*** (final 1988)
chlordimeform, **6.A09*** (final 1987)
dalapon, **6.296** (rev. 1987; final 1987)
dicofol, **6.B22***
fluazifop-butyl, **6.353** (final 1985)
metolachlor, **6.A14*** (final 1988)
propachlor, **6.B17*** (final 1988)
Organophosphorus pesticide residues, acephate, **29.A01*** (final 1986; rev., 1987)
chlorpyrifos, **29.A01*** (final 1986; rev. 1987)
methamidophos, **29.A01*** (final 1986; rev. 1987)
monocrotophos, **29.A01*** (final 1986; rev. 1987)
omethoate, **29.A01*** (final 1986; rev. 1987)
Organophosphorus pesticides, fenitrothion, **6.A19***
fensulfothion, **6.B28***
isofenphos, **6.C01***
Oxamyl, residues in fruits and vegetables, **29.A05*** (final 1986)
Oxythioquinox, in formulations, **6.B34***
Oysters, poliovirus 1 in, **46.A12***
V. cholerae in, **46.D09***

Pacifiers, nitrosamines in latex infant pacifiers, **5.B01***
Packaging materials, excrement (bird and insect) on, **44.186** (rev. 1986), **44.B07***
insect penetration, **44.162** (final 1988)
Papain, filth in crude and refined products, **44.161** (final 1988)
Paprika, filth in, **44.145** (final 1988)
Paralytic shellfish poison, **18.086** (rev. 1988)
Parasites, in fish muscle, **18.A01** (procedure 1985)
PCBs, residues in fish, **29.029** (final 1985)
Peanuts and peanut products, acrylonitrile in peanut butter, **21.A01***
aflatoxins in, **26.026** (final 1988), **26.032** (final 1988)
aflatoxins in peanuts, **26.020** (final 1988)
aflatoxins in peanuts, peanut butter, and peanut meal, **26.014** (final 1988)
filth in peanut butter, **44.033** (final 1988)
Pebulate, in formulations, **6.581** (final 1988)
Pecans, filth in, **44.030** (final 1988)
Penicillin, in milk, **16.135** (rev. 1986)
Penicillin G, in milk, **16.152** (rev. 1987), **16.D01***
Pentachlorophenol, residues in gelatin, **29.A14***
Pepper, filth in, **44.148** (final 1988)
piperine in, **30.C01***
Salmonella in, **46.A06*** (final 1986)
Peppermint, filth in whole leaves, **44.A06***
Perfenazine, microchemical test, **36.A07***
Permanganate oxidation number, of vinegars, **30.083** (rev. 1988)
Permethrin, in formulations, **6.B06*** (final 1988)
Pesticide formulations, alachlor, **6.A05*** (final 1987), **6.D07***
aminocarb, **6.A25*** (final 1987)
anilazine, **6.D13***
bendiocarb, **6.B40***
γ-BHC, **6.221** (final 1986)
butachlor, **6.B12*** (final 1988)
butylate, **6.581** (final 1988)
carbofuran, **6.B45***
chlordimeform, **6.A09*** (final 1987)
cycloate, **6.581** (final 1988)
cyhexatin, **6.D01***
cypermethrin, **6.A01*** (final 1987), **6.B01***
dalapon, **6.296** (rev. 1987; final 1987)
diazinon, **6.425** (corr. 1985; final 1988)
dicofol, **6.B22***
EPTC, **6.581** (final 1988)
ethion, **6.436** (corr. 1985)
fenitrothion, **6.A19***
fensulfothion, **6.B28***
fluazifop-butyl, **6.353** (final 1985)
fumigant mixtures, **6.159** (final 1985)
isofenphos, **6.C01***
methiocarb, **6.546** (final 1987)
metolachlor, **6.A14*** (final 1988)
metribuzin, **6.553** (final 1986)
molinate, **6.581** (final 1988)
oxythioquinox, **6.B34***
pebulate, **6.581** (final 1988)
permethrin, **6.B06*** (final 1988)
propachlor, **6.B17*** (final 1988)
propoxur, **6.565** (final 1987)
triadimefon, **6.A31*** (final 1987)
vernolate, **6.581** (final 1988)
Pesticide residues, acephate, **29.A01*** (final 1986; rev. 1987)
aldicarb, **29.A05*** (final 1986)
α-BHC, **29.A01*** (final 1986; rev. 1987)
bufencarb, **29.A05*** (final 1986)
carbamates, **29.A05*** (final 1986)
carbaryl, **29.A05*** (final 1986)
carbofuran, **29.A05*** (final 1986)
chlorpyrifos, **29.A01*** (final 1986; rev. 1987)
DDT, **29.A01*** (final 1986)
dieldrin, **29.A01*** (final 1986; rev. 1987)
ethylene dibromide, **29.B01*** (final 1987)
methamidophos, **29.A01*** (final 1986)
methiocarb, **29.A05*** (final 1986)
methomyl, **29.A05*** (final 1986)
monocrotophos, **29.A01*** (final 1986; rev. 1987)
multiresidue methods, **29.029** (final 1985), **29.A01*** (final 1986; rev. 1987), **29.A05*** (final 1986)
omethoate, **29.A01*** (final 1986; rev. 1987)
organochlorine pesticides, **29.029** (final 1985)
organochlorine pesticides in beef and swine fats, **29.037** (1985*; final 1986)
organochlorine pesticides in poultry fat, **29.037** (final 1985)
oxamyl, **29.A05*** (final 1986)
pentachlorophenol, **29.A14***
Phenethylamine drugs, **36.176** (corr. 1985)
Phenothiazine drugs, microchemical test, **36.A07*** (final 1988)
Phenprocoumon, in tablets, **37.D10***
Phenylpropanolamine, in drug dosage forms, **36.176** (corr. 1985)
Phosphatase (residual), in casein, **16.127** (rev. 1986), **16.B03***
Phosphorothioic acid O-O-diethyl O-[4-(methylsulfinyl)phenyl] ester (fensulfothion), **6.B28***
Phosphorothioic acid *O,O*-dimethyl *O*-(3-methyl-4-nitrophenyl) ester (fenitrothion), **6.A19***
Phosphorus in feeds, **7.125** (rev. 1988)
in milk-based infant formula, **43.B12***
in plants, **3.006** (final 1988), **3.A01*** (final 1988)
Physostigmine, in drug dosage forms, **38.074** (final 1987)
Phytate, in dried cereal foods, **14.B01*** (final 1988)
Pilocarpine, isopilocarpine, and pilocarpic acid, in drugs, **38.144** (final 1987)
Piperine, in pepper preparations, **30.C01***
Pistachio nuts, aflatoxins in, **26.014** (final 1988), **26.067** (final 1988)
Plants, fluoride in, **3.075** (final 1988)
metals and other elements in, **3.006** (final 1988), **3.013** (final 1988), **3.075** (final 1988), **3.A01*** (final 1988)
Plate counts, dry rehydratable film method for bacteria and coliform counts in milk, **46.B05***
Polenske value, of oils and fats, **28.039** (rev. 1985)
Potassium in beer, **10.C01***
in fertilizers, **2.097** (final 1985), **2.108** (surplus 1986), **2.114** (surplus 1986)
in milk-based infant formula, **43.A37*** (final 1988)

in plants, **3.006** (final 1988), **3.013** (final 1988), **3.A01*** (final 1988)
Potato chips, filth in, **44.101** (final 1988)
Potatoes, filth in dehydrated products, **44.117** (final 1988)
pesticide multiresidue methods, **29.A05*** (final 1986)
total dietary fiber in, **43.A14*** (rev. 1986; final 1986)
total sulfites in dehydrated product, **20.C01***
Poultry, calcium in deboned products, **24.062** (final 1985; rev. 1987)
crude fat in poultry products, **24.A04***
moisture in poultry products, **24.A01***
organochlorine residues in fat, **29.037** (final 1985)
Salmonella in raw meat, **46.A06*** (final 1986)
species identification for detection of adulteration, **24.C01***
Prednisolone, in drug tablets and bulk, **39.B01***
Primidone, in drug tablets, **37.A17** (interim 1985; 1986*; final 1988)
Promethazine, microchemical test, **36.A07***
Propachlor, in formulations, **6.B17*** (final 1988)
Propoxur, technical and formulations, **6.565** (final 1987)
Protein, crude protein in feeds, **7.021** (rev. 1987), **7.D01***
in milk, **16.083** (rev. 1988)
in milk-based infant formula, **43.B03***
Protein nitrogen unit precipitation method, **38.205** (final 1985; amended 1986), **38.B01***
Proximate analysis, of milk-based infant formula, **43.B01*** (final 1988)
Pyridoxine, pyridoxal, and pyridoxamine, in milk-based infant formula, **43.A24*** (amended 1986; final 1988)

Quinic acid, in fruit juices, **22.B01***

Radioactivity, iodine-131 and cesium-137 in foods, **48.B01*** (rev. 1987)
Raisins, filth in, **44.097** (final 1988)
RDX, as waste in waters, **33.B01***
Refractive index, for dry substance of corn syrups, **31.226** (rev. 1985*; rev. final 1986)
Reichert-Meissl value, of oils and fats, **28.039** (rev. 1985)
Riboflavin, in milk-based infant formula, **43.A23*** (final 1988)
Rice, filth in cereal, **44.070** (final 1988)
filth in rice flours (powders), extruded rice products, and rice paper, **55.055** (final 1988)
Roberts copper method, dextran in raw cane sugar, **31.D01***
Romer minicolumn method, **26.014** (final 1988)
Rot, in comminuted tomato products, **44.224** (rev. 1985)
Roxarsone, in feeds, **42.B01***

Saccharides, in corn syrups and sugars, **31.245** (corr. 1986)
Saccharin, in foods, **20.204** (corr. 1985)
Saccharomyces uvarum, as test organism, **43.A24(k)**
Sage, filth in rubbed and ground products, **44.A07***
Salmon, canned, ethanol in, **18.B01***
Salmonella, in foods, **46.117** (rev. 1986), **46.A06***, **46.B21*** (rev. 1987; final 1988), **46.C07***, **46.C17***
in nonfat dry milk, **46.117** (rev. 1986)
Salt, in meat, **24.010** (rev. 1986; final 1987)
Sample preparation, meat and meat products, **24.001** (rev. 1985)
milk-based infant formula, **43.A21*** (final 1988)
Sampling, barrel cheese, **16.B01***
fertilizers, **2.001** (rev. 1987)
Sauerkraut, filth in, **44.118** (final 1988)
Sediment, in dairy products, **44.021** (final 1988)
Selenium, trace levels in food, **25.001** (final 1988)
Sencor (metribuzin), technical and formulations, **6.553** (final 1986)
Serum, copper in, **49.003** (final 1986)
Shampoo, γ-BHC in, **6.221** (final 1986)
Shrimp, filth in canned product, **44.083** (final 1988)
total sulfites in, **20.C01***
Sodium, in beer, **10.C06***
in milk-based infant formula, **43.A37*** (final 1988)
Sodium chloride, in canned vegetables, **32.032** (rev. 1987)
in meat, **24.010** (rev. 1986; final 1987)
Solids, in canned tomato products, **32.A01***
in liquid and pressed yeast, **10.222** (final 1988)
in milk-based infant formula, **43.B04***
in vinegars, **30.063** (repealed 1987), **30.C06***
Somatic cells, in milk, **46.171** (rev. 1987)
Soybeans, aflatoxins in, **26.069** (final 1988)
Soy flour, filth in, **44.060** (final 1988)
Soy protein, in meat products, **24.D01***
Specific gravity, in beer and wort, digital density meter method, **10.D01***
of distilled liquors, **9.020** (rev. 1987)
of oils, **28.006** (repealed 1985), **28.008** (repealed 1985)
of oils and fats, **28.A01***
Spices and other condiments, see also specific product
filth in, **44.125** (final 1988), **44.129** (final 1988), **44.130** (final 1988), **44.131** (final 1988), **44.140** (final 1988), **44.142** (final 1988), **44.145** (final 1988), **44.148** (final 1988), **44.155** (final 1988), **44.156** (final 1988)
filth, in rubbed and ground sage, **44.A07***
in unground marjoram, **44.A04***
in whole peppermint leaves, **44.A06***
in whole spearmint leaves, **44.A06** (1988*)
moisture in, **30.B01*** (final 1988)
moisture in spices, **30.005** (repealed 1987)
mold in ground spices, **44.213** (corr. 1986)
permanganate oxidation number of vinegars, **30.083** (rev. 1988)
piperine in pepper preparations, **30.C01***
Salmonella in pepper, **46.A06*** (final 1986)
solids in vinegar, **30.063** (repealed 1987), **30.C06***
Sporeformers, in low-acid canned foods, **46.A01***
Sporicidal activity, of disinfectants, **4.033** (rev. 1985)
Staphylococcus aureus, in food ingredients and nonprocessed foods, **46.062** (repealed 1987), **46.C01***
Starch, filth in, **44.062** (final 1988)
in flour, **14.031** (surplus 1987)
Sterigmatocystin, in barley and wheat, **26.132** (final 1988)
Strawberries, pesticide multiresidue method, **29.A01*** (final 1986; rev. 1987)
Streptomycin, in milk, **16.D01***
Strontium, in plants, **3.006** (final 1988)
Sugars and sugar products, acrylonitrile in honey butter, **21.A01***
Canadian lead number of maple products, **31.191** (repealed 1988)
color classification of honey, **31.A01*** (final 1986)
corn syrup and cane sugar in maple syrup, **31.185** (final 1988)
corn syrups in honey, **31.156** (rev. 1988), **31.158** (rev. 1988)
dextran in raw cane sugar, **31.D01***
diastatic activity of honey, **31.162** (rev. 1987)
dry substance in corn syrups, **31.226** (rev. 1985*; rev final 1986)
filth in sirups, molasses, and honey, **44.105** (final 1988)
filth in sugars, **44.106** (final 1988)
high fructose corn syrup in honey, **31.156** (rev. 1988), **31.158** (rev. 1988)
in presweetened cereals, **14.075** (rev. 1985)
in wines, **11.A01***
lactose in milk, **16.059** (final 1985)
lactose purity, **31.064** (final 1988)
saccharides in corn syrups and sugars, **31.245** (corr. 1986)
sugars in honey, **31.128** (rev. 1987)
Winton lead number of maple products, **31.192** (repealed 1988)
Sulfamethazine, in milk, **16.D01***
Sulfamethoxazole, in drug tablets, **37.A07*** (final 1987)
Sulfaquinoxaline, in feeds, **42.179** (final 1988)
Sulfisoxazole, in drug dosage forms, **37.166** (final 1986)
Sulfites (total), in foods, **20.C01***
Sulfur, in fertilizers, **2.182** (final 1985)
Suppliers and manufacturers, pp. xxiii and xxiv (14th ed.) (rev. 1987)

Tea, filth in, **44.014** (final 1988)
Tetracycline, in milk, **16.D01***
1,3,5,7-Tetranitro-1,3,5,7-tetraazacyclooctane (HMX), as waste in waters, **33.B01***
Thiamine, in milk-based infant formula, **43.B29***
Thiethylperazine, microchemical test, **36.A07***
Thiocarbamates, in formulations, **6.581** (final 1988)
Tin, trace levels in food, **25.161** (surplus 1986), **25.A01*** (final 1988)
Tissue culture methods, *E. coli* invasiveness of mammalian cells, **46.020** (final 1987)
Tobacco, glycerol and glycols in, **3.155** (final 1985)
Tocopherols, mixed concentrate, **43.D01***
Tomatoes, filth in tomato products, **44.119** (final 1988)
mold count in dehydrated powder, **44.211** (final 1988)
pesticide multiresidues in, **29.A01*** (final 1986; rev. 1987)
rot, in comminuted products, **44.224** (rev. 1985)
tin in canned paste, **25.A01*** (final 1988)
total solids in canned products, **32.A01***
Triadimefon, technical and formulations, **6.A31*** (final 1987)
Triamino-s-triazine, in fertilizers, **2.D01***
Triazine pesticides, triadimefon, **6.A31*** (final 1987)
Tricyclohexylhydroxystannane (cyhexatin), **6.D01***

Trier, for sampling fertilizers, **2.001** (rev. 1987)
Triflupromazine, microchemical test, **36.A07***
Triglycerides, in oils and fats, **28.B01***
Trimethobenzamide, in drug dosage forms, **36.A01*** (final 1988)
Trinitrotoluene (TNT), as waste in waters, **33.B01***
1,3,5-Trinitro-1,3,5-triazacyclohexane (RDX), as waste in waters, **33.B01***
Tryptophan, in foods and food and feed ingredients, **43.D08***
Tuberculocidal activity, of disinfectants, **4.036** (rev. 1987; repealed 1988), **4.039** (rev. 1987; repealed 1988)

Uric acid, as indicator of excrement on foods and containers, **44.186** (rev. 1986), **44.B07***
Urine, on foods and containers, **44.163** (rev. 1987)
Use-dilution methods, disinfectant testing, **4.007** (rev. 1987; repealed 1988), **4.010** (rev. 1987; repealed 1988), **4.011** (rev. 1987)
U.S. Pharmacopeia, Food Chemicals Codex–U.S. Pharmacopeia–AOAC method for mixed tocopherols concentrate, **43.D01***

Vegetable products, processed, filth, in canned corn, **44.109** (final 1988; corr. 1988)
filth, in dehydrated potato products, **44.117** (final 1988)
in potato chips, **44.101** (final 1988)
in sauerkraut, **44.118** (final 1988)
in tomato products, **44.119** (final 1988)
mold count, in canned products, **44.219** (final 1988)
in comminuted products, **44.220** (final 1988)
in cream style corn, **44.223** (final 1988)
in dehydrated tomato powder, **44.211** (final 1988)
sodium chloride in canned products, **32.032** (rev. 1987)
tin in, canned products, **25.A01***
total solids in canned tomato products, **32.A01***
total sulfites in dehydrated products and peas, **20.C01***
vitamin C in, **43.076** (final 1985)
Vegetables, aflatoxins in soybeans, **26.069** (final 1988)
filth in green leafy vegetables, **44.110** (final 1988)
pesticide multiresidue methods, **29.A01*** (final 1986; rev. 1987), **29.A05*** (final 1986)
total dietary fiber in, **43.A14*** (rev. 1986; final 1986)
Vernolate, in formulations, **6.581** (final 1988)
Veterinary analytical toxicology, arsenic in liver tissue, **49.B01***
copper in liver, **49.A01*** (final 1987)
copper in serum, **49.003** (final 1986)
nitrate in forages, **49.B05***
Vibrio cholerae, in oysters, **46.D09***
Vinegars, permanganate oxidation number, **30.083** (rev. 1988)
solids in, **30.063** (repealed 1987), **30.C06***
Virus, poliovirus 1 in oysters, **46.A12***
Vitamins and other nutrients, ascorbic acid in foods, **43.076** (final 1985)
ascorbic acid in milk-based infant formula, **43.A28*** (final 1988)
B_1 in milk-based infant formula, **43.B29*** (final 1988)
B_6 in milk-based infant formula, **43.A24*** (amended 1986; final 1988)
B_{12} in milk-based infant formula, **43.B11*** (final 1988)
C in foods, **43.076** (final 1985)
C in milk-based infant formula, **43.A28*** (final 1988)
cobalamin in milk-based infant formula, **43.B11*** (final 1988)
D in AD concentrates, **43.A01***
in milk-based infant formula, **43.A21*** (amended 1986; final 1988), **43.B01*** (final 1988)
minerals in milk-based infant formula, **43.A37*** (final 1988)
mixed tocopherols concentrate, **43.D01***
niacin and niacinamide in milk-based infant formula, **43.A32*** (final 1988)
nutrients in infant formula, elemental analysis, **43.292** (final 1986)
phosphorus in milk-based infant formula, **43.B23*** (final 1988)
proximate analysis of milk-based infant formula, **43.B01*** (final 1988)
pyridoxine, pyridoxal, and pyridoxamine in milk-based infant formula, **43.A24*** (amended 1986; final 1988)
riboflavin in milk-based infant formula, **43.A23*** (final 1988)
sulfur amino acids in food and feed ingredients, **43.A08*** (final 1986)
in processed foods, **43.A08** (1988*)
thiamine in milk-based infant formula, **43.B29*** (final 1988)
total dietary fiber in foods, **43.A14*** (rev. 1986; final 1986; rev. 1987)
tryptophan in foods and food and feed ingredients, **43.D08***

Warfarin sodium, in tablets, **37.D10***
Wastewater, 2,4-DNT, TNT, RDX, and HMX in, **33.B01***
Water, see also Moisture
added in milk, **16.101** (surplus 1987)
2,4-DNT, TNT, RDX, and HMX in, **33.B01***
Weight per unit volume, oils and fats, **28.A01***
Wheat, deoxynivalenol in, **26.B07***, **26.B13***
filth in whole wheat cereals, **44.071** (final 1988)
insect infestation (internal), **44.040** (final 1988)
sterigmatocystin in, **26.132** (final 1988)
Wheat flour, total dietary fiber in, **43.A14*** (rev. 1986; final 1986; rev. 1987)
Wheat gluten, filth in, **44.061** (final 1988)
Whisky, color units, **9.002** (final 1985)
Wines, alcohol in, **11.008** (rev. 1985; rev. final 1988), **11.012** (final 1988)
caloric content, **11.022** (final 1988)
carbohydrate content, **11.A05*** (final 1988)
carbon dioxide in, **11.D01***
citric acid in, **11.A06***
citric and malic acids in, **11.052** (final 1988)
fructose and glucose in, **11.A01***
volatile acidity, **11.046** (final 1988)
Wort, specific gravity, **10.D01***

Yeast, total solids in liquid and pressed products, **10.222** (final 1988)

Zearalenone, in corn, **26.139** (final 1988)
Zearalenone and α-zearalenol, in corn, **26.A09*** (final 1988)
Zinc, in milk-based infant formula, **43.A37*** (final 1988)
in plants, **3.006** (final 1988), **3.013** (final 1988), **3.A01*** (final 1988)
trace levels in food, **25.001** (final 1988)
Zoalene, in feeds, **42.077** (final 1988)

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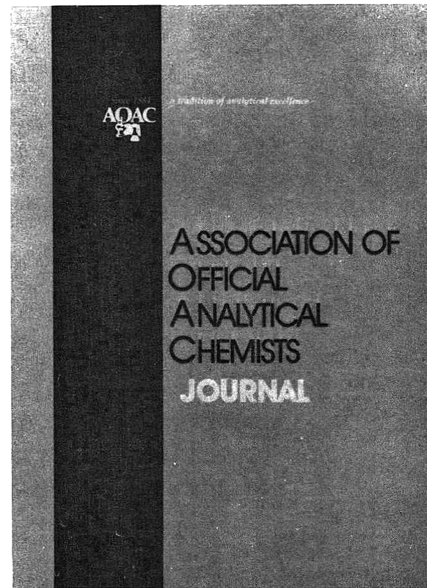

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