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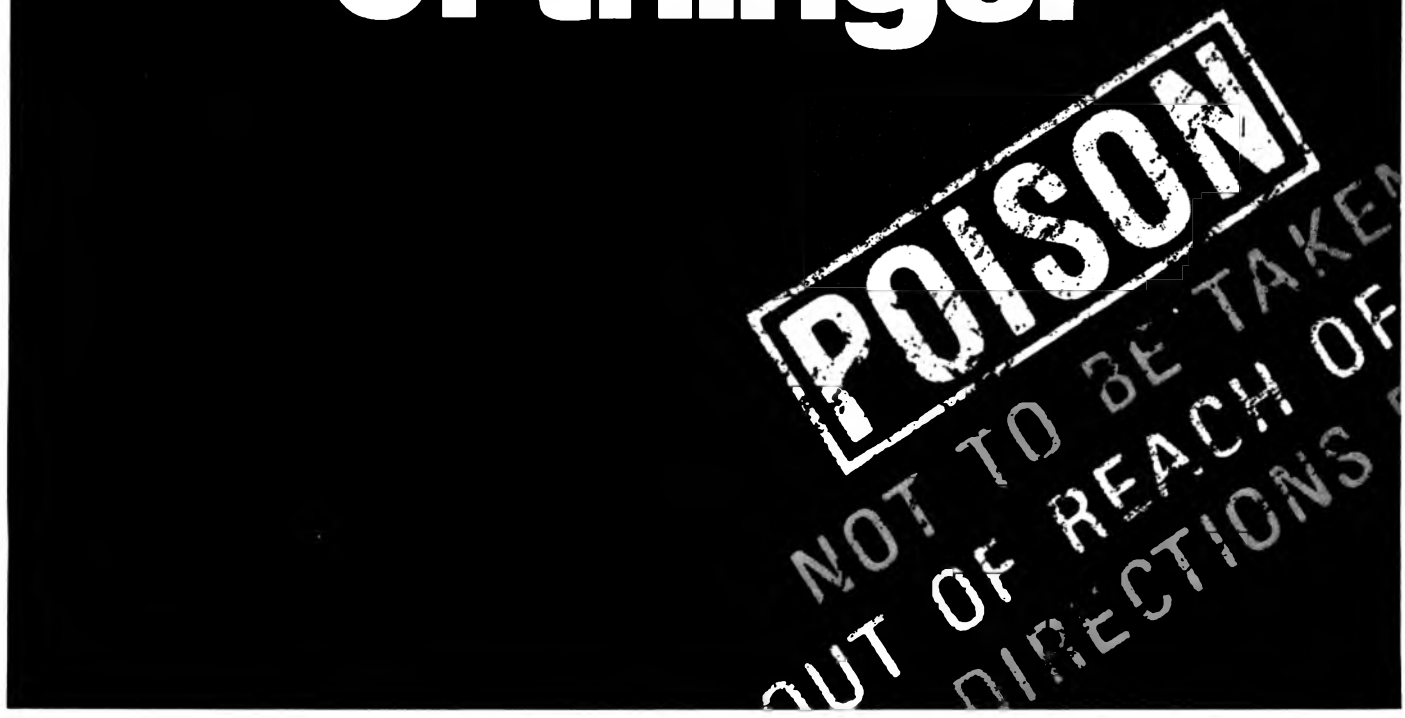

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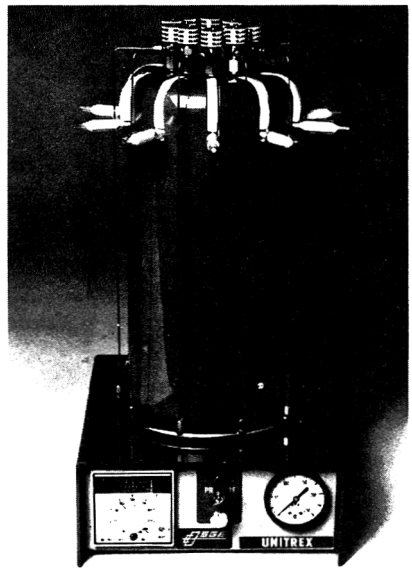

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Basic & Applied Research in the Analytical Sciences Related to Agriculture and the Public Health

MARCH/APRIL 1984
VOL. 67, NO. 2

General Referee Reports: Committee F

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Transactions of the Association of Official Analytical Chemists

Changes in Official Methods of Analysis

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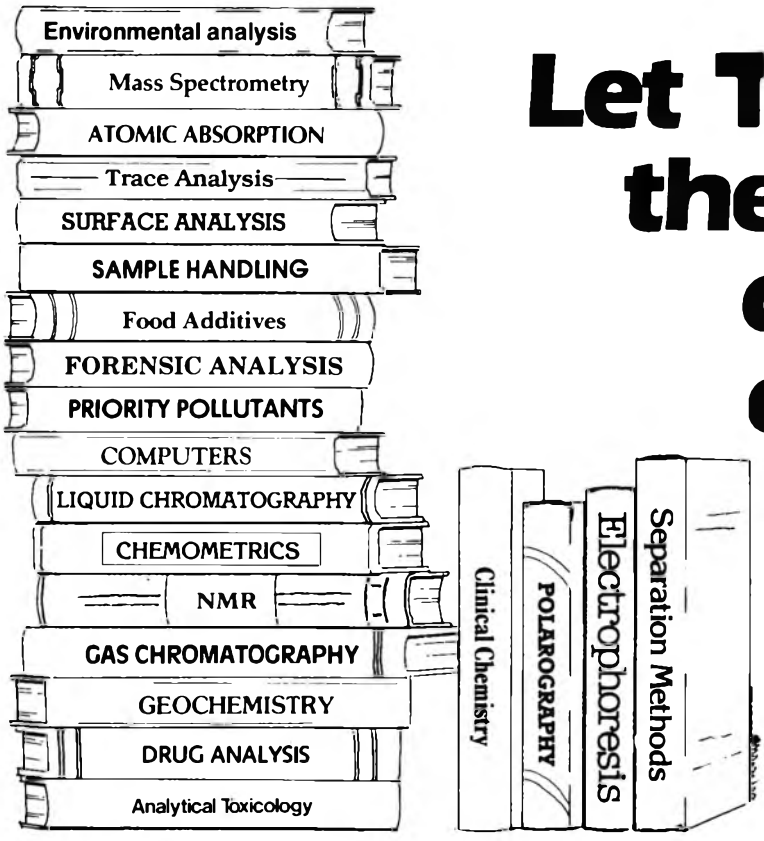

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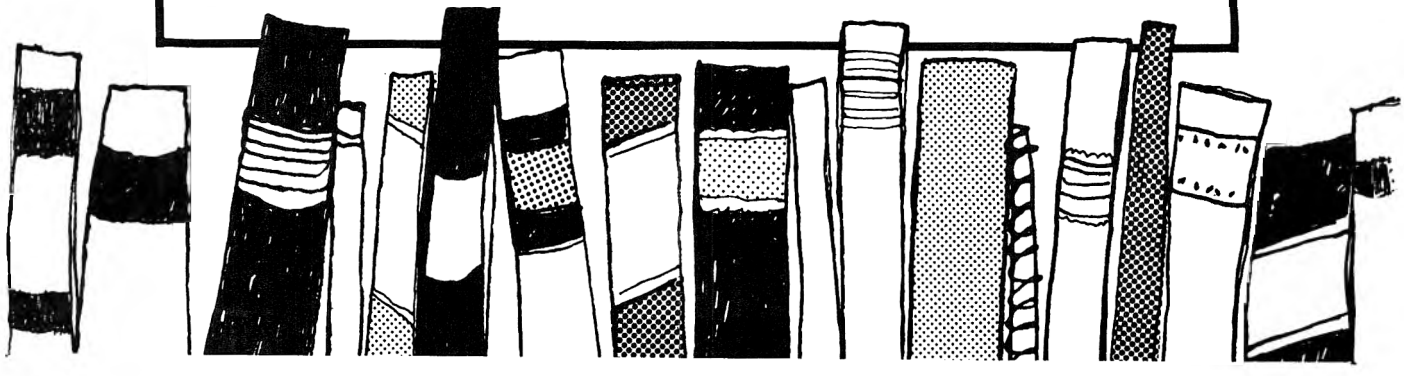

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Circle No. 309.

GC Brochure

Varian Associates, Inc., has published a 16-page brochure describing the VISTA Model 6000 and Model 6500 gas chromatographs. Key features of both models are covered, along with photographs and illustrations. For a copy, write to: Varian Instrument Group, 220 Humboldt Ct, Sunnyvale, CA 94089, or contact: J. Aeschliman, 408/734-5370.
Circle No. 310.

Geiger Counter

RDX nuclear introduces the new DX-1 pocket geiger counter. Lightweight and battery powered, the DX-1 has several meter scales, including a new overscale that causes beeping at high levels of beta, gamma, and X-radiations. For information, contact: Kathy Torrence, RDX nuclear, 2003 Canyon Dr, Los Angeles, CA 90068. Phone: 213/466-8833.
Circle No. 311.

Information Network

The American Chemical Society and West Germany's Fachinformationszentrum Energie, Physik, Mathematik GmbH (FIZ Karlsruhe) are linking their on-line computer information services to form an international scientific and technical information network. Chemical Abstracts Services's CAS ONLINE substance search service will be accessible to European users early in 1984, and FIZ Karlsruhe's *Physics Briefs* will be accessible to North American users by mid-1984. For more information, contact Edward P. Donnell, Chemical Abstracts Service, Public Information, 2540 Olentangy River Rd, PO Box 3012, Columbus, OH 43210. Phone: 614/421-3624; WUI Telex 684-2086.
Circle No. 312.

Surface Area Analyzer

Erba Instruments introduces the SORPTY 1750, a totally automated, accurate surface area analyzer. The SORPTY 1750 will measure surface areas from 1 m^2/g upwards in less than 20 min, is fitted with 2 furnaces to outgas the sample, and has a full vacuum range to 10^{-2} mm Hg with variable settings. For information, contact: Erba Instruments, Inc., 4 Doulton Pl, Peabody, MA 01960. Phone: 617/535-5986.
Circle No. 313.

Freeze Dryer and Brochure

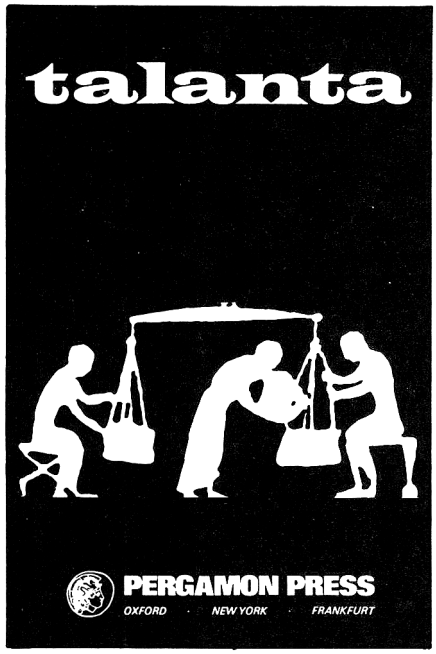
Labconco has published a new brochure describing the new Purifier Class II, Type A laminar flow safety cabinet. This 4-page, 4-color brochure contains specifications, features, and performance tests, with photos of different applications. Labconco has also upgraded its Freeze Dryer-3 with a new interface opening and an additional ½ in. valve. For more information on the freeze dryer and a free copy of the brochure on the cabinet, contact: Labconco Corp., 8811 Prospect, Kansas City, MO 64132. Phone: 1-800/821-5506.
Circle No. 314.

Spectrophotometer Brochure

Varian Associates has published a 12-page color brochure describing the linkage of the DMS 100 UV/VIS spectrophotometer with a video monitor or the DS 15 Command Station. The brochure includes photographs, spectra, and graphics to delineate features, as well as a complete listing of specifications and ordering information. For information, contact: Nina Hadden, Varian Instrument Group, 220 Humboldt Ct, Sunnyvale, CA 94089. Phone: 408/734-5370.
Circle No. 315.

Electrochemical Detector

LKB introduces a new electrochemical detector. The cell combines a large electrode surface area with low internal volume (1.5 μL), is easy to clean, and can be detached from the instrument and placed in a column oven. For information, contact: LKB Instruments, Inc., 9319 Gaither Rd, Gaithersburg, MD 20877. Phone: 301/963-3200; Telex: 64634.
Circle No. 316.



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Organic complexing agents in atomic-absorption spectrometry — a review, J KOMAREK and L SOMMER.
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BOOKS IN BRIEF

Trace Element Speciation in Surface Waters and Its Ecological Implications Edited by Gary G. Leppard. Published by Plenum Press, 233 Spring St, New York, NY 10013, 1983. 320 pp. Price: $45.00 (U.S. & Canada).

As part of the NATO Conference: Series 1 on Ecology, Volume 6 provides a critique of analytical approaches to the problem of trace element speciation and reports on interactions of trace elements with aquatic species. Recommendations are made and future directions for research are suggested.

Progress In Pesticide Biochemistry: Volume 2. Edited by D. H. Hutson and T. R. Roberts. Published by John Wiley & Sons, One Wiley Dr, Somerset, NJ 08873. 226 pp.

Recent developments in the conjugation of pesticides and their primary metabolites is the focus of this volume. Volume 2 is part of a series which reviews selected areas on the biochemistry of pesticides, including mode of action, biotransformation in target species, environmental effects, environmental chemistry, and biochemical toxicology in mammals, including humans.

Advances in Cereal Science and Technology: Volume V. Edited by Y. Pomeranz. Published by the American Association of Cereal Chemists, 3340 Pilot Knob Rd, St. Paul, MN 55121, 1982. 294 pp. Price: $32.50 (members); $36.00 (nonmembers).

This volume brings together information on the identification of cereal varieties by gel electrophoresis, and development of low-erucic acid rapeseed cultivars, as well as new information on wheat genetics and enzymes, oats, and buckwheat.

Chemical Methods in Gas Chromatography. By V. G. Berezkin. Published by Elsevier Science Publishing Co., Inc., PO Box 1663, Grand Central Station, New York, NY 10163. Also available from Elsevier Science Publishers, PO Box 211, 1000 AE Amsterdam, The Netherlands, 1983. Approx. 314 pp. Price: U.S. & Canada $80.75/Dfl. $190.00. ISBN 0-444-41951-9.

This book contains detailed coverage of the entire field of reactionary gas chromatography, including descriptions of a large number of original techniques currently in use throughout the world. Describing the joint use of chemical and chromatographic methods, its 9 chapters include the following topics: derivitization methods for the preliminary treatment of samples for analysis, kinetic methods, pyrolysis-gas chromatographic methods, determination of the carbon skeleton in organic compounds, and others. It is supplemented by a bibliography of more than 1000 references.

Federal Food, Drug, and Cosmetic Act, 1978–1980. Edited by Vincent A. Kleinfeld and Alan H. Kaplan. Published by the Food and Drug Law Institute, Washington, DC 20036, 1983. 1118 pp. Library of Congress Card Catalog No. 83-81287.

This volume is the twentieth research book in the Food and Drug Law Institute series. It contains judicial decisions pertaining to the Federal Food, Drug, and Cosmetic Act for the years 1978–1980 and is the tenth book in the judicial record series spanning the years 1938–1980. For easy reference, it includes an alphabetical list of the cases, a list of case references to sections of the Act, and an index of terms used in the decisions. In addition, a separate category of cases and references is included pertaining to the Freedom of Information Act, 5 U.S.C. §552 (1976 & Supp. II 1978) as it applies in the context of food, drug, and cosmetic law.

McGraw-Hill Dictionary of Scientific and Technical Terms. Editor in Chief, Sybil P. Parker. Published by McGraw-Hill Book Co., 1221 Sixth Ave, New York, NY 10020, 1983. 1846 pp. Price: $70.00. ISBN 0-07-045269-5.

This third edition covers every major field of science, engineering, and technology, with the current usage of specialized terminology of 100 different disciplines, and contains more than 115 000 definitions, including 7500 new terms. The dynamic areas of electronics and computer science are emphasized. Illustrated with more than 3000 photographs, drawings, tables, and diagrams, the dictionary is thumb-indexed and has an extensive appendix.

Revision of the AACC Approved Methods of Analysis. Available from the American Association of Cereal Chemists, 3340 Pilot Knob Rd, St. Paul, MN 55121, 1983. Contact: Raymond J. Tarleton, 612/454-7250. 1084 pp. Price: with 2 hardcover ringbinders, $140.00 AACC members, $190.00 nonmembers; without ringbinders, $96.00 members, $136.00 nonmembers. (Hardcover ringbinders sold separately for $25.00/set of 2.)

The first major revision of the AACC *Approved Methods of Analysis* in 21 years, the 8th edition, has been realigned and re-edited for clarity and consistency, and 5 new methods have been added. The book features full color in one section and loose leaf format to allow addition of revisions, which will be made annually. Contents include: acidity, acids, admixture of flours, total ash, baking quality, carbon dioxide, color and pigments, drugs, egg solids, enzymes, crude fat, crude fiber, inorganic constituents, and many others.

Analysis and Chemistry of Water Pollutants. Edited by R. W. Frei and U. A. Th. Brinkman. Published by Gordon and Breach Science Publishers, One Park Ave, New York, NY 10016, USA. Also available from Gordon and Breach Science Publishers, 42 William IV St, London, WC2N 4DE, UK, 1983. 302 pp. Price: $55.00. ISBN: 0-677-06150-1. ISSN: 0275-2581.

This volume contains selected papers presented at the 12th Annual Symposium on the Analytical Chemistry of Pollutants, Amsterdam, The Netherlands, April 14–16, 1982. Focusing on organic pollutants, this new volume reflects the latest research on water pollution, as well as policy matters and more interdisciplinary aspects of pollution control, including testing procedures for mutagenic pollutants. This collection will be a useful and accessible tool for scientists concerned with the research, control, management and legislation of water pollution, and for environmentalists in general.

Quantitative Analysis of Steroids. Volume 5: Studies in Analytical Chemistry. By S. Görög. Published by Elsevier Science Publishers, PO Box 211, 1000 AE Amsterdam, The Netherlands. Also available from Elsevier Science Publishing Co., Inc., PO Box 1663, Grand Central Station, New

York, NY 10163, 1983. 440 pp. Price: U.S. & Canada $86.50/Dfl. $225.00. ISBN: 0-444-99698-2.

This is the first book to deal with the quantitative analysis of steroids and all the important groups of steroids are discussed: sex and adrenocortical hormones, sterols, the D vitamins, bile acids, cardiac glycosides, sapogenins, etc. All analytical methods are discussed which are used for the determination of these groups of compounds, e.g. classical spectroscopic, chromatographic, electroanalytical, radioanalytical, protein-binding, enzymatic methods, etc. Detailed description is given of how these methods may be used for the determination of steroids in the pharmaceutical industry and research, in biochemistry, in the analysis of food products, and so on. Some 2500 references complete the book.

Methods in Food and Dairy Microbiology. By Leo R. Diliello. Published by AVI Publishing Co., Inc., 250 Post Rd E., PO Box 831, Westport, CT 06881, 1983. 142 pp. Price: U.S. $16.50, elsewhere $18.00. ISBN 0-87055-411-5.

This new manual presents laboratory methods intended for technicians employed in the field of quality control and for students enrolled in food microbiology courses. Standardized laboratory methods, designed to establish the sanitary quality of milk, food and dairy products in accordance with accepted standards of bacteriology are discussed within a simple format combining schematic diagrams and illustrations.

FOR YOUR INFORMATION

AOAC 97th Annual International Meeting and Exposition

Again this year, the 97th AOAC Annual International Meeting was held at its former meeting place, the elegant and spacious Shoreham Hotel in Washington, DC. About 1500 analytical scientists attended the successful meeting, held October 3–6, 1983.

Many attendees enjoyed a wine and cheese party on Sunday evening, but the meeting officially opened with the General Session on Monday morning. Warren R. Bontoyan, the outgoing president, gave his presidential address, "On the Eve of Our 100th Anniversary." He stressed the importance of continued development and implementation of methods to ensure a safe environment for the public, and emphasized that AOAC must demonstrate flexibility in working with all representative groups toward achieving this end. President Bontoyan considered future involvement of AOAC in training through workshops, and in taking a more international approach to the development of standardized methods.

Incoming President Charles W. Gehrke presented the Presidential plaque to Mr. Bontoyan, who in turn, presented Walter E. Hill, Food and Drug Administration, Division of Microbiology, Washington, DC, with the 1983 award for the best Associate Referee Report of the Year. Mr. Bontoyan presented certificates to the 1983 Fellows of AOAC:
Ted M. Hopes, Food and Drug Administration, Brooklyn, NY; Edmond J. Baratta, Food and Drug Administration, Winchester, MA; Randolph H. Dyer, Bureau of Alcohol, Tobacco and Firearms, Rockville, MD; Peter M. Scott, Health and Welfare Canada, Ottawa, Ontario, Canada; Alan R. Hanks, Purdue University, West Lafayette, IN; Edwin R. Jackson, State Chemical Laboratory, Mississippi State, MS; Frank J. Johnson, Tennessee Valley Authority, Muscle Shoals, AL; and Stanley M. Cichowicz, Food and Drug Administration, Washington, DC.

The AOAC Wiley Scholarship winners, Bradley M. Brock of the Pennsylvania State University, and Loretta Colbert of Union College, were announced.

For outstanding services to AOAC in planning and conducting the 1982 Spring Training Workshop and Exhibition, special awards for meritorious service were awarded to Lawrence Sullivan, General Chairman; Paul Quinney, Treasurer and Registration Chairman; Herbert H. Baux, Exhibit and Local Arrangements Chairman; and Roger F. McClain, Publicity Chairman.

The final item on the General Session agenda was the Wiley Award Winner's Address: "Concept, Birth, and Development of Successful 'High Tech' Analytical Instrumentation," by Velmar A. Fassel, Deputy Director of the Energy and Mineral Resources Research Institute, Ames Laboratory, Iowa State University, Ames, IA. Dr. Fassel elaborated on the basic steps involved in conceiving and developing the 3 analytical methodologies for which he and his colleagues are known: Inductively Coupled Plasma–Atomic Emission Spectroscopy, Atomic Fluorescence Spectroscopy, and Mass Spectroscopy.

The Harvey W. Wiley Award banquet closed the first meeting day. Senator Robert T. Stafford gave the Banquet Address, which traced a brief history of our pollution problems in terms of origins and legislative actions, and discussed the present deliberations over the acid rain issue before the Committee on Environment and Public Works, of which he is a participant. For the balance of Monday and the next 3 days and evenings, attendees had their choice of over 200 papers, 122 poster sessions, 9 symposia, 51 equipment and supply exhibits, as well as many varied special sessions, meetings, and workshops.

The 9 symposia included: Instrumental Methods for the Analysis of Vitamins; Hospital Disinfectants and Sterilization Tests; Food Microbiology: Update on Foodborne Pathogens of Recent Significance; Detection and Quantitation of Protein Additives in Foods; Mutagenicity Screening—Microbiological Systems; Instrumental Methods and Data Handling; and Sulfite Analysis Methods for Food Products.

A short course, Maintaining and Troubleshooting Chromatographic Systems, sponsored by the American Chemical Society, was held on the 2 days preceding the meeting.

1983 Associate Referee Report of the Year Award

Walter E. Hill, Food and Drug Administration, Associate Referee for Genetic Methods for the Determination of Microbial Pathogens, won the 1983 award for the best Associate Referee Report of the Year. Dr. Hill, the first microbiologist to win this award, was nominated by Committee F for the report, co-authored by William L. Payne, "A Collaborative Study to Identify Enterotoxigenic *Escherichia coli* by DNA Colony Hybridization."

Every year, each committee is asked to nominate one referee for this award. Other nominees for the year were: Committee A—Peter F. Kane, Purdue University, West Lafayette, IN; Committee B—Michel Margosis, Food and Drug Administration, Washington, DC; Committee C—Dick H. Kleyn, Rutgers University, New Brunswick, NJ; Committee D—no nomination; Committee E—James A. Ault, ABC Labs, Columbia, MO; Committee G—no nomination.

Course Offered

Varian Associates Instrument Group will offer courses on capillary techniques in gas chromatography at 4 sites during the next 12 months.

The two-day courses, which emphasize personal instruction and are limited in size, will be available 3 times during the year at each location. Courses qualify for college credit and CEUs.

For more detailed course information, contact: Training Dept, Varian Instrument Group, PO Box 9020, Walnut Creek, CA 95498. Phone: 415/939-2400, Ext. 2533.

New Private Sustaining Member

The Bristol-Myers Company, Syracuse, NY, joins the growing list of firms aware of the need to support an independent methods validation association. AOAC welcomes and recognizes the importance of continued industry support, and will continue to provide beneficial services to the entire scientific community.

Workshop

The American Association of Cereal Chemists (AACC), in cooperation with the Environmental Protection Agency (EPA), the Food and Drug Administration (FDA), and the U.S. Department of Agriculture (USDA), announces a workshop on "Integrated Pest Management for the Food Industry," to be held February 14 and 15, 1984, at the Sheraton Atlanta Airport Hotel in Atlanta, GA.

Three committees will discuss the 3 separate facets of pest management: improving designs of structures and equipment, nonchemical, and chemical means of pest control, in terms of defin-

ing the problems, how to deal with these problems, and future courses of action.

The workshop will benefit people in industries such as, cereal grain processing, dairy processing, meat and poultry processing, and any other food processing operation.

AACC will publish a summary of the proceedings, as well as a series of guidelines on Integrated Pest Management, sometime in the future.

For information, contact Raymond J. Tarleton, AACC, 3340 Pilot Knob Rd, St. Paul, MN, 55121. Phone: 612/454-7250.

Meetings

April 10–13, 1984: ANALYTICA 84, Munich Fair Center, Munich, Germany. The latest developments in analytical instrumentation and laboratory equipment are featured by more than 400 exhibiting firms. U.S. manufacturers can participate by joining the "Made in America" pavilion being organized by Kallman Associates, U.S. representative of the Munich Fair Authority. Complete details on the fully furnished booth provided, and other aspects of the trade fair can be obtained from: Kallman Associates, 5 Maple Ct, Ridgewood, NJ 07450. Phone: 201/652-7070.

April 29–May 2, 1984: AOAC 9th Annual Spring Training Workshop and Exposition, Philadelphia Marriott Hotel, Philadelphia, PA. Technical sessions will provide the latest information on the following subjects: Immunochemistry, Genetic Toxicology, Electroanalytical Techniques, Drugs, Pesticides, Thin Layer Chromatography, Liquid Chromatography, Electrochemical Detectors, Gas Chromatography, Toxicology, Nutrient Analysis, Adulteration by Migration of Packaging Material, Drug Metabolism, Environmental Contamination, Liquid Chromatography, Disinfectants, Collaborative Study Procedures, Robotics in Laboratory Automation, Trace Metal Analysis, and Macromolecular Separations.

An exhibition of state-of-the-art scientific equipment will be held in conjunction with this conference. For information, contact: James J. Karr, Pennwalt Technological Center, 900 First Ave, Box C, King of Prussia, PA 19406 (215/337-6560); or Harvey Miller, FDA, Customhouse, 2nd & Chestnut Sts, Philadelphia, PA 19106 (215/597-4375).

April 29–May 3, 1984: 75th Annual Meeting of the American Oil Chemists' Society, Fairmont Hotel, Dallas, TX. For information, contact: Meetings Coordinator, AOCS, 508 S Sixth St, Champaign, IL 61820. Phone: 217/359-2344. Telex: 404472 OIL CHEM CHN.

June 25–29, 1984: Chemrawn III–World Conference on Resource Material Conversion, Congress Center, The Hague, The Netherlands. Sponsored by the International Union of Pure and Applied Chemistry and the Royal Netherlands Chemical Society, the main theme of Chemrawn III is the transformation of raw materials into products. It will discuss new chemical routes and technologies, and as in previous conferences, the present one will suggest a research program for chemical conversions needed for our future. For information, contact: Chemrawn III Congress Bureau, QLT Convention Services, Keizersgracht 792, 1017 EC Amsterdam. Telex: 31578 inter nl att qlt, 020-26 13 72.

September 23–26, 1984: AOCS Short Course on Fatty Acids, Kings Island, OH. For information, contact: Meetings Coordinator, AOCS, 508 S Sixth St, Champaign, IL 61820. Phone: 217/359-2344. Telex: 404472 OIL CHEM CHN.

October 8–11, 1984: XIII International Symposium of Agrochimica on "Lipids in Plants and Soils." Symposium topics include: lipid metabolism and function in plants; effects of cultivation systems and environment on lipid metabolism in plants; lipids in soil; qualitative and quantitative aspects of plant lipid production; plant lipids as foods. For information on deadlines and registration fees, contact: Organizing Committee: Agrochimica, % Institute of Agricultural Chemistry, University of Pisa, Via S. Michele degli Scalzi, 2-56100 Pisa (Italy). Telex: 571 577.

October 28–Nov 1, 1984: AOAC 98th Annual International Meeting, Shoreham Hotel, Washington, DC. Abstracts (on special forms) of papers and reports for the meeting must be received by the AOAC office by July 6, 1984. For information, please contact: Cathy Anderson, AOAC, 1111 N 19th St, Suite 210, Arlington, VA 22209. Phone: 703/522-3032.

April 8–11, 1985: AOAC 10th Annual Spring Training Workshop, Downtown Sheraton Hotel, Dallas, TX. For information, contact: M. Virginia Gibson, FDA, 332 Bryan, Dallas, TX 75204 (214/767-0312); or Molly Ready, Alcon Labs, 620 S Freeway, Fort Worth, TX 76134. Phone: 817/293-0450.

ISO Standards Published

The following standards have been published by the International Organization for Standardization (ISO), Technical Committee 34—Agricultural Food Products. The standards are available, at prices indicated, from the Food and Drug Administration, Bureau of Foods, HFF-7, 200 C St, SW, Washington, DC 20204.

ISO 6489-1983 Animal feeding stuffs—Preparation of test samples—$12.00

ISO 5739-1983 Caseins and caseinates—Determination of scorched particles content—$15.00

ISO 7560-1983 Cucumbers—Guide to storage and refrigerated transport—$8.00

ISO 6560-1983 Fruit and vegetables products—Determination of benzoic acid content (benzoic acid contents >200 mg/L or mg/kg)—Molecular absorption spectrometric method—$11.00

ISO 6561-1983 Fruits, vegetables and derived products—Determination of cadmium content—Flameless atomic absorption spectrometric method—$11.00

ISO 5492/5-1983 Sensory analysis—Vocabulary—Part 5—$15.00

ISO 6490/2-1983 Animal feeding stuffs—Determination of calcium content—Part 2: atomic absorption spectrometric method—$11.00

THANKS TO REVIEWERS OF JOURNAL MANUSCRIPTS

We gratefully acknowledge the scientists who have served as reviewers of manuscripts for the 1983 *Journal*. Reviewers read and comment on the manuscripts, and the Editor's decision to accept or reject the paper is based on their recommendations. This is a time consuming task and one that reviewers are asked to fit into already busy schedules so that the publication process is carried out as rapidly as possible. We extend our appreciation to all the reviewers of *Journal* manuscripts.

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A CENTURY OF ANALYTICAL EXCELLENCE

Pesticides, Their Analysis and the AOAC: An Overview of a Century of Progress (1884–1984)

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The Early Years (1884–1942)

In this centennial year of the founding of AOAC, it is almost impossible for us to comprehend just what it was like to live in the year 1884. Therefore, the reader is asked to digress briefly to reflect on events and conditions in the United States at the time our Association was founded. Such reflections will emphasize the incredible growth of science and technology as well as the rapid development of the American nation and world civilization.

Visualize the year 1884: The population of the United States was about 55 million and the national debt stood at the staggering figure of 2 billion dollars. Grover Cleveland had just been elected to succeed Chester A. Arthur in the White House, and there were 38 stars in our flag. There was talk about opening up the Oklahoma Territory to settlement, but this would not occur for 5 more years. The Industrial Revolution had gained full momentum, and a modern engineering marvel, the Brooklyn Bridge, was opened last year. Only 5 years had passed since Alexander Graham Bell had carried on the first telephone conversation, and 13 more years would pass before Marconi could successfully send short-distance radio messages. Although railroads were fully operational, automobiles were still in the experimental stage (Daimler; Benz). Some recent inventions were barbed wire (Glidden), the phonograph and incandescent lamp (Edison), electric fan (Wheeler), electric flat iron (Seeley), steam turbine (Parsons), and the fountain pen (Waterman) (1). These developments are mentioned to emphasize the fact that the technology and apparatus required for modern pesticide research were just beginning to emerge; only a very small percentage of the devices currently used in research existed in the year 1884. Broad advances in the areas of chemistry, physics, medicine, communications and information, engineering, and transportation slowly and steadily provided the building blocks for our present capabilities. Also, during the past several decades we have seen that requirements for national defense, space exploration, competition among nations for world markets, and public demand for an improved quality of life and a safe environment have accelerated the advancement of science.

In 1884, pesticide formulations were concoctions containing one or more of the following ingredients: tobacco, soap, turpentine, various oils, kerosene, sulfur, lime, phosphorus, mercuric chloride, arsenic, hydrocyanic acid, phenol, cresol, pyrethrum, and naphthalene (2). Paris green, an aceto-meta-arsenite of copper [$Cu(CH_3COO)_2{\cdot}3Cu(AsO_2)_2$], had come into common usage as an insecticide. Bordeaux mixture, a combination of lime and copper sulfate, was discovered to have fungicidal properties by Millardet in France in 1883. Bordeaux mixture and Paris green could be safely combined to produce a spray with both insecticidal and fungicidal properties. London purple, a mixture of calcium arsenate, calcium arsenite, and other products including a small amount of dye, was recommended as a substitute for Paris green. The name "London purple" was given to the material because it was originally an arsenical by-product of the magenta dye industry in London (2). Oddly enough, DDT had been synthesized by Zeidler in Germany in 1874 but its insecticidal properties would not be discovered until 1939. This list of pesticides appears completely inadequate when compared with the many products available to us today. However, if we begin with the earliest records of pesticides found on stone tablets which described the use of red squill (dried bulbs of the lily family) as a rat poison, include Homer's description of the use of sulfur for fumigation and other forms of pest control in about 1000 BC, and all other discoveries up to the use of turpentine to repel and kill insects in 1787 (2), it becomes obvious that more progress was made during the nineteenth century than in all previously recorded history. In the year 1984, even a cursory view of the history of pesticides shows that the knowledge, development, and usage of these substances are increasing exponentially with time.

Conditions which led to the organization of the Association of Official Agricultural Chemists (AOAC) centered around the new chemical fertilizer industry which had begun to flourish late in the nineteenth century. Several states passed legislation which required that the N-P-K content be placed in each bag. Consequently, chemists from government and industry made three separate attempts in 1880 and 1881 to coordinate their work; however, all failed because of disputes over the choice of methods. In 1884, J. T. Henderson, Commissioner of Agriculture, State of Georgia, called a conference in Atlanta to organize an association to adopt uniform analytical procedures. This group wrote a constitution which allowed only regulatory chemists to vote on the adoption of methods; however, others could participate in discussions and investigations. The fertilizer industry subsequently recognized the urgent need for uniform methods and recommended that the constitution be adopted (3). Thus, AOAC was born later that same year at Philadelphia. From this simple beginning, AOAC (known as the Association of Official Analytical Chemists since 1965) has grown to encompass the development and adoption of uniform methods for virtually every field of the analytical sciences pertaining to agriculture, public health and safety, consumer protection, and quality of the environment. The Association has quickly outgrown its present name and is no longer just an organization for chemists but is truly an Association of all analytical scientists.

Prior to writing this article, the author searched the archives of AOAC at Arlington, VA, and found that the early activities of the Association were published as proceedings by the U. S. Department of Agriculture. Although nothing pertaining to the analysis of pesticides could be found before 1899, some of the entomology reports were quite interesting and should be briefly shared with the reader. An extensive report was presented in 1886 concerning the production and use of the insect powder "Buhach" (4). The product was produced at Stockton, CA, from dried flowers of *Pyrethrum cinerariafolium* grown on 300 acres in Merced County, CA, and sold wholesale for 45 to 50 cents a pound. The application of the

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material as a powder, tea, or tincture was vividly described along with its insecticidal effects. Several testimonials were presented concerning its low mammalian toxicity as follows: 1) "Workmen in the manufacturing plant continuously breathed the fine dust for several hours at a time and never suffered adverse effects," 2) "One teaspoonful of the alcoholic extract of Buhach was administered to a certain person afflicted with tape-worm; the dose was repeated each hour for ten consecutive hours, with the effect of removing the tape-worm without in the least degree injuring the patient." 3) At the stable of the Buhach plantation several tons of the dried stems were fed to the horses; "the latter appeared to relish it very much" and no injury could be discovered. Although these testimonials were useful in 1886 and contained many basic elements of modern toxicology, they would hardly be admissable by regulatory agencies as good laboratory practice experiments in 1984.

In entomology reports of 1886 (5), the blister beetle (*Cantharis nuttalli* Say) was reported to cause great damage to bean crops in North Dakota; the remedy was to drive them into windrows of straw which were then burned. In another report, the wooly aphis (*Schizoneura lanigera*) was causing extensive damage to apple trees in California; the remedy was to spray with a mixture containing one-half pound of tobacco and one-half pound of whale oil soap per gallon of water. The mixture was to be applied at about 130°F and the process repeated in about one week. A comparison of these remedies with current practices will emphasize the progress that has been made in pest control during the past century.

At the 15th Annual Convention of the AOAC in 1898, the president of the Association recognized that the value of products such as tobacco powder and extracts, which were coming into extensive use, was chiefly dependent on the nicotine content and called for methods to determine this ingredient. Furthermore, methods for arsenical powders and fungicides were also needed; therefore, he called for the appointment of a referee to investigate methods for the valuable ingredients in these products (6). Thus, the following year (1899), the first AOAC report on fungicides and insecticides was presented to the convention by Referee L. A. Voorhees. This report included methods for determining nicotine, arsenic, cyanogen, copper, and formaldehyde which specified volumetric or gravimetric techniques after various chemical reactions and manipulations (7). With this modest beginning, a continuous effort has been sustained by AOAC to obtain, develop, validate, and adopt analytical procedures for pesticides that are legally and mutually acceptable to both government and industry. These methods were initially published annually in the *Proceedings of the Annual Meeting of the AOAC* and subsequently in the *Journal of the AOAC* which succeeded it in 1915 and has appeared continuously since its inception. The *Journal* began accepting contributed articles in 1923, and since 1920, AOAC has also published *Official Methods of Analysis* every 5 years. Through the years, these publications have been invaluable as references to accepted procedures for pesticide analysis; the *Journal* has also provided for the rapid publication of new methods not yet subjected to the rigorous evaluation required for official adoption.

From the turn of the century until the beginning of the synthetic organic pesticide era in 1942, many new products and ideas were advanced for pest control. Various new compounds of arsenic came into use and new fumigants included *p*-dichlorobenzene, ethylene oxide, ethylene dichloride, and methyl bromide. Geraniol was found to attract the Japanese beetle, and alkyl phthalates to repel insects. Cubé was being tested as an insecticide, and sesame oil was discovered to synergize pyrethrum. The airplane was first used at Troy, OH, to distribute insecticides in 1921. The age of synthetic organic pesticides was beginning to dawn with the commercial production of pentachlorophenol in 1936 and the discovery of hexachlorobenzene in 1941 (2). The development of new analytical chemical techniques during this period proceeded at a snail's pace; only the electrolytic determination of copper was added to the conventional volumetric and gravimetric methods for pesticide analysis.

On the regulatory scene, an arsenic poisoning incident involving some 6000 persons in England who had consumed contaminated beer resulted in about 70 deaths. This prompted the British to establish a tolerance of 0.01 gr (grains) As_2O_3/lb of solid food (1 ppm = 0.007 gr/lb) in 1903. A Federal Pure Food and Drug Act was passed in 1906; however, proof that an adulterated food was a potential health hazard was required prior to corrective action. The Federal Insecticide Act was passed in 1910, and in 1927, the Food and Drug Administration established a tolerance of 0.025 gr As_2O_3/lb for fruits and vegetables in interstate commerce. Later, in 1923, accurate methods for low levels of lead became available and a tolerance of 0.025 gr Pb/lb of commodity was also set because of the widespread use of lead arsenate. The Food, Drug and Cosmetic Act of 1938 provided more extensive protection to the consumer than the previous act of 1906, because provision was made to establish safe tolerances for deleterious substances added to foods (2).

It should be pointed out that Harvey W. Wiley was a key figure in the founding and development of AOAC. Dr. Wiley, supported by the official methods of AOAC, was primarily responsible for the passage of the Pure Food and Drug Act of 1906. As the first administrator of this Act, he came under severe pressure from industry; however, even after his retirement from the Department of Agriculture in 1912, he continued to speak out for consumer protection until his death in 1930 (3).

As we entered World War II and the era of synthetic organic pesticides, most of the analytical chemical procedures were still confined to the analysis of formulations except for the As and Pb residue determinations previously mentioned. Analytical technology required for trace-level analyses was simply not available to keep pace with our knowledge of chemistry and the development of pesticides.

The Era of Synthetic Organic Pesticides (1942–Present)

DDT is probably the most widely known, used, and abused pesticide in all recorded history. Although first synthesized in 1874, it was not tested as an insecticide until 1939. A small amount of the compound was brought into the United States in 1942 and tests soon revealed its outstanding insecticidal properties. The author has selected this event as the beginning of the era of synthetic organic pesticides. Production was begun almost immediately and essentially the entire output was used by the armed forces to control body lice, mosquitoes, and other pests during World War II. Large quantities of the pesticide were produced for post-war use, and soon an entire family of synthetic organochlorine compounds was in widespread use. In addition to DDT, this class of chemicals included chlordane, toxaphene, lindane, methoxychlor, aldrin, dieldrin, heptachlor, and others. Residues of many of these compounds were highly persistent in the environment. Then in about 1946, synthetic organophosphorus insecticides such as parathion and TEPP, produced by German research, were also introduced into the United States.

Many compounds of this general class also quickly came into general use. These chemicals differed from organochlorine compounds in that they were generally more toxic to warm-blooded animals and less persistent in the environment. In the 1950s, another class of synthetic insecticides known as carbamates came into use, and a large family of these compounds soon developed.

Concurrent with the development of organochlorine, organophosphorus, and carbamate insecticides, many classes of herbicides were also being produced. These included chlorinated phenoxy acids, *S*-triazines, phenylureas, carbamates, phenols, dinitroanilines, chlorinated benzoic acids, and others. During this same period of about two decades, many other compounds of pesticidal activity came into use as rodenticides, repellants, attractants, sexual sterilants, fungicides, nematocides, acaricides, bactericides, miticides, synergists, defoliants, fumigants, growth regulators, algicides, mulloscicides, etc. All of these chemicals, when added to those already in use prior to 1942, constitute a plethora of compounds available for pesticidal evaluation and use. Hence, there was an exponential increase in the demand for analytical chemical procedures which were both specific and sensitive for determining residues of these individual chemicals and their admixtures.

As we entered the era of synthetic organic pesticides in 1942, the development of analytical chemical technology had not kept pace with the requirements for it. Furthermore, with the addition of several hundred new pesticides of many classes during the 1940s and 1950s, problems facing analytical scientists seemed almost insurmountable, particularly in the area of residue analysis for regulatory purposes.

Instrumentation for infrared, visible, and ultraviolet absorption spectrometry became generally available during the 1940s and was very useful for the analysis of pesticide formulations. Polarography was also used in limited applications. However, these methods were not easily adapted to the analysis of residues in food, feed, and environmental samples. The most widely used residue methods of this period were based on the formation of a colored product followed by a spectrophotometric measurement of its intensity in the visible portion of the spectrum. Examples of these are the classical Schechter-Haller method for DDT (8) and the Averell-Norris method for parathion (9). Another useful procedure developed during this period, which remains equally useful today, is the enzymatic method based on chlolinesterase inhibition (10). This method, which may now be quantified by change in pH, colorimetrically or manometrically, is useful for the determination of organophosphorus insecticides and to measure the extent of any exposure of humans or animals to such compounds.

Most analyses for residues of pesticides consist of a three-step process involving, 1) the extraction of residues from the substrate, 2) separation of the residues from coextractives (known as cleanup), and 3) measurement of the extracted residues. Considerable progress was made in all three of these steps during the 1940s and 1950s; however, experiments with rudiments of chromatography primarily for use in the cleanup step would prove to be the key to successful analyses of residues in the future. Tswett (11), whose name in Russian means color (12), is usually credited with the process known as chromatography. His experiments, reported in 1906, dealt with the separation of solutions of colored plant pigments by percolating them through columns of solid adsorbents. The colored plant pigments were separated into distinct colored bands by a kind of liquid-solid or liquid-adsorption chromatography. Whether Tswett's coining of the term chromatography (color writing) alluded to the colored bands on the column or to his own writings is not known.

As early as 1512, Brunschwig (13) had described a primative form of gas chromatography for purifying ethyl alcohol (14, 15). Also, in this very brief review of chromatography, the author is compelled to cite instructions on this subject given by the *Supreme Scientist of the Universe*, in about 1491 BC; "So Moses brought Israel from the Red Sea, and they went out into the wilderness of Shur; and they went three days in the wilderness, and found no water. And when they came to Marah, they could not drink of the waters of Marah, for they were bitter; therefore the name of it was called Marah. And the people murmured against Moses, saying 'What shall we drink'? And he cried unto the Lord; and the Lord shewed him a tree, which when he had cast into the waters, the waters were made sweet: there he made for them a statute and an ordinance, and there he proved them" (16).

Regardless of where the reader chooses to put the origin of the chromatographic process, it is clear that the concept, as applied to analytical chemistry, was dormant until the mid-1940s when adsorption columns came into use for separating pesticide residues from coextractives which interfered with the analysis. Liquid-liquid chromatography, described in 1941 (17), was later developed into paper chromatography (18). An early application of paper chromatography to pesticide analysis was that of Metcalf and March (19) for parathion and related phosphate esters. Although Martin and Synge (18) had presented the basic principles of gas chromatography (GC) in their 1948 paper, little was done to develop the procedure before the work of James and Martin (20) in 1952. GC then developed very rapidly and became one of the outstanding methods for analysis. Its usefulness for the analysis of pesticides was limited to formulations because the detectors were not specific; however, a combustion furnace, titration cell, and coulometer were soon placed in tandem with the gas chromatograph for the analysis of pesticides which contained chlorine or sulfur (21).

In brief summary of the events during the 20-year period leading into the 1960s, hundreds of synthetic organic pesticides of many chemical classes became available for use; sensitive and specific methods were required for their analysis, particularly from the residue aspect. Infrared, visible, and ultraviolet spectrometry became common, and many colorimetric methods were developed. Outstanding progress was made in the area of separation science with the development of liquid-solid column chromatography, paper chromatography, and gas-liquid chromatography. Sensitivity for analyzing residues was brought from the milligram to the microgram level. A popular guide to the analysis of such residues during that period was the classical textbook of Gunther and Blinn (22). In reviewing the archives for this period at AOAC, the author found that the names of the Associate Referees would literally constitute a Who's Who in pesticide analysis. AOAC had indeed been hard at work, and many procedures based on new technology had appeared in the *Official Methods of Analysis* of the AOAC by 1960. It was also noted that the old section heading "Insecticides and Fungicides" was replaced by "Economic Poisons" in 1950. However, this term was short-lived and, in 1955, under the editorship of William Horwitz, the present category "Pesticides" was adopted. The *Journal* of the AOAC and meetings and workshops of the Association continued to provide forums for the development of analytical procedures for pesticides.

By the mid 1960s, several events had occurred which would change the future of pesticide analysis. First, a highly sensi-

tive detector based on the ability of certain functional groups, such as halogens, nitro groups, peroxides, and others, to capture low energy electrons was reported by Lovelock and Lipsky (23). The detector was almost immediately coupled with a gas chromatograph and used for the analysis of chlorinated pesticides (24, 25). This system, known as electron capture gas chromatography (EC-GC), was shown to increase the sensitivity for analyzing halogenated pesticides by a factor of 10,000, making the detection of picogram amounts possible. Second, Rachel L. Carson, an American biologist and science writer, published her book *Silent Spring* in 1962, which aroused public and government concern about the adverse effects of environmental pollutants. Susequently, large sums of public funds were made available for research, monitoring, and regulation of such materials which included pesticides. These two events more than any others set the scene for extensive work in the area of pesticide analysis.

In the 1950s, thin layer chromatography (TLC), which combines desirable features of liquid-solid column chromatography with paper chromatography, was introduced as an analytical procedure. At first, the method was considered only qualitative or semiquantitative at best. However, pioneering work by Beroza et al. (26) and others has brought the technique to acceptable levels of accuracy and precision for quantitative analysis. The method is also well suited for confirmatory tests. Also, beginning in 1965, Bowman and Beroza published a series of papers which described the usefulness of partition values (p-values) in selecting solvents for pesticide extraction, cleanup, and confirmation of identity of the residues (27–29). The EC-GC system previously described for halogenated pesticides responded poorly to the general class of organophosphorus pesticides which were widely used. This gap in methodology was bridged by the development of the alkali flame ionization detector (AFID) of Giuffrida (30) and the flame photometric detector (FPD) of Brody and Chaney (31), which was sensitive and specific for phosphorus or sulfur. The FPD was extensively evaluated for gas chromatographic analysis of phosphorus and sulfur-containing pesticides by Bowman and Beroza, and in 1968, they reported an improvement of the detector which simultaneously presented phosphorus- and sulfur-specific chromatograms on separate channels and also provided for an estimation of the molecular P and S content of the gas chromatographic peak (32). Thousands of publications based on gas chromatographic analysis of pesticide residues appeared in the scientific literature during the ensuing decade. Many of the compounds not directly amenable to gas chromatography were derivatized or otherwise modified prior to analysis.

One of the major deficiencies in gas chromatographic methodology for pesticides had been the absence of detectors sensitive and specific for nitrogen-containing compounds. Coulson (33) addressed this need by placing an electrolytic conductivity cell in tandem with a reduction furnace (nickel catalyst; hydrogen carrier gas) which converted the N-compounds to ammonia. This system was evaluated by Patchett (34) and others and further developed by Hall (35). Although sensitivities to 0.1 ng organic nitrogen with about a 10,000:1 selectivity have been reported (34), operation of the system in the N-mode has not been as popular as the Cl-mode. Another detector useful for the analysis of N-containing compounds is the "rubidium-sensitized" system which responds to both N- and P- containing compounds. After experimental use for several years, the detector became sufficiently reliable for routine use after the rubidium salt was placed into an electrically heated bead and the low hydrogen flow (1–3 mL/min) was precisely controlled (36). Both the electrolytic conductivity and rubidium-sensitized detectors are useful for the analysis of residual N-containing pesticides such as carbamates; however, the instability of many of these compounds may require derivatization or an alternative means of analysis.

A comprehensive series of reviews of the state-of-the-art of pesticide analysis was edited by Bowman in 1975 (37–39). Experts in the field dealt with the analytical chemistry of the various classes of chemicals, types of methods, sampling, and alternative means to achieve the desired biological control. In writing on the subject of carbamate analysis, which remains one of the most difficult classes, H. W. Dorough said, "One of the most disturbing aspects of carbamate residues analysis involves what might be referred to as laboratory-specific methods." Dorough went on to explain that while the laboratory which developed the method continued to report good results, others simply could not make it work. This phenomenon was attributed primarily to the omission of a vital point in the residue procedure which, on the surface, might appear insignificant (40). Most analytical chemists have encountered this problem many times, and its continued presence emphasizes the need for wide participation in the interlaboratory activities of AOAC. Because of the extensive collaborative tests among laboratories which are required for the adoption of a procedure, there are no "laboratory-specific" official methods of AOAC. The adoption of official methods does take time and, of necessity, lags the state-of-the-art; however, this period can be held to a minimum by wide laboratory participation in the process.

In this same series of reviews (37–39), the editor emphasized two problems that still require our attention (41). First, residues of pesticides in environmental samples cannot be analyzed by present methods unless they are extracted from the substrate, and the concern for efficient extraction of such residues has not been commensurate with its importance. Through the years, extraction techniques have progressed from "surface stripping" with nonpolar solvents, through blending with solvents of medium polarity, to exhaustive procedures using more polar mixtures via reflux or Soxhlet extraction. Recoveries of biologically incorporated or weathered residues have increased as techniques improved. However, results from tests with radiolabeled pesticides have revealed that significant amounts of the residual radioactivity remained unextracted even with use of the most rigorous techniques (42, 43). Every effort should be made to develop and use efficient extraction procedures; the nature and toxicological significance of the bound residues should also be determined. Second, tentative identification of pesticide residues in samples of unknown treatment history is often made on the basis of one determinative procedure. It is imperative that such identifications be confirmed (or rejected) by several alternative procedures. Many residues reported in the past could be erroneous because the analyst failed to perform the confirmations. Techniques useful for confirmatory tests are gas chromatography (GC) employing a variety of detectors and/or columns of different polarities, TLC, p-values, derivatization, liquid chromatography (LC), GC or LC instruments coupled to a mass spectrometer (MS), and others. An excellent text which addresses most of the aspects of pesticide residue analysis is that of Moye published in 1981 (36).

Recent advances in the analysis of pesticide residues include increased use of LC, attempted inclusion of polar compounds in multiresidue schemes, greater emphasis on analysis of metabolites and conjugates, development and use of GC-MS systems, and methods development for a relatively new class

of pesticides, the synthetic pyrethroids (44). Capillary GC is also becoming widely accepted in residue analysis because of the high quality columns now available which yield a high level of resolution and longevity not previously attainable. An example of the highly sophisticated methods now being developed is the isomer-specific determination of 2,3,7,8-tetrachloro-*p*-dioxin (2,3,7,8-TCDD) by capillary GC/atmospheric pressure negative chemical ionization/MS (45). It should be noted that 22 TCDD isomers are possible and many other chemicals such as DDT, DDE, PCBs, toxaphene, benzyl phenyl ethers, and chlorinated methoxybiphenyls have been known to interfere in previous methods; yet in this procedure the 2,3,7,8-TCDD can be specifically determined in tissue at the low parts-per-trillion level. On the other hand, the availability of methodology to determine such infinitesimal levels of residues imposes an almost insurmountable task on toxicologists, i.e., the task to determine their toxicological significance to humans and the environment.

An excellent review of governmental regulations pertaining to pesticides and their residues was presented by Leng in 1981 (46). Pesticide Assessment Guidelines revised in 1982 are available at moderate cost through the Environmental Protection Agency, Washington, DC. Extensive research is now required for pesticides before consideration for registration; therefore, it is anticipated that only effective and safe materials will gain approval in the future.

The members and officials of AOAC can take great pride in the accomplishments of the Association during the past century. Many of the significant accomplishments in pesticide analysis previously cited were first reported as meetings of the AOAC and/or in the *Journal*. Similar accomplishments were simultaneously being made in many other areas of the analytical sciences. The *Official Methods of Analysis of the AOAC* will continue to provide a source of tried and proven methods recognized by government, industry, and the courts. Continued support of the Association by its members and sustaining members, and an even more active participation in the laboratory collaborative process, will ensure the availability of reliable and accepted methods for all of the analytical sciences in the future.

Epilog: A Time Capsule for 2084

With faith that humankind will somehow gain the wisdom to live and work together in peace to allow our civilization to survive and develop, the author of this centennial paper and the members of AOAC (1984) send greetings and congratulations to the members of AOAC in their bicentennial year (2084). The current rapid development of electronics, computer science, communication and information systems, and space-age technology in general, makes it impossible for us to even imagine what the state-of-the-art in pesticide science will be a century from now. Undoubtedly, your technology will be highly sophisticated compared with ours. We sincerely hope that you have achieved a high quality of life, and that most of the problems related to the analysis and use of pesticides and all biologically active substances are solved.

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PRESIDENT'S ADDRESS

On the Eve of Our 100th Anniversary

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Every year at this time the president delivers his address and hopes he says something interesting or imparts wisdom or some significant insights. I have thought of many things to say but after reading the previous 10 presidential addresses I have found, not to my surprise, there isn't very much left to say. Every president has been concerned with the Association's financial condition; every president has been concerned with state, federal, and industry support; every president likes to recount the forward steps or major decisions which occurred during his or her presidency. I too was tempted to do this, but for better or worse I have decided not to follow. I will say, however, that the general health of the Association was never better.

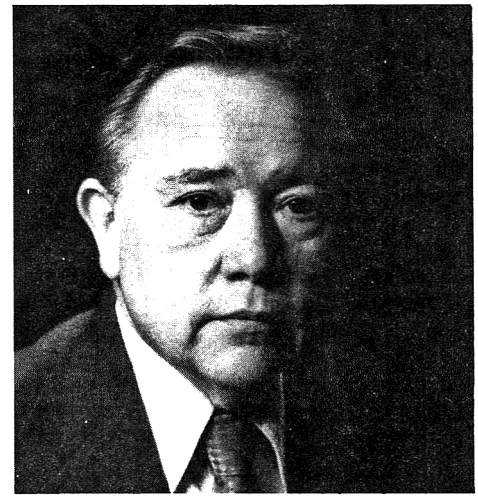
I am going to give what I hope is a relatively short address which may at times be rambling, but you may find that we share or have shared similar perspectives of the AOAC.

As an undergraduate student taking analytical chemistry, I remember a professor saying that AOAC methods are outdated and behind the times. It did not bother me that he said this. I knew nothing of the AOAC and in the eyes of a chemistry student, the professor seemed all so wise and knowledgeable of analytical chemistry. After all, we students, near the end of the course, would use the very sophisticated Beckman DU Spectrophotometer, and the professor indicated that such instrumentation did not appear in the AOAC methods. Looking back, I have found the professor's criticism interesting and amusing. He graded the students on their ability to analyze individual sample unknowns prepared by him for each student. What the student reported, and what the professor found by his own duplicate analysis of each unknown, determined the student's grade. If my memory serves me correctly, you received an A if your analysis was within 1 part per thousand of his; a B if you were within 2 parts per thousand; and a C if you were within 3 parts per thousand. Anything reported which did not fall within these limits of the duplicate analysis performed by him were incorrect and received a failing grade. How strange his criticism was of AOAC. Perhaps at some time during his career, one of his unknowns was part of a collaborative study, and his analytical results were the outliers. Under his grading system he would have failed or, better still, all the other collaborators would have failed.

Since then, I have become a member of AOAC, but my joining was a result of an observation that if I was to be considered a serious analytical chemist by my employer, I had better participate in AOAC collaborative studies and attend the Annual International Meeting. It was this attitude which launched me into the sea of the Association's many activities and committees.

On the eve of AOAC's 100th Anniversary, it is an examination of my former employer's attitude, my perception of present federal, state, and industry attitudes, and the recollections of the professor's grading system which cause apprehension.

Presented at the 97th Annual International Meeting of AOAC, Oct. 3–6, 1983, Washington, DC.



As the Association is about to begin its next 100 years, what should its role be in relating to analytical science and its impact on the regulator and the regulated? I believe we all agree that AOAC has done as much as or more to protect the pocketbooks and health of the consuming public than any other scientific organization. Through the development of sound scientific method, AOAC should and will continue in this direction; but, the question is *how* and under what climate?

There has been a tendency on the part of some regulatory agencies to promulgate rules and regulations and to enforce these with in-house methods of analysis. Enforcement by using in-house methods of analysis arises out of public and government concern over almost daily reports in the news media of potential cancer-causing chemicals in food, water, and air, both in the home and workplace. Such concern is justified, and strong expeditious regulatory actions are often needed. However, such actions, which result from the analysis of food and environmental samples, may, at times, be an over-reaction. How then should organizations such as AOAC respond to the trend of agencies using uncollaborated in house methods of analysis? If anyone were to ask high ranking managers in various federal and state regulatory agencies about this, they would probably all agree that collaborated methods should be used when possible, but, if such methods do not exist then they must rely on in-house methods of analyses. Unfortunately, such an answer is correct; and it is this correctness that causes me concern.

Most of the AOAC methods are applicable and serve to protect the public. There is no question as to the relevance of these methods; but it is the lack of methods for pesticides, toxic chemicals, and the metabolites in many different matrixes of the environment, food, and human body secretions, which is of concern.

At times the present collaborative regime is too slow for regulatory agencies, which must respond to crises arising on an almost weekly basis. Regulatory agencies cannot afford the luxury of waiting the outcome of 2- and 5-year collaborative studies. I am not advocating the abolishment of

our present collaborative study approach, but I am saying we must respond by developing scientific methods which will stand cross-examination in courts of law and have not been subjected to a full-blown collaborative study. AOAC must be careful that, in defense of our present system, we are not flexible enough to be relevant for the future. I believe the Association has taken a step in a new direction. 2,3,7,8-TCDD (Dioxin) in environmental samples has been a problem of great concern since the latter part of the 1960s. During this time there has not been a collaborative study in this area. However, at last year's meeting, a fish sample containing 2,3,7,8-TCDD was sent to five different laboratories using different cleanup methods. The reported results from the different laboratories were in good agreement, even though they used different methods. Perhaps we should explore this and other approaches in establishing acceptable methods based on performance standards. Although I have sounded critical of the Association, it is because I believe AOAC is composed of a tight and cohesive group of international regulatory and industry analytical scientists with great experience, expertise, and knowledge, and has the will to explore and respond in a way to develop new avenues for developing sound methods. AOAC must not limit itself to only one approach of establishing official methods of analysis.

If AOAC is to demonstrate flexibility in exploring new approaches without abandoning its goal of good science, it is the responsibility of the federal, state, and international regulators and the regulated industries to establish the climate for good science. If the regulators and the regulated do not demonstrate a willingness to develop sound defensible methods regardless as to whether they are the result of full-blown collaborative study or something less, then we are doomed. Therefore, it is urgent that each of us: the bench chemist or microbiologist, the first line-supervisor, division directors, state chemists, etc., make known to management that a significant and specific amount of time and resources be set aside for AOAC studies. AOAC must use every *ethical* means to influence decision makers and controllers of the purse strings in state and federal legislative bodies of the need for such resources. I am not advocating partisan politics, but I am saying that AOAC and its sister organizations, such as AOCS (American Oil Chemists' Society), AACC (American Association of Cereal Chemists), ASBC (American Society of Brewing Chemists), ASTM (American Society for Testing and Materials), etc., must unite and explore ways to make legislatures and representatives cognizant of the need for the development of good analytical science and how it provides protection to the public and regulated industries.

How should AOAC and other groups present their case? They must clearly demonstrate the great need to define accuracy, limits of detection, and precision of analytical methods in order to assess the significance of analytical results with respect to the certainty and significance of health effects data. And this can be obtained only by the development of sound analytical methods.

If associations such as AOAC, with a wealth of scientific resources through a network of thousands of analytical scientists, are to be used for maximum benefit to the public, then regulatory bodies must be shown that panic response to a crisis using data derived from uncollaborated methods is counterproductive causing confusion, political rhetoric, and costly corrective action. Regulators and the regulated industry must be made to understand that communications between themselves and AOAC and similar bodies are essential. Regulators and the regulated industry must, on an annual basis, indicate short-term and future needs in the area of analytical science to ensure regulatory decisions based on reliable scientific data.

In addition to the development of methods, there is a problem of credibility with respect to the many laboratories using standardized methodology. Regulators and the regulated are contracting certain laboratory functions to outside laboratories. Recent disclosures have indicated that such contracting has, at times, resulted in the reporting of unreliable data. How can the contractee know with confidence that the contractor is reliable? I believe that AOAC has the credentials to be of service by establishing itself in the area of laboratory certification. Such a service would help both the regulator and the regulated by ensuring the scientific credibility of the contracted laboratory. AOAC should move forward in this area.

AOAC should also consider future involvement in training, which is a natural and logical compliment to method development and laboratory certification. The spring training workshops, which have drifted away from the principle of "workshop," could perhaps serve as a vehicle for training.

AOAC must also be more vigorous in its approach to becoming more international. It must convince other countries that it stands for the development of standardized methods, and that it is not a North American institution. It must find and explore ways to receive and review the many international studies conducted outside of North America for possible adoption as AOAC methods. It must incorporate scientists outside of North America into its decision-making process, its committees, and eventually the Board of Directors.

In closing, I wish to thank the AOAC membership for the priviledge and honor to serve as President, the last President of AOAC's first one hundred years.

WILEY AWARD ADDRESS

Conception, Birth, and Development of Three New Successful Analytical Concepts

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According to the Harvey W. Wiley Award announcement, this award is bestowed annually to recognize "contributions to analytical methodologies in areas pertaining to agriculture and public health" and to "emphasize the role of the scientist in protecting the consumer and the quality of the environment." Although I am not aware on what basis I was fortunate enough to be selected as the 1983 recipient, I strongly suspect that a major consideration was the pioneering role that my associates and I played in conceiving and developing 3 analytical methodologies for performing elemental determinations at the ultratrace, trace, minor, and major constituent levels. These methodologies are inductively coupled plasma–atomic emission spectroscopy (ICP-AES); inductively coupled plasma–atomic fluorescence spectroscopy (ICP-AFS); inductively coupled plasma–mass spectroscopy (ICP-MS).

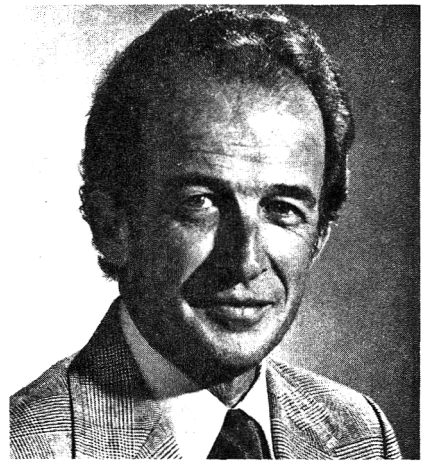
Individually or collectively, these methodologies either have had a major impact on the way elemental analyses are performed, or are destined to do so in the future, not just in public health, agriculture, and consumer and environmental protection, but also in virtually all technological endeavors where elemental analytical data are essential.

Because an award address provides the recipient with an opportunity to offer personal commentary on the history and nature of the science in which he has been involved, it seems particularly appropriate to me to take a narrative walk back into the corridors of time and re-visit the conception and development of these 3 concepts. The scenarios for the evolution of successful, high-technology analytical instruments are basically the same, no matter whether they are based on spectroscopic, electrometric, chromatographic, or other principles. The basic steps of these scenarios are as follows:

1. Documentation of sustained need
2. Prior fundamental and applied research on scientific principles
3. Conception of the idea
4. Design and construction of first operating apparatus to verify scientific principles
5. Design and construction of a finely tuned facility to identify parameters that lead to useful analytical figures of merit, e.g., accuracy, sensitivity, and precision
6. Field or laboratory studies to demonstrate acceptable sensitivity, selectivity, accuracy, and precision for various samples matrixes
7. Acceptance by the analytical community
8. Design and construction of engineering prototypes
9. Design and manufacturing of production models
10. Marketing and sales

Steps 5–10 of this scenario typically involve the investment of large sums of venture capital, an investment that must eventually be recovered as profit and as a return on investment. It is therefore essential that there is clearly a sustained need for a new analytical instrument, not just for the next few years, but for a sufficient time interval to make the development of a new instrument commercially attractive.

What needs did we identify? To answer that question, I should identify the high priority needs in the general field of elemental analysis that prevailed at the time explorations



were initiated on the ICP-AES concept. It may surprise some to learn that our and Stanley Greenfield's ICP studies were initiated as early as 1962. To identify the elemental analysis needs at that time, it is instructive to take a walking tour through a versatile analytical chemistry laboratory of 1962 vintage, equipped for elemental determinations at the major, minor, trace, and ultratrace concentration levels in all types of matrixes imaginable. Such a tour would have revealed facilities and competencies for performing analyses by many if not most of the commonly used analytical methodologies, namely, gravimetric, titrimetric, electrometric, spectrophotometric, X-ray fluorescence, atomic absorption, flame, arc, or spark emission spectroscopy, and others. These methodologies complimented each other nicely in that a weakness inherent in one approach was often balanced by a strength in one of the other methods. This however, often presented the analyst with constraints. For example, many of the techniques were useful only for the determination of one element at a time, or in sequence with substantial changes in experimental conditions from one element to another. Some of the methods were best suited for major constituent determinations; others were best for trace constituents, but if they were, their linearity of response, as in atomic absorption spectroscopy, for example, was often rather restrictive. Finally, several of the methods had limited element coverage, and others required lengthy sample pretreatment and separations to reduce interferences. What was needed in the early 1960s was just one analytical approach that fulfilled the following requirements to an acceptable degree.

Requirements of an Ideal Analytical Method for the Determination of the Elements

1. Applicable to all elements
2. Simultaneous or rapid sequential multielement determination capability at the major, minor, trace, and ultratrace concentration level without change of operating conditions
3. No interelement interference effects
4. Applicable to the analysis of microliter or microgram sized samples
5. Applicable to the analysis of solids, liquids, and gases with minimal preliminary sample preparation or manipulation

6. Capable of providing rapid analyses; amenable to process control
7. Acceptable precision and accuracy

The goal of eventually devising an analytical approach that would come as close as possible to meeting these requirements was always lurking in the recesses of my mind. I was convinced that if such a search was successful, the way elemental determinations would be performed in the future would be greatly impacted, for many years, if not forever. Therefore, the need was clear, so we may proceed to steps 2 and 3 of the scenario. I can't imagine anyone conceiving a high-technology analytical concept that is not built on prior fundamental and applied research and an accumulation of prior scientific experiences and realizations. With reference to the defined need, a very significant realization in the early 1960s was that in the steel and aluminum industries, a single analytical instrument, usually operated by a technician, provided simultaneous quantitative elemental determinations at the major, minor, and trace concentration levels for some 10 to 20 elements, under one set of experimental conditions, at a rate of one sample analysis every one to two minutes, adding up to approximately 500 quantitative determinations per hour, with a coefficient of variation of 5–10 parts per thousand. These analyses were and still are being performed by a highly developed technology of using controlled, high-frequency spark discharges to vaporize and atomize sample material from metal self electrodes and to excite the atomic spectra of the released atoms by the same discharge. The simultaneous, multielement capability was provided by multichannel spectrometers (polychromators), based on the principle of isolating the analyte lines of interest by precisely located exit slits along the focal plane. Thus, spark discharge vaporization-excitation combined with AES fulfilled many of the stated requirements of an ideal analytical method.

The notion of turning to atomic emission spectroscopy as an approach to meeting the needs defined above was appealing because among all of the known analytical approaches, it had basic qualities, which, upon refinement, had the potential of satisfying all of the stated criteria listed above to an unusually high degree. Indeed, during the period of the mid-1930s to the early 1960s, various versions of flame, arc, and spark atomic emission spectroscopy were often, if not usually, the methods of choice for multielement determinations at the minor or trace constituent levels. Unfortunately, any serious thought of relying on some form of atomic emission spectroscopy was considered ill-advised by many analysts in the early 1960s, for several important reasons. First, although flame, arc, or spark emission spectroscopy had been used for 3 decades with reasonable success, many perceptive analytical spectroscopists had long recognized that these excitation sources were not performing the vaporization, atomization, excitation, and ionization processes in a satisfying manner, especially for applications involving elemental determinations in solutions and in other liquid materials. As a consequence, there was, in the early 1960s, a general waning of interest in AES.

The decline in popularity of AES was further exacerbated by one of the most exciting developments in the history of analytical chemistry, namely, the emergence of flame atomic absorption spectroscopy (FAAS) as a practical analytical tool (1, 2). The recognition by analysts that FAAS provided a simple, sensitive, and highly specific means for elemental determinations at the minor or trace concentration level contributed so much to its popularity that the use of alternative techniques for performing the same tasks suffered precipitous declines. The technique that experienced the greatest decline in usage was AES. The appeal of AES to analytical chemists and spectroscopists was further undermined by the promulgation, primarily by AAS enthusiasts, of theoretically unsound claims regarding the alleged superiority in analytical figures of merit of observing free atoms in absorption, rather than in emission. The publication of these scientific misdemeanors ceased rather abruptly after the appearance in 1968 of C. J. Alkemade's perceptive paper entitled "Science vs Fiction in Atomic Absorption Spectroscopy" (3). Unfortunately by that time, the reputation of AES as a general analytical tool was sufficiently tarnished that its attractiveness as a research field in academia and as a commercial venture by instrument entrepreneurs continued to decline.

Decline in usage and waning of interest, however, do not necessarily imply total stagnation in activity. One of the spin-offs of the extensive research in FAAS was the development of improved flame and burner systems. The surprisingly good analytical figures of merit exhibited by some of these flames, e.g., oxygen-acetylene and nitrous oxide-acetylene, when the free atoms formed were observed in emission, posed a logical question in the minds of a number of investigators still active in AES. The question? "Would it not be possible to devise electrically generated flames or plasmas, whose higher temperature and less active chemical environment would lead to vast improvements in free atom formation and excitation, while still allowing the precise control of sample injection typically achieved for flame atomization-excitation cells? The search for flame-like plasmas during the period of 1959 to 1970 led to the development of transferred DC plasmas of various characteristics, 3-electrode DC plasmas, and capacity coupled, microwave-frequency powered plasmas (4, 5). A common deficiency among all of these plasmas was inefficient injection of sample material into the plasma core and less than desired residence time in the plasma.

The prior fundamental and applied research that led to the initial explorations of ICPs as vaporization, atomization, excitation, and ionization cells (VAEI), takes us back to 1942, when G. Babat published his first paper on some properties of ICPs (6). Babat continued his investigations at the "Svetlana" plant in Leningrad even while that city was experiencing a severe blockade during the early years of World War II. Babat's studies eventually ceased when the entire power system of the city failed and some of his colleagues perished during the hostilities (7). In spite of these adversities, Babat submitted an English manuscript to the *Journal of the Institute of Electrical Engineering* in 1942. Four years elapsed before the revision was received and the paper was eventually published in 1947 (8). The importance of Babat's papers was that he apparently was the first individual to sustain electrodeless ICPs at atmospheric pressure. Approximatley 20 years later, T. B. Reed described some exceptionally effective ways of forming and stabilizing Ar-supported inductively coupled plasmas (9, 10). Normally, neither the *Journal of Applied Physics* nor these papers would attract the attention of an analytical chemist. Because I majored in physics in undergraduate college, I did then—and still do—scan the physics journals. When I read Reed's papers, I recognized that these plasmas offered several unusually attractive possibilities as VAEI cells for analytical atomic spectroscopy. These attractive possibilities were, first, their high temperatures, which should make them effective VAEI cells; second, because the plasmas are sustained by pure Ar, the free atom lifetimes should be longer than in other atomization cells used for that purpose; and finally, because the discharges are electrodeless, there should be no contamination from electrode materials. The same thought processes must have gone through

the mind of Stanley Greenfield at Albright and Wilson, Ltd, Oldbury, England. As best as we can ascertain, we independently initiated analytical studies on these plasmas in early 1962, within a few weeks of each other. It was not until late 1964 and early 1965, when our first publications appeared (11, 12), that we became aware of each others' activities. These publications constituted completion of step 4 of the scenario discussed above.

The powers of detection reported in these early papers were not particularly encouraging; they were, in fact, considerably inferior to those being reported in the FAAS literature at that time. Obviously, Step 5 of the scenario had to be implemented. These fine-tuning operations were spectacularly successful, as attested by the striking improvements in powers of detection achieved during the next decade (13–17). These improvements resulted primarily from progressive refinements in the following: (a) reduction of radiofrequency interference with the measurement electronics; (b) more effective impedance matching between the high frequency generator-coil combination and the plasma; (c) greater control of forward power regulation; (d) improvements in techniques for generating aerosols of analyte solutions and in their injection into properly shaped plasmas; and (e) optimization of torch design.

During the period of 1969 to 1974, the current state-of-the-art performance of ICP-AES was amply demonstrated (13–18) and repeated application studies in our and Greenfield's laboratories, as well as by others, confirmed that this analytical approach fulfilled the requirements of an ideal analytical method described above to an unusually high degree. Step 6 of the scenario was therefore completed during the 1969-to-1974 interval.

The fulfillment of Step 7 of the scenario in terms of acceptance and endorsement of the new concept by analysts in industry, or the instrument makers, or professors in academia was slow in materializing for several subtle reasons. First, it should be recalled that AAS was at that time enjoying remarkable acceptance as a means for elemental determinations. In view of its popularity, it was not in keeping with human nature to turn to new, somewhat untried, methodologies. Then, as now, when presently used analytical methods are able to provide the required analytical data, albeit in a less satisfactory manner than new approaches, the analyst was not inclined to thoroughly evaluate new approaches in terms of immediate needs. Understandably, the industrial analytical chemist was also quite unwilling to assume a personal risk in committing company's resources to expensive facilities when the instrumentation had not been thoroughly evaluated and the new approach had not gained endorsement by leaders in the field. Unfortunately, several leaders had, at that time, published negative conclusions about the technique. Finally, there was relatively little action by the scientific instrument community to provide the type of keyboard operated, highly automated facilities that many industrial analytical chemists deemed necessary.

In contrast to their very active present participation in ICP-AES research, professors in academia in the USA were very slow in entering this field. Only 4 papers were published (19, 20) by these individuals during the first decade after the publication of the pioneering papers. The instrument makers, too, were slow in implementing steps 8–10 of the scenario. In the absence of strong acceptance by the academic and industrial community, the instrument makers were understandably reluctant to assume the high risk of launching an expensive instrument design, engineering, and manufacturing effort.

I believe that another more subtle factor was also involved. Starting in the early 1970s, the top management of several major instrument companies shifted markedly from scientist-engineer entrepreneurs to financial type managers whose goals and styles are usually more strongly oriented towards improving short-term profits than to investments in long-term promise.

As a result of the slow response of the academic, industrial, and instrument maker communities, only approximately 10 home-built ICP-AES facilities, scattered around the world, were in operation 10 years after the first ICP-AES papers were published. Now, 8 years after the first commercial instruments were marketed, there are approximately 20 vendors internationally; the number of operating facilities is approximately 3000 and increasing at a rate of approximately 500/year, which represents a percentage growth rate higher than for any other analytical instrument (21–23).

With the completion of all 10 steps of the scenario in 1975 and the subsequent rapid growth of ICP-AES, the analytical capabilities of ICP-AES, as summarized below are receiving broad recognition.

1. Applicable to the determination of all elements except Ar
2. Simultaneous or rapid sequential multielement determination of major, minor, trace, and ultratrace elemental constituents without change in experimental parameters
3. Unusually low interelement effects
4. Applicable to the analysis of liquids including microliter volumes, solids, and gases with minimal preliminary sample preparation
5. Acceptable precision and accuracy
6. Amenable to process control applications

The extensive utilization of ICPs as VAEI cells during the past decade has highlighted a number of their attractive physical properties. These properties are summarized below:

1. Plasma can be shaped to allow efficient sample injection into the heart of the plasma.
2. Residence time of sample material can be extended to time periods sufficient to achieve a high degree of atomization and excitation.
3. High temperature, unreactive Ar environment is conducive to longer lifetimes of free atoms.
4. Vaporization process can be spatially separated from the excitation-ionization zones.
5. Unusually small interelement effects exist in the vaporization, atomization, excitation, and ionization processes.
6. Atmospheric pressure operation is possible.
7. Response of atomic line intensities is linear over exceptionally wide concentration ranges.

These properties make ICPs relatively simple but remarkably effective devices for transforming gases and aerosols generated from liquid and solid samples into free atoms, excited atoms, ions, excited ions, molecular ions, and molecular fragments. Once these species are formed, they may provide the analyst with a wealth of information through their emission, absorption, fluorescent, and mass spectra. Because these species are formed at atmospheric pressure, sample interchange and retrieval of spectral information are simplified compared with many other VAEI and molecular fragmentation cells. It is therefore not surprising that these plasmas are finding appplications in analytical tasks that transcend conventional ICP-AES.

Seven years ago, A. Montaser and I published the initial paper on using ICPs as atomization cells for atomic fluorescence spectroscopy (AFS) (24). In prosecuting our ICP-AFS research, we were guided by the pioneering suggestions of Alkemade (25), by the pioneering papers of Winefordner and

associates on combustion flame-AFS (26, 27), and by the extensive subsequent literature in this field (28, 29). In our ICP-AFS paper, we enunciated the obvious advantages of ICPs over combustion flames as atomization cells for AFS. These advantages were (a) greater freedom from interelement effects in the atomization process; (b) minimization of scattering processes from unvaporized particles because of the high degree of atomization achieved; and (c) potentially improved powers of detection because of the lower quenching cross section of Ar as compared to flame combustion products.

Several additional advantages accrue when free atoms are observed in the atomic fluorescence mode. These are reduced probability of spectral coincidences as compared with atomic emission, and the rejection of many of the background shifts observed in atomic emission. In view of these collective advantages, it is only logical that ICP-AFS eventually would make market penetration, which it has with the introduction of the Baird plasma AFS instrument (30). For ICP-AFS, the completion of steps 5–10 of the scenario outlined above required only 5 years.

Many of the properties of ICPs that have vaulted them to supremacy as VAEI cells for atomic spectroscopy are also desirable as an ion source for mass spectroscopy. A review of these characteristics is appropriate. In addition to the attractive properties of ICPs summarized earlier, the following additional attributes are worth noting. First, the localization of analyte ions in the axial channel region is conducive to high ion extraction efficiency. Second, the ions are formed at close to thermal energies (as opposed to much higher energies in many conventional MS ionization sources, e.g., spark ion sources) in an environment where the electrical field to pressure ratio is relatively low. As a result, the lines are sharp and symmetrical. In 1975, we initiated our first explorations on the direct probe sampling of the ion laden, axial channel of ICPs. Successful mass spectroscopic detection of extracted metal ions at trace concentration levels occurred in 1978 and our first paper was published in 1980 (31). Our experimental plan in prosecuting this work relied heavily on the voluminous mass spectroscopic literature and on the prior descriptions of experiences by others on the probe-orifice, high vacuum sampling of very hot gases. Extensive references to these accounts are listed in our first papers (31). In this context, the papers (32–34) by A.L. Gray are particularly worthy of mention. Only 3 years after the publication of our papers, and within the past 6 months, 2 commercial instruments have been introduced, the first by SCIEX, a Canadian firm, and the second by VG-Isotopes, a British firm. So far, no American firm has entered the market. The brief time interval of 3 years from publication of the first ICP-MS paper to the marketing of commercial instruments suggests that the future promise of this version of ICP spectroscopy is viewed as very bright, so much so that at least one observer (R.Barnes) has forecast the possibility that ICP-MS may eventually replace the ICP/optical system (35). ICP-MS does provide a solution to a long-standing problem, namely, the capability of measuring both elemental concentration ratios and isotopic abundance ratios, from trace to major constituent levels, in liquid or solid samples, without the necessity of inserting the sample into the high vacuum environs of the mass spectrometer.

Conclusion

The 3 ICP optical and mass spectroscopic modes of analyses that I have discussed will no doubt continue to exert a major impact on the way elemental determinations are made in the future, but this expectation should not lull us into the notion that these new concepts will be the last word in elemental analyses. There is nothing as constant as change, or, as stated by the historian Tacitus, "Tomorrow's fish is wrapped in paper that contains today's wisdom." Somewhere in the atomic and molecular physics, laser physics, plasma physics or some other topical journal in our canonical scientific literature, the germs of still better ideas are awaiting nurturing by someone who is not completely satisfied by presently available methods.

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BANQUET ADDRESS

Role of Scientific Data in Political Considerations Concerning Pollution

SENATOR ROBERT T. STAFFORD
United States Senator from Vermont

I come here this evening with mixed emotions. It always delights me to have the opportunity to participate in conferences such as this where distinguished individuals from around the world come together to exchange ideas that have the power to enhance the quality of life on this planet. But, it is always with considerable fear that any politician accepts the challenge of addressing a group of scientists and other specialists on a subject that falls within their field of expertise. If I were wiser and more prudent, I would probably limit my discussion with you to some of the issues that have been dominating the headlines and otherwise attracting the attention of our nation in recent months.

Surely there are many pressing problems: shooting wars in our own hemisphere and in tinderbox parts of the world; the series of direct and indirect confrontations of the Soviet Union; unbalanced federal budgets, and still-nagging unemployment and ailing economy. Those issues occupy much of our time and energy because they deal with a past that demands an explanation and with a present that defies easy solution. Experience warns us, however, that challenges of the past and of the present have a tendency to divert our attention from the future. It is a tendency we must guard against. So, if you will permit me, I will talk about the future—and specifically of the urgent need to protect the quality of the environment that is required to sustain our lives if we are to have a future. Thus, it is important to examine what we are doing and where we are trying to go. We have choices to make and challenges to face. The choices are more limited than they were thirty years ago. That means the challenges are greater.

If we are to have a livable environment in the future, we must all clearly understand how much we will have to depend upon each other to achieve that goal. Clearly we need the best efforts and the concerned involvement of scientists and specialists like you who have gathered here. I will have more to say about your role later. But, we must also attract the attention of workers, managers, union leaders, business owners, bureaucrats, politicians and both public and private interest groups. Are there goals common to such varied interests in our quest? Surely there are. And, surely the most basic of the goals are these: Safe and adequate supplies of food and water; safe and healthy housing and workplaces; disposal systems for liquid and solid wastes that perform adequately, and air quality that enhances, rather than diminishes, life. In the United States, our own and previous generations have made substantial gains in the effort to achieve those basic goals. We must clearly protect those gains already recorded. And, we must understand that we and our children and their children face environmental hazards far greater than those of the past.

Before we examine the prospects for the future, it may be worthwhile to review the past. During this nation's period of new frontiers and apparently unlimited resources, there was little perception that we had to restrain the processes that were damaging and diminishing the air, the water, and the land. With few such notable exceptions as the Donora killer smog and the near-death of Lake Erie, there was little apparent evidence to suggest that the deterioration of our environment was a problem. We had only a suspicion, supported by a few scattered facts and some nagging doubts, that a problem of major proportions was building up all around us. Yet, that suspicion set in motion a process designed to gather information about our environment and about ourselves.

That process began to pick up momentum about the time I entered the House of Representatives more than 22 years ago. What we found stunned the Congress and the nation. There were few clean streams and millions of scarred acres in our country. Smoke from industrial plants was poisoning millions of us. Invisible pollutants were causing damage—to health, to crops, and to property. We took the scanty information that had been gathered, and we embarked upon a moderate course designed to reduce the pollution where we could demonstrate a cause and effect relationship. That earlier effort failed to achieve much success—mainly because of the difficulty we had in establishing a causal relationship between pollution sources and environmental effects. We also learned that, when negotiation was the method used, it was no contest in a showdown between environmental and economic factors. Clean air was no match for the dollar, even in good times. The result was that the Congress provided the nation with the rhetoric of law contrasted with the reality of increasing pollution across our nation. But, it was only as pollution became more evident—as beaches were posted for swimming, even in Vermont, for instance—that pollution finally attracted the attention of the Congress. More important, it captured the attention of the public. As so often is the case, once the people of our nation showed the way, Congress began to respond to the public demand for better pollution control efforts.

In the early 1970s, after nearly a decade of experimentation, and about the time I moved to the Senate and became a member of the Environmental Pollution Subcommittee, the Congress made a dramatic decision. The Congress decided that the deterioration of our environment was a national problem and that our nation could no longer afford to accept the massive losses that our national policy—or lack of policy—was costing. It was also noted at this time that there was no naturally occurring force in our society that would protect the environment against the forces that had been insulting it. Thus, it was decided that environmental security would require a federal interest to protect the public interest. The Congress followed different paths in its efforts to control air pollution and to control water pollution—but each strategy was designed to avoid economic discrimination and to permit economic planning. Although the legislative action was taken with the awareness that there was some scientific disagreement—even controversy—over the facts, it was a dramatic and rewarding beginning.

While the action of the Congress was only the first step down a long road, there are many who believe history will record there have been few laws as important or as far-reaching as the Clean Air and Clean Water Acts of the 1970s. They did not, of course, constitute the final solution to the problems they confronted. Adjustments were made as we learned more about the nature of the problem and about the nature of the solutions. We have followed the same general course of action in dealing

with threats to public health and the environment posed by other pollutants that result from industrial activity and the general advances of science—including those posed by synthetic poisonous chemicals.

In all of these instances, we in the Congress depend heavily on the advice of scientific and technical advisers, both in assessing the problems and in seeking solutions. Most of you here are scientists accustomed to the precision and certainty of your professions and disciplines. Unfortunately, those of us who practice the art of politics often are denied the comfort provided by the certainty of science. This is particularly true in the field of pollution control legislation, where decisions must be made in the face of uncertainties because the public health or welfare demand decisions.

Indeed, I am deeply involved at the present time in just such a state of affairs as my Senate Committee on Environment and Public Works struggles with reauthorization of the Clean Air Act. The issue before our committee deals with efforts to control acid rain, an issue that I am certain is familiar to at least some of you. I don't have to tell this gathering that there are uncertainties surrounding the issue of acid rain. We have regulated the emissions of sulfur and nitrogen under the Clean Air Act for nearly 13 years because we know they are hazards to health. What we did not realize until relatively recently is that there is another good reason to regulate the oxides of sulfur and nitrogen—for, once emitted, they can be transformed into acids. Those acids can, in turn, damage lakes, streams, crops, forests and soils. Our scientific community already knows a great deal about this process, and within 3 to 5 years will know much more. But, even a strong advocate of acid rain control like myself will concede that there are gaps in our knowledge. We know, for example, that oxides of sulfur and nitrogen are transported in the atmosphere, sometimes several hundreds of miles but, the pathways of transport are less clear. Emissions can be pinpointed with relative ease and great accuracy but, determining their exact fate is much more difficult. Similarly, we have learned that the oxides of sulfur and nitrogen can be—and often are—transformed during their transportation into acids and acid-forming compounds. The National Academy of Science has recently provided us with information that indicates the transformation takes place at a linear rate, but we are still uncertain about the exact rate of this transformation. Finally, while we have learned that acid deposition is almost certainly causing serious damage to lakes and rivers, our knowledge of its effects on soils, forests and crops is only beginning to evolve.

About a year ago, a letter I received from an official of the U.S. Environmental Protection Agency (EPA) highlighted most of those uncertainties and followed with this conclusion: "These questions and others regarding transformation must be better understood before reasonable scientific judgments can be made with any accuracy." The official who wrote that letter demonstrated that there is a vast difference between judgments that are responsible in science and those that are responsible in politics. When I talk of being politically responsible, I am not talking about pork barrel politics or the selfish protection of vested parochial interests. I am talking about the obligation of an elected public official to make prudent decisions that protect public interests.

You have heard me outline some of the uncertainties that exist in the debate over acid rain. Let me add at this point what, in my view, at least, we have learned with a high degree of confidence:

(1) First, it is clear that acid deposition is occurring.
(2) It is equally clear that a major source of acid deposition is the industrialized Midwest region of our nation.
(3) That acid deposition may or may not cause harm when it falls to earth, but when it falls on soil which is sensitive, it almost always causes some harm.
(4) This harm includes reduced productivity of forests and crops. which may be large.
(5) Virtually the entire Northeast is sensitive to acid rain, and acid rain falls regularly over this region. In short, acid rain is falling on ground where it can cause harm and that ground includes two entire regions of the United States.
(6) In addition to damaging life on or in the ground, acid rain can damage things on and in lakes and streams. This harm is occurring at the present time.

Thus, it is clear to me, the stakes in the acid rain debate are enormous. Failing to act now places entire regions of our nation at risk and threatens the economic well being of farmers, foresters, fishermen and others. Refusal to act because there is a level of uncertainty can be as unwise and foolish as acting too hastily on the basis of too little information. There are many times when it would be irresponsible for a public official to give that uncertainty too much weight in deciding public policy. It is my view that we are in one of those times in our deliberations over the issue of acid rain.

There is some evidence that W. Ruckelshaus, the new and gifted administrator of the Environmental Protection Agency, understands the responsibilities of managing risk in our society. Let me quote from his statement to the Senate Committee on Environment and Public Works during his confirmation hearing. Here's what he, Ruckelshaus had to say on this issue: "The problems EPA confronts are hard ones. Throughout—from the definition of the problem to its solution—the Agency confronts enormous scientific uncertainty. It often must act before it is clear what the optimum solution would be." Because of that circumstance, it is important that we learn how to accommodate environmental requirements even where the costs are uncomfortable and the benefits are distant.

An additional burden we must carry is the fact that necessary environmental investments will not necessarily deliver results that can be readily measured and identified. The benefits of our environmental investment may be nothing more visible than the failure of elements of the food chain to disappear under the pressure of an ill-defined—or even unidentified—threat. All of which is to say that the protection of our environment often requires investment based on suspicion and speculation. If we wait always for absolute knowledge, it will likely be too late to avert disaster.

There are those who oppose the suggestion that the United States should commit itself to a program to reduce sulfur emissions, but, they will also concede that within 3 to 5 years, we will know much more about the subject. When pressed further, they will admit that the design and implementation of a sulfur emissions reduction program will take 3 to 5 years, even if we begin today.

Thus, it is my view that there is no irreconcilable conflict between developing more knowledge and committing ourselves to action. Indeed, the politically responsible course is to take both actions—and to take them now. I intend to continue my efforts to persuade the Congress to do just that. I hope that I am successful. I hope also that you and your colleagues in the scientific community are successful in your efforts to unlock the remaining secrets of the acid rain issue.

DRUGS

Liquid Chromatographic Determination of Hydrocortisone in Bulk Drug Substance and Tablets: Collaborative Study

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A normal phase liquid chromatographic (LC) method for determining the hydrocortisone content of bulk drug substance, tablet composites, and individual tablets was subjected to a collaborative study by 6 laboratories. The results showed a mean recovery of 98.5% for an authentic tablet formulation and reproducibility coefficients of variation of 0.97, 1.6, and 2.7% for bulk drug substance, tablet composites, and individual tablets, respectively. Infrared (IR) and thin layer chromatographic (TLC) identification tests, also included in the collaborative study, were satisfactory. The LC method for determining hydrocortisone in bulk drug substance, tablet composites, and individual tablets, with IR and TLC identification, has been adopted official first action.

A liquid chromatographic (LC) system capable of resolving hydrocortisone from its major degradation products and related corticosteroids was developed (1). The system is applicable to the determination of hydrocortisone in various pharmaceutical dosage forms. The methods for bulk drug substance, tablet composites, individual tablets, and enema samples have been subjected to a collaborative study, and the results for the first 3 products are included in this report. Problems with the uniformity and stability of hydrocortisone enema samples submitted to collaborators indicate a need for further study of that product.

The USP (2) identification tests for hydrocortisone have been evaluated and the proposed modifications have been included in the collaborative study. The major changes consist of improvements in the tablet sample preparation for infrared (IR), substitution of KBr for mineral oil dispersion, and the addition of a thin layer chromatographic (TLC) identification test.

Collaborative Study

Six laboratories participated in the study, with each analyzing 10 tablet and 6 bulk drug samples. The samples consisted of 2 dosage levels of intact commercial tablets and blind duplicate samples from each of 3 commercial tablet composites, an authentic tablet formulation, and 3 lots of bulk hydrocortisone. The collaborators were instructed to perform a single assay on each of the bulk and composite samples, to individually analyze 10 intact tablets of each dosage level, and to perform the specified qualitative tests on 7 of the samples. The assay results for tablet composites were to be reported in terms of mg hydrocortisone/g sample to avoid revealing the average tablet weights, which would have invalidated the blind duplicate system. The potency and uniformity of the samples were determined by the Associate Referee before submission to collaborators. The authentic and commercial tablet composites were passed through a 60 mesh sieve, placed in a jar containing porcelain balls, and mixed on a rotary mixer for several hours. Uniformity was considered acceptable when the coefficient of variation (CV) of 6 assay results obtained with samples taken from different areas of the container did not exceed 1%. The products selected for testing the individual tablet method had the least variation (CV = 0.68% and CV = 1.5%) in individual tablet potencies determined by multiple analyses in the Associate Referee's laboratory.

Hydrocortisone in Drugs
Liquid Chromatographic, Infrared Spectroscopic, and Thin Layer Chromatographic Methods
First Action

Method Performance

Av. rec. at 26–89 mg/g = 98% (S_x = 0.25–1.39)

Principle

Sample is dissolved in MeOH and CH_2Cl_2, and hydrocortisone is detd by liq. chromatography using acetaminophen internal std. Identity is confirmed by IR or TLC.

Apparatus and Reagents

(a) *Liquid chromatograph.*—Equipped with sampling valve capable of introducing 10 μL injections, 25 cm × 4.6 mm id stainless steel column packed with spherical 5–6 μm diam. porous silica particles, and 254 nm UV detector set at sensitivity to produce ca ½ full scale peak ht on suitable recorder for 10 μL injection of std soln. Mobile solv. flow rate 1.5 mL/min, ambient temp.

(b) *Water-washed, 1,2-dichloroethane.*—Shake 500 mL LC grade 1,2-dichloroethane with 250 mL H_2O for 1 min, let layers sep., and filter lower layer thru 0.5–1 μm porosity polytetrafluoroethylene (PTFE) membrane.

(c) *Mobile solvent.*—Mix 55 mL H_2O–LC grade MeOH (5 + 95) soln with 1.0 mL glacial HOAc and dil. to 1 L with H_2O-washed 1,2-dichloroethane. Degas mixt. Adjust H_2O and MeOH content to obtain suitable retention times.

(d) *Internal std soln.*—Dissolve 200 mg acetaminophen (100% pure, Aldrich Chemical Co.) in 4 mL MeOH and dil. to 200 mL with H_2O-washed 1,2-dichloroethane.

(e) *Std soln.*—Accurately weigh ca 10 mg USP Ref. Std Hydrocortisone, add 2 mL MeOH and 4.0 mL internal std soln, and dil. to 50 mL with LC grade CH_2Cl_2.

Chromatographic System Suitability Test

Equilibrate LC system and inject 10 μL portions of std soln. Retention times for hydrocortisone and acetaminophen should be ca 8 and 10.5 min, resp. Column efficiency, n, calcd using hydrocortisone peak, should be ≥ 5000 theoretical plates; resolution, R_s, between hydrocortisone and acetaminophen peaks should be ≥ 2.5. Inject 6 replicate 10 μL portions of std soln and calc. response ratios, R', of hydrocortisone peak relative to acetaminophen peak. Relative std deviation for six R' values should be ≤ 1.0%.

The recommendation of the Associate Referee was approved by the General Referee and Committee B and was adopted by the Association. See the General Referee and Committee reports, *J. Assoc. Off. Anal. Chem.* 1984 **67**, March issue.

This report of the Associate Referee was presented at the 96th Annual International Meeting of the AOAC, Oct. 25–28, 1982, at Washington, DC.

Sample Preparation

(a) *Bulk material.*—Accurately weigh ca 50 mg previously dried (3 h at 105°) sample, add 10 mL MeOH and 20.0 mL internal std soln, and dil. to 250.0 mL with CH_2Cl_2.

(b) *Tablet composite.*—Weigh and finely powder ≥ 20 tablets. Accurately weigh portion of powder equiv. to 1 tablet and transfer to vol. flask of a size to yield final hydrocortisone concn of 0.2 mg/mL. Add 2 mL MeOH for each 10 mg of labeled hydrocortisone content. Place flask in ultrasonic bath 2 min. Add CH_2Cl_2 until flask is ca ½-full and return to ultrasonic bath 1 min. Add 4.0 mL internal std soln for each 10 mg labeled hydrocortisone content. Dil. to vol. with CH_2Cl_2, mix, and filter thru 0.5–1.0 μm porosity PTFE membrane.

(c) *Individual tablets.*—Place 1 tablet in g-s or vol. flask of a size to yield final hydrocortisone concn of ca 0.2 mg/mL. Place 100 μL H_2O for each 10 mg hydrocortisone directly on tablet and let soak 30 min. Add 2 mL MeOH for each 10 mg of labeled hydrocortisone content and place flask in ultrasonic bath 10 min or until tablet disintegrates. Proceed as in *Tablet composite,* starting with "Add CH_2Cl_2 until. . . ."

Determination

Equilibrate LC system by passing mobile phase thru column ca ½ h. Inject 10 μL portions of std soln until R' values for 3 consecutive std chromatograms agree within 1%, then inject 10 μL sample prepn.

$$\text{mg Hydrocortisone in sample} = (R/R') \times (V/V') \times W$$

where R and R' = response ratios of hydrocortisone peak relative to internal std peak for sample and std, resp.; V' and V = mL internal std soln in std and sample solns, resp.; W = mg hydrocortisone in std soln.

Infrared Spectroscopic Identification

(a) *Bulk material.*—IR spectrum of KBr dispersion of sample, previously dried 3 h at 105°, exhibits max. at same wavelengths as that of similar prepn of USP Ref. Std Hydrocortisone.

(b) *Tablets.*—Powder tablets equiv. to ca 50 mg hydrocortisone and digest 5 min with 15 mL hexane. Decant hexane, ext residue with 15 mL peroxide-free ether in same manner as before, and discard ext. Digest residue with 25 mL dehydrated alcohol for 5 min, filter, and evap. alcohol ext on steam bath to dryness. Add 10 mL H_2O, mix, let residue settle, and decant H_2O. Dry residue at 105°. Dissolve USP Ref. Std Hydrocortisone in alcohol and treat as sample prepn, beginning with "evap. alcohol ext. . . ." The IR spectrum of KBr dispersion of sample residue exhibits absorbance maxima at the same wavelengths as that of similar USP Ref. Std Hydrocortisone prepn.

Thin Layer Chromatographic Identification

Apparatus and Reagents

(a) *Thin layer plate.*—20 × 20 cm, coated with 0.25 mm thick layer of chromatographic silica gel with fluorescent indicator.

(b) *Developing solvent.*—$CHCl_3$–MeOH–H_2O (180 + 15 + 1).

(c) *Spray reagent.*—H_2SO_4–EtOH (1 + 4).

(d) *Std soln.*—0.2 mg USP Ref. Std Hydrocortisone/mL CH_2Cl_2.

Procedure

Apply 10 μL portions of sample prepn from LC *Determination* and std soln (d) to TLC plate 2 cm from bottom edge. Develop plate in suitable tank equilibrated with developing solv. Let plate dry and examine under shortwave UV light. Spray plate with H_2SO_4–EtOH (1 + 4), heat 5 min at 120°, and examine under longwave UV light. Hydrocortisone has R_f of ca 0.2, appearing as dark spot under UV before spraying and as bright yellow spot in final step.

Results and Discussion

The collaborators used 2 different brands of LC pumps, 3 different detectors, and both manual and automated sample injectors. A 25 cm × 4.6 mm id column packed with 5–6 μm spherical silica particles was specified and all collaborators used the same brand (Zorbax SIL, DuPont Co., Wilmington, DE 19891). Although either peak area or peak height ratios are allowed by the method, all collaborators used electronically integrated areas obtained with 4 different types of equipment.

The chromatographic system suitability requirements were met without apparent difficulties and the results are summarized in Table 1. Based on the reported differences in column efficiency and resolution, it appears that columns of varying age and degree of activation were used but all were suitable. The peak area ratio precision was well within the specified CV ≤ 1% limit. A typical chromatograph is shown in Figure 1.

The collaborative study data were statistically evaluated by using the procedures described by Youden and Steiner (3). Results of the hydrocortisone bulk drug substance assay are summarized in Table 2. Data from Collaborator 3 were eliminated on the basis of Steiner's ranking test and one set of data from Collaborator 1 was dropped as an outlier using the Dixon test.

The CVs for the 3 samples ranged from 0.28 to 1.52%, while the reproducibility between collaborators and repeatability within laboratories both had CVs ≤ 1%. The mean results obtained by the collaborators were in good agreement with the Associate Referee's results for the respective samples.

Tablet composite assay results are presented in Table 3. The results obtained by Collaborator 3 again showed a high bias and were dropped on the basis of Steiner's ranking test. Examination of this collaborator's data indicated abnormally low relative response for the hydrocortisone standard used to obtain this set of data. This could be attributed to a weighing or dilution error; however, it was not possible to resolve this question. Three individual results (Collaborator 1, Samples 7/8 and 11/12, Collaborator 2, Sample 13/14) were eliminated by the Dixon and modified Cochran tests. The mean recovery for the authentic sample was 98.5% with a CV of 1.1%. The 3 commercial samples had CVs ranging from 0.78 to 1.56%, and agreed closely with the Associate Referee's results. The between-laboratory reproducibility and the within-laboratory repeatability of the pooled data were satisfactory, CV = 1.6 and 1.0%, respectively.

The results of individual tablet analyses are presented in Table 4. Two outliers were found by applying Thompson's test (3) and were eliminated. The mean for the 5 mg dosage level was 101.9% of the labeled amount with a CV of 2.5%. The results for the 10 mg tablets had a mean of 97.2% of

Table 1. LC system suitability

	Collaborator					
Requirement	1	2	3	4	5	6
Column efficiency, $N \geq 5000$	6626	6529	6529	5246	11360	6850
Resolution, $R_s \geq 2.5$	6.2	6.9	6.7	8.9	9.1	3.4
Precision, RSD ≤ 1.0%	0.41	0.2	0.57	0.1	0.5	0.33

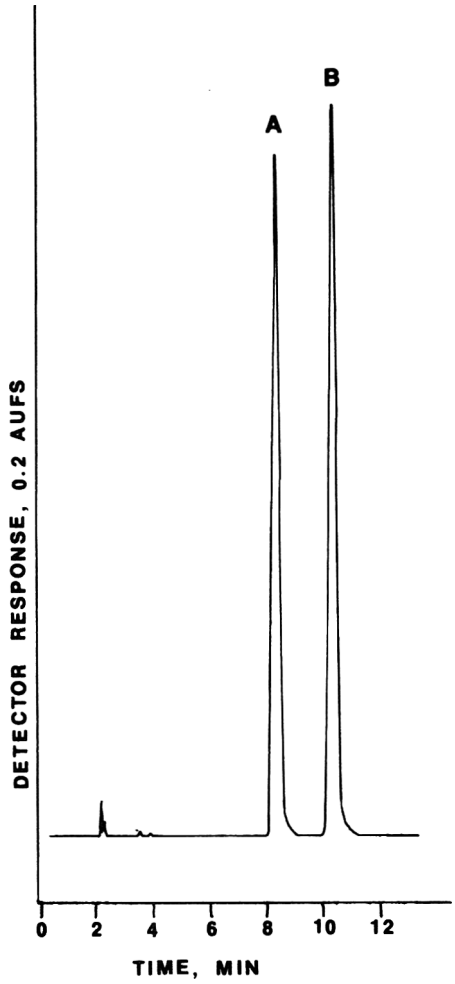

Figure 1. Typical liquid chromatogram of tablet sample preparation. A, hydrocortisone; B, acetaminophen internal standard. AUFS = absorbance unit full scale.

declared with a 3.2% CV. The precision of the data in this case represents not only the method but also includes the nonuniformity of the product between individual tablets. In an attempt to partially compensate for the product variability, the results were normalized by dividing each value by the individual tablet weight and multiplying by the average tablet weight. The precision of the data was not significantly affected; the new CVs were 2.66 and 3.04% for the 5 and 10 mg tablets, respectively. Since it is not possible to completely eliminate the product variability effect from the data, the actual method

Table 2. Collaborative results of hydrocortisone bulk drug substance assay (% by weight on dried basis)

	Sample/blind duplicate					
Coll.	1/2		3/4		5/6	
(0)[a]	(98.6)		(98.5)		(97.2)	
1	99.8,	98.4	99.6,[b]	99.2[b]	97.5,	100.4
2	98.6,	98.6	97.8,	98.6	96.3,	95.9
3[c]	100.7,	100.0	100.7,	101.5	99.4,	100.0
4	98.8,	99.7	98.1,	98.0	96.4,	95.8
5	98.0,	98.7	97.7,	98.2	95.4,	98.3
6	99.3,	99.3	98.2,	98.0	97.4,	97.1
Mean	98.9		98.0		97.1	
SD	0.58		0.28		1.47	
CV, %	0.59		0.28		1.52	
Pooled mean = 98.1						
Reproducibility SD = ± 0.95, CV = 0.97%						
Repeatability SD = ± 0.88, CV = 0.90%						

[a]Associate Referee's results, not included in statistical analysis.
[b]Outlier by Dixon test.
[c]Collaborator's data rejected based on Steiner's ranking test.

Table 3. Collaborative results of tablet composite assay (mg/g)

	Sample/blind duplicate			
Coll.	7/8[a]	9/10[b]	11/10[c]	13/14[d]
(0)[e]	(26.04)	(26.48)	(48.29)	(88.38)
1	27.6,[f] 27.6[f]	26.3, 26.5	51.6,[f] 51.7[f]	90.4, 89.6
2	25.3,[f] 26.2	25.8, 26.1	48.0, 47.3	88.4,[g] 85.2[g]
3[h]	27.5, 28.2	28.0, 27.9	51.4, 50.8	94.4, 92.6
4	25.6, 25.8	26.1, 26.0	47.5, 47.8	87.1, 88.6
5	25.9, 25.5	26.6, 26.4	48.3, 48.4	91.0, 90.3
6	25.9, 25.5	26.2, 26.4	48.0, 47.8	87.5, 88.0
Mean	25.7	26.2	47.9	89.0
SD	0.29	0.25	0.37	1.39
CV, %	1.1	0.94	0.78	1.56
Recd, %	98.5%			
Decld, %		99.8	95.6	99.1
Pooled mean = 47.2 mg/g. 98.0%				
Reproducibility SD = ± 0.76, CV = 1.6%				
Repeatability SD = ± 0.46, CV = 1.0%				

[a]Dosage level 5 mg/tablet; average weight 0.1917 g/tablet; authentic.
[b]Dosage level 5 mg/tablet; average weight 0.1898 g/tablet; commercial.
[c]Dosage level 10 mg/tablet; average weight 0.1995 g/tablet; commercial.
[d]Dosage level 20 mg/tablet; average weight 0.2229 g/tablet; commercial.
[e]Associate Referee's results, not included in statistical analysis.
[f]Outlier determined by Dixon test.
[g]Outlier determined by Cochran test.
[h]Collaborator's data rejected based on Steiner's ranking test.

precision (reproducibility) is expected to be better than suggested by the data, although even based on these results the method is considered acceptable for individual tablet analysis.

All collaborators reported positive results for the IR and TLC identification tests performed on Samples 1, 3, 5, 7, 9, 11, and 13. Typical standard and tablet sample IR spectra are shown in Figure 2. No problems were reported with the IR test and all of the submitted spectra were of acceptable quality. Collaborator 3 reported a curved solvent front in the TLC plate development. This problem was not encountered by any other collaborators and is attributed to an isolated problem related to that analyst's equipment or technique. Collaborator 3 was able to obtain positive TLC results in spite of the curved development problem by bracketing sample spots with standards.

The collaborators were encouraged to submit comments regarding the methodology or problems they encountered. The majority of the comments were received from Collaborator 2, who expressed concern about the normal phase LC system relative to the more commonly used reverse phase systems. The main advantage of normal phase LC for corticosteroids is its superior selectivity (1). The various concerns regarding potential difficulties in working with normal phase LC were considered during development of the method and in actual practice serious problems did not materialize.

The technical difficulties encountered by some of the collaborators were apparently overcome and satisfactory results were obtained. These problems, involving temporary baseline instability (Collaborators 2 and 3) and discoloration of the internal standard solution (Collaborators 1 and 3), had been encountered and resolved during method development. The baseline problems are typical of those observed when using one supplier's 1,2-dichloroethane, which contains an impurity. The impurity must be removed by water-washing as specified in the method. The contaminated columns can be reconditioned by washing with acetonitrile or methanol. Baseline problems were not encountered by other collaborators and the 2 reports are very possibly due to inadvertent contamination of the column or possible omission of the water-washing step. The discoloration of the internal standard solution is caused by impure acetaminophen, impure

Table 4. Collaborative results of individual tablet analysis (% of declared)

Coll.										
					Sample 15, labeled 5 mg/tablet					
1	106.0,	102.0,	114.0[a]	104.0,	102.0,	108.0,	114.0,[a]	108.0,	104.0,	106.0
2	100.0,	100.0,	99.6,	101.4,	100.8,	102.0,	103.2,	100.0,	107.2,	107.6
3	100.0,	100.2,	100.2,	101.4,	100.8,	100.4,	101.6,	102.4,	102.4,	103.0
4	103.2,	100.0,	101.6,	99.0,	100.8,	102.2,	102.0,	101.2,	99.2,	101.6
5	101.8,	104.4,	100.8,	102.8,	101.8,	101.8,	104.0,	102.2,	109.4,	103.2
6	100.2,	99.0,	98.4,	99.4,	99.8,	97.8,	100.6,	99.2	98.8,	100.4
	Mean =	101.9								
	SD =	2.57								
	CV, % =	2.52								
					Sample 16, labeled 10 mg/tablet					
1	101.0,	102.0,	101.0,	99.0,	101.0,	102.0,	98.0,	101.0,	99.0,	99.0
2	94.0,	98.4,	93.3,	91.9,	98.4,	94.6,	94.3,	96.3,	92.8,	98.8
3	95.1,	94.9,	95.6,	99.8,	102.4,	97.9,	95.8,	103.4,	102.4,	101.4
4	93.5,	99.8,	93.8,	99.0,	96.3,	97.4,	96.9,	92.6,	96.0,	95.7
5	95.2,	97.3,	94.0,	97.2,	96.0,	95.6,	95.8,	102.2,	98.9,	92.7
6	95.0,	94.0,	96.8,	95.1,	95.6,	95.2,	97.1,	95.3,	96.6,	96.4
	Mean =	97.2								
	SD =	3.1								
	CV, % =	3.2								
Pooled mean = 99.6%										
Reproducibility SD = ± 2.70, CV = 2.7%										
Repeatability SD = ± 2.32, CV = 2.3%										

[a]Outliers by Thompson's test.

1,2-dichloroethane, detergent residue in the glassware, or a combination of more than one of these factors. Color in the internal standard solution did not affect the assay results; however, the use of water-washed 1,2-dichloroethane to prepare the internal standard is specified in the final method.

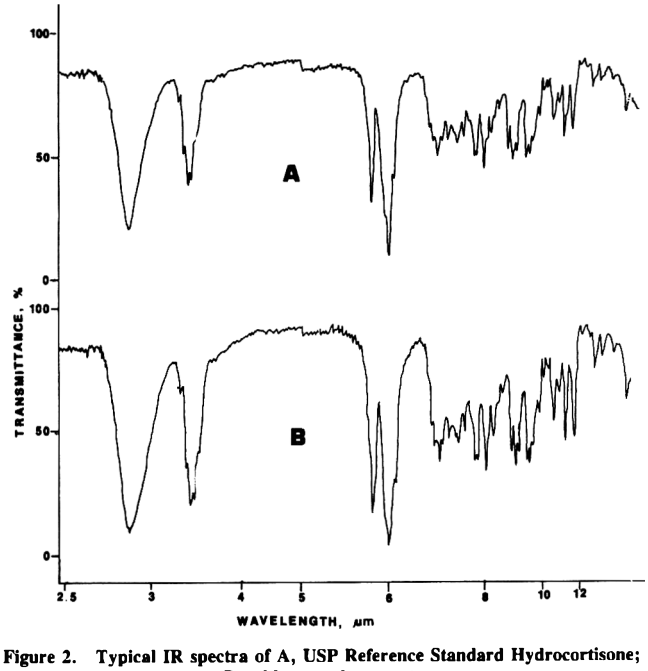

Figure 2. Typical IR spectra of A, USP Reference Standard Hydrocortisone; B, tablet sample extract.

Recommendation

It is recommended that the LC method for determining hydrocortisone in bulk drug substance, tablet composites, and individual tablets, with identification by IR and TLC, be adopted official first action.

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Kenryu Tsuru, FDA, Los Angeles, CA

Nicholas Falcone, FDA, Philadelphia, PA

Everett H. Jefferson, FDA, St. Louis, MO

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Nuclear Magnetic Resonance Spectroscopic Determination of Dicyclomine Hydrochloride in Tablet, Capsule, and Injection Dosage Forms

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A rapid and specific nuclear magnetic resonance (NMR) spectroscopic method was developed for determining dicyclomine hydrochloride in tablet, capsule, and injection dosage forms. The method consists of an extraction step with chloroform, evaporation of the solvent, addition of maleic acid as an internal standard, dissolution of the mixture in deuterated chloroform-deuterated acetone (40 + 60), NMR spectral determination, and integration of the peaks of interest. The concentration of dicyclomine hydrochloride in the dosage form was calculated from the integral values for the peaks of the test compound and the internal standard. The average recovery value ± the standard deviation (n = 5) of dicyclomine hydrochloride added to synthetic samples was 99.7 ± 0.9% (coefficient of variation 0.9%). The assay values for various commercial tablets, capsules, and injectables analyzed by using the proposed method differed in all cases by <1% from those obtained by using the USP XX titrimetric method. There was no interference from stearate, an excipient found in tablets and capsules, or from chloral hydrate, a preservative found in injectables.

Dicyclomine hydrochloride (2-(diethylamino)ethyl (bicyclohexyl)-1-carboxylate hydrochloride) is an anticholinergic agent which, by virtue of its antispasmodic effect, decreases the motility of the gastrointestinal tract without affecting gastric secretion (1). This drug is used for the treatment of a variety of gastrointestinal disorders such as irritable colon, spastic constipation, spastic colitis, mucous colitis, pylorospasm, and biliary dyskinesia, as well as for delaying gastric emptying during therapy for peptic ulcers (1).

In the USP XX method (2) dicyclomine hydrochloride in dosage forms is determined titrimetrically. In this method, methyl yellow, a known carcinogen (3), and 0.004M sodium lauryl sulfate are added as the indicator and titrant, respectively, to the sample in a chloroform–water solvent system. Several commercial dosage forms of dicyclomine hydrochloride contain dyes that make detection of the end point difficult (4). Furthermore, the titrant tends to develop a precipitate (4) and it must be restandardized before each analysis (2). As an alternative, several gas chromatographic (GC) methods have been reported (5–7), but even when automated (4) or semiautomated (6), these methods are lengthy and require the preparation of several reagents.

This paper describes a nuclear magnetic resonance (NMR) spectroscopic method for the determination of dicyclomine hydrochloride in dosage forms that avoids many of the problems encountered with the titrimetric and GC methods, and which at the same time can be used for the positive identification of this drug in the sample tested.

Experimental

Apparatus

All NMR spectra were recorded on a 90 MHz Varian EM-390 spectrometer at an ambient probe temperature of 35°C, using a sweep time of 5 min and a sweep width of 10 ppm.

Reagents

(a) *Dicyclomine hydrochloride.*—Laboratory working reference standard.

(b) *Maleic acid.*—Eastman Kodak Co., Rochester, NY.

(c) *Deuterated chloroform and deuterated acetone.*—+99.5% isotopic purity (Aldrich Chemical Co., Milwaukee, WI).

(d) *Tetramethylsilane (TMS).*—Mallinckrodt Chemical Works, St. Louis, MO.

Samples

Dicyclomine hydrochloride tablets, capsules, and injectables were obtained from commercial sources.

Procedure

Tablets and capsules.—Weigh and finely powder ≥20 tablets, or weigh and combine contents of ≥20 capsules. Accurately weigh portion of powder equivalent to ca 200 mg dicyclomine hydrochloride, transfer to 60 mL separatory funnel containing cotton plug in stem, and extract with 3 ca 5 mL portions of chloroform. Quantitatively transfer chloroform extracts to 25 mL glass-stopper flask and evaporate to dryness with aid of stream of air. To residue add ca 200 mg maleic acid, accurately weighed, 1 mL deuterated chloroform, and 1.5 mL deuterated acetone. Stopper flask and effect solution by means of vortex mixer, keeping solution from touching stopper. Transfer ca 0.4 mL solution to analytical NMR tube containing 1 drop of TMS. Place tube in NMR spectrometer and record spectrum, using spin rate that will produce no interfering spinning side bands between 3.0 and 4.0 ppm and between 6.0 and 7.0 ppm. Assign all chemical shifts with reference to TMS taken as 0 ppm. Integrate overlapping quartet and triplet centered at ca 3.5 ppm (dicyclomine hydrochloride) and singlet at ca 6.46 ppm (maleic acid) at least 5 times. Obtain average integration values and calculate amount of dicyclomine hydrochloride per dosage unit, using equation given below.

Injectables.—Combine sufficient number of dosage units to obtain aliquot of sample solution equivalent to ca 200 mg dicyclomine hydrochloride. Pipet this aliquot into 60 mL separatory funnel, and proceed as described under *Tablets and Capsules,* starting with ". . . extract with 3 ca 5 mL portions of. . . ".

Freeze-dry method for injectables.—Transfer volume of injectable solution equivalent to ca 200 mg dicyclomine hydrochloride to vacuum jar, add ca 200 mg maleic acid, accurately weighed, mix, and freeze solution at ca −40°C. Freeze-dry frozen sample to dryness at ca 10^{-3} torr. Add ca 1 mL deuterated chloroform and ca 1.5 mL deuterated acetone to residue and effect solution by means of vortex mixer. Then proceed as described under *Tablets and Capsules,* starting with "Transfer ca 0.4 mL solution to. . . ".

Calculations

Amount of dicyclomine hydrochloride (as $C_{19}H_{35}NO_2HCl$) in dosage form is calculated as follows:

$$\text{mg/tablet or capsule} = (A_{sp}/A_{st}) \times (EW_{sp}/EW_{st}) \times (C_{st}/C_{sp}) \times \text{av. tablet or capsule wt}$$

$$\text{mg/mL injectable} = (A_{sp}/A_{st}) \times (EW_{sp}/EW_{st}) \times (C_{st}/V)$$

where A_{sp} is average value of integration of overlapping quartet and triplet centered at ca 3.5 ppm dicyclomine hydrochlo-

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ride; A_{st} is average value of integration of singlet at ca 6.46 ppm due to olefinic protons of maleic acid; EW_{sp} is formula weight of dicyclomine hydrochloride (molecular weight divided by number of absorbing protons, 345.94/6 = 57.66); EW_{st} is formula weight of maleic acid (molecular weight divided by number of absorbing protons, 116.07/2 = 58.04); C_{st} is weight of maleic acid used, mg; C_{sp} is weight of sample used, mg; and V is volume of injectable used, mL.

Results and Discussion

Figure 1 shows the 90 MHz spectra of dicyclomine hydrochloride and maleic acid recorded under the suggested conditions. Dicyclomine hydrochloride displayed the following resonance signals relative to TMS serving as the internal reference: broad multiplet between about 0.73 and 2.19 ppm due to the 21 protons of the cyclohexyl groups, triplet at about 1.43 ppm corresponding to the 6 protons of the methyl groups, quartet centered at about 3.43 ppm ascribable to the protons of the 2 methylenes on the *N*-ethyl groups, triplet at about 3.63 ppm due to the 2 protons of the methylene group beta to the oxygen atom, and triplet centered at about 4.57 ppm from the 2 protons of the methylene group alpha to the oxygen atom. The resonance signals selected for calculations were the overlapping triplet and quartet centered at about 3.50 ppm, and corresponding to 6 protons.

Maleic acid was selected as the internal standard on the basis of its singlet due to the olefinic protons, which appeared at the convenient position of about 6.46 ppm. Based on the reported solubilities of dicyclomine hydrochloride and maleic acid, water appeared to be a good solvent; however, the resonance signal of water showed approximately the same chemical shift as that of the triplet due to the methylene alpha to the oxygen atom of dicyclomine hydrochloride. Furthermore, the slight amount of residual HDO that is normally present in deuterated water would interfere with the integration of the peaks of interest on account of the broadness of its resonance signal and the intensity of its side bands. Although this interfering peak could be shifted further downfield by acidification of an aqueous solution of the test sample, the acid caused a parallel conversion of maleic acid to its more stable trans form, fumaric acid. Since fumaric acid is far less soluble than maleic acid, the former started to separate out of solution and to introduce error into the results. Although the specimen of maleic acid used in this study had a declared fumaric acid content of <0.5%, the integral value for these 2 acids was used in the calculations without affecting the accuracy of the assay. If desired, a purity correction factor for maleic acid may be included in the calculations.

Deuterated chloroform readily dissolved dicyclomine hydrochloride but not maleic acid, unless a certain amount of deuterated acetone was added. Neither the weak signal of the residual chloroform at about 7.27 ppm nor the multiplet from deuterated acetone (CD_3COCD_2H) at about 2.00 ppm interfered with the peaks of interest. Thus, a mixture of deuterated chloroform-deuterated acetone (40 + 60) was used as the NMR solvent.

Stearates are present as excipients in certain tablets and capsules of dicyclomine hydrochloride. The methylene protons of the long hydrocarbon chain resonate at about 1.27 ppm, upfield from the signals of interest, and therefore do not interfere with the assay. However, if the need arises, stearates can easily be eliminated by treating an aqueous solution of the sample with mineral acid, removing the insoluble stearic acid by filtration, and then proceeding with the extraction of the test compound into chloroform.

Chloral hydrate is added to dicyclomine hydrochloride injectables as a preservative. Although this compound gives a singlet at about 5.30 ppm, it does not interfere with the

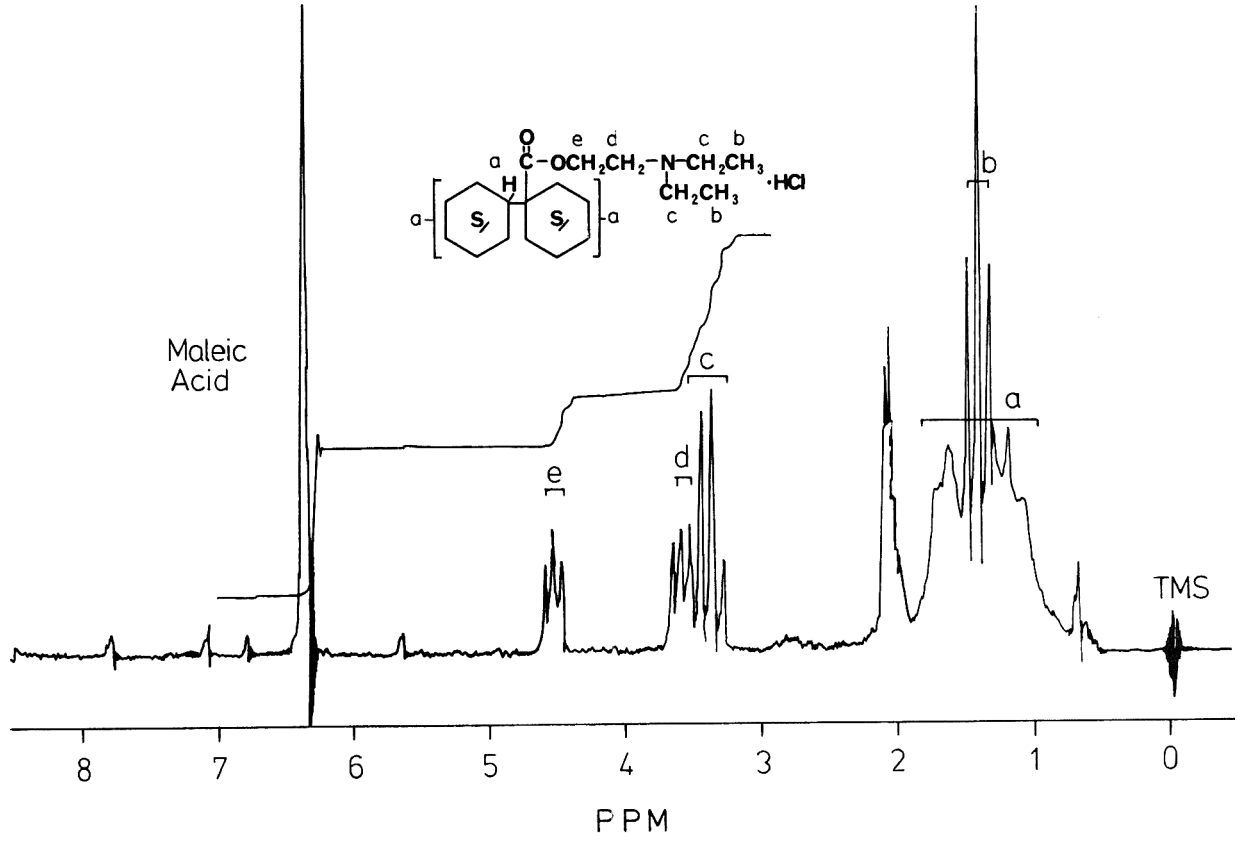

Figure 1. 90 MHz spectrum of dicyclomine hydrochloride and maleic acid in $CDC_3-(CD_3)CO$ (40 + 60).

analysis because it is lost during the evaporation of the chloroform extract or the freeze-drying step due to its high volatility.

Results of the NMR analysis of several mixtures of dicyclomine hydrochloride with varying amounts of maleic acid are summarized in Table 1. The relative proportions of maleic acid to dicyclomine hydrochloride had no significant effect on the accuracy of the determination within the range of concentrations shown in Table 1.

Various lots of commercial tablets, capsules, and injectables were analyzed by both the proposed NMR method and the compendial method. Table 2 summarizes the results and indicates that the values obtained by the NMR method differed by only 1% or less from the corresponding values obtained by the compendial method.

In summary, the NMR method described is simple, rapid, and specific. It is reproducible, precise, and sensitive enough to measure as little as 10 mg dicyclomine hydrochloride/tablet, capsule, or milliliter of injectable. In addition the method can serve as a conclusive identification test for this drug.

Table 1. Determination of dicyclomine hydrochloride in standard mixtures by NMR

		Dicyclomine hydrochloride		
Std mix.	Maleic acid added, mg[a]	Amt added, mg	Amt found, mg	Rec., %
1	200.0	252.8	253.7	100.4
2	20[-].2	183.9	183.6	99.8
3	194.9	205.0	206.4	100.7
4[b]	198.7	198.7	196.7	98.9
5[b]	200.0	195.3	192.3	98.5
Av.				99.7
SD				0.9
CV, %				0.9
USP XX		20.00	19.98	99.98

[a]Internal standard.
[b]Drug and internal standard were dissolved in 10 mL water and solution was freeze-dried.

Table 2. Determination of dicyclomine hydrochloride in commercial dosage forms by proposed and USP XX methods

	NMR		USP XX	
Sample	Amt found, mg	Amt found, %	Amt found, mg	Amt found, %
Tablets, 20 mg				
1	20.40	102.0	20.30	101.5
2	20.40	102.0	20.30	101.5
3	20.50	102.5	20.30	101.5
Capsules, 10 mg				
1	9.88	98.8	10.00	100.0
2	9.86	98.6	9.99	99.9
3	10.03	100.3	9.95	99.5
4	9.89	98.9	9.97	99.7
5	9.97	99.7	9.98	99.8
Injectable, 10 mg/mL				
1	10.06	100.6	10.01	100.1
2	10.06	100.6	10.05	100.5
3	10.11	101.1	10.02	100.2

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Liquid Chromatographic Determination of Methocarbamol in Injection and Tablet Dosage Forms: Collaborative Study

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Collaborators: J. Allgire; R. L. Ballbach; J. Booth; D. Johnson; M. Lookabaugh; B. C. Tyson

A reverse phase liquid chromatographic method was developed for determining methocarbamol in injection and tablet dosage forms. The injections require dilution only; the tablets require a filtration step before introduction into the chromatograph. Response for methocarbamol was linear over the range 0–18 μg, using an ultraviolet detector at 274 nm. Recoveries by the author ranged from 96.1 to 101.9% for authentic injection formulations and 98.0 to 101.0% for authentic tablet formulations. A collaborative study of the method by 6 laboratories resulted in standard deviations of 1.70 and 2.22 for injection and tablet dosage forms, respectively. The method has been adopted official first action.

Methocarbamol is a carbamate structural analog of the aryl glyceryl ethers, which have muscle relaxant effects (1), and is used to treat skeletal muscle spasms (2).

Present manufacturing technology uses guaifenesin (3-(*o*-methoxyphenoxy)-1,2-propanediol) as the starting material in the synthesis of methocarbamol to form a cyclic carbamate intermediate (4-(*o*-methoxyphenoxymethyl)-1,3-dioxolone-2). This cyclic carbonate is reacted with ammonia, which ruptures the ring to form the primary carbonate methocarbamol (3-(*o*-methoxyphenoxy)-1,2-propanediol 1-carbamate) (3). Two known side reactant products can form: the secondary isomer of methocarbamol (3-(*o*-methoxyphenoxy) 2-carbamate propanol) and *N*-[2-hydroxy-3-(2-methoxyphenoxy)propyl] urea (3).

The earlier USP XX assays for methocarbamol injections and tablets (4) employed separatory funnel and Celite chromatographic column separations, respectively, followed by an infrared (IR) spectrophotometric determinative step. The IR absorbance was measured at about 5.78 μm, which is the characteristic wavelength of the methocarbamol carbonyl functional group. All of the known possible impurities with the exception of guaifenesin also contain this functional group and would contribute to the IR absorbance.

In addition to the nonspecificity of both assays, an interference of unknown origin was consistently obtained in all sample IR spectra in the 5.93–6.19 μm region, using the procedure for tablets. This peak contributed to sample absorbance, causing a potential 5% error. The earlier USP methods required methocarbamol concentrations of 20 mg/mL and an IR cell path length of 0.1 mm. Because of the volatility of chloroform, the need for exhaustive cleaning of the small path length size cells, and the relatively high concentrations of methocarbamol specified, meticulous attention to technique was necessary to produce reliable results.

This study was initiated as a result of the Compendial Monograph Evaluation and Development program established by the Food and Drug Administration (FDA). The purpose was to develop specific and stability-indicating assays for the determination of methocarbamol in liquid and solid dosage forms. The proposed liquid chromatographic (LC) method uses a C_{18} reverse phase bonded column, a mobile phase of water and methanol, acetanilide as an internal standard, and a detector operated at 280 nm. (If a variable wavelength detector is available, it is optimally operated at 274 nm.) Response for methocarbamol was linear from 0 to 18 μg. All known impurities are separated from methocarbamol at the specified retention times and resolution. This proposed assay has been adopted by the USP (5, 6).

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The recommendation of the author was approved by the General Referee and Committee B and was adopted by the Association. See the Referee and Committee reports, *J. Assoc. Off. Anal. Chem.* (1984) **67**, March issue.

Collaborative Study

A collaborative study was designed to evaluate the proposed LC method. A total of 12 samples (6 each in blind duplicates) were provided to 6 collaborators. The samples were as follows:

Injection dosage forms:

(1) an authentic mixture formulated to contain 97.5 mg methocarbamol and 2.1 mg guaifenesin/mL.

(2) 2 commercial lots each labeled to contain 100 mg methocarbamol/mL. (The manufacturer formulates this product with a 4% overage.)

Tablet dosage forms:

(1) an authentic mixture formulated to contain 85.36 mg methocarbamol/100 mg.

(2) 1 commercial lot labeled to contain 500 mg methocarbamol/tablet.

(3) 1 commercial lot labeled to contain 750 mg methocarbamol/tablet.

(Samples 2 and 3 were from different manufacturers.)

Methocarbamol in Drugs
Liquid Chromatographic Method
First Action
(Applicable to injectables and tablets)

Method Performance

Injection av. rec. at 100 mg/mL = 98.3% (S_x = 1.36)

Tablet av. rec. at 95.0 mg/g = 98.9% (S_x = 4.33)

Principle

Methocarbamol is dissolved in aq. MeOH and detd by liq. chromatography with acetanilide internal std, H_2O–MeOH mobile phase, and detection at 280 nm.

Apparatus

(a) *Liquid chromatograph.*—Equipped with Model U6K injector, Model 6000A solv. delivery system, and Model 440 detector (Waters Associates, Inc., Milford, MA 01757), or equiv. Operating conditions: flow rate 1.8 mL/min; 280 nm detector at 0.10 AUFS; temp. ambient; vol. injected 20 μL.

(b) *LC column.*—Zorbax ODS, 5–6 μm particle size, 4.4 mm id by 25 cm long (E. I. DuPont), or equiv.

(c) *Integrator.*—HP 3380A (Hewlett Packard, Avondale, PA), or equiv.

(d) *Filters.*—Millipore type HAWP, pore size 0.45 μm (Millipore Corp., Bedford, MA 01730), or equiv.

Reagents

(a) *Solvents.*—H_2O and MeOH, each of suitable LC grade; degas before use.

(b) *Internal std.*—Acetanilide, 6 mg/mL H_2O-MeOH (75 + 25).

(c) *Methocarbamol std soln.*—Transfer ca 50 mg, accurately weighed, USP Methocarbamol Ref. Std to 100 mL vol. flask, add ca 23 mL MeOH and 10.0 mL internal std, dil. to vol. with H_2O, and mix thoroly.

(d) *LC mobile phase.*—H_2O–MeOH (75 + 25); degas.

Sample Preparation

(a) *Injections.*—Transfer accurately measured vol. of methocarbamol injection equiv. to ca 500 mg to 100 mL vol. flask, add 25 mL MeOH and dil. to vol. with H_2O. Transfer 10.0 mL of this diln and 10.0 mL internal std to second 100 mL vol. flask, add 20 mL MeOH, and dil. to vol. with H_2O (sample soln).

(b) *Tablets.*—Transfer accurately weighed portion of finely ground tablet composite equiv. to 250 mg methocarbamol to 200 mL vol. flask, add 50 mL MeOH, mix thoroly, and add H_2O to vol. Transfer 20.0 mL of this initial diln plus 5.0 mL internal std to 50 mL vol. flask, add 6–7 mL of MeOH, and dil. to vol. with H_2O (sample soln).

Filter sample soln thru Swinny filter (Millipore Cat. No. XX 30 012 00) contg 0.45 μm Millipore filter (HAWP 01300) and attached to Hamilton gas-tight syringe (No. 1010 W) fitted with Luer-Lok tip.

Standardization

Set mobile phase flow rate at 1.8 mL/min and inject 20 μL methocarbamol std soln. Retention time for methocarbamol (2nd peak) should be 19 ± 2 min. Resolution, $R = 2(t_2 - t_1)/(W_2 + W_1)$, should be not < 6 for methocarbamol and internal standard peak, where t_1 = retention of internal std; t_2 = retention of methocarbamol; W_1 = width of base of internal std peak; W_2 = width of base of methocarbamol peak.

Adjust mobile phase ratios and/or flow rates as necessary to give required retention times and/or resolution.

Determination

Make 20 μL injections of std and sample solns. Measure peak areas and calc. response ratios (methocarbamol peak to internal std peak) for std and sample solns. Calc. methocarbamol:

$$\text{Liqs, mg/mL} = (R/R') \times (C/V) \times 1000$$

$$\text{Tablets, mg/tab.} = (R/R') \times (C/W) \times 500 \times T$$

where R and R' = response ratios for sample and std solns, resp.; C = concn methocarbamol std soln, mg/mL; V = mL injection taken for analysis; T = av. tablet wt, g; W = sample wt, g.

Results and Discussion

Tables 1–4 show the methocarbamol assay results achieved by the author for various authentic and commercial injection and tablet samples, using the proposed LC and former USP methods.

Table 1. Results (mg/mL) of analysis of authentic injections, using LC method[a]

Parameter	Lot 1	Lot 2	Lot 3
Known methocarbamol content	102.0	97.6	76.0
LC assay mean	102.5	97.2	74.9
Std dev.	1.36	1.09	1.20
Coeff. of var.	1.32	1.12	1.61
Highest value	103.9	99.0	76.2
Lowest value	100.9	96.4	73.0
Range	3.0	2.6	3.2
USP assay mean	105.9	99.3	82.2
No. of assays	5	5	5

[a]Recoveries ranged from 96.1 to 101.9%.

Table 2. Results (mg/mL) of analysis of commercial injections (declared 100 mg/mL), using LC and USP methods

	Lot 4		Lot 5		Lot 6	
Parameter	LC	USP	LC	USP	LC	USP
Std dev.	0.74	1.28	1.06	1.92	0.95	1.44
Coeff. of var.	0.72	1.24	1.03	1.84	0.92	1.37
Mean	102.9	103.7	103.5	104.2	103.7	104.8
Highest value	103.8	105.4	104.9	106.5	105.6	106.5
Lowest value	101.5	102.2	101.6	101.5	102.3	103.4
Range	2.3	3.2	3.3	5.0	3.3	3.1
No. of assays	10	6	10	6	10	4

Table 3. Results (mg/g) of analysis of authentic tablet preparations, using LC method[a]

	Lot				
Parameter	7	8	9	10	11
Known methocarbamol content	925.6	934.7	946.8	904.9	890
Std dev.	4.33	2.97	4.52	2.30	3.68
Coeff. of var.	0.47	0.32	0.48	0.26	0.42
Mean	924.5	930.5	949.6	898.4	877.2
Highest value	931.8	932.6	956.1	900.5	880.9
Lowest value	921.2	925.4	944.1	895.5	872.3
Range	10.6	7.2	12.0	5.0	8.6
No. of assays	5	5	6	5	5
Mean rec., %	99.9	99.6	100.3	99.3	98.6

[a]Recoveries ranged from 98.0 to 101.0%.

Authentic Lot 3 in Table 1 was deliberately formulated as a mixture, including guaifenesin, a secondary isomer of methocarbamol, and the cyclic carbonate intermediate. By total weight the concentration of this mixture was 85.8 mg/mL. The methocarbamol content was 76 mg/mL. This authentic mixture was prepared to demonstrate that the proposed assay could give accurate results when possible impurities were present. Detailed results of impurities found in various commercial samples have been previously reported by the author (7).

Collaborator 1 used a Sperisorb S10-ODS column, Collaborators 2, 3, 4, and 5 used Zorbax-ODS, and Collaborator 6 used Ultrasphere 5 ODS. Collaborators 1, 4, and 5 used 280 nm and 2, 3, and 6 used 274 nm. The resolution factors reported by the 6 collaborators were 5.99, 8.90, 6.40, 7.60, 6.00, and 7.20, respectively. The results obtained by the collaborators using these conditions are given in Table 5 (see also ref. 7).

The statistical evaluation of the collaborative results was made using the general procedure described in the *Statistical Manual of the AOAC* (8). Outlying individual results were eliminated by using both the Dixon test and the modified Cochran test. A 2-way analysis of variance (ANOVA) was performed on the adjusted data and the standard deviations for repeatability and reproducibility were calculated for each experiment.

The proposed LC method is specific and stability indicating. Recovery and collaborative results indicate good accuracy and precision.

Recommendation

It is recommended that the LC method for the determination of methocarbamol in injection and tablet dosage forms be adopted official first action.

Table 4. Results (mg/tablet) of analysis of commercial tablets (declared 500 mg/tablet), using LC and USP methods

	Manufacturer									
	A		B		C		D		E	
Parameter	LC	USP	LC	USP	LC	USP	LC	USP	LC	USP
Std dev.	2.41	7.19	2.84	13.9	4.29	2.71	3.86	11.4	3.43	10.8
Coeff. of var.	0.48	1.45	0.58	2.85	0.88	0.56	0.83	2.29	0.72	2.21
Mean	502.8	494.3	489.6	488.5	489.5	485.5	462.7	496.6	477.3	489.1
Highest value	505.9	503.8	494.0	97.1	496.4	489.1	471.7	510.9	481.8	503.1
Lowest value	498.4	488.1	486.3	467.7	484.4	482.6	456.2	483.1	470.7	479.9
Range	7.5	15.7	7.7	29.4	12.0	6.5	15.5	27.8	11.1	23.2
No. of assays	10	4	10	4	10	4	10	4	10	4
Percent of declared (mean)	100.6	98.9	97.9	97.7	97.9	97.1	92.5	99.3	95.5	97.8

Table 5. Collaborative study results as percent of declared or percent of theoretical for injections and tablets

Coll.	Authentic	Lot 2	Lot 3
	Injections		
1	95.7, 96.7	101.5, 101.8	103.2, 103.0
2	98.8, 99.5	104.8, 105.2	107.1, 106.1
3	97.2, 96.4	102.9, 101.1	103.7, 104.1
4	101.5, 100.3	105.8, 105.5	106.6, 107.8
5	98.1, 96.9	104.2, 102.6	105.1, 104.5
6	99.3, 97.8	104.3, 105.1	105.7, 106.2
Pooled mean, %	98.2	103.7	105.0
% Declared + 4% overage	—	99.7	101.0
Std dev. (reproducibility) = ± 1.70			
Std dev. (repeatability) = ± 0.69			
	Tablets		
1	98.8, 99.2	97.8, 97.3	100.3, 100.8
2	95.9, 94.6	89.5,[a] 100.6[a]	98.9, 97.0
3	98.0, 97.8	96.4, 95.9	100.2, 99.4
4	87.1,[a] 100.6[a]	94.6, 100.0	101.1, 98.6
5	105.4,[a] 94.2[a]	96.2, 97.7	99.2, 100.0
6	104.5, 102.3	101.4, 99.5	104.1, 100.8
Pooled mean, %	98.9	97.7	100.0
Std dev. (reproducibility) = ± 1.70			
Std dev. (repeatability) = ± 1.70			

[a]Statistical outlier.

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Separation and Identification of Nine Penicillins by Reverse Phase Liquid Chromatography

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A simple and rapid high performance liquid chromatographic method was developed for the separation and identification of amoxicillin, ampicillin, cloxacillin, dicloxacillin, methicillin, oxacillin, nafcillin, penicillin G potassium, and penicillin V potassium. The antibiotics were separated at ambient temperature on a Chromegabond 10 μm C_{18} column with acetonitrile–methanol–0.01M potassium dihydrogen phosphate buffer, pH 4.7 (19 + 11 + 70), at 1 mL/min. A variable wavelength detector set at 225 nm, 0.16 AUFS, and a recorder set at 0.25 cm/min were used for the detection. Individual antibiotics and their mixtures were dissolved in the mobile phase and injected into the chromatograph through a 20 μL injection loop. Baseline separation was observed for virtually all 9 antibiotics. The entire mixture was resolved in less than 30 min. The method was sensitive, reproducible, and applicable to the qualitative analysis of commercial dosage forms.

Since 1979, New York Regional Laboratory had been conducting post-certification studies as well as stability testing on commercial dosage forms of a variety of penicillins. Because of the nature of this program and the large number of samples and analyses involved, a rapid and accurate method of assay was needed. Although the iodometric and hydroxylamine methods are rapid for potency determinations (1) and are easily automated (2, 3), they fail to distinguish the intact penicillin molecule and the corresponding degradation products. Liquid chromatography (LC) combines the necessary specificity with suitable accuracy and speed. Based on these attributes and demands on time, LC has gained wide recognition in recent years as a superior approach to the analysis of antibiotics (1, 4, 5).

Current LC methods for the analysis of penicillins have, for the most part, dealt with single drugs (6–18). Those applied to mixtures have been limited to simple mixtures (19–26) or to more complex mixtures whose resolution has necessitated alterations in the composition of the original mobile phase (6, 7, 10), the use of a gradient device (27), or the use of more than one chromatographic column (22) to achieve optimum separation among certain penicillins. Since this laboratory routinely analyzes 9 different penicillins, namely amoxicillin, ampicillin, cloxacillin, dicloxacillin, methicillin, oxacillin, nafcillin, penicillin G, and penicillin V, we proceeded to develop a general LC method for their identification in pharmaceuticals.

By combining the use of a C_{18} reverse phase column with an acetonitrile–methanol–0.01M potassium dihydrogen phosphate (19 + 11 + 70) mobile phase and photometric detection at 225 nm, the proposed method resolved a mixture of the 9 penicillins in less than 30 min. The utility of this method for the identification of selected penicillins in commercial dosage forms was also demonstrated.

METHOD

Apparatus

(a) *Liquid chromatograph.*—Consisting of Model 950 solvent pump, Model 970A variable wavelength detector, and Model TS-10 recorder (Tracor Instruments Inc., Austin, TX 78721). Samples were injected through 20 μL Rheodyne Model 7125 loop injector (Rheodyne Inc., Berkeley, CA 94710).

(b) *LC column.*—Stainless steel, 30 cm × 4.6 mm id, prepacked with 10 μm particle size Chromegabond C_{18} (E. S. Industries, Marlton, NJ 08053), preceded by 70 mm Co:Pell ODS LC guard column (Whatman Inc., Clifton, NJ 07014).

(c) *Spectrophotometer.*—Cary 118 UV-VIS, double beam, with automatic recorder (Varian Instruments Group, Palo Alto, CA 94303).

Reagents

(a) *LC solvents*—Acetonitrile (LC grade, Fisher Scientific Co. Fair Lawn, NJ 07410), methanol (LC grade, Fisher Scientific Co.), and deaerated glass-distilled water.

(b) *Potassium dihydrogen phosphate solution.*—0.01M KH_2PO_4 (Baker Analyzed Reagent), 1.361 g/L, giving a pH of 4.7.

(c) *LC mobile phase.*—Acetonitrile-methanol-0.01M KH_2PO_4 (19 + 11 + 70, by volume).

(d) *Standard samples.*—Methicillin sodium and penicillin V potassium were USP reference standards. Amoxicillin trihydrate, ampicillin trihydrate, cloxacillin sodium, dicloxacillin sodium, nafcillin sodium, oxacillin sodium, and penicillin G potassium were working standards from the National Center for Antibiotics Analysis (Washington, DC 20204).

(e) *Mixed standard preparation.*—Individual standards were dissolved in mobile phase to give mixture containing the following concentrations of antibiotic (mg/mL): amoxicillin 0.030, ampicillin 0.060, cloxacillin 0.100, dicloxacillin 0.090, methicillin 0.070, nafcillin 0.050, oxacillin 0.080, penicillin G 0.170, and penicillin V 0.110.

Sample Preparation

(a) *Tablets and capsules.*—Blend dosage form with water in high-speed blender ca 5 min. Filter into volumetric flask, and dilute with mobile phase to obtain appropriate concentration of antibiotic giving discernible peak on the chromatogram.

(b) *Oral suspensions and injections.*—Pipet volume of dosage form into volumetric flask and dilute with mobile phase to obtain appropriate concentration of antibiotic giving discernible peak on the chromatogram.

LC Procedure

Inject 20 μL portions of mixed standard preparation and of sample preparation into chromatograph, using mobile phase flow rate of 1 mL/min, inlet pressure of 790 psi, detector set at 225 nm and 0.16 AUFS, and chart speed of 0.25 cm/min. Operate column at ambient temperature. Compare retention time of sample peak to those of mixed standard and identify penicillin present.

Results and Discussion

In the development of the LC method presented here for the separation of the 9 penicillins shown in Figure 1, 4 major chromatographic parameters were taken into consideration: column, detection wavelength, mobile phase, and flow rate of the mobile phase.

(a) *Column.*—Ion-exchange columns have been found to provide separations that are too slow to be of practical value

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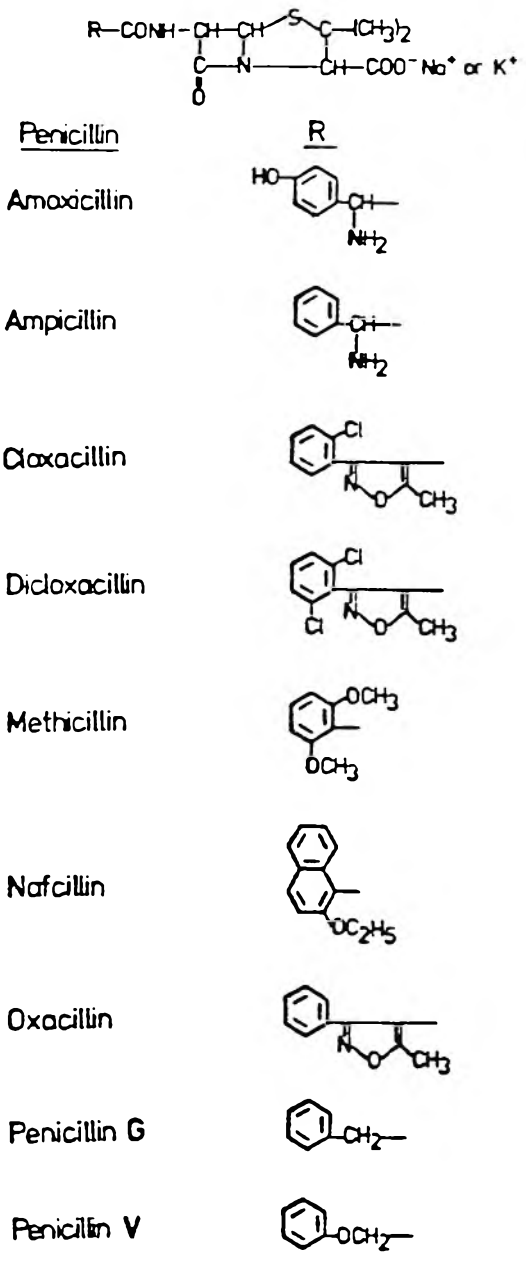

Figure 1. Penicillins separated by proposed LC method.

in multiple analysis (15, 16) and to represent potential cause for the degradation of penicillins (16); therefore, this type of column was not evaluated. Reverse phase columns, on the other hand, have proven very useful in the LC analysis of penicillins, cephalosporins, and related families of antibiotics (7, 11, 22, 25, 26).

(b) *Detection wavelength.*—Penicillins have been analyzed by using photometric detectors set at a number of wavelengths between 204 and 310 nm (1, 4, 5–9, 11, 17, 21, 22, 26). At 225 nm, discernible detector responses were observed for all 9 penicillins even though the respective ultraviolet absorbances and absorptivities at equimolar concentrations varied somewhat from penicillin to penicillin (Table 1). White et al. (26) have shown that at 225 nm the detector response for several penicillins may be as much as 10 times that obtained between 254 and 276 nm.

(c) *Mobile phase.*—The majority of LC methods for penicillins use a reverse phase column and a mobile phase consisting of an aqueous solution of an inorganic salt plus one or more organic modifiers. In most cases, the inorganic component has been a phosphate buffer with a pH between 3.3 and 8.0 (1, 6, 8, 9, 11, 16–19, 23, 25). In other cases, an acetate buffer of various pH values has been suggested (11, 20). The organic modifier has usually been methanol (16, 20, 21, 23), or acetonitrile (11, 16, 23, 25), but occasionally isopropanol (27). White et al. (26) used a mobile phase that consisted of 65% 0.01M KH_2PO_4–35% methanol for the separation of a mixture of ampicillin, penicillin G, methicillin, penicillin V, and oxacillin on a C_{18} column, and at a flow rate of 1 mL/min. Under these conditions on our mixture of 9 penicillins, peaks appeared broad, occasionally asymmetric, and with a few retention times in excess of 60 min. In addition, this mobile phase was unable to resolve nafcillin from dicloxacillin.

Attempts to improve peak shapes, retention times, and resolution by altering the molar concentration of the inorganic solvent and the proportion of methanol were unsuccessful. Since all binary solvent systems tested failed to satisfactorily resolve the mixture of 9 penicillins, a ternary mobile phase containing 0.01M KH_2PO_4-methanol-acetonitrile was prepared. By varying the proportions of these components, a solvent system offering the chromatographic separation shown in Figure 2 and Table 2 was developed.

(d) *Flow rate of mobile phase.*—The flow rate was varied between 0.50 and 2.00 mL/min to determine the best detector responses, peak shapes, and retention times. Slower flow rates yielded greater resolution and sensitivity than faster ones, but with a sacrifice in elution times. In practice, however, when dealing with individual penicillins, faster flow rates can be used to speed up the analysis.

Stability Study

Solutions of the various penicillins were prepared by dissolving the corresponding compound in the mobile phase adopted for the LC separation (pH 4.7). Samples were kept refrigerated at about 4°C when not in use. At this pH, all of

Table 1. Absorption maxima and molar absorptivities of penicillins in (A) 35% methanol in water and (B) 35% methanol in 0.01M KH_2PO_4 solutions

	Solvent A			Solvent B		
Compound	Concn, $\times 10^{-4}$M	λmax, nm	Molar absorptivity	Concn, $\times 10^{-4}$M	λmax, nm	Molar absorptivity
Amoxicillin	3.29	272	1167.0	3.34	270	1261.4
Ampicillin	3.54	256	490.9	3.32	255	358.3
Cloxacillin	3.49	ND[a]	NC[b]	3.54	ND	NC
Dicloxacillin	3.64	274	615.4	3.68	269	646.7
Methicillin	3.73	279	2193.0	3.78	279	2195.8
Nafcillin	3.62	279	5221.0	3.74	280	4866.3
Oxacillin	3.71	ND	NC	3.87	ND	NC
Penicillin G	3.56	256	508.9	3.63	255	338.8
Penicillin V	3.50	267	1430.7	3.60	265	1276.1

[a]ND = not distinct.
[b]NC = not calculated.

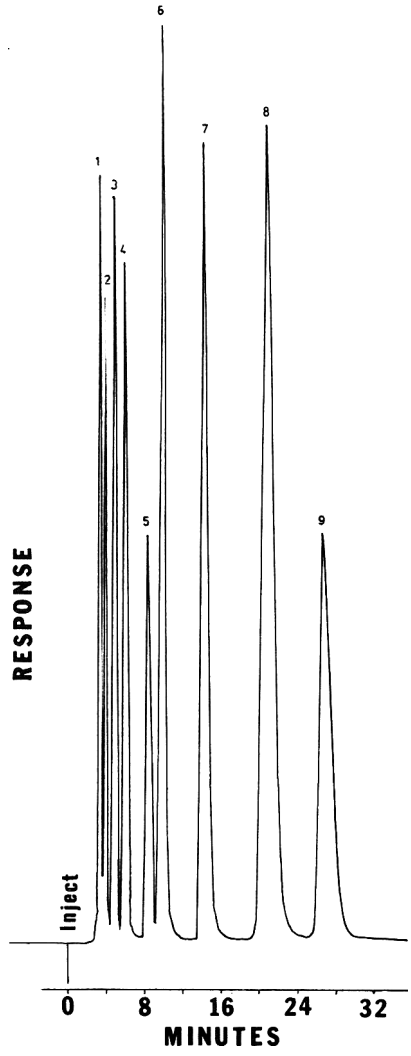

Figure 2. Liquid chromatogram of standard mixture: 1, amoxicillin; 2, ampicillin; 3, methicillin; 4, penicillin G; 5, penicillin V; 6, oxacillin; 7, cloxacillin; 8, nafcillin; and 9, dicloxacillin.

the penicillins, except methicillin and penicillin G, gave chromatograms whose peak heights and shapes were identical for up to 7 days with those obtained for the drugs in fresh solutions. In contrast, the chromatograms for methicillin and penicillin G each displayed an additional peak, which because of its increase in height with time was ascribed to a degradation product.

Sensitivity Study

Stock solutions of each of the 9 penicillins in the mobile phase and containing approximately the same concentrations found in the mixed standard preparation were diluted with mobile phase to concentrations which, when injected into the chromatograph, produced detector responses at least 3 times as great as the noise level at a sensitivity of 0.01. The minimum detectability limits obtained for triplicate injections of each dilution of antibiotic are presented in Table 3.

Table 2. Retention times, relative retention times, and resolution factors of penicillins in standard mixture

Compound	Retention time[a] Min	Retention time[a] Rel.[b]	Resolution factor[b]
Amoxicillin	3.6	0.90	1.2
Ampicillin	4.0	1.00	0.0
Methiciliin	5.2	1.30	3.1
Penicillin G	6.2	1.55	5.1
Penicillin V	8.4	2.10	7.1
Oxacillin	10.0	2.50	9.4
Cloxacillin	14.4	3.60	12.7
Nafcillin	21.0	5.25	14.6
Dicloxacillin	26.8	6.70	19.5

[a]Average of 2 runs.
[b]Relative to ampicillin.

Analysis of Dosage Forms

Samples of penicillin G potassium capsules and injections and of penicillin V potassium tablets and oral suspensions were analyzed qualitatively by the proposed LC method with the results shown in Figure 3. In all cases, the results obtained

Table 3. Detection limits for individual penicillins by proposed LC method

Compound	Limit of detection, μg/mL	Flow rate, mL/min[a]
Amoxicillin	0.342	1.0
Ampicillin	0.699	1.0
Cloxaciliin	1.890	2.0
Dicloxacillin	3.640	2.0
Methicillin	0.629	1.0
Nafcillin	0.659	2.0
Oxacillin	0.200	1.0
Penicillin G	1.700	1.0
Penicillin V	1.020	1.0

[a]A higher flow rate was used for penicillins that had retention times exceeding 15 min at a flow rate of 1.0 mL/min.

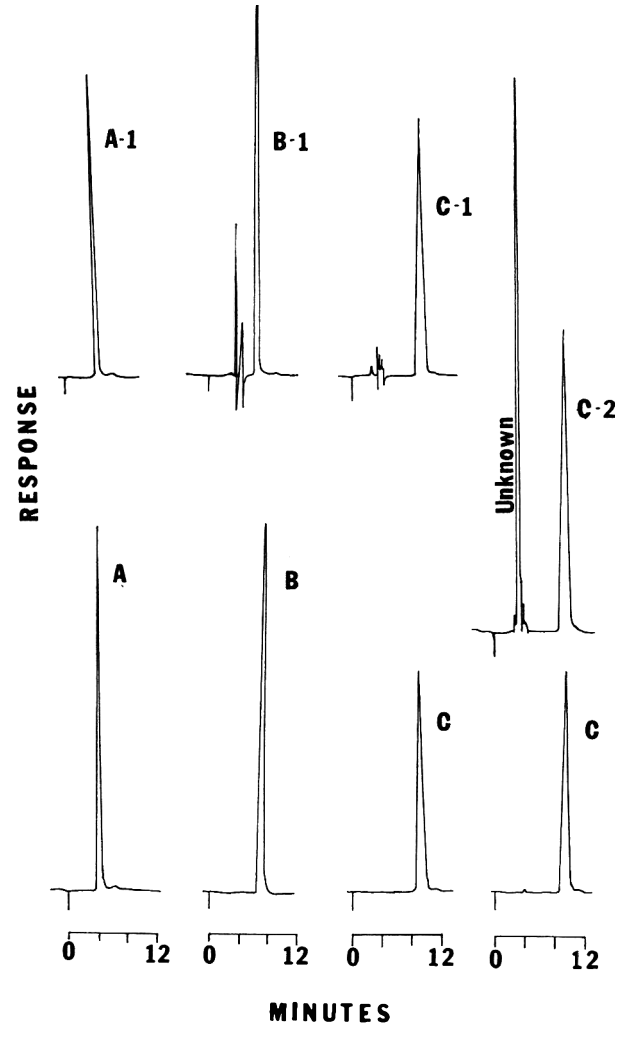

Figure 3. Liquid chromatograms of penicillins in commercial dosage forms: A, ampicillin; A-1, ampicillin capsule; B, penicillin G potassium; B-1, penicillin G potassium injectable; C, penicillin V potassium; C-1, penicillin V potassium tablet; C-2, penicillin V potassium oral suspension.

for these samples agreed with those obtained for solutions of standard samples of penicillin G potassium, penicillin V potassium, and ampicillin.

Conclusions

The results of this study demonstrate that the proposed LC method is an efficient and reliable technique for the qualitative analysis of penicillins in various dosage forms. Work is currently in progress on the quantitative aspects of the method.

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FOOD ADDITIVES

Determination of *N*-Nitrosodimethylamine in Nonfat Dry Milk: Collaborative Study

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Ten laboratories participated in a collaborative study of a method for the determination of *N*-nitrosodimethylamine (NDMA) in nonfat dry milk. NDMA is eluted with dichloromethane from a mixture of Celite, acidic sulfamic acid, and nonfat dry milk (all packed in a chromatography column), concentrated in a Kuderna-Danish concentrator, and finally analyzed by a GC-thermal energy analyzer technique. Ten samples were studied: 6 were naturally contaminated (NDMA levels 0.38–3.56 ppb) and 4 were spiked with known levels (0.96 and 3.2 ppb) of NDMA. The coefficients of variation (CV) of the complete data for the naturally contaminated samples (excluding the 2 samples containing the lowest levels) were 8.5% and 22.5% for repeatability and reproducibility, respectively. The corresponding CVs for the spiked samples were 14.4% and 20.4%, respectively. The percent recoveries of the added NDMA in the spiked samples (at the 2 levels indicated above) were 101.6 ± 3.2 (omitting 1 outlier) and 95 ± 2.1, respectively. The method has been adopted official first action.

The occurrence of traces of *N*-nitrosodimethylamine (NDMA) in nonfat dry milk (NFDM) has been well established (1–4). The concentration of NDMA in NFDM usually varies between 0.1 and 5 ppb; the average level is < 1 ppb. Because NDMA is a highly potent carcinogen (5) and because NFDM is consumed by young children, even the presence of such low levels of NDMA in NFDM should be viewed with concern. Every effort should be made to reduce its concentration to as low as technically feasible; thus, the availability of an accurate method for its determination in NFDM is essential. Of all the methods reported in the literature (1–4), the method of Havery et al. (2) seems to be the simplest and most rapid and is ideal for rapid screening of a large number of samples. At the 1981 Spring AOAC Workshop held in Ottawa, the Committee on *N*-Nitrosamines recommended (6) that the above method (2) be studied collaboratively. This report presents the result of that collaborative study.

Collaborative Study

Each laboratory received a set of 11 NFDM samples (some in screw-cap bottles and some in sealed plastic bags), instructions for storage at 4°C and analysis, and reporting forms. Of the 11 samples, one was a practice sample whose estimated NDMA content, as found in the Associate Referee's laboratory, was given to each laboratory. Collaborators were instructed to analyze this sample first and not to analyze the rest of the samples until they obtained a value that was within ± 20% of the expected NDMA content. The other 10 samples (all randomly numbered) were 5 pairs of blind duplicates whose identities are given at the bottom of Table 1. The collaborators were asked to carry out a single determination on each sample on a pre-assigned schedule (to avoid analyzing the replicates on the same day) and report the results within 1 month. They were also requested to send copies of the chromatograms and details of chromatographic conditions used to analyze the samples. Each laboratory procured and used its own reagents.

As can be seen from Table 1, both naturally contaminated samples and those spiked with known amounts of NDMA were used in the study. The concentration range chosen was representative of that normally found in commercial NFDM samples (1–4). Before analysis, each sample was poured into a 4 L beaker and mixed thoroughly with a large flat spatula. Homogeneity of the samples was confirmed by 3–5 replicate analyses. Because of lack of sufficient sample, it was not possible to carry out such replicate analyses for sample pair 21 and 85 (containing a high natural level of NDMA). However, it was mixed thoroughly in the same manner as the other samples and the other samples were found to be homogeneous on replicate analysis; therefore, it is highly likely that this sample was also homogeneous in nature. Spiking was done by weighing exactly 25.0 ± 0.1 g sample in a screw cap (Teflon liner) bottle and adding exactly 4.0 mL NDMA standard (ethanol) of appropriate strength. This method of spiking was preferred to that of spiking a large batch of NFDM (and taking an aliquot) because of the danger of improper mixing which might be a problem with the latter technique. For these samples, as well as for all others (except the practice samples), which were also sent in exactly 25.0 ± 0.1 g quantities, laboratories were asked to use the entire contents for analysis with appropriate rinsing of the containers. Initially, water and 50% ethanol were tried as the solvents for the spiking standard, but in both cases the spiked samples, on storage at room temperature, transformed into hard lumps which were difficult to break and mix with Celite. For this reason, ethanol was chosen as the solvent. Studies were also carried out to determine the stability of added NDMA after storing spiked samples for 2 weeks at room temperature and for up to 4 weeks at 4°C. No decrease in NDMA levels could be detected under the above storage conditions.

Statistical Methods

The study yielded a set of data, x_{ijk}, where i denotes the laboratory, j denotes the level of NDMA contamination, and k denotes the individual sample within a level. The analysis consisted of identification of outliers, estimation of repeatability and reproducibility, and estimation of percent recovery for spiked samples. Analysis was carried out on both the reported data and on the natural logarithms of the data.

Tests for outliers were done on the means of duplicates $(x_{ij1} + x_{ij2})/2$, on the differences between duplicates $(x_{ij1} - x_{ij2})$, and on the overall results of each laboratory. Methods used are those of Cochran (7), Dixon (8), and Thompson and Wilke (9) and are described in Steiner (10).

Tests for homogeneity of variances were based on Bartlett's test (11) and *F*-tests (11). As with previous data of this nature it appears that variances are more homogeneous on

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The recommendation of the Associate Referee was approved by the General Referee and Committee C and was adopted by the Association. See the General Referee and Committee reports, *J. Assoc. Off. Anal. Chem.* (1984) **64**, March issue.

Table 1. Collaborative study results for *N*-nitrosodimethylamine in nonfat dry milk

Sample[a]	NDMA levels (ppb) found by various laboratories									
	A	B	C	D	E	F	G	H	I	J
P	0.95	0.98	0.82	0.85	0.84	0.95	0.95	0.78	0.86	0.94
8	0.34	0.34	0.48	0.15	0.30	0.32	0.24	0.32	0.17	0.49
40	0.31	0.42	0.45	0.20	1.09	0.74	0.23	0.26	0.33	0.35
9	1.41	1.49	1.45	1.14	1.23	1.52	1.07	—	1.10	2.55
83	1.38	1.20	1.58	1.20	1.98	1.78	1.07	1.2	1.25	2.12
14	3.39	4.04	3.22	3.12	3.35	3.66	2.86	2.6	3.25	3.62
99	3.22	2.69	3.74	3.57	4.66	3.51	3.06	3.5	3.44	4.04
21	4.02	4.08	3.06	3.67	3.91	3.47	3.23	2.8	3.09	3.98
85	3.49	4.28	2.90	3.87	3.58	3.95	2.89	3.1	3.34	4.47
24	0.91	1.00	0.92	0.88	1.76	1.18	0.82	0.81	0.96	1.34
39	1.04	1.03	1.08	0.80	1.58	1.50	0.70	1.0	0.94	1.29

[a]The samples identities are as follows:
P = Practice sample; expected level of NDMA (0.84 ppb) given in advance to each laboratory.
8 & 40 = blind duplicates of naturally contaminated sample extremely low in NDMA.
9 & 83 = blind duplicates of above spiked with 0.96 ppb NDMA.
14 & 99 = blind duplicates of low NDMA sample spiked with 3.2 ppb NDMA.
21 & 85 = blind duplicates of naturally contaminated sample high in NDMA.
24 & 39 = blind duplicates of naturally contaminated sample with medium NDMA content.

the logarithmic scale. Hence, analyses of variance (11) were carried out on the logarithmic scale for appropriately grouped data sets and estimates of the components of variance within laboratories (σ_E^2), between laboratories (σ_L^2) and for laboratory-sample interaction (σ_{LS}^2) were calculated as described by Steiner (10). Coefficients of variation (CV) for repeatability and reproducibility were calculated as $\sigma_E \times 100$ and $\sqrt{\sigma_E^2 + \sigma_{LS}^2 + \sigma_L^2} \times 100$, respectively. The CV values can be calculated in this manner since standard deviations on the logarithmic scale can be translated to CV values on the original scale by merely multiplying by 100.

Percent recoveries were calculated within each laboratory for the 0.96 ppb and 3.2 ppb spiked samples as follows:

$$\text{Recovery, } \% = (\bar{x}_{ij} - \bar{x}_{il}) \times 100/A$$

where $\bar{x}_{ij}$ = average in the spiked sample; $\bar{x}_{il}$ = average in the unspiked low NDMA sample; and A = amount used in spiking. Average percent recoveries were calculated to assess the overall degree of bias in the method.

N-Nitrosodimethylamine in Nonfat Dry Milk Gas Chromatographic Method First Action

Principle

Sample is mixed with Celite, ammonium sulfamate, and dil. H_2SO_4 and packed in chromatgc column contg anhyd. Na_2SO_4. *N*-Nitrosodimethylamine (NDMA) is eluted with CH_2Cl_2, concd, and analyzed by GC with thermal energy analyzer (TEA).

Caution

NDMA is potent carcinogen; use extreme caution in handling compd. Preferably, purchase dild std available from com. supplier and carry out all work in fume hood. Use mech. pipetting aids for measuring all solns and wear protective gloves while handling NDMA std. Before disposal, destroy stds according to method of Williams (Food Cosmet. Toxicol. **13.** 302(1975)).

Reagents

(a) *Celite 545.*—Not acid-washed (Fisher Scientific Co. No. C-212). Heat overnight at 700°, cool, and store in tightly sealed jar.

(b) *Ammonium sulfamate–sulfuric acid soln.*—Dissolve 1 g ammonium sulfamate in 100 mL 1N H_2SO_4.

(c) *Dichloromethane.*—Distd in glass. Test for NDMA contamination before use: Conc. 200 mL to 1 mL as described under concentration and then analyze 10 μL aliquot by GC-TEA. Test must show absence of NDMA.

(d) *Water.*—Double-distd, or equiv. Test for NDMA contamination before use; use thruout: Take 50 mL H_2O, add 2 mL 10N KOH, ext with two 50 mL portions of CH_2Cl_2, and test for NDMA contamination as under (c).

(e) *NDMA std.*—Serially dil. previously prepd stock soln (100 μg/mL) to working std (0.1 μg/mL) with CH_2Cl_2.

Reagent Blank

To ensure absence of contamination, carry reagent blank, excluding nonfat dry milk, thru all steps. If contamination is indicated, test each reagent sep. CH_2Cl_2 and H_2O are most likely sources.

Apparatus

(a) *Chromatographic tube.*—Glass, 32 mm id × 400 mm long, with stopcock.

(b) *Tamping rod.*—19 mm diam. disk.

(c) *Powder funnel.*—Top diam. 140–150 mm, stem 25–28 mm od.

(d) *Evaporative-concentrator.*—Kuderna-Danish, 250 mL with 4 mL concentrator tube and 0.1 mL subdivisions from 0 to 2.0 mL, and 3-chamber Snyder column (Kontes Glass Co. K-570001, K-570050, and K503000, resp.). Check accuracy of graduations in tube before use.

(e) *Gas chromatograph-thermal energy analyzer.*—Hewlett Packard Model 5710A gas chromatograph (or equiv.) interfaced to TEA Model 502L (Thermo Electron Corp., Waltham, MA) and with 2.7 m × 4 mm id glass column packed with 10% Carbowax 1540 (Analabs) and 5% KOH on 100–120 mesh Chromosorb WHP. Program column from 100 to 180° at 4°/min for each analysis with carrier gas (Ar) flow rate set at 40 mL/min. Injection port, 200°.

Operate TEA at ca 1.1 mm Hg vac., 450° furnace, and ×2 to ×4 attenuation ranges, with liq. N trap. Connect output of TEA to 1 mV recorder (chart speed, 1.3 cm/min, or equiv.).

Sample Analysis

Weigh 25.0 ± 0.1 g nonfat dry milk into tared 600 mL beaker. Add 40 g Celite, 25 mL ammonium sulfamate–H_2SO_4 soln, and mix using s-s spatula until homogeneous and fluffy in consistency. Place small glass wool plug at bottom of

chromatgc tube with end of tamping rod extending thru funnel opening and cover with 20 g anhyd. Na_2SO_4. Slowly pour nonfat dry milk–Celite mixt. (thru funnel) into column, a little at a time, and tamp down firmly (avoid packing too tightly) to depth of 16–20 cm. Rinse beaker with 125 mL CH_2Cl_2 and pour rinse on column thru funnel before removing tamping rod. Remove tamping rod and collect eluate in 250 mL K-D evaporative-concentrator fitted with 4 mL concentrator tube. If necessary, adjust column flow to 1–2 mL/min. (Failure to obtain adequate solv. flow indicates that column is too tightly packed.) End collection when column stops dripping.

Add 2–3 carborundum boiling chips, fit Snyder column, and conc. CH_2Cl_2 eluate to ca 4 mL in 55–60° H_2O bath. Raise concentrator out of bath, let Snyder column drain completely, and disconnect concentrator tube. Further conc. ext in tube to 1.0 mL under gentle stream of N at ambient temp. Final concn must be done slowly, over ca 30 min.

Inject 8 μL NDMA std (0.1 μg/mL) into GC-TEA app. under conditions stated above. Similarly, inject 8 μL sample ext and repeat analysis. Measure peak hts (± 1 mm) of both std and sample ext, and calc. NDMA concn in sample using following formula:

$$\text{ppb (μg/kg) NDMA in NFDM} = (PH/PH') \times C \times V \times (1000/W) \times Z$$

where C = concn of NDMA std (μg/mL); V = final vol. of sample ext (mL); PH = NDMA peak ht of sample (mm); PH' = NDMA peak ht of std (mm); W = sample wt (g); Z = attenuation factor (1 if both std and sample are analyzed at same attenuation).

Results and Discussion

The data reported by the 10 participating laboratories are presented in Table 1. All laboratories analyzed the practice sample successfully and reported values within ± 20% of the expected value, 0.84 ppb, established by the Associate Referee. The mean value for the practice sample was 0.89 ppb.

All the laboratories followed the method closely with only minor modifications introduced. The most common variation was the use of different GC columns and different GC oven temperatures than those called for in the method. Laboratory A added an internal standard (*N*-nitroso-di-*n*-propylamine) to the sample extract during analysis and used electronic integration for quantitation of peak areas, whereas all others used peak height measurements and carried out the analysis without any added internal standard (as recommended in the method). Laboratory G used a micro Snyder column for the final concentration step (from 4 to 1 mL) instead of evaporation under N as suggested in the method; this laboratory also eluted the NFDM–Celite column with an extra 25 mL CH_2Cl_2. However, all used some sort of Carbowax columns and obtained satisfactory chromatograms for most of the samples. Only Laboratories A and H obtained large tailing solvent peaks for Sample 9, which interfered with the detection of NDMA. Using an electronic integrator, Laboratory A was able to obtain a value (NDMA content) for this sample but Laboratory H was unable to detect any NDMA peak because the extract had to be analyzed (to bring down the solvent tailing on scale) on an attenuation of × 64 on TEA to reduce solvent tailing. Although the reason for this is not entirely clear, the bad solvent tailing could have resulted from the use of 4 mL ethanol (as a solvent) for spiking this sample. Similar solvent tailings, although on a much smaller scale, were also observed occasionally on the chromatograms of Samples 9, 83, 14, and 99 which were also spiked with ethanol-containing NDMA standard. Laboratories D and G as well as the Associate Referee did not observe any detectable solvent tailings on the chromatograms for these samples. This drawback should not, however, deter the use of the method because such solvent tailings were not observed in any of the naturally contaminated unspiked samples.

The Thompson and Wilke test (9) on overall laboratory results indicated that Laboratory G is an outlier. Although its results did not differ from those of other laboratories by any appreciable amount, its values were consistently low.

Dixon's test (8) on the means of duplicates indicated that Laboratory J had atypically high values for Samples 9 and 83. In fact, its estimated percent recovery for these samples is 199.5%.

The mean values, within-laboratory standard deviations ($\sigma_{\bar{E}}$), and corresponding coefficients of variation (CV) for each NDMA level are given in Table 2. The CV values in Table 2 can be regarded as standard deviations on the logarithmic scale. Based on Bartlett's test (11) and *F*-tests (11), the within-laboratory variation is not sufficiently homogeneous to allow pooling of data across NDMA levels on the original scale. However, on the logarithmic scale, data can be pooled for the spiked samples (Samples 9, 83, 14, 99) and for the 2 naturally contaminated samples with medium and high NDMA levels (Samples 21, 85, 24, 39). It is believed that the increased variation in the spiked samples is due to the tailing solvent peaks observed in these samples. The lowest NDMA levels (Samples 8, 40) had the largest CV, 55.1%. This is partially due to the large difference between duplicates in Laboratories E and F which are considered to be outliers by Dixon's test (8). In fact, Laboratory E had carried out the final GC analysis for Samples 8 and 40 on the same day but the column elution and concentration for Sample 8 had been done on the previous day. Exclusion of these possible outliers leads to a more acceptable CV. However, examination of the peak heights for Samples 8 and 40 indicates that peaks are barely detectable in many cases. Therefore, a realistic detection limit of the method (after injection of 8 μL/1 mL extract) would be in the range of 0.3–0.4 ppb.

Analysis of variance results on the logarithmic scale are presented in Table 3. For the naturally contaminated samples, the pooled data analysis indicates a significant laboratory-sample interaction. This implies that the ratio of NDMA levels in these samples is not consistent across laboratories, and hence the reproducibility measure may fluctuate as different NDMA samples are studied. Analysis of variance results after deleting suspected outliers discussed above are presented in Table 4.

Estimates of percent recovery for the 2 spiked samples are given in Table 5. Laboratory J had 199.5% recovery for the 0.96 ppb NDMA sample and was considered as an outlier on the basis of Dixon's test (8). Recovery of NDMA was slightly better at the 0.96 ppb level than at the 3.2 ppb level, but there was no noticeable bias in percent recovery at either level.

Collaborator Comments

There were few comments from the collaborators, suggesting that the method worked well in their hands. Only 2

Table 2. Analysis of each NDMA level on original scale (complete data)

Samples	Mean, ppb	σ_E, ppb	CV, %
8, 40	0.377	0.207	55.1
9, 83	1.459	0.229	15.7
14, 99	3.427	0.506	14.8
21, 85	3.559	0.248	7.0
24, 39	1.077	0.109	10.1

Table 3. Repeatability and reproducibility estimates based on logarithmic scale analysis (complete data)[a]

Samples	Repeatability CV, %	Lab-sample interaction CV, %	Laboratory effect CV, %	Reproducibility CV, %
8, 40	39.4	—	25.8	47.1
9, 83, 14, 99 (Spiked samples)	14.4	5.4	13.5[b]	20.4
21, 85, 24, 39 (Naturally contaminated samples)	8.5	13.5[b]	15.9[b]	22.5

[a]Tabulated values are actually "standard deviations on the logarithmic scale × 100" but can be regarded as CV values on the original scale.
[b]Variance component significantly greater than zero ($P < 0.05$).

Table 4. Repeatability and reproducibility estimates based on logarithmic scale analysis after deleting suspected outliers[a]

Samples	Repeatability CV, %	Lab-sample interaction CV, %	Laboratory effect CV, %	Reproducibility CV, %
8, 40 excluding Labs E, F, G	22.9	—	30.2[b]	37.9
9, 83, 14, 99 excluding Labs G, J	15.7	<0	6.1	16.8
21, 85, 24, 39 excluding Lab. G	8.4	13.7[b]	14.6[b]	21.7

[a]The tabulated values are actually "standard deviations on the logarithmic scale × 100" but can be regarded as CV's on the original scale.
[b]Variance component significantly greater than zero ($P < 0.05$).

laboratories reported some difficulties in using the method. Laboratory G reported that 125 mL CH_2Cl_2 was not adequate for eluting all NDMA from the NFDM–Celite column; as mentioned above, they eluted with an additional 25 mL solvent. Laboratory J failed to get adequate solvent flow through the NFDM–Celite column; slight pressure (using nitrogen gas) was applied on top of the column to collect the eluate. This laboratory probably packed the columns too tightly and hence failed to obtain proper solvent flow. A warning has been included in the present version of the method to avoid such problems.

A few laboratories also reported the presence of traces of other volatile nitrosamines (mainly *N*-nitrosopiperidine and *N*-nitrosopyrrolidine) in some samples. Havery et al. (2) also reported finding traces of these 2 nitrosamines, although in much lower average concentrations than that of NDMA, in commercial NFDM samples. Since NDMA is the predominant volatile nitrosamine present in NFDM, the study was limited to its determination, and therefore, the data for the other volatile nitrosamines are not discussed further in this report.

Table 5. Estimates of percent recovery for NDMA spiked in nonfat dry milk

Lab.	Recovery, % 0.96 ppb NDMA	3.2 ppb NDMA
A	111.5	93.1
B	100.5	93.3
C	109.4	94.2
D	103.6	99.1
E	94.8	103.4
F	116.7	95.5
G	87.0	85.2
H	94.8	86.3
I	96.4	96.7
J	199.5[a]	106.6
Mean ± SE:		
All labs	111.4 ± 10.2	95.3 ± 2.1
Omitting Lab. J	101.6 ± 3.2	—

[a]Significant outlier ($P < 0.05$) by Dixon's test (8).

Conclusions and Recommendation

The detection limit of the method appears to be in the 0.3–0.4 ppb range. At this level, satisfactory results were obtained with coefficients of variation of 39.4% for repeatability and 47.1% for reproducibility (reduced to 22.9% and 37.9%, respectively, if outliers are omitted).

At higher NDMA levels, results were excellent with repeatability coefficients of variation of 8.5% for the naturally contaminated samples and 14.4% for the spiked samples. The increased variability in the spiked samples appears to be due to the ethanol solvent used in spiking. Laboratories were also in good agreement; estimated reproducibility coefficients of variation were 22.5% for naturally contaminated samples and 20.4% for the spiked samples. The 22.5% CV includes a laboratory-sample interaction component which was significant for the naturally contaminated samples. Percent recoveries for the 2 spiked samples were also excellent, suggesting that the overall method gives unbiased results.

It is recommended that the method be adopted official first action for determining NDMA content of NFDM.

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was used in the study) is also gratefully acknowledged. Finally, the authors thank R. W. Dabeka of this laboratory for helpful advice in carrying out the statistical analysis.

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Comparison of Three Methods for Determination of *N*-Nitrosopyrrolidine in Fried Dry-Cured Bacon

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The recently developed Eastern Regional Research Center (ERRC) dry column chromatographic procedure for determining *N*-nitrosopyrrolidine (NPYR) in fried cure-pumped bacon was evaluated for its applicability to fried dry-cured bacon. The method was then compared with 2 established procedures for volatile nitrosamine analysis in cured meat products: the multidetection thermal energy analyzer (MD) method and the mineral oil distillation (MOD) screening procedure. No significant difference ($P < 0.05$) in NPYR values was found between the ERRC and MD procedures, but significant differences were found between the ERRC and MOD procedures and between the MOD and MD procedures. No artifactual nitrosamine formation was found in the ERRC procedure, but significant amounts were found in samples analyzed by the MOD procedure. The ERRC method was demonstrated to be rugged and very rapid. It is proposed that the ERRC method replace the MOD method as the official screening procedure for NPYR in fried bacon.

In 1981, approximately 20 million pounds of dry-cured bacon was manufactured in the United States, mostly in the South (C. S. Custer, USDA, Food Safety and Inspection Service, Processed Products Inspection Division, personal communication). Dry-cured bacon is produced by the direct application of the curing ingredients to the pork belly, which results in slow diffusion of the curing salts into the meat. This produces bacon that is lower in water activity, more variable in in-going nitrite levels, higher in salt concentration, and with greater shelf-life stability than cure-pumped bacon. In addition, the increased curing time yields greater chemical degradation of the product, particularly the lipids, which imparts a distinctive flavor to dry-cured bacon. Many people consider this flavor highly desirable. The difference in curing may influence nitrosamine formation and may also interfere with current nitrosamine analytical techniques.

Recently, attention has been focused on nitrosamine formation in fried dry-cured ham and bacon as a result of surveys of these products for *N*-nitrosopyrrolidine (NPYR) (1, 2), the nitrosamine found consistently in fried cure-pumped bacon. Thirty-eight of 55 bacon samples and 21 of 124 ham and shoulder samples surveyed had NPYR values over the 10 ppb nitrosamine violation level established for cure-pumped bacon in 1978 by the Food Safety and Inspection Service (FSIS) (3); several samples had exceptionally high levels, up to 320 ppb (2). These surveys have prompted FSIS to establish a monitoring system designed to collect a data base for dry-cured bacon. This will enable FSIS to determine the extent of nitrosamine occurrence in this product and evaluate the possibility that current regulations on cure-pumped bacon may be extended to dry-cured bacon. The FSIS survey results (1) also prompted research to develop methods to inhibit nitrosamine formation in fried dry-cured bacon. One such method was recently reported by Reddy et al. (4).

The analytical methods most commonly used for monitoring volatile nitrosamines in fried bacon are the multidetection (5) and the mineral oil distillation (6, 7) procedures. However, only products with apparent nitrosamines above the violation level are subjected to the lengthy multidetection method for subsequent mass spectral confirmation. The mineral oil procedure developed by Fine et al. (6) is used to screen all samples for possible violative levels, but this procedure has 2 disadvantages: possible artifactual formation and a lengthy analysis time for a screening procedure. A new rapid method for screening cure-pumped bacon, which reduces the possibility of artifactual nitrosamine formation, was developed by Pensabene et al. (8, 9) at the USDA, Eastern Regional Research Center (ERRC). The applicability of the ERRC dry-column method for screening dry-cured bacon was evaluated by comparative analysis with the 2 currently used procedures, the mineral oil screening and the more elaborate multidetection-thermal energy analyzer (TEA) procedure. Results of the 3-way comparison and their treatment are reported here.

METHODS

Note: Nitrosamines are potential carcinogens, therefore, care should be exercised in handling these materials.

Reagents and Apparatus

Reagents and apparatus used were previously described (5, 7, 8), except for the following:

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

(b) *N-Nitrosodipropylamine (NDPA) internal standard.*—0.25 μg NDPA/mL DCM.

(c) *N-Nitrosopyrrolidine (NPYR) standard.*—0.25 μg NPYR/mL DCM.

Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

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(d) *Dry-cured bacon.*—Random samples obtained from local producers or FSIS, and fried according to protocol set forth in FSIS survey (1).

(e) *Other reagents.*—From local suppliers. Used without further purification.

Procedures

Sodium nitrite was determined as described in **24.041–24.042** (10).

In all procedures, nitrosamines were quantitated by using a gas chromatograph interfaced to a thermal energy analyzer set to parameters specified in the ERRC procedure (8), except the GC temperature was programmed from 140 to 220°C at 4°/min.

The ERRC procedure was described in detail by Pensabene et al. (8). A flow diagram of this method is shown in Figure 1.

The multidetection method described by Fazio et al. (5) was used, with modification. NPYR was quantitated by GC-TEA prior to the column chromatography step, which was omitted.

The mineral oil distillation procedure for *N*-nitrosamines in fried bacon was used as described in **24.CO1–24.CO7** (7).

To check for artifactual nitrosamine formation, a reagent blank was performed for each method; no nitrosamines were detected.

NAZET was used as an internal standard in the ERRC procedure because NDPA is not recovered with this method. It was found to be highly correlated to NPYR (8). NAZET is decomposed by distillation techniques, so NDPA was used in the other 2 procedures. NDPA was selected because it is listed as the internal standard in the AOAC official method (7).

Statistical Analysis

Nitrosopyrrolidine results were corrected (normalized) for recovery of the specific internal standard. Analysis of variance (ANOVA) and least significant difference testing were performed on the measured nitrosamine according to the methods of Snedecor and Cochran (11). The ruggedness test was patterned after the AOAC procedure for ruggedness evaluation (12). Where only a statistical summary is presented, the more extensive raw data are available on request.

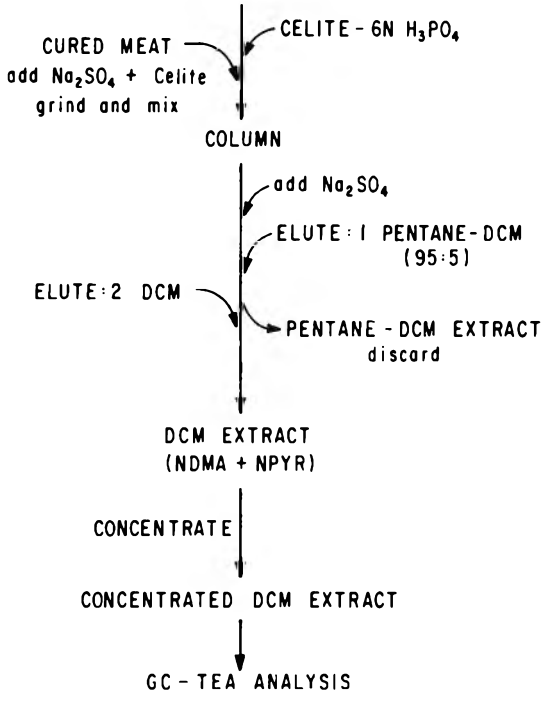

Figure 1. Diagram of ERRC dry column method for determining *N*-nitrosopyrrolidine in fried bacon.

Results and Discussion

A ruggedness test of the ERRC procedure was performed on dry-cured bacon containing 8.4 ppb NPYR. Extreme deviations in the normal grinding, packing, and solvent elution steps were employed and most variations did not result in significant differences in NPYR determinations. However, when columns were allowed to run dry for 15 min before dichloromethane elution, NPYR values varied 15% from those obtained when columns were run normally.

Sodium nitrite was determined in all samples of dry-cured bacon before frying. After frying, 16 dry-cured bacon samples were analyzed in duplicate for NPYR by each of 3 methods: mineral oil distillation (MOD), multidetection (MD), and the ERRC dry column procedure that is designated "ERRC." The results averaged over 2 determinations are shown in Table 1. Individual NPYR values ranged from 0.62 to 12.78 ppb for MOD, none detected (ND) to 9.62 ppb for the MD method, and ND to 13.21 ppb for the ERRC procedure. The minimum detectable level for MOD and MD was 0.2 ppb and for ERRC 0.5 ppb. This is because MOD and MD methods use a 25 g sample and the ERRC method uses only 10 g. Mean recoveries of the internal standards were 93.7, 94.4, and 97.9% for the MOD, MD, and ERRC methods, respectively.

Contrary to reports of correlations in cure-pumped bacon (13), no significant correlation ($P < 0.05$) was found between residual nitrite and NPYR values (r = 0.354, 0.285, and 0.496 for the MOD, MD, and ERRC methods, respectively). The number of variables involved and the heterogeneous nature of the dry-cured bacon may preclude any simple linear correlations.

Repeatability (within-laboratory variation) of NPYR determinations obtained from a 1-way analysis of variance (Table 2) is 0.62 ppb for the MOD and MD methods and 0.43 ppb for the ERRC method. The repeatability of the internal standard recoveries was 6.5, 6.2, and 5.4% for the MOD, MD, and ERRC procedures, respectively.

Table 1. Determination of *N*-nitrosopyrrolidine in fried dry-cured bacon by 3 methods[a]

	Mineral oil		Multidetection		ERRC	
$NaNO_2$, ppm	NPYR, ppb	NDPA, % rec.	NPYR, ppb	NDPA, % rec.	NPYR, ppb	NAZET, % rec.
ND[b]	2.85	72.9	3.76	87.5	1.10	100.4
8	1.33	104	ND	103.7	ND	85.7
8	4.84	95.2	4.62	94.6	3.79	107.3
10	1.78	75.6	1.37	98.1	1.64	94.1
14	2.15	103.3	1.45	88.8	1.84	97.3
24	0.67	101.6	ND	90.3	ND	97.8
24	9.69	87.2	9.53	89.7	6.32	91.2
28	4.25	96.6	3.26	107.5	3.10	103.6
38	3.73	95.0	4.03	86.8	3.41	96.6
40	6.15	88.8	5.40	83.6	4.38	94.1
40	10.28	82.3	6.97	90.7	6.86	96.6
46	10.94	93.6	7.27	101.0	5.77	112.2
76	3.28	98.8	2.52	98.9	3.36	88.2
90	6.21	107.5	5.23	94.3	4.88	113.9
—[c]	9.44	100.2	5.96	100.5	8.44	95.5
—	12.35	96.7	8.71	95.0	12.50	92.5
Mean	5.62	93.7	4.38	94.4	4.21	97.9

[a]Results are averages of duplicate determinations.
[b]ND = none detected.
[c]Nitrite analysis not performed.

Table 2. Statistical analysis for repeatability of 3 methods for determining *N*-nitrosopyrrolidine in fried dry-cured bacon

Source	DF	Mineral oil SS	Mineral oil MS	Mineral oil F	Multidetection SS	Multidetection MS	Multidetection F	ERRC SS	ERRC MS	ERRC F
ANOVA of Corrected NPYR Values										
NPYR	15	431.92	28.79	75.89**	251.77	16.78	43.48**	322.37	21.49	116.72**
Error	16	6.07	0.379	—	6.18	0.386	—	2.95	0.184	—
Total	31	437.99	—	—	257.94	—	—	325.31	—	—
Repeatability[a] (ppb)			0.62			0.62			0.43	
ANOVA of Internal Standard Recovery Values										
Internal std	15	1997.4	199.8	4.65**	1356.0	90.4	2.32	1888.2	124.9	4.39**
Error	16	686.9	42.9	—	623.0	38.9	—	458.6	28.7	—
Total	31	3684.2	—	—	1979.0	—	—	2346.8	—	—
Repeatability (%)			6.5			6.2			5.4	

[a]Repeatability = $\sqrt{\text{MS error}}$.
**$P < 0.01$.

Two-way ANOVA of the NPYR values, assuming random samples, is shown in Table 3. Differences among samples are highly significant ($F = 196.90$, $P < 0.01$) as is to be expected from random sampling of different manufacturers. Differences among methods ($F = 7.98$, $P < 0.01$) and interactions between samples and methods ($F = 7.50$, $P < 0.01$) were also highly significant, possibly due to artifactual nitrosamine formation.

Further investigation into the differences between procedures, using the least significant difference (LSD) between NPYR means, yielded significant differences (>LSD = 0.786 ppb, $P < 0.05$) between the MOD, MD pair and between the MOD, ERRC pair. No significant difference was found between the MD, ERRC pair.

NPYR values tend to be higher with the mineral oil distillation method than with either of the other 2 methods. This may be due to artifactual formation of NPYR during analyses. To determine whether NPYR would form artifactually in the ERRC procedure, Pensabene et al. (8, 9) added up to 100 ppm sodium nitrite to fried nitrate-free bacon and up to 10 ppm morpholine, an easily nitrosatable amine, to fried nitrite-free and nitrite-cure-pumped bacon. Neither NPYR nor *N*-nitrosomorpholine (NMOR) were detected in any of the samples. In more recent studies, sodium nitrite, sodium nitrate, sodium chloride, and morpholine were added to fried nitrite-free bacon in various combinations; nitrosamines were not detected except in those samples in which sodium nitrite and morpholine were added simultaneously prior to analysis. (J. W. Pensabene, USDA, ARS, ERRC, 1982, unpublished data).

A further study of artifactual NPYR formation was conducted using fried dry-cured bacon with normally incurred levels of sodium nitrite and sodium nitrate. Adding 10 ppm morpholine to the pentane wash solvent of the ERRC procedure resulted in no detectable NMOR in the samples. When morpholine was added directly to the meat, rapid nitrosation occurred. Morpholine added to the solvent simulates an environment rich in amines available for nitrosation on the column. No NMOR was produced from solvent-added morpholine, indicating little chance of artifactual nitrosamine formation. Mineral oil distillation was performed on the identical samples as above to which the same level of morpholine was added to the mineral oil. High amounts of NMOR, from 10 to 24 ppb, were found in the mineral oil distillation extracts, suggesting that artifactual formation was probable in samples containing residual nitrite after cooking. Artifactual nitrosamine formation has been observed by several laboratories using the MOD method. As a result, a few laboratories add ascorbate and α-tocopherol inhibitors. While this is effective for most cured meat products, where relatively high levels of nitrite may be present, this is not the case for all samples of fried dry-cured bacon. FSIS has recently found evidence of artifactual NPYR formation in 8 of 37 samples tested (A. J. Malanoski, USDA, FSIS, personal communication).

In conclusion, the ERRC dry column method is particularly applicable to screening dry-cured bacon for NPYR. Using the ERRC procedure, which requires a minimal amount of equipment and solvents, a single analyst in our laboratory has analyzed 18 samples in a single work day, limited only by the sample input rate of the GC-TEA system. Compared with the MOD procedure, the more rapid ERRC method is much less susceptible to artifactual nitrosamine formation, which can occur in fried dry-cured bacon with high residual nitrite levels. In addition, the ERRC method is quantitatively comparable to the lengthy multidetection procedure. It is proposed that the ERRC method replace the MOD method as the screening procedure for NPYR in both fried cure-pumped and dry-cured bacon.

Table 3. Analysis of variance among 3 methods for determining *N*-nitrosopyrrolidine in fried dry-cured bacon

Source	DF	SS	MS	F
Method	2	37.86	18.93	7.98**
Samples	15	934.87	62.32	196.90**
Interaction	30	71.18	2.37	7.50**
Error	48	15.19	0.317	
Total	95	1059.10		

**$P < 0.01$.

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COLOR ADDITIVES

Liquid Chromatographic Determination of Intermediates, Subsidiary Colors, and Two Reaction By-Products in FD&C Yellow No. 6: Reverse Phase Method

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A reverse phase liquid chromatographic (LC) method is presented for determining intermediates, subsidiary colors, and 2 reaction by-products in FD&C Yellow No. 6. Gradient elution from 0.02M ammonium acetate to 60% methanol in 0.02M ammonium acetate is performed on a C-8 column. Recoveries of the analytes from samples of FD&C Yellow No. 6 were as follows: 82 to 129% for the intermediate sulfanilic acid added at levels ranging from 0.04 to 0.2%; 85 to 107% for the intermediate Schaeffer's salt added at levels ranging from 0.03 to 0.3%; 91 to 107% for the reaction by-product 4,4′-(diazoamino)-dibenzenesulfonic acid added at levels ranging from 0.01 to 0.1%; 67 to 108% for the reaction by-product 6,6′-oxybis(2-naphthalenesulfonic acid) added at levels ranging from 0.1 to 1%; 88 to 100% for the subsidiary color 3-hydroxy-4-[(4-sulfophenyl)azo]-2,7-naphthalenedisulfonic acid added at levels ranging from 0.7 to 5%; and 43 to 106% for the subsidiary color 6-hydroxy-5-(phenylazo)-2-naphthalenesulfonic acid added at levels ranging from 0.1 to 1%. To further demonstrate the capabilities of the method, results of multiple analyses of several FD&C Yellow No. 6 samples are presented, and results from corresponding analyses by an AOAC ion exchange LC method and by a thin layer chromatographic method are compared with results found with the new reverse phase LC method.

FD&C Yellow No. 6 (Colour Index No. 15985, Sunset Yellow FCF) is one of the colors allowed by the Food and Drug Administration (FDA) for use in foods, drugs, and cosmetics (1). Each batch of this color manufactured for use in foods, drugs, or cosmetics in the United States must be analyzed and approved by the Division of Color Technology (DCLT), FDA. Among the analyses performed are the determinations of intermediates, certain reaction by-products, and subsidiary colors. The intermediates, 4-aminobenzenesulfonic acid (commonly known as sulfanilic acid (SA)) and 6-hydroxy-2-naphthalenesulfonic acid (commonly known as Schaeffer's salt (SS)), are currently determined with an ion exchange (IE) liquid chromatographic (LC) method (2) that was adopted official first action by the Association of Official Analytical Chemists (AOAC) in 1979. This method is also used to determine 2 reaction by-products: 4,4′-(diazoamino)-dibenzenesulfonic acid (DAADBSA) and 6,6′-oxybis(2-naphthalenesulfonic acid) (DONS). The subsidiary colors of FD&C Yellow No. 6 are currently determined by a thin layer chromatographic (TLC) method reported by Bell (3). Private communications from several dye industry chemists indicate that a higher sulfonated subsidiary color, 3-hydroxy-4-[(4-sulfophenyl)azo]-2,7-naphthalenedisulfonic acid, commonly known as R-salt dye (RS dye), is frequently added to FD&C Yellow No. 6 to improve the solubility properties of the color. The TLC method (3) is also used to determine lower sulfonated subsidiary colors, one of which is speculated by Bell to be 4-[(2-hydroxy-1-naphthalenyl)azo]benzenesulfonic acid, the color formed from the coupling reaction of diazotized SA with 2-naphthol. Another likely lower sulfonated subsidiary color, also suggested by Bell, is 6-hydroxy-5-(phenylazo)-2-naphthalenesulfonic acid (SSA), which is formed from the coupling reaction of SS with diazotized aniline. Our unpublished work indicates that, in the TLC method, these 2 lower sulfonated subsidiary colors cannot be distinguished. However, the literature about the levels of contaminants in the intermediates indicates that the predominant lower sulfonated subsidiary color is that formed from the coupling reaction of SS with diazotized aniline (4, 5).

Using a single method to replace the IE LC and the TLC determinations would result in a significant saving of time. In DCLT, reverse phase (RP) LC has been useful for determining intermediates and subsidiary colors (6–8). Also, RP LC is represented by many column manufacturers as giving more reproducible results than IE LC. For these reasons and particularly because reproducibility is very important in a method that is used by a regulatory agency, an RP LC method was developed to determine SA, SS, DAADBSA, DONS, RS dye, and a lower sulfonated subsidiary color calibrated as SSA.

METHOD

Apparatus

(a) *Liquid chromatograph.*—With gradient elution capability and automatic sampler, e.g., Hewlett-Packard Model 1084B with variable volume injector and automatic sampling system or DuPont 850 with Model 834 automatic sampler with 50 μL loop. Operating conditions: chart speed 0.5 cm/min; eluant flow rate 2 mL/min; oven temperature 40°C; injection volume 50 μL. Gradient profile for Hewlett-Packard Model 1084B: 0–7% B in 2 min; 7–12% B in next 5 min; 12–60% B in next 6 min; hold at 60% B for 5 min; return to 0% B in 0.1 min; hold at 0% B for 5 min before next injection sequence is started. Gradient profile for DuPont 850: segment 1, 0–7% B in 2 min; segment 2, 7–12% B in 5 min; segment 3, 12–60% B in 6 min; segment 4, hold at 60% B for 5 min. DP 850 automatic sampler Model 834 settings: stop integrate time 18 min; total time 24 min; sample pumping time 60 s.

(b) *Detectors.—(1)* Hewlett-Packard Model 79875A variable wavelength detector (deuterium lamp) set at 232 nm, attenuation 0.0128 AU/cm, for one run and at 358 nm, attenuation 0.0064 AU/cm, for a second run. Set reference wavelength at 600 nm for both runs. *(2)* DuPont UV spectrophotometer detector set at 232 nm, attenuation 0.32 AUFS, for one run and at 358 nm, attenuation 0.16 AUFS, for a second run.

(c) *Integrator—(1)* Hewlett-Packard Model 1084B (integrator capability built-in). Set slope sensitivity to 0.3. *(2)* DuPont 850 with VISTA CDS 401 (Varian Associates, Inc., Palo Alto, CA 94303) connected to integrator outlet of spectrophotometer detector through 10:1 attenuator (1800 and 200 ohms voltage divider, Datel Systems, Inc.). Set signal to noise ratio (used by VISTA CDS 401 to set slope sensitivity) equal to 8.

(d) *Column.*—Reverse phase C-8, e.g., Zorbax C-8, 4.6 mm × 25 cm (Cat. No. 850952-706, DuPont Instruments, Wilmington, DE 19898).

[1]Division of Mathematics.

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(e) *Filter disk.*—0.45 μm pore size (Cat. No. HATF 047 00, Millipore Corp., Bedford, MA 01730).

(f) *Spectrophotometer.*—Visible and UV range.

Reagents

(a) *Eluants.*—*(1)* Eluant A, 0.02M ammonium acetate prepared with water from Milli-Q water purification system (Millipore Corp.). Filter eluant through 0.45 μm pore filter disk. *(2)* Eluant B, methanol. For the DuPont 850, bubble He gas through both eluants. For the Hewlett-Packard Model 1084B, heat eluant A to 85°C and eluant B to 50°C and degas with vacuum.

(b) *Standard solutions.*—*(1)* SA, 0.15 mg/mL. *(2)* SS, 0.3 mg/mL. *(3)* DONS, 1.0 mg/mL. *(4)* DAADBSA, 0.06 mg/mL. *(5)* RS dye, 4.0 mg/mL. *(6)* SSA, 1.0 mg/mL. Prepare all solutions in water except *(4)*, which is prepared in 0.004M NaOH. Determine exact concentrations of standard solutions *(1)–(3)* from UV spectra of diluted aliquots in water; determine concentration of standard solution *(4)* from visible spectra of diluted aliquots in 0.02M NaOH; determine concentrations of *(5)* and *(6)* from visible spectra of diluted aliquots in water. Approximate absorptivities (mg/mL, 1 cm) are *(1)* 81.6 at 250 nm, *(2)* 292 at 232 nm, *(3)* 178.6 at 241 nm, *(4)* 86.5 at 409 nm, *(5)* 49 at 488 nm, and *(6)* 59.7 at 482 nm. Preparation and purification of these compounds are presented in references (9) and (10).

(c) *FD&C Yellow No. 6.*—Available from Warner-Jenkinson Manufacturing Co., 2526 Baldwin St, St. Louis, MO 63106. For calibration solutions, select a batch of FD&C Yellow No. 6 that contains only a very small amount of higher sulfonated subsidiary color (an amount found to be less than 0.5% by the TLC method in reference (3) or an amount causing a peak with an area (measured in integrator counts) less than 3000 (Hewlett-Packard Model 1084B) or less than 9000 (DuPont 850) when using this method). Recrystallize by dissolving ca 20 g in 100 mL boiling water; while stirring and heating, add 200 mL methanol; let cool (FD&C Yellow No. 6 will precipitate); filter (Whatman Qualitative No. 3 filter paper) with vacuum; wash filter cake twice with methanol and let dry in air with vacuum applied. Repeat recrystallization with dried filter cake and dry overnight in vacuum oven at 100°C. Chromatograph the FD&C Yellow No. 6 sample under the conditions of this method to check its purity. Repeat recrystallization if any peaks are observed for SA, SS, DAADBSA, or DONS. Small amounts of RS dye and SSA that may remain do not interfere with calibration data.

Suitability Test

Prepare test solution containing 0.5 g FD&C Yellow No. 6 per 100 mL 0.02M ammonium acetate and, relative to FD&C Yellow No. 6, 0.2% SA, 0.3% SS, 0.5% DONS, 0.1% DAADBSA, 0.5% SSA, and 3% RS dye. Set operating conditions and run 2 blank gradients and test solution at 232 nm. Compare resulting chromatogram to that in Figure 1. If similar resolution is not attained, adjust operating conditions to those needed to resolve these compounds. Running a mixture of 25% acetic acid in water–methanol (1 + 1) through the Zorbax C-8 column at an oven temperature of 50°C has been found with 4 Zorbax C-8 columns to improve resolution of the compounds determined in this method.

Calibration

For each calibrating solution, dissolve 0.500 g FD&C Yellow No. 6 (free of compounds being determined) in ca 50 mL water and add 10 mL 0.02M ammonium acetate. Add aliquots of standard solutions needed to calibrate for SA from 0.02 to 0.25%, for SS from 0.03 to 0.35%, for DONS from 0.1 to 1.0%, for SSA from 0.1 to 1.2%, for DAADBSA from 0.01 to 0.15%, and for RS dye from 0.5 to 5.5%. Dilute to 100 mL with water. For each compound in solution, calculate C, % by weight relative to the amount of FD&C Yellow No. 6 in the solution:

$$C = V \times C' \times 0.2$$

where V = volume of aliquot of standard solution (mL) taken; C' = concentration of standard solution (mg/mL) as determined spectrophotometrically; and 0.2 = 100 (%)/500 (mg). Using integrator, measure peak areas on the chromatogram for SA, SS, DONS, and SSA from the 232 nm detector setting and, for DAADBSA and RS dye, from the 358 nm detector setting. For each compound, plot C, % by weight, vs peak area.

Determination

Start instrument pumps and operate at 2 mL/min, 0% B, until flow and baseline are stable. Run at least 2 gradients with injection of 0.02M ammonium acetate until baseline is reproducible. To identify retention time of each component, analyze 6 calibration solutions, each containing only 1 added component of interest plus FD&C Yellow No. 6.

For sample solutions, dissolve 0.500 g sample in water, add 10 mL 0.2M ammonium acetate, and dilute to 100 mL with water. Analyze samples interspersed among additional calibration solutions covering concentration range outlined in *Calibration*. Analyze each calibration solution and each sample solution at detector settings of 232 and 358 nm.

Obtain retention times for identification of compounds of interest in samples from analyses of calibration solutions interspersed among samples. The variation in retention times for calibration solutions run within 24 h should not exceed 0.5 min for any compound and is frequently less. Use range of retention times plus or minus 0.05 min to identify peaks in chromatograms of samples of FD&C Yellow No. 6. Use chromatograms at 232 nm (Figure 1) to calculate SA, SS, DONS, and SSA. Use chromatograms at 358 nm (Figure 2) to calculate RS dye and DAADBSA. Use plots of C versus area to determine % by weight of each compound in the samples.

Note: If the liquid chromatograph is not to be used, i.e., eluants will not be flowing, for more than 1 or 2 h, replace eluant A with water and flush water through instrument and column. Then run a gradient from 0 to 100% B (methanol) and then from 100 to 65% B, leaving instrument and column in 65% methanol in water.

Results and Discussion

Preliminary method development was done primarily with the Hewlett-Packard Model 1084B liquid chromatograph.

A pH of 7 for the aqueous component of the mobile phase was selected because DAADBSA decomposes at lower pH levels and the silica-based reverse phase column cannot be used for long periods at higher pH levels. Two buffering solutions were tested: dipotassium hydrogen phosphate plus potassium dihydrogen phosphate (K_2HPO_4 plus KH_2PO_4) and ammonium acetate (NH_4OAc). Ammonium acetate was selected because it is simpler to prepare and is much more soluble in methanol than the potassium phosphate salts. Several concentrations of ammonium acetate were tested; a 0.02M solution was selected because it gave the best resolution of the FD&C Yellow No. 6 components.

During development of the method, problems with variation in retention times were alleviated by controlling the

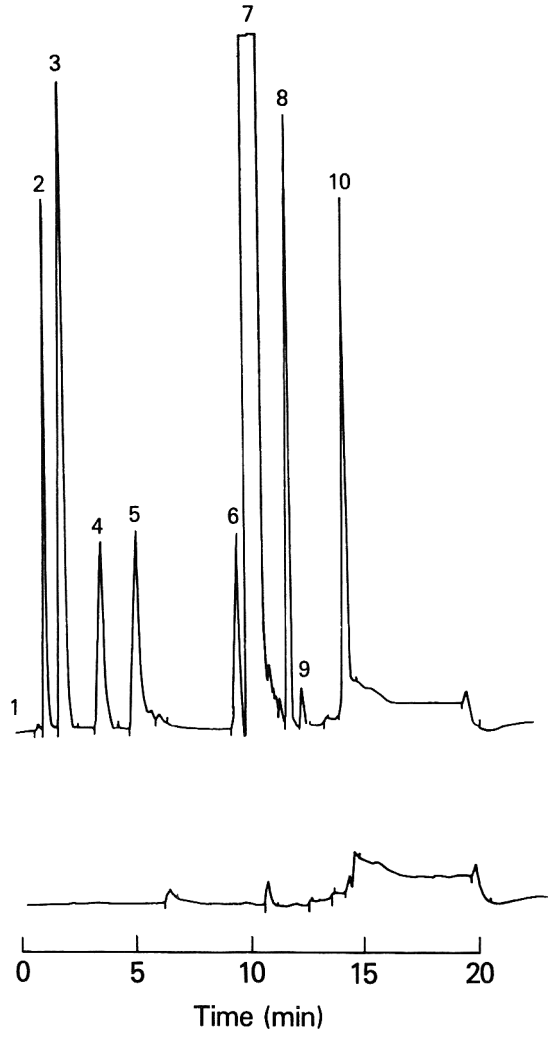

Figure 1. Chromatogram of FD&C Yellow No. 6 (peak 7) at 232 nm. Top tracing: 1 = injection; 2 = SA; 3 = RS dye; 4 = DAADBSA; 5 = SS; 6 = unknown; 8 = DONS; 9 = unknown; 10 = SSA. Bottom tracing = blank. Hewlett-Packard Model 1084B chromatograph used for both tracings.

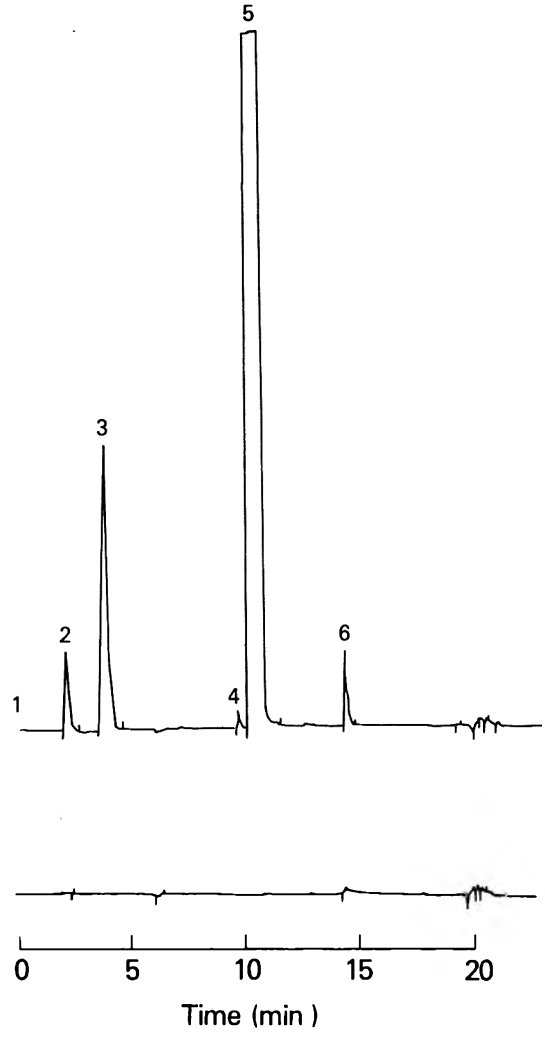

Figure 2. Chromatogram of FD&C Yellow No. 6 (peak 5) at 358 nm. Top tracing: 1 = injection; 2 = RS dye; 3 = DAADBSA; 4 = unknown; 6 = SSA. Bottom tracing = blank. Hewlett-Packard Model 1084B chromatograph used for both tracings.

column temperature. The oven temperature of 40°C is used in the method.

Controlling the exact length of the equilibration time between gradient runs was not as critical to retention times as it is in IE LC. When the Hewlett-Packard Model 1084B is operated automatically, the exact length of the equilibration time varies slightly because of variations in the lengths of the retention time and area reports that are printed at the completion of each chromatographic analysis. With the DuPont 850, equilibration time is controlled and repeated exactly when the instrument is operated automatically.

The 2 possible lower sulfonated subsidiary colors, 4-[(2-hydroxy-l-naphthalenyl)azo]benzenesulfonic acid and SSA, are not resolved by this method; they elute at the same retention time. No attempt was made to resolve them because the specification limit is general for subsidiary dyes and the absorptivities of the 2 dyes are close enough so that calibrating with one dye when determining the other causes only a small error. The SSA subsidiary dye was selected for calibration purposes because it was considered to be the subsidiary color more likely to occur in commercial samples.

Several different wavelengths were tested during development of the method. The 6 analytes dissolved in 0.02M ammonium acetate were examined spectrophotometrically from 540 to 205 nm by using a deuterium lamp in a Cary 118 spectrophotometer. The absorption spectrum of SA has a maximum at 248 nm with significant absorption from about 225 to 265 nm. The absorption spectrum of SS has a maximum at 232 nm with significant absorption from about 215 to 245 nm and very little absorption at 254 nm. The wavelength of maximum absorption in the spectrum of DAADBSA is 358 nm; DAADBSA has significant absorption from about 230 to 250 nm and from about 310 to 400 nm. The spectrum of DONS has maximum absorption at 240 nm with significant absorption from 205 to 280 nm. The spectra of RS dye and SSA have significant absorption over the entire range scanned with the maximum absorption at 238 nm and minimum absorption at about 345 nm for both compounds.

The wavelengths 232, 235, 240, 248, 254, and 358 nm were tested by chromatographing calibration solutions and evaluating the resulting data according to the statistical scheme in reference (11). The SA calibration was best at 248 nm and adequate at all the other wavelengths tested except 358 nm, where SA has no absorption. The SS calibration was best at 232 nm but was unacceptable at 248 or 254 nm. The poor calibration data for SS are most likely due to the low absorption of SS at those wavelengths. The DAADBSA calibration was good at all wavelengths tested but was best at 358 nm. The DONS calibration was good at 248 and 254 nm, acceptable at 232 nm, and inadequate at 235 and 240 nm; DONS does not absorb at 358 nm. The SSA calibration was good at all the wavelengths tested. The RS dye calibration was

acceptable at 358 nm but was inadequate at 232 or 254 nm. The unacceptable calibration data for DONS at 235 and 240 nm and RS dye at 232 and 254 nm were surprising because both have strong absorption at those wavelengths. When the unacceptable data were plotted, the plots of area vs amounts added were not linear for the higher amounts; a line connecting the points tended to plateau. This observation appears to demonstrate a limitation of the applicability of Beer's Law.

Because there was no single wavelength at which the calibration of all 6 analytes was adequate, the method was subsequently developed with detection at 2 wavelengths: 232 nm for SA, SS, DONS, and SSA and 358 nm for RS dye and DAADBSA. Each solution of FD&C Yellow No. 6 is chromatographed at both wavelengths. With the DuPont 850, all the analyses are made at one wavelength; then the spectrophotometer is reset manually to the other wavelength and the solutions are re-analyzed. With the Hewlett-Packard Model 1084B, the spectrophotometer can be made to change wavelengths automatically so that there is a choice of operating modes: either performing the analysis as with the DuPont 850 with automatic switching of the wavelength or analyzing a solution at one wavelength, automatically changing wavelength, and making a second injection from the same vial. Both procedures work well.

A problem that may occur with RS dye when this method is used over an extended period of time is peak splitting, i.e., RS dye elutes as 2 just barely resolved peaks. During method development, 2 different situations were found to cause the RS dye peak to split. When solutions of FD&C Yellow No. 6 prepared in water were injected, the RS dye peak frequently split. Thus, preparing FD&C Yellow No. 6 in 0.02M ammonium acetate is essential. With the Hewlett-Packard Model 1084B, RS dye peak splitting was apparently related to some change, occurring with sustained use, in the Teflon cone of the injector check valve (Hewlett-Packard part number 79841-62303). Twice, in the course of developing this method, changing the injector check valve corrected the problem of RS dye peak splitting.

There is some indication that a precolumn between the pump and the injector is needed on the DuPont 850. No precolumn was used nor was its use indicated on the Hewlett-Packard Model 1084B. Further work in this area is planned.

This method was tested from written instructions with a second Hewlett-Packard Model 1084B liquid chromatograph and a different Zorbax C-8 column. The separation was reproduced and the written instructions needed to be only slightly modified for clarity.

Batches of FD&C Yellow No. 6 received for certification from many different companies were analyzed with the Hewlett-Packard Model 1084B liquid chromatograph to test the method, and 2 different Zorbax C-8 columns were used. The method was further tested with the DuPont 850 liquid chromatograph by a recovery study and analyses of certain FD&C Yellow No. 6 samples. Again, 2 different Zorbax C-8 columns were used. Thus, a total of 4 different Zorbax C-8 columns were used for the analysis of FD&C Yellow No. 6 samples during the testing of this method. The testing covered a period of 11 months.

During testing, 11 sets of calibration data were generated. Sets A, B, and C, from the Hewlett-Packard Model 1084B liquid chromatograph with column L1325 (number assigned by DuPont), were collected for the purpose of analyzing certification samples. Sets D, E, F, and G, also from the Hewlett-Packard Model 1084B liquid chromatograph but with column L2769, were collected for the same purpose. Sets H and I, from the DuPont 850 liquid chromatograph with column L3069, were collected for the purpose of analyzing the recovery study solutions. Sets J and K, from the DuPont 850 liquid chromatograph with column L3270, were collected for the purpose of analyzing certification samples. Each set of calibration solutions was chromatographed within a 24 h period except that for set A, which covered 4 days.

The average retention times and ranges for the analytes in the calibration chromatograms are presented in Table 1. All of the ranges are less than 0.5 min wide and frequently are less than 0.1 min wide. Within a set of data, no ranges overlap. However, an examination of the whole body of data reveals that the DAADBSA and the SS retention time ranges do overlap—the retention time of 5.48 min for DAADBSA in one calibration run from set A overlaps the retention time of 5.34 min for SS in one calibration run from set I. Thus, it is necessary to chromatograph calibration and sample solutions within a short time interval if retention time is to be used to identify the peaks. The retention times of the analytes chro-

Table 1. Average retention times (and ranges) found in chromatograms of sets of calibration solutions obtained during testing of this method

Set[a]	Average retention times, min (range)					
	SA	RS dye	DAADBSA	SS	DONS	SSA
A	1.49 (1.48–1.52)	2.38 (2.36–2.39)	5.36 (5.23–5.48)	6.87 (6.80–6.95)	12.59 (12.55–12.63)	15.10 (15.07–15.15)
B	1.46 (1.46–1.46)	2.35 (2.33–2.37)	5.20 (5.17–5.25)	6.79 (6.76–6.81)	12.54 (12.53–12.55)	15.06 (15.05–15.07)
C	1.48 (1.46–1.51)	2.43 (2.39–2.49)	5.14 (5.09–5.24)	6.80 (6.68–6.97)	12.54 (12.50–12.58)	14.97 (14.93–15.03)
D	1.52 (1.51–1.53)	2.43 (2.38–2.52)	5.19 (5.10–5.35)	6.71 (6.67–6.76)	12.54 (12.52–12.56)	15.05 (15.03–15.08)
E	1.46 (1.39–1.52)	2.37 (2.29–2.45)	4.94 (4.85–5.04)	6.65 (6.60–6.73)	12.49 (12.46–12.51)	14.97 (14.95–14.99)
F	1.40 (1.39–1.42)	2.29 (2.21–2.38)	4.75 (4.58–4.93)	6.43 (6.36–6.58)	12.36 (12.32–12.47)	14.88 (14.84–14.94)
G	1.42 (1.40–1.44)	2.28 (2.21–2.35)	4.67 (4.56–4.77)	6.47 (6.41–6.52)	12.39 (12.35–12.42)	14.86 (14.84–14.89)
H	1.37 (1.36–1.38)	1.90 (1.87–1.94)	4.06 (4.05–4.09)	5.38 (5.36–5.41)	13.14 (13.12–13.16)	15.82 (15.79–15.84)
I	1.36 (1.36–1.37)	1.88 (1.85–1.92)	3.98 (3.97–3.98)	5.35 (5.34–5.37)	13.12 (13.10–13.16)	15.80 (15.78–15.80)
J	1.37 (1.37–1.38)	2.18 (2.15–2.20)	4.38 (4.35–4.41)	5.46 (5.44–5.47)	13.31 (13.29–13.32)	15.98 (15.97–15.99)
K	1.36 (1.35–1.37)	2.14 (2.12–2.16)	4.26 (4.22–4.29)	5.33 (5.30–5.42)	13.25 (13.20–13.30)	15.91 (15.89–15.96)

[a]The sets of calibration data, A through K, are further identified in the text.

matographed at closely spaced times can be seen to fall within a small range (Table 1).

The calibration data, amounts of the analytes added, and the areas of the resulting peaks (in integrator counts) were evaluated according to the statistical scheme presented in reference (11). Additional suggestions have been published subsequently for statistical evaluation of calibration data (12). Because 2 of the authors of reference (11) are also co-authors of this paper, and because we have had extensive experience using the statistical scheme in reference (11), we take this opportunity to revise some of the recommendations made in that paper. When calibration data are statistically evaluated, we recommend further investigation of the data for any of the following conditions: *(1)* if a correlation coefficient of less than 0.98 is calculated for the calibration data; *(2)* if a limit of detection, X_{LD} (X_{LD} is the concentration whose lower prediction limit (99%) for peak area is equal to the upper prediction limit of the peak area for zero concentration (11)), is found to be greater than one-half the specification limit; *(3)* if a prediction limit at the specification concentration is calculated to be greater than one-quarter of the specification limit.

The summaries of the calibration data evaluations are presented in Tables 2–7. As can be seen in Table 2, the calculated X_{LD} values for SA range from 0.03 to 0.14%. There is no specification limit in the Code of Federal Regulations (CFR) for SA but 0.2% has been proposed. All X_{LD} values for SA, except that in set E, are below one-half the proposed specification limit, and all the prediction limits, except that in set E again, are below one-quarter of the proposed specification limit. All the correlation coefficients are above 0.99.

Table 3 presents the evaluations of the SS calibration data. The proposed specification limit for SS is 0.3%. All the calculated X_{LD} values, which range from 0.02 to 0.13%, are below one-half the proposed specification limit. Only in set K is the prediction limit greater than one-quarter of the proposed specification limit. All the correlation coefficients are above 0.98.

The proposed specification limit for DAADBSA is 0.1%. The evaluations of DAADBSA calibration data are presented in Table 4. All the calculated X_{LD} values, which range from 0.004 to 0.05%, are equal to or less than one-half the proposed specification limit. All the prediction limits at the specification concentrations are less than one-quarter of the proposed specification limit. All the correlation coefficients are greater than 0.99.

The evaluations of the DONS calibration data are presented in Table 5. The proposed specification limit for DONS is 1.0%. All the calculated X_{LD} values, which range from 0.06 to 0.28%, are well below one-half the proposed specification limit. All the prediction limits are well below one-quarter of the proposed specification limit. All the correlation coefficients are above 0.99.

The specification limit in the CFR is 5% for subsidiary dyes. RS dye was calibrated through 5%, and evaluation of the calibration data is presented in Table 6. All the calculated X_{LD} values are well below one-half the specification limit and range from 0.1 to 1.7%. All the prediction limits are well below one-quarter of the specification limit. The correlation coefficients are all above 0.99.

Although the 5% limit on subsidiary colors also applies to SSA, the method calls for calibration of SSA only up through about 1% because SSA is very rarely found above that level in the batches of FD&C Yellow No. 6 submitted for certification. Also, amounts of lower sulfonated subsidiary color at levels of approximately 1% would be of concern if the amounts of RS dye were over 4%. The evaluation of SSA calibration data is presented in Table 7. All the calculated X_{LD} values are well below one-half the surrogate specification limit of 1% and they range from 0.04 to 0.31%. The prediction limits are all less than one-quarter of the surrogate specification limit. All the correlation coefficients are above 0.99.

We would like to point out that the limits of detection calculated here display a wide variation as demonstrated in the ranges cited in the above paragraphs. This variation is most likely due to instrumental variation, column aging, and/or fluctuation in the number of points and levels in the calibration data sets.

The calibration data evaluations indicate that most of the data collected were consistent and yielded good linear fits. All calibration data were adequate except possibly those for SA in set E. SA is the most difficult analyte in the method to determine because it elutes close to t_0 and the wavelengths

Table 2. Summary of statistical evaluations of calibration data for SA

Set	n^a	r^b	$X_{LD}{}^c$	Prediction limits[d]
A	10	0.9957	0.06	±0.03
B	5	0.9995	0.04	±0.02
C	7	0.9970	0.08	±0.04
D	8	0.9989	0.05	±0.02
E	8	0.9907	0.14	±0.08
F	9	0.9994	0.03	±0.01
G	11	0.9985	0.05	±0.02
H	11	0.9958	0.05	±0.03
I	16	0.9940	0.05	±0.03
J	10	0.9966	0.05	±0.03
K	10	0.9989	0.03	±0.02

[a] *n* is the number of points in the set of data.
[b] *r* is the correlation coefficient.
[c] X_{LD} is the limit of detection with 99% confidence in units of % dye.
[d] Prediction limits calculated on a new observation at the 0.2% level.

Table 3. Summary of statistical evaluations of calibration data for SS

Set	n^a	r^b	$X_{LD}{}^c$	Prediction limits[d]
A	10	0.9967	0.04	±0.02
B	5	0.9998	0.02	±0.01
C	7	0.9985	0.07	±0.04
D	8	0.9994	0.04	±0.02
E	8	0.9917	0.12	±0.07
F	9	0.9992	0.03	±0.02
G	11	0.9985	0.04	±0.02
H	11	0.9997	0.02	±0.01
I	16	0.9990	0.03	±0.02
J	10	0.9993	0.03	±0.02
K	8	0.9868	0.13	±0.08

[a]–[c] See footnotes to Table 2.
[d] Prediction limits calculated on a new observation at the 0.3% level.

Table 4. Summary of statistical evaluations of calibration data for DAADBSA

Set	n^a	r^b	$X_{LD}{}^c$	Prediction limits[d]
A	9	0.9991	0.02	±0.01
B	6	0.9994	0.02	±0.01
C	6	0.9986	0.03	±0.02
D	8	0.9996	0.01	±0.005
E	11	0.9967	0.02	±0.01
F	10	0.9991	0.02	±0.01
G	9	0.9990	0.02	±0.01
H	11	0.9999	0.004	±0.002
I	8	0.9997	0.01	±0.004
J	10	0.9991	0.04	±0.02
K	11	0.9983	0.05	±0.02

[a]–[c] See footnotes to Table 2.
[d] Prediction limits calculated on a new observation at the 0.1% level.

Table 5. Summary of statistical evaluations of calibration data for DONS

Set	n^a	r^b	$X_{LD}{}^c$	Prediction limits[d]
A	10	0.9968	0.19	±0.11
B	5	0.9999	0.07	±0.04
C	6	0.9999	0.06	±0.03
D	8	0.9998	0.07	±0.04
E	8	0.9949	0.21	±0.13
F	9	0.9996	0.08	±0.05
G	11	0.9996	0.07	±0.04
H	11	0.9977	0.19	±0.11
I	16	0.9981	0.15	±0.08
J	10	0.9947	0.28	±0.15
K	10	0.9976	0.19	±0.10

[a-c]See footnotes to Table 2.
[d]Prediction limits calculated on a new observation at the 1% level.

used are not close to its wavelength of maximum absorption. SA is rarely seen in batches of FD&C Yellow No. 6 at levels above 0.1%.

The recovery study solutions were prepared as directed in the calibration portion of the method, submitted to the analyst as unknowns, and analyzed by using the DuPont 850 liquid chromatograph and the calibration data in sets H and I. The recovery data and individual percent recoveries are presented in Table 8. In general, there were 4 levels of each component with each level added, arbitrarily, to 5 different recovery solutions, for a total of 20 recovery solutions. Only 19 recoveries are presented in Table 8 for each component because the buffer was accidentally not added to one recovery solution and the resulting chromatogram was unacceptable. The data in Table 8 are organized with the amounts found for the same levels grouped together. For most of the components, 2 different standard solutions of slightly different concentrations were used to prepare the recovery solutions. For 3 of the SS and 3 of the DONS recovery study levels, these differences in concentration prevented grouping for statistical calculations. Table 9 presents the averages and coefficients of variation (CVs) (standard deviation multiplied by 100 and divided by the average), organized by spiking level, of the amounts found in the recovery study. The fact that the CVs are highest at the lowest levels for most components (DAADBSA and DONS are the exceptions) indicates that relative variation is greatest at the lowest levels, as expected. The CVs for all components at the spiking levels closest to the surrogate specification limits are all low, indicating good repeatability.

The width (or really the half-width) of a 99% confidence interval (appropriate Student's *t*-value multiplied by the standard deviation divided by the square root of the number of recoveries) was calculated from amounts found at each spiking level. The average found at each spiking level plus or minus the width of the confidence interval was examined to determine if the known value for the amount added was included within the calculated range.

The amounts added for SS, DAADBSA, and SSA were encompassed at all spiking levels by the averages found plus or minus the corresponding confidence interval width. For SA the average found plus or minus the confidence interval width at the 3 lowest spiking levels did enclose the amounts added; but at the 0.229% spiking level, the average found minus the 99% confidence interval width was still higher than the amount added, suggesting a bias at this concentration.

For DONS the averages found plus the confidence interval width did exceed the amounts added at the 0.204 and 1.11% spiking levels. The average amount found plus the confidence interval width at the 0.093% spiking level (considered to be below the limit of detection) was below the amount added. However, at the 0.557% spiking level, the average found minus the confidence interval width was above the amount added. The lack of a consistent relationship between the average found and the spiking level for the DONS recovery ranges suggests a bias that varies randomly rather than one that is systematic.

For RS dye, only at the 2.91% spiking level did the average found plus and minus the confidence interval width encompass the amount added. At the other 3 levels the average found plus the corresponding confidence interval width was below the amount added, suggesting a negative bias of approximately 0.05%.

In testing this method 31 certification samples and the 2 samples currently undergoing pharmacological testing were analyzed. The concentrations of the analytes in these samples were estimated by using the regression line equations from the calibration solutions that were chromatographed along with the samples. The results of the RP LC analyses are compared in Figures 3–8 with those found by IE LC for SA, SS, DAADBSA, and DONS and by TLC for RS dye and SSA. Also shown in these figures are the average X_{LD} values calculated from the 11 sets of calibration data. Most of the results for SA (Figure 3) are below the average X_{LD}, 0.06%. For 6 of the 33 samples, both methods indicated that no SA was present in the samples. The 7 samples containing 0.06% or more SA display a marked difference in amounts of SA found by the 2 methods. Figure 4 presents the results for SS. Approximately one-half of the samples are above the average X_{LD} of 0.05%, and the amounts of SS found in these samples by the 2 methods correspond to each other closely with 1 exception (0.08% by RP LC and 0.03% by IE LC).

For 19 of the 33 samples, both methods indicated that no DAADBSA was present in the samples. In one sample (DCLT No. 159) a peak of unknown origin interfered with the

Table 6. Summary of statistical evaluations of calibration data for RS dye

Set	n^a	r^b	$X_{LD}{}^c$	Prediction limits[d]
A	9	0.9908	1.7	±1.0
B	6	0.9999	0.4	±0.2
C	6	0.9992	0.7	±0.4
D	8	0.9999	0.3	±0.1
E	11	0.9997	0.3	±0.2
F	10	0.9986	0.8	±0.4
G	9	0.9997	0.4	±0.2
H	11	0.9999	0.2	±0.1
I	8	1.0000	0.1	±0.1
J	10	0.9998	0.3	±0.1
K	11	0.9999	0.2	±0.1

[a-c]See footnotes in Table 2.
[d]Prediction limits calculated on a new observation at the 5% level.

Table 7. Summary of statistical evaluations of calibration data for SSA

Set	n^a	r^b	$X_{LD}{}^c$	Prediction limits[d]
A	10	0.9954	0.18	±0.10
B	5	0.9999	0.04	±0.02
C	6	0.9991	0.15	±0.08
D	8	0.9997	0.07	±0.04
E	8	0.9941	0.31	±0.21
F	9	0.9988	0.12	±0.07
G	11	0.9982	0.14	±0.08
H	11	0.9994	0.10	±0.05
I	16	0.9960	0.24	±0.13
J	10	0.9989	0.14	±0.08
K	10	0.9993	0.11	±0.06

[a-c]See footnotes to Table 2.
[d]Prediction limits calculated on a new observation at the 1% level.

Table 8. Recovery of components added to FD&C Yellow No. 6

SA		SS		DAADBSA		DONS		RS dye		SSA	
Found,%	Rec.,%	Found,%	Rec.,%	Found,%	Rec.,%	Found,%	Rec.,%	Found,%	Rec.,%	Found,%	Rec.,%
(Added: 0.038%)		(Added: 0.031%)		(Added: 0.012%)		(Added: 0.093%)		(Added: 0.728%)		(Added: 0.095%)	
0.031	81.6	0.028	90.3	0.012	100	0.062	66.7	0.644	88.5	0.061	64.2
0.049	128.9			0.012	100	0.063	67.7	0.644	88.5	0.063	66.3
0.043	113.2	(Added: 0.027%)		0.012	100	0.064	68.8	0.643	88.3	0.096	101.1
0.049	128.9	0.023	85.2	0.012	100			0.678	93.1	0.041	43.2
		0.029	107.4	0.012	100	(Added: 0.102%)				0.078	82.1
(Added: 0.076%)		0.024	88.9			0.076	74.5	(Added: 2.91%)			
0.092	121.1			(Added: 0.023%)		0.076	74.5	2.87	98.6	(Added: 0.190%)	
0.095	125.0	(Added: 0.109%)		0.023	100			2.87	98.6	0.186	97.9
0.091	119.7	0.108	99.1	0.023	100	(Added: 0.204%)		2.90	99.7	0.143	75.3
0.083	109.2	0.104	95.4	0.023	100	0.199	97.5	2.87	98.6	0.170	89.5
0.073	96.1	0.108	99.1	0.021	91.3	0.191	93.6	2.87	98.6	0.151	79.5
		0.107	98.2			0.193	94.6			0.170	89.5
(Added: 0.095%)		0.107	98.2	(Added: 0.046%)		0.197	96.6	(Added 2.98%)			
0.095	100.0			0.047	102.2			2.93	98.3	(Added: 0.571%)	
0.091	95.8	(Added: 0.163%)		0.042	91.3	Added: 0.557%)		2.95	99.0	0.559	97.9
0.096	101.1	0.167	102.5	0.046	100	0.595	106.8	2.94	98.7	0.566	99.1
0.098	103.2	0.160	98.2	0.045	97.8	0.598	107.4	2.96	99.3	0.567	99.3
0.096	101.1	0.169	103.7	0.047	102.2	0.603	108.3	2.95	99.0	0.582	101.9
		0.157	96.3			0.588	105.6				
(Added: 0.229%)				(Added: 0.104%)				(Added: 5.10%)		(Added: 1.14%)	
0.253	110.5	(Added: 0.183%)		0.107	102.9	(Added: 0.613%)		5.04	98.8	1.11	97.4
0.248	108.3	0.181	98.9	0.109	104.8	0.648	105.7	5.03	98.6	1.11	97.4
0.248	108.3			0.111	106.7			5.03	98.6	1.12	98.2
0.256	111.8	(Added: 0.272%)		0.103	99.0	(Added: 1.11%)		5.01	98.2	1.19	104.4
0.254	110.9	0.269	98.9	0.106	101.9	1.09	98.2	5.04	98.8	1.21	106.1
		0.269	98.9			1.09	98.2				
		0.275	101.1			1.13	101.8				
		0.268	98.5			1.10	99.1				
						1.10	99.1				
		(Added: 0.305%)									
		0.301	98.7								

DAADBSA peak in the IE LC analysis, making determination impossible; 0.02% DAADBSA was found in the RP LC analysis of this sample. Another sample (DCLT No. 30) is not plotted in Figure 5 because the values found for DAADBSA were so high that their inclusion reduced the plot of the other values to a small part of the figure. Further discussion of this sample occurs later in this report. Of the remaining samples, 6 contain amounts found by RP LC to be above the average calculated X_{LD} of 0.02%. The amounts of DAADBSA found by the 2 methods in these samples agree fairly well.

Figure 6 is the plot of the amount of DONS found by one method vs the amount found by the other method. Both methods indicated that no DONS was present in 10 of the 33 samples. Five samples contained amounts of DONS found by RP LC to be above the average calculated X_{LD} of 0.14%; the results of 4 of the 5 IE LC analyses agree well, the exception being the sample found to contain 0.37% DONS by RP LC and 0.25% DONS by IE LC.

The amounts of RS dye found by TLC and RP LC are plotted in Figure 7. All the amounts found by RP LC are above the average calculated X_{LD} of 0.49%. A significant amount of variation is displayed in this figure.

For 4 of the 33 samples, both RP LC and TLC indicated that no lower sulfonated subsidiary was present in the samples. Nineteen samples were found by RP LC to contain amounts of lower sulfonated subsidiary calculated as SSA at or above the average calculated X_{LD} of 0.15%. These amounts vary significantly in some samples from the amounts found by TLC as can be seen in Figure 8.

In a collaborative study (2), the 2 pharmacology samples, AA3003 and AA8634, were analyzed by IE LC for SA, SS, DAADBSA, and DONS. AA3003 and AA8634 were analyzed 3 times by RP LC; the results are presented in Table 10. A Student's *t*-test was performed to compare the results from the collaborative study to the RP LC results. In the study, sample AA3003 was analyzed in duplicate by the collaborators; only the results reported first (sample 1a in reference (2)) were used in the present calculations. For both samples, results given as "less than" a given amount were treated in the statistical calculations as the given amounts. Results of

Table 9. Averages and coefficients of variation (CV) of amounts found in recovery study, presented by level

Spike level	Av.	CV, %	Spike level	Av.	CV, %	Spike level	Av.	CV, %
	SA			SS			DAADBSA	
0.038	0.043	19.7	0.027	0.025	12.9	0.012	0.012	0.0
0.076	0.087	10.3	0.109	0.107	1.5	0.023	0.023	4.4
0.095	0.095	2.7	0.163	0.163	3.5	0.046	0.045	4.6
0.229	0.252	1.4	0.272	0.270	1.2	0.104	0.107	2.8
	DONS			RS dye			SSA	
0.093	0.063	1.6	0.728	0.652	2.6	0.095	0.068	30.2
0.204	0.195	1.9	2.91	2.88	0.5	0.190	0.164	10.2
0.557	0.596	1.1	2.98	2.95	0.4	0.571	0.569	1.7
1.11	1.10	1.5	5.10	5.03	0.2	1.14	1.15	4.2

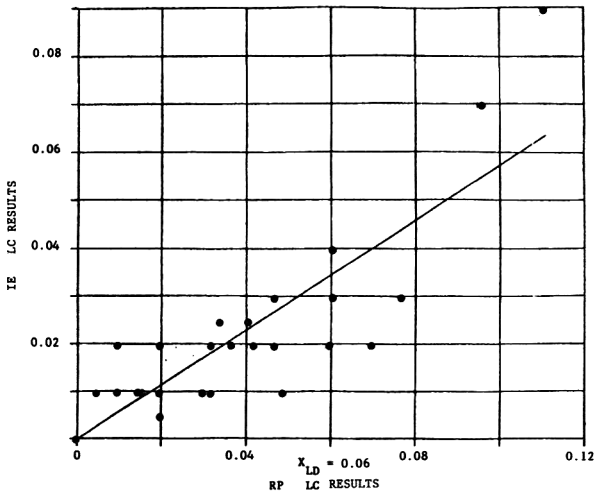

Figure 3. Amounts of SA found as wt % in samples of FD&C Yellow No. 6 by RP LC and IE LC methods. X_{LD} is average limit of detection calculated from calibration data.

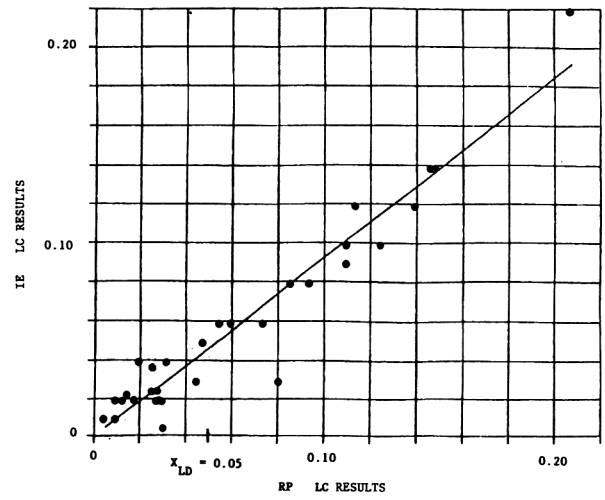

Figure 4. Amounts of SS found as wt % in samples of FD&C Yellow No. 6 by RP LC and IE LC methods. X_{LD} is average limit of detection calculated from calibration data.

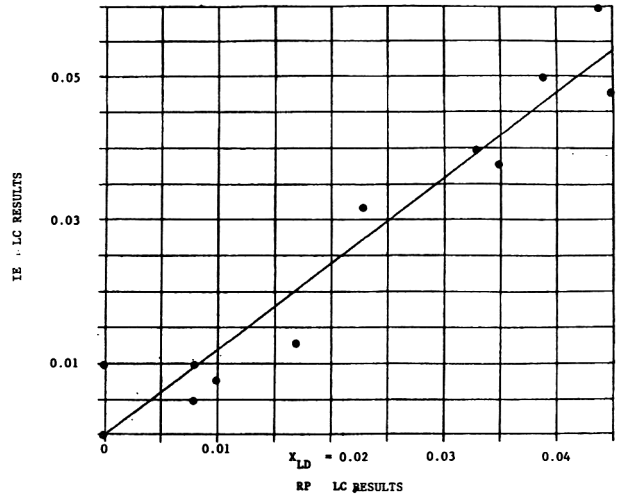

Figure 5. Amounts of DAADBSA found as wt % in samples of FD&C Yellow No. 6 by RP LC and IE LC methods. X_{LD} is average limit of detection calculated from calibration data.

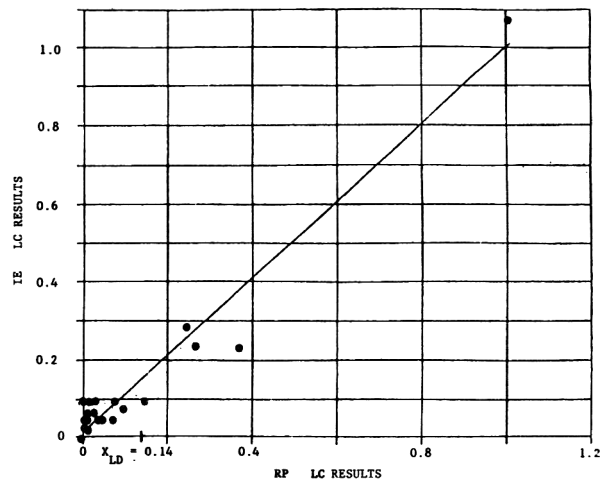

Figure 6. Amounts of DONS found as wt % in samples of FD&C Yellow No. 6 by RP LC and IE LC methods. X_{LD} is average limit of detection calculated from calibration data.

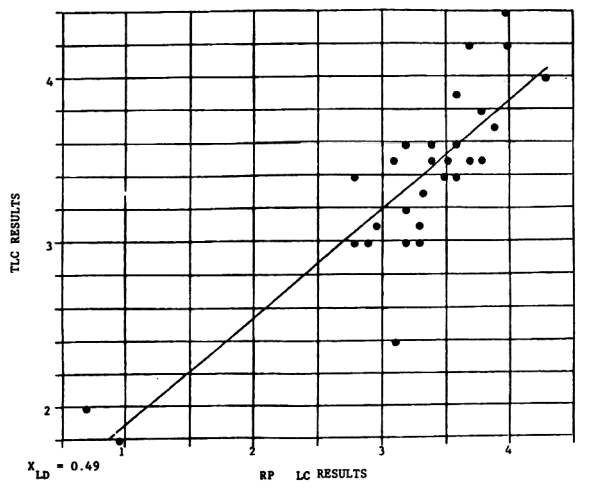

Figure 7. Amounts of RS dye found as wt % in samples of FD&C Yellow No. 6 by RP LC and TLC methods. X_{LD} is average limit of detection calculated from calibration data.

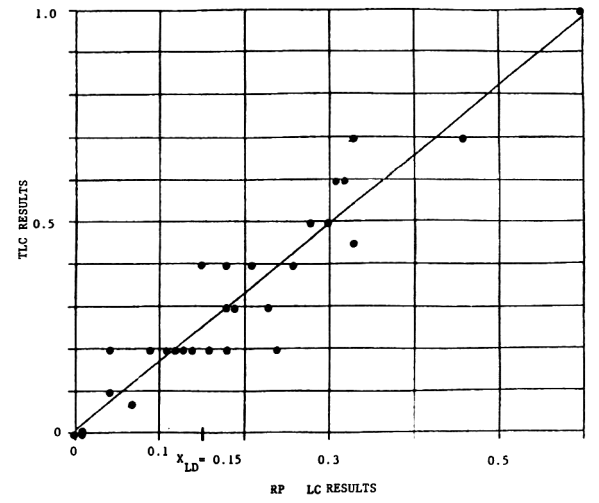

Figure 8. Amounts of lower sulfonated subsidiary color found as wt % in samples of FD&C Yellow No. 6 by RP LC and TLC methods. X_{LD} is average limit of detection calculated from calibration data.

Table 10. Amounts of contaminants found in multiple analyses of 8 different samples of FD&C Yellow No. 6, in units of % (w/w) of dye

	Sample No.							
	30	75	82	154	308	445	AA3003	AA8634
				SA				
	0.102	0.027	0.047	0.001	0.006	0.097	0.035	0.040
	0.101	0.053	0.054	0.012	0.009	0.083	0.027	0.037
	0.104	0.045	0.073	0.017	0.02	0.092	0.040	0.046
	0.138	0.025	0.072	0.014	0.017	0.107		
	0.110	0.020	0.056	0.003	0.023	0.101		
		0.019	0.061	0.011				
Av.	0.111	0.032	0.061	0.010	0.015	0.096	0.034	0.041
CV	14.0	44.1	16.9	63.1	48.3	9.5	19.3	11.2
				SS				
	0.027	0.141	0.014	0.073	0.108	0.011	0.027	0.033
	0.033	0.162	0.020	0.081	0.125	0.014	0.013	0.027
	0.031	0.153	ND[a]	0.075	0.113	0.015	0.004	0.019
	0.005	0.146	0.012	0.081	0.112	ND		
	0.005	0.145	0.011	0.061	0.111	ND		
		0.148	0.005	0.016[b]				
Av.	0.020	0.149	0.010	0.074	0.114	0.008	0.015	0.026
CV	70.2	5.0	70.0	11.1	5.7	93.1	77.3	27.0
				DAADBSA				
	0.288	0.042	0.052	0.053	0.016	ND	0.022	ND
	0.297	0.036	0.043	0.038	0.015	ND	0.022	ND
	0.238	0.036	0.044	0.047	0.017	ND	0.025	ND
	0.214	0.029	0.047	0.040	0.016	ND		
	0.178	0.034	0.039	0.039	0.022	ND		
		0.035	0.044	0.045				
Av.	0.243	0.035	0.045	0.044	0.017		0.023	
CV	20.6	11.9	9.68	13.1	16.2		7.5	
				DONS				
	0.355	0.025	0.023	1.10	0.05	0.048	0.274	0.039
	0.362	0.01	0.020	0.99	0.06	0.046	0.250	ND
	0.369	0.004	0.023	0.94	0.05	0.054	0.286	0.013
	0.374	0.007	0.025	1.03	ND	ND		
	0.403	ND	ND	1.06	0.02	0.015		
		ND	ND	0.96				
Av.	0.373	0.008	0.015	1.01	0.036	0.033	0.270	0.017
CV	4.9	117	79.0	6.1	69.7	71.8	6.8	116
				RS dye				
	3.76	3.68	3.46	3.95	2.88	1.00	3.19	2.97
	3.73	3.57	3.49	3.97	3.05	1.02	3.44	3.17
	3.62	3.63	3.58	3.76	2.95	0.88	3.35	3.22
	3.67	3.58	3.52	4.04	3.00	0.98		
	3.73	3.60	3.55	4.06	2.99	0.99		
		3.61	3.58	4.15				
Av.	3.70	3.61	3.53	3.99	2.97	0.97	3.33	3.12
CV	1.5	1.1	1.4	3.3	2.1	5.6	3.8	4.2
				SSA				
	0.45	0.064	0.13	0.050	0.24	0.34	0.23	0.12
	0.46	0.03	0.07	0.03	0.24	0.32	0.24	0.14
	0.44	0.041	0.07	0.04	0.23	0.33	0.26	0.15
	0.47	0.07	0.09	0.06	0.23	0.34		
	0.47	0.023	0.08	0.025	0.22	0.32		
		0.032	0.07	0.051				
Av.	0.46	0.043	0.09	0.043	0.23	0.33	0.24	0.14
CV	2.8	44.9	28.1	31.3	3.6	3.0	6.4	10.9

[a]ND = non detected.
[b]This result not included in calculations; explanation in text.

none detected (ND) were calculated as zero. For the SA results in the 2 samples at the 95% confidence level, the Student's *t*-test yields probabilities of 0.020 (AA3003) and 0.00075 (AA8634), indicating a significant difference between the average amounts of SA found by the 2 methods.

For the SS results in the 2 samples, the Student's *t*-test yields probabilities of 0.103 (AA3003) and 0.109 (AA8634), indicating no significant difference at the 95% confidence level between the averages found by the 2 methods.

Similarly for the DAADBSA results, the probabilities calculated were 0.064 (AA3003) and 0.117 (AA8634), indicating no significant difference between the averages found by the 2 methods.

For the DONS results, the probabilities calculated were 0.282 (AA3003) and 0.299 (AA8634), indicating no significant difference between the averages found by the 2 methods.

The plots shown in Figures 3–8 and the SA results for the pharmacology samples indicate that the amounts found by

RP LC frequently do not closely agree with the amounts found by IE LC and TLC. This variation could be best evaluated with a collaborative study in which the same analysts would analyze the same samples by all 3 methods. The collaborative study could also allow a comparison of the accuracy, repeatability, and reproducibility of the 3 methods.

Of the 31 certification samples, 6 were selected for multiple analyses by RP LC because they contained significant amounts of one or more of the analytes. For each sample, each of the analyses was performed from a different weighing of the sample, at a different time, and with a different set of calibration data. The concentrations of the analytes were estimated by using the regression line equation calculated from analyses of calibration solutions chromatographed with the samples. The results of these analyses along with averages and CVs are presented in Table 10. All results are reported, although some results are lower than the calculated X_{LD} for the appropriate calibration data set. Results were reported as ND when either no peak was detected or the number of integrator counts for the peak was so small that the concentration calculation resulted in a negative number. For results of ND, zero was used in calculating the averages and CVs.

The amounts of SA found in samples 75, 154, and 308 are clearly below the limit of detection of the method. The variation found, as expressed by the CV of the multiple determinations, is high. In sample 82, the amounts of SA found range from 0.047 to 0.073% with a CV of 17%, a high variation. The determination of SA in sample 445 gave results ranging from 0.083 to 0.107% with a CV of 9.5%. For sample 30, the amounts of SA found range from 0.101 to 0.138% with a CV of 14%. Sample 30 results will be discussed further in connection with the DAADBSA results. As mentioned earlier in the text, SA is the most difficult analyte. This method would need some modification if analytical capability below 0.1% was necessary.

For SS, the amounts found in samples 30, 82, and 445 are clearly below the limit of detection of the method and display high variation. The amounts found in samples 75 and 308 show reasonably good repeatability as measured by the CVs, 5.0 and 5.7%, respectively. In sample 154, the last result of 0.016% was excluded from calculations because the SS peak appeared to split into 2 peaks in the chromatogram. This problem may be due to column aging, although the last determinations on samples 75 and 308, 0.148 and 0.111%, respectively, were made at the same time with no apparent peak splitting. Additional work should be done on this problem.

Sample 30 was found by RP LC to contain an average of 0.243% DAADBSA, whereas 0.39% DAADBSA was found in the IE LC analysis—a clearly discrepant result. These values were not plotted in Figure 5. DAADBSA is known to be stable under basic conditions and to decompose under acidic conditions, producing SA as a decomposition product. Therefore, DAADBSA is probably more stable under the conditions in the IE LC method than it is under those in the RP LC method. Further evidence of DAADBSA stability can be seen when the determinations of SA in sample 30 are compared with the determinations of DAADBSA by RP LC. These determinations are complementary, i.e., when DAADBSA is high, SA is low, and vice versa. The amount of DAADBSA found in sample 30 is well above the proposed specification limit of 0.1%. At the proposed specification limit and below, there seems to be much less of a problem with repeatability, and RP LC and IE LC values are in better agreement. If the proposed specification limit for DAADBSA is set higher, the RP LC method would need modification.

The amounts of DONS found in samples 75, 82, 308, and 445 are clearly below the limit of detection of the method. The amounts found in samples 30 and 154 show reasonably good repeatability as indicated by the CVs of 4.9 and 6.1%, respectively.

For RS dye, all of the amounts found are above the limit of detection, and good repeatability is indicated by the low CVs.

The amounts of SSA found in samples 75, 82, and 154 are clearly below the limit of detection of the method and the CVs indicate high variation. For samples 30, 308, and 445, the CVs indicate reasonably good repeatability.

Summary

A reverse phase method has been presented for determining intermediates, subsidiary colors, and 2 reaction by-products in samples of the color additive FD&C Yellow No. 6.

This method was tested by using liquid chromatographs from 2 manufacturers and 4 different Zorbax C-8 columns. Collected data include 11 sets of calibration data, a recovery study, multiple analyses of 6 samples, and comparison with the IE LC and TLC methods it was designed to replace. The data indicate that the method is adequate for determinations at the levels tested. Use of this method to replace the 2 current methods would result in a significant saving of time. A collaborative study of this method is planned.

Acknowledgments

We are grateful to John Bailey, DCLT, for preparation and purification of the analytes used in the development and testing of this method and to Frances Clark, DCLT, for performing the TLC determinations.

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Reverse Phase Liquid Chromatographic Screening Procedure for Parts-Per-Million Levels of *p*-Toluidine in D&C Red No. 6 and D&C Red No. 7

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A reverse phase liquid chromatographic method is described for the determination of *p*-toluidine in D&C Red No. 6 and D&C Red No. 7. The procedure employs octylsilanized silica gel as the stationary phase and gradient elution with a mobile phase of aqueous acetonitrile solutions containing ammonium sulfate-sulfuric acid electrolytes (pH 2). The dye is first mixed with an aqueous extraction solution containing tetrabutyl ammonium sulfate and sulfuric acid and is then extracted with chloroform. The dye migrates to the organic layer, leaving the *p*-toluidine in the aqueous layer as the protonated amine. The limit of determination for the method is 1.9 ppm. Recoveries of *p*-toluidine averaged 91.8% from samples of D&C Red No. 6 and 94.6% from samples of D&C Red No. 7.

D&C Red No. 6 and D&C Red No. 7 are regulated color additives which are currently permitted for use in drugs and cosmetics (1). The primary commercial application of these dyes is the coloring of lipsticks, nail polish, and enamels, for which the "lake" of the color is usually employed.

D&C Red Nos. 6 and 7 are manufactured by coupling diazotized 2-amino-5-methylbenzenesulfonic acid, commonly known as *p*-toluidine-*m*-sulfonic acid (PTMS), with 3-hydroxy-2-naphthalenecarboxylic acid. D&C Red No. 6 is the disodium salt (Figure 1), and D&C Red No. 7 is the calcium salt (Figure 2).

According to manufacturers' specifications (2), technical grade PTMS may contain up to 0.75% *p*-toluidine. When *p*-toluidine occurs as a contaminant in PTMS, it may be diazotized and coupled with 3-hydroxy-2-naphthalenecarboxylic acid to yield the unsulfonated subsidiary color 3-hydroxy-4-[(4-methylphenyl)azo]-2-naphthalenecarboxylic acid. Indeed, the proposed regulations for D&C Red Nos. 6 and 7 limit the unsulfonated subsidiary color to no more than 0.5% by weight. Analytical records for recently certified D&C Red No. 6 show that the level ranges from 0.05 to 0.11% with an average of 0.07% (3).

If diazotization of PTMS and *p*-toluidine is incomplete, it is possible to find these materials in D&C Red Nos. 6 and 7. Limits for each are included in newly proposed specifications for these color additives.

No definitive analytical data have been presented to establish the levels of occurrence of *p*-toluidine in D&C Red Nos. 6 and 7. With the promulgation of the FDA constituents policy (4) for regulating carcinogenic substances in food additives, a method of analysis for *p*-toluidine and data quantitating the levels of occurrence are needed to set reasonable limits for this contaminant in these drug and cosmetic color additives.

The reverse phase liquid chromatographic (LC) method described here provides a relatively simple procedure for screening D&C Red Nos. 6 and 7 samples for *p*-toluidine with a limit of determination of less than 5 ppm. In addition, several batches of the color additive have been analyzed. It should be noted that the procedure was developed only for the straight color additive. D&C Red Nos. 6 and 7 lakes may require modification of the procedure because these color additives differ chemically from the straight dye.

Received April 1, 1983. Accepted August 24, 1983.

Experimental

Apparatus and Reagents

(a) *Liquid chromatograph.*—Altex Model 312MP equipped with 2 Model 110A pumps and Model 420 microprocessor-controller (Altex Scientific, Inc., Berkeley, CA 94710) and Rheodyne Model 7125 loop injector (Rheodyne, Inc., Cotati, CA 94928) fitted with 250 μL loop.

(b) *Detector.*—Altex Model 155-10 UV/VIS variable wavelength detector set at 210 nm, attenuation 0.02 AUFS, and time constant 0.3 s.

(c) *Liquid chromatographic column.*—Altex Ultrasphere octyl, 25 cm × 4.6 mm id., 5 μm particle size, Part No. 256-08 and Serial No. UEO-77.

(d) *Recording integrator.*—Shimadzu Model C-R1A (Shimadzu Scientific Instruments, Inc., Columbia, MD 21045). Signal for detector is attenuated 10:1 through a Cahn Recorder Control, Cat. No. 1490 (Cahn Instruments, Inc., Cerritos, CA 90701).

(e) *Acetonitrile.*—LC grade (Burdick & Jackson Laboratories, Inc., Muskegon, MI 49442).

(f) *Ammonium sulfate.*—Mallinckrodt analytical reagent grade (Scientific Products, Inc., Columbia, MD 21045).

(g) *Sulfuric acid.*—Fisher, ACS reagent grade (Fisher Scientific Co., Pittsburgh, PA 15219).

(h) *Chloroform.*—LC grade (Burdick & Jackson Laboratories, Inc.).

(i) *Tetrabutylammonium hydrogen sulfate (TBA-sulfate).*—Cat. No. 15,583-7 (Aldrich Chemical Co., Inc., Milwaukee, WI 53233).

(j) *Water.*—Distilled and treated with Milli-Q water purification system (Millipore Corp., Bedford, MA 01730).

(k) *LC eluants.*—*(1) Solvent A.*—0.3M ammonium sulfate–0.045M sulfuric acid–1.5% (v/v) acetonitrile in water (1 + 1 + 1). *(2) Solvent B.*—50% (v/v) acetonitrile in water.

(l) *p-Toluidine.*—Eastman Laboratory and Specialty Chemicals (Rochester, NY 14650). Dissolve ca 50 mg *p*-toluidine in 95% ethanol in 100 mL volumetric flask. Prepare stock solutions by placing 1 mL aliquots of ethanolic solution in 100 and 250 mL volumetric flasks and dilute to volume with distilled water. Prepare calibration solutions by taking aliquots from these solutions. Check solution strength daily by obtaining UV spectrum.

(m) *Extraction solution.*—Place 10.0 g TBA-sulfate and 7.8 g sulfuric acid in 2 L volumetric flask and dilute to volume with distilled water.

Instrument Parameters

(a) *LC gradient elution profile.*—0–50% solvent B in A (linear) in 15 min; 100% solvent B at 15 min and hold at 100% B for 15 min; 0% solvent B in A at 30 min and hold at 0% B in A for 10 min to equilibrate column before next injection. Run 2 blank gradients before analyzing first sample. Flow rate is 1 mL/min.

(b) *Integrator settings.*—Lock, 4 (integration inhibit, minutes from T = 0); stp tm, 16 (end of integration, min); attn, 0 (integrator attenuation, exponent of 2); speed, 5 (chart speed, mm/min). These conditions provide a chart speed of

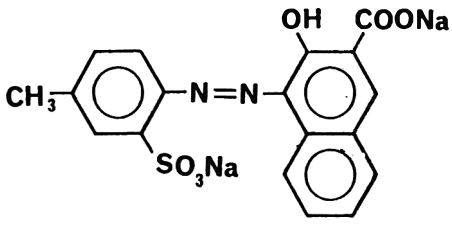

Figure 1. D&C Red No. 6.

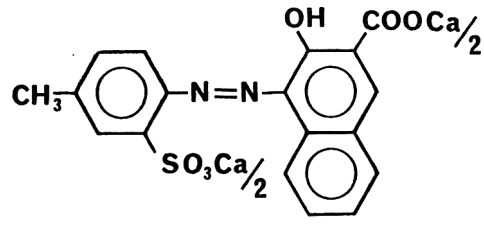

Figure 2. D&C Red No. 7.

0.5 cm/min and inhibit peak detection for the first 4 min of the analysis. The chromatograms are recorded at an attenuation of 0.02 AUFS.

Calibration

Prepare calibration curve for *p*-toluidine by placing aliquots of stock solution in separatory funnels and adding extraction solution so that total volume in each separatory funnel equals 100 mL.

Calculate C, ppm *p*-toluidine relative to the amount of D&C Red No. 6 or D&C Red No. 7 in sample:

$$C = V \times C' \times 4.0$$

where V = volume of aliquot of stock solution (mL) taken; C' = concentration of stock solution (μg/mL); and 4.0 = 1.0/0.250 (g). Measure *p*-toluidine response by first drawing a baseline; then measure from peak maximum to baseline, using a caliper. Measure peak height to the nearest 0.1 mm. Obtain 8 calibration points that span the desired range (e.g., ca 4.0–40.0 ppm *p*-toluidine). Also run a blank extraction to determine background levels, which are subtracted from response obtained. Calculate regression line equation for *p*-toluidine according to the method reported by Bailey et al. (5).

Sample Extraction and Determination

Weigh 0.250 g dye and transfer to 250 mL separatory funnel. Measure 100 mL extraction solution in graduated cylinder and add to separatory funnel. Shake vigorously 60 s. Add 50 mL chloroform from graduated cylinder, shake vigorously 60 s, and let layers separate. The lower chloroform layer should contain most of the dye and be free of suspended matter. The aqueous layer should be somewhat cloudy and light red. Drain lower layer, being careful not to lose any insoluble material that may be at the interface. Repeat extraction by shaking 15 s with 2 additional 50 mL portions of chloroform. The remaining aqueous layer should be colorless and somewhat cloudy. The aqueous layer becomes clear after standing ca 30 min. Inject 250 μL aqueous layer and chromatograph according to parameters described for LC gradient elution profile. Calculate ppm *p*-toluidine in sample from regression line equation.

Recovery Study

Recoveries were obtained for *p*-toluidine added to samples of both D&C Red No. 6 and D&C Red No. 7. For each analysis, 0.250 g dye was weighed and transferred to a 250 mL separatory funnel. The dry dye samples were spiked with the *p*-toluidine stock solutions, and extraction solution was added so that the total volume in each separatory funnel equaled 100 mL. The extraction and LC analysis were then performed as described above.

Calculations

All calculations used in calibration and in the statistical analysis of the data were made according to the method reported by Bailey et al. (5), and the responses observed in the recovery study and sample survey were quantitated by using the regression line equation.

Results

LC Separation

A typical LC chromatogram for a spiked commercial sample of D&C Red No. 6 is shown in Figure 3 with a blank baseline (water injected). The *p*-toluidine peak, which occurs just after the peak corresponding to PTMS, is a sharp, well resolved response. The use of a large injection volume does not seem to affect the peaks in the latter part of the chromatogram although the peaks in the earlier part are somewhat broadened and a large "vacancy" peak can be observed. The chromatographic profile was reproducible throughout the investigation.

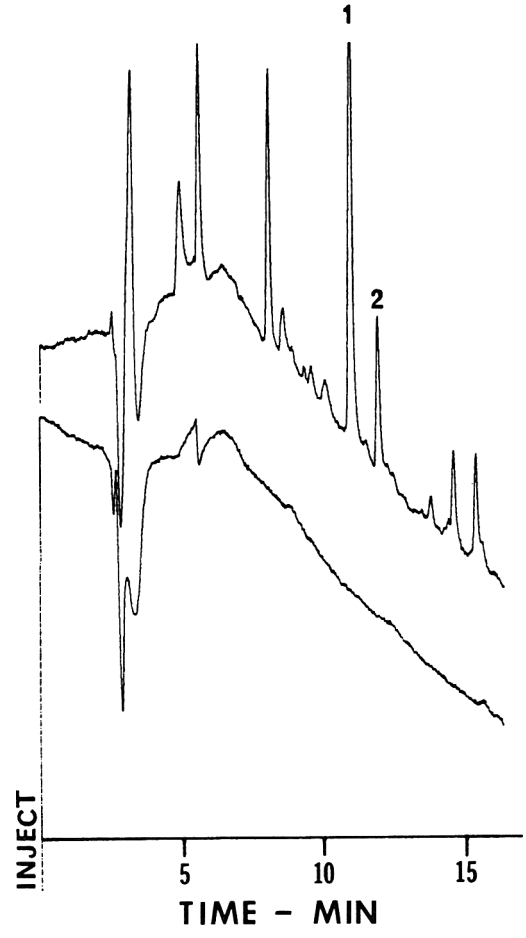

Figure 3. LC chromatogram of extraction solution from D&C Red Nos. 6 and 7. 1 = PTMS (10.77); 2 = *p*-toluidine (11.69). Numbers in parentheses are elution times (min).

Calibration Curve

Table 1 contains the calibration and regression analysis data. The correlation coefficient of 0.9998 indicates a linear relationship between the points. The lower limit of determination and prediction interval (calculated at 20 ppm) are acceptable for a reliable screening procedure for *p*-toluidine in D&C Red Nos. 6 and 7.

Recovery Study

The recovery data for *p*-toluidine added to D&C Red Nos. 6 and 7 are presented in Tables 2 and 3, respectively.

For D&C Red No. 6, recoveries of *p*-toluidine were measured at 6 levels ranging from 8.0 to 39.8 ppm with multiple analyses at each level. A total of 20 recovery solutions were analyzed. The average recovery was 91.8% and the coefficient of variation (CV) was 2.4%. At the 19.9 ppm level, recoveries averaged 93.5% with a CV of 2.3%, and at the 15.9 ppm level, recoveries averaged 92.7% with a CV of 2.8%.

For *p*-toluidine recoveries from D&C Red No. 7, dye sample was insufficient for multiple analyses at several levels. For this reason, multiple analyses were performed at only 1 level; however, a total of 8 *p*-toluidine levels ranging from 4.0 to 39.8 ppm were measured. The average recovery was 94.6% and the CV was 2.6%. At the 19.9 ppm level, recoveries averaged 95.0% with a CV of 3.1%.

Sample Survey

Commercial samples of D&C Red Nos. 6 and 7 were analyzed to determine the levels of *p*-toluidine, if present. The only available sample of a D&C Red No. 7 straight, pharmacology sample W4602, and several commercial samples of D&C Red No. 6 (AA6705, AA6706, and AA9657) were analyzed according to the procedure described above. In all cases, the responses obtained for *p*-toluidine were below the 1.9 ppm limit of determination for the method, although some of the samples did produce a response at the retention time for *p*-toluidine.

Table 2. Recovery of *p*-toluidine added to D&C Red No. 6

Added, ppm	Found, ppm	Rec., %
8.0	7.4	92.5
	7.2	90.0
15.9	14.4	90.6
	14.2	89.3
	14.9	93.7
	15.0	94.3
	15.2	95.6
19.9	18.2	91.4
	18.8	94.5
	18.1	91.0
	19.0	95.5
	18.9	95.0
23.8	21.3	89.5
	21.3	89.5
	21.5	90.3
31.8	28.6	89.9
	29.2	91.8
	28.7	90.2
39.8	36.5	91.7
	35.8	89.9
Mean		91.8
SD		2.18
CV, %		2.38

Table 3. Recovery of *p*-toluidine added to D&C Red No. 7

Added, ppm	Found, ppm	Rec., %
4.0	3.6	90.0
8.0	7.4	92.5
10.0	9.5	95.0
15.9	15.1	95.0
19.9	19.8	99.5
	18.8	94.5
	18.4	92.5
	19.1	96.0
	18.4	92.5
23.8	22.6	95.0
31.8	30.6	96.2
39.8	38.2	96.2
Mean		94.6
SD		2.45
CV, %		2.6

Discussion

Liquid chromatographic separations of organic amines, including *p*-toluidine, have been achieved on unbonded silica gel with organic (6) and aqueous (7) solvent systems as well as on silica gel bonded with hydroxylated (8), copper II (9), and hydrocarbonaceous (10, 11) ligands. With hydrocarbonaceous bonded phases, excessive retention and peak tailing are often observed and are attributed to interaction of the amine function with residual silanol groups of the stationary phase (7). The chromatographic behavior of *p*-toluidine on silica gel columns using a chloroform-cyclohexane eluant yields acceptable retention and peak shape (6). However, in the case of D&C Red Nos. 6 and 7, several factors combine to make silica gel an unsuitable support. These dyes are only sparingly soluble in water at pH 7 or greater and are insoluble in most organic solvents. This prohibits a direct analysis or extraction with an organic solvent, since the *p*-toluidine may be occluded in the undissolved dye. The dye is reasonably soluble in aqueous acid; however, the *p*-toluidine is protonated under these conditions and cannot be extracted into organic solvents. It is possible that the acidified solution of the dye could be chromatographed on silica gel, using reverse phase solvent systems (7), but it is likely that the response of the dye that is present would swamp the *p*-toluidine response. This is particularly true because *p*-toluidine absorbs only at low wavelengths in the UV region in acid solution (λ_{max} = 205 nm at pH 2.0), and the dye would be expected to absorb strongly at this wavelength.

Because of the factors discussed above, the possibility of using phase transfer reagents (PTRs) was investigated. The dye is initially mixed well with a solution containing the PTR at acid pH (<2). Under these conditions, the dye is at least partially solubilized. Extraction with chloroform permits removal of the dye as an ion-pair with the PTR, leaving behind

Table 1. *p*-Toluidine calibration data and statistical analysis

Added ppm	Peak ht, mm
31.8	57.4
23.8	44.2
15.9	29.5
39.8	72.7
19.9	37.1
8.0	14.8
10.0	18.5
4.0	7.9
Slope, mm/ppm	1.8082
Intercept, mm	0.6349
Corr. coeff.	0.9998
Y_{UB}[a]	2.3513
X_{LD}[b]	1.9
Prediction interval, ppm	20.0 ± 0.98

[a] Y_{UB} is the 99% upper prediction limit on the blank (ht).
[b] X_{LD} is the limit of detection with 99% confidence (in ppm).

the *p*-toluidine as the *p*-toluidinium ion in the aqueous layer. The PTR acts both as a pairing agent to permit extraction of the dye into the organic solvent and as a wetting agent to aid in the solubilization of the dye.

The effectiveness of the extraction is demonstrated by the fact that the first chloroform extraction is highly colored. Some residual insoluble matter is observed in the aqueous layer, particularly with D&C Red No. 7, but this is eliminated in the subsequent extractions with chloroform. Performing the extraction with aqueous acid without added PTR resulted in the migration of only a small amount of the dye into the chloroform layer; it is likely that this material is the unsulfonated subsidiary color mentioned earlier.

The tendency of the *p*-toluidine to remain in the aqueous layer as the *p*-toluidinium ion during the extraction procedure was determined by comparing the LC response of the aqueous layer to that of a *p*-toluidine stock solution prepared so as to contain the identical *p*-toluidine concentration. LC analysis of the 2 solutions produced nearly identical responses for *p*-toluidine, suggesting that once the *p*-toluidinium ion is formed, it quantitatively remains in the aqueous layer. However, when the *p*-toluidine was first added to the chloroform and then vigorously extracted with the PTR solution, none of the *p*-toluidine was observed in the aqueous layer after extraction with chloroform, suggesting that there is little tendency of the acid to enter the chloroform to form the water-soluble form of the *p*-toluidine. Thus, in order for the *p*-toluidine in D&C Red Nos. 6 and 7 to be observed, the dye must be well mixed with the PTR solution, and the solubilization of the dye must occur in the aqueous portion of the system. If solubilization occurs in the chloroform, any *p*-toluidine that is present will not be observed. In the case of D&C Red No. 6, the bulk of the dye, which is solubilized upon mixing with the PTR solution, is readily extracted into the chloroform layer. However, with D&C Red No. 7, which is the less soluble calcium salt, a larger portion of the dye remains undissolved when it is initially mixed with the PTR solution. However, the first chloroform extract yields a clear solution of the dye with residual undissolved solids remaining in the aqueous layer. Subsequent treatments with chloroform remove the remaining dye as clear solutions in the organic layer, suggesting that solubilization is occurring in the aqueous layer. With both dyes, the aqueous layer is colorless after 3 chloroform extractions. A residual cloudiness of the aqueous layer is attributed to emulsification caused by the PTR since cloudiness was also observed in the blanks and calibration analyses. The cloudiness dissipates after 30 min. The injection of a cloudy solution for LC analysis had no adverse effects on the chromatographic results.

Once the *p*-toluidine was isolated in the aqueous layer, several chromatographic alternatives were available for its quantitation. The selection of an acid (pH 2) solvent system with an octyl-bonded phase does not mean that other systems might not perform equally well or better. As mentioned earlier, analysis could be performed on silica gel, using either organic (6) or aqueous (7) solvent systems. However, if an organic solvent system was employed, then the aqueous extract would have to be made alkaline and the *p*-toluidine would have to be extracted into an organic solvent and dried before analysis. This would add extra steps but would permit determination at a more desirable wavelength (230 nm). The same would be true of aqueous eluants with silica gel (7) or C_8- and C_{18}-bonded phases.

The use of an acid solvent system implies that the species being chromatographed is the *p*-toluidinium ion. The protonated, ionic form of *p*-toluidine is less lipophilic and, therefore, less retained on reverse phase ligands than the neutral form. The protonated form is also less influenced by residual silanol groups on the stationary phase. However, the use of an acid system requires measurement at 210 nm, a more difficult wavelength at which to work. The use of ammonium sulfate-sulfuric acid electrolytes did not pose any significant problems with regard to measurement and baseline stability, but it did require that 2 blank injections be made to stabilize the system before beginning the analysis of dye samples.

Perhaps the primary advantages of the system presented here are the efficiency of the procedure in terms of labor and equipment and the fact that it can also be used for screening for *p*-toluidine in other matrices. The use of an acid solvent system shows promise for the direct quantitation of *p*-toluidine at a specification level of 15 ppm in aqueous solutions of D&C Green No. 5 (work in progress). Also, the extraction procedure using PTR solution and chloroform may be applicable to the lakes of D&C Red Nos. 6 and 7. Initial results using the PTR technique suggest that the lakes are readily solubilized and the dyes are extracted into chloroform.

Several comments relating to the calibration and recovery data generated in this report are appropriate. It may be better to calibrate the procedure by spiking a dye sample rather than by performing the calibration extractions in the absence of the dye; this would improve the recovery of *p*-toluidine from commercial samples by taking into account the matrix effect. Also, in the work reported here, the data were collected over several weeks with the calibration data collected first, followed by recovery data for D&C Red No. 7 and then by recovery data for D&C Red No. 6. Examination of the data shows poorer recovery for D&C Red No. 6, suggesting a matrix effect. However, at the onset of the investigation, a new UV lamp was installed in the detector, and it is possible that the differences in recovery may be due to equilibration of the lamp. Of course, working at 210 nm may cause an inherent drift that is observed in results obtained over a period of time. It is suggested that new calibration points be added at the time of each set of analyses to take into account any changes in the system. In the case of a sample that may contain *p*-toluidine at rejectable levels, it would be appropriate to recalibrate the system with the calibration points clustered around the level of interest.

The sample survey failed to find *p*-toluidine at levels greater than the limit of determination although several of the samples did produce responses at the retention time of *p*-toluidine. It is not surprising that the contamination of D&C Red Nos. 6 and 7 with free *p*-toluidine is low. *p*-Toluidine is a relatively low level contaminant of the intermediate PTMS, which is present during the diazotization step. Thus most of the *p*-toluidine couples to produce an unsulfonated azo dye contaminant of the color additive. In addition, since it is a frequent practice in the commercial manufacture of color additives to include the treatment of the crude dye with a carbon adsorbent to remove volatile contaminants, such a step would greatly lower the levels of *p*-toluidine in the colorants.

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VITAMINS AND OTHER NUTRIENTS

Net Protein Ratio Data: AACC–ASTM Collaborative Study

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Seven- and 14-day net protein ratio (NPR) data were obtained from 7 laboratories for 6 protein sources: ANRC casein, lean beef, lactalbumin, textured vegetable protein, and peanut flour were fed as 10% protein (N × 6.25) in the test diet. Wheat flour, casein, and textured vegetable protein were fed as 6% protein (N × 6.25) in the test diet. Weighed dry ingredients for each diet were sent to each collaborator, who mixed the dry ingredients, then added specified amounts of corn oil and water and mixed each complete diet thoroughly. Rats were adapted for 0, 2, or 4 days, and then were fed the test diets for 28 days for protein efficiency ratio (PER) diets. The animal weight gain and feed consumption data obtained after 7 or 14 days of feeding were used to calculate NPR values. Analyses of data were done before [net protein ratio (NPR)] and after (R-NPR [relative-NPR]) adjustment of the data from each laboratory by its results for the reference protein casein. From the analysis of variance for NPR, significant ($P < 0.05$) interactions were observed among laboratories, protein sources, and adaptation times of the animals (0, 2, or 4 days). Inter- and intralaboratory variability were decreased by use of 14-day values compared with 7-day values. Adjustment of the NPR data to R-NPR did not lower the intralaboratory variability but did lower the interlaboratory variability of the data. Increasing adaptation time did not consistently decrease interlaboratory or intralaboratory variability or decrease coefficients of variation (CV) of R-NPR values. The 14-day NPR inter- and intralaboratory variations for the 10% protein diet over all factors (5 protein diets, 3 adaptation periods, and 7 laboratories), as measured by CV values, were 13.2 and 7.7%, respectively. The corresponding R-NPR values were 9.2 and 8.0%, respectively.

Determination of net protein ratio (NPR), a 10-day rat bioassay proposed by Bender and Doell in 1957 (1) for estimation of protein quality, has potential as a standard method. Therefore, NPR with a time modification also was determined during the AACC–ASTM collaborative study on protein efficiency ratio (PER) (2). Hackler et al. (3) have reported the results of the collaborative study on PER. The NPR bioassay is similar to a shortened PER test, except in the NPR bioassay, 1 group of rats is fed a nonprotein diet. The weight loss of the animals fed the nonprotein diet is assumed to be equivalent to the requirements for maintaining rats of the age tested (1, 4–8). This adjustment addresses one of the most serious criticisms of the AOAC PER method (9) for measuring protein quality, which is that the PER assay evaluates proteins for growth (weight gain) only and does not allow for maintenance (10–13).

McLaughlan and co-workers (4, 5, 14, 15) found that the NPR method of Bender and Doell (1) gave essentially the same values as the slope ratio (SR) multiple dose assay of Hegsted and Chang (11, 16, 17) when expressed as a percentage of the values of casein, except for lysine-deficient proteins. The SR assay method produces a relative nutritive value and is considered superior to the PER method (4, 11, 12, 14–16). It was found that the SR procedure may either overestimate [relative nutritive value (RNV)] (18) or underestimate [relative protein value (RPV)] (19) the nutritional quality of lysine-deficient proteins. The NPR procedure yielded higher values than did the SR assay for lysine-deficient proteins (4, 12, 14, 17); the PER assay yielded low values. There is a controversy over which method gives the most appropriate value for lysine-deficient proteins (17, 20).

NPR values have a high correlation with net protein utilization (NPU) values obtained by the method of Bender and Miller (21) for a variety of proteins (22–24). Theoretically, the NPU procedure is probably the most satisfactory of the rat assays because the nitrogen retained in the body is measured directly by nitrogen analyses of the carcass at the termination of the test (22). Henry (23) concluded, however, that for a rapid routine assessment of protein quality, the simpler determination, NPR, is an adequate replacement for NPU.

Lachance et al. (25, 26) reported on studies at Institute of Nutrition of Central America and Panama (INCAP) that indicated a high correlation between NPR and slope ratio data and the PER data obtained by Mertz et al. (27) from 18 samples of grain cereal. He also reported results from similar studies at INCAP, using PER, NPU, and NPR data from 21 legume samples (28). The same approach, comparison of data from PER, NPR, and SR methods, was used for 7 high protein foods under development at INCAP. The correlation coefficients were highly significant, indicating a close relationship between these methods (25, 26).

Two previous collaborative studies on NPR have been reported (10, 17, 29). One, conducted by Samonds and Hegsted (17, 29), compared data for 7 protein sources (lactalbumin, casein, defatted meat, soy flour, a soy protein isolate, wheat gluten, and white flour) from 7 laboratories. The second study, reported by McLaughlan et al. (10), compared data for 6 protein diets (casein plus L-methionine as the reference protein, lactalbumin, egg white, wheat gluten, soybean protein isolate, and wheat gluten and soybean protein isolate) from 6 laboratories.

One of the overall objectives of a third study, the AACC–ASTM collaborative study (2, 3), which is reported here, was to compare NPR values and the precision of the NPR method

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with similar data from the PER method on the same protein sources. The primary objective of the study as reported in the PER section (2, 3) was to better define the specifications of the procedure for determining PER, with the ultimate goal of making some useful changes in the assay. The determination of interlaboratory variation of the data was also of major interest.

Experimental

Seven laboratories collaborated by conducting rat bioassays. The test diets contained either 10 or 6% protein (N × 6.25) (Table 1). The sources and composition of the diets, information on the selection and distribution of the rats, and the environmental conditions of the study were described in an earlier preliminary publication (2) and by Hackler et al. (3).

There were 2 differences between the protocols for determining NPR (Table 1) and PER (3) in this collaborative study: (a) The feeding period for NPR was 7 and 14 days [instead of 7, 14, 21, and 28 days as for PER; 10 days as described by Bender and Doell (1); or 14 days only as used by McLaughlan et al. (10)]. (b) A nonprotein diet was fed to one group of rats for 14 days. NPR calculations were made using the 7- and 14-day weight gain and protein consumption data from each animal fed the test diets and the average weight loss of the 10 animals fed the nonprotein diet, using the following equation (30):

$$\text{NPR} = \frac{\text{wt gain of test animal} + \text{av. wt loss of nonprotein group}}{\text{wt protein consumed by test animal}}$$

The above equation was used to calculate the NPR for each rat individually within a trial [instead of for 10 rats collectively (1)], so the intralaboratory variation could be calculated. Thus, it was necessary to use the average weight of 10 animals from the nonprotein diet because an individual animal on the nonprotein diet could not be linked to a specific animal on a test diet.

ANRC casein, the reference standard for the determination of PER by the official method (AOAC) in the United States and Canada, was chosen as the reference protein for calculating relative NPR (R-NPR) and relative PER (R-PER). Casein is readily available worldwide and is standardized for use as the reference protein for PER determinations. The following equations were used to calculate the R-NPR and the R-PER:

$$\text{R-NPR} = \frac{\text{NPR of protein source}}{\text{NPR of ANRC casein (for same week and adaptation period within 1 lab.)}} \times 100$$

Table 1. Protocol for net protein ratio (NPR) study

Variable	10% Protein diets[a]	6% Protein diets[a]
Adaptation period	0, 2, 4 days	2 days
Adaptation diet	10% casein	10% casein
Animals	10 rats/trial	10 rats/trial
Protein sources	ANRC casein	ANRC casein
	freeze-dried lean beef	
	lactalbumin	
	text. veg. protein peanut flour	text. veg. protein wheat flour
	Nonprotein[b]	Nonprotein[b]
Test period	14 days	14 days

[a]Referred to as Test I (10%) and Test II (6%) by Hackler et al. (3).
[b]Nonprotein diet fed for 14 days after 0-, 2-, or 4-day adaptation period on adaptation diet.

$$\text{R-PER} = \frac{\text{PER of protein source}}{\text{PER of ANRC casein (for same week and adaptation period within 1 lab.)}} \times 100$$

Statistical analyses including analysis of variance were carried out, and the intralaboratory variability (repeatability) and interlaboratory variability (reproducibility) were determined by the methods of Youden and Steiner (31) for protein diet (protein sources), adaptation time, for each week individually, and for 7 laboratories. Examination of the data for outlying results yielded no evidence of a consistent bias for any of the 7 laboratories. The intralaboratory variability is a measure of the variability between individual rats, and not between 10-rat NPR replications. Inter- and intralaboratory variability was also determined for each week and adaptation time over all protein diets and for each week over all variables for 7 laboratories.

Results and Discussion

Nonprotein Diet

The data from feeding the nonprotein diet (Figure 1) indicated an increasing mean weight loss of 10 animals over the 7 laboratories with increasing length of the adaptation period of either 0, 2, or 4 days. Mean weight loss standard deviations were similar except for the 2-day-adaptation 14-day-weight-loss data. This is due to a reported mean weight loss of only 45 g per 10 animals from Laboratory 2. The next lowest reported value for this same adaptation–time test period was 106 g. Removing the value of 45 g as an outlier increased the mean for 6 laboratories to 134 g. The SD (standard deviation) value, 17.0 g, and the CV value, 13% (cross-hatched areas on Figure 1), were similar to the data from the other time periods. The CV values for the adaptation periods 0 and 4 days of the second week were generally lower than those of the first week. This was also true of the 2-day adaptation when the 45 g outlier was removed.

Individual Laboratory Data

The NPR mean values for the 10% protein diets for 7 and 14 days (means for 10 rats), intralaboratory SD of the mean, and CV values are given in Table 2 for each laboratory, protein diet, and adaptation period. In general, the 14-day NPR means were lower than those for 7 days. Exceptions (higher 14-day NPR means) can be found in the data reported from Laboratory 2 in the 0-day adaptation group and for Laboratory 6 in the 4-day adaptation group. The CV value is lower for the 14-day mean data for 84% of the values (88 are lower out of 105 comparisons). The 14-day CV values are

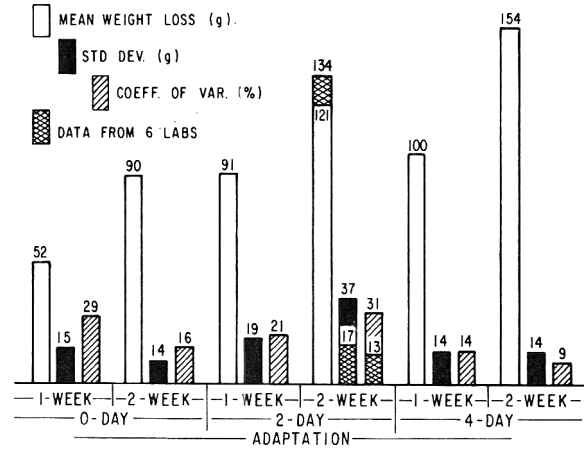

Figure 1. Data from feeding nonprotein diet. Mean weight loss (g) for groups of 10 rats over 7 laboratories.

Table 2. Net protein ratio (NPR) data for 10% protein diets from 7 laboratories[a]

Adapt. time, days	Lab. No.	Casein						Beef					
		7 days			14 days			7 days			14 days		
		Mean	SD[b]	CV,[c] %	Mean	SD	CV, %	Mean	SD	CV, %	Mean	SD	CV, %
0	1	5.83	0.23	4.0	4.82	0.18	3.7	6.09	0.16	2.7	5.03	0.10	2.1
	2	3.02	2.17	72.0	3.56	0.50	14.0	3.14	2.32	73.8	3.96	0.29	7.3
	3	5.68	0.85	14.9	3.89	0.43	10.9	6.51	0.73	11.2	4.62	0.28	6.1
	4	4.76	0.62	13.0	3.60	0.40	11.1	5.05	1.13	22.3	4.16	0.33	7.9
	5	4.99	0.67	13.5	3.97	0.26	6.5	5.20	0.37	7.0	4.42	0.39	8.8
	6	4.87	0.46	9.4	4.03	0.60	14.9	4.18	2.01	48.2	4.08	0.39	9.6
	7	5.53	0.37	6.7	4.52	0.19	4.1	5.63	0.23	4.1	4.71	0.17	3.6
	Mean	4.9		19.1	4.1		9.3	5.1		24.2	4.4		6.5
2	1	5.75	0.18	3.1	4.47	0.18	4.0	6.19	0.27	4.4	4.88	0.51	10.4
	2	3.90	0.50	12.9	3.22	0.20	6.3	4.35	0.43	10.0	3.67	0.15	4.2
	3	6.48	0.34	5.2	3.84	0.45	11.7	6.94	1.08	15.6	4.72	0.34	7.2
	4	4.50	0.46	10.2	3.81	0.29	7.5	4.90	0.30	6.1	4.02	0.33	8.2
	5	4.72	0.47	10.1	4.02	0.22	5.4	4.53	0.69	15.2	4.18	0.18	4.3
	6	4.06	1.05	25.9	3.79	0.49	12.9	4.37	0.52	11.8	4.07	0.22	5.3
	7	5.67	0.43	7.7	4.55	0.16	3.5	5.69	0.23	4.1	4.48	0.20	4.4
	Mean	5.0		10.7	4.0		7.3	5.3		9.6	4.3		6.3
4	1	5.71	0.31	5.4	4.54	0.18	4.0	6.01	0.20	3.3	4.76	0.16	3.4
	2	4.11	0.66	16.2	3.64	0.23	6.4	4.40	0.50	11.4	4.02	0.15	3.7
	3	5.05	0.70	13.8	3.63	0.30	8.4	5.74	0.43	7.5	4.29	0.15	3.6
	4	4.38	0.49	11.2	3.67	0.13	3.5	5.18	0.36	7.0	4.17	0.22	5.4
	5	4.92	0.27	5.4	3.74	0.23	6.0	4.92	0.45	9.2	3.80	0.35	9.1
	6	4.18	0.70	16.8	3.63	0.34	9.3	3.83	0.77	20.2	4.01	0.40	10.0
	7	5.41	0.23	4.2	4.32	0.09	2.0	5.47	0.24	4.3	4.29	0.14	3.3
	Mean	4.8		10.4	3.9		5.7	5.1		9.0	4.2		5.5
	Overall Mean	4.9		13.4	4.0		7.4	5.2		14.3	4.3		6.1

Table 2. Continued.

Lactalbumin						Peanut flour						Textured vegetable protein					
7 days			14 days			7 days			14 days			7 days			14 days		
Mean	SD	CV, %	Mean	SD	CV, %	Mean	SD	CV, %	Mean	SD	CV, %	Mean	SD	CV, %	Mean	SD	CV, %
6.01	0.32	5.3	5.27	0.21	4.1	3.38	0.67	19.8	3.00	0.24	8.1	4.84	0.29	6.0	4.23	0.23	5.3
3.56	0.59	16.6	3.98	0.32	8.0	2.38	0.49	20.6	2.33	0.16	6.8	2.85	1.82	63.7	3.25	0.40	12.2
6.19	1.04	16.8	4.68	0.35	7.5	2.67	0.55	20.7	2.27	0.24	10.4	4.88	0.51	10.4	3.75	0.18	4.9
4.03	0.39	9.8	4.04	0.34	8.4	2.96	0.77	26.2	2.21	0.22	9.7	3.31	0.31	9.5	3.11	0.19	6.1
5.05	0.45	8.9	4.49	0.34	7.5	3.09	0.36	11.5	2.53	0.14	5.4	3.95	0.28	7.2	3.50	0.23	6.7
4.19	2.34	55.9	4.39	0.52	11.8	1.96	1.32	67.4	2.24	0.26	11.4	3.58	0.53	14.8	3.46	0.22	6.2
5.56	0.29	5.3	4.95	0.11	2.1	3.06	0.39	12.8	2.95	0.13	4.3	4.35	0.38	8.6	3.93	0.18	4.6
4.9		16.9	4.5		7.1	2.8		25.6	2.5		8.0	4.0		17.2	3.6		6.6
5.96	0.25	4.1	4.98	0.15	3.0	3.62	0.37	10.1	2.83	0.15	5.3	5.00	0.23	4.5	4.00	0.19	4.9
3.72	0.36	9.8	3.49	0.24	7.0	2.34	0.52	22.4	1.77	0.27	15.1	3.58	0.54	15.1	2.98	0.30	9.9
7.12	0.96	13.5	4.71	0.17	3.6	3.25	0.67	20.7	2.49	0.23	9.2	4.99	0.70	14.1	3.79	0.28	7.3
4.43	0.29	6.5	4.28	0.14	3.3	2.40	0.39	16.2	2.03	0.18	9.0	3.24	0.33	10.1	3.06	0.23	7.4
4.44	0.75	16.9	4.40	0.24	5.4	2.92	0.31	10.8	2.47	0.17	7.1	3.81	0.44	11.5	3.47	0.24	6.9
3.56	1.08	30.4	4.01	0.46	11.5	1.92	0.36	18.8	1.99	0.31	15.4	2.90	1.00	34.6	2.92	0.49	16.8
5.75	0.28	4.8	4.90	0.19	3.8	3.29	0.25	7.7	2.87	0.14	4.9	4.59	0.24	5.2	3.74	0.25	6.6
5.0		12.3	4.4		5.4	2.8		15.2	2.3		9.4	4.0		13.6	3.4		8.5
5.93	0.22	3.7	5.06	0.13	2.6	3.54	0.34	9.7	2.89	0.27	9.5	4.81	0.25	5.2	3.96	0.14	3.5
4.40	0.46	10.5	4.17	0.34	8.2	2.59	0.44	16.8	2.41	0.22	9.2	3.80	0.60	15.8	3.37	0.16	4.8
5.88	0.54	9.3	4.41	0.23	5.2	2.82	0.48	16.9	1.98	0.32	16.0	4.62	0.70	15.1	3.25	0.51	15.8
4.84	0.31	6.4	4.12	0.22	5.4	2.47	0.47	19.0	2.24	0.20	8.7	3.57	0.39	11.0	3.06	0.19	6.3
4.87	0.57	11.6	4.01	0.47	11.8	2.89	0.25	8.7	2.19	0.17	8.0	4.10	0.49	11.9	3.12	0.25	8.0
3.69	1.11	30.2	3.86	0.46	12.0	1.66	0.89	53.4	2.35	0.28	11.8	2.51	1.01	40.0	2.89	0.55	19.1
5.46	0.46	8.4	4.61	0.19	4.1	3.27	0.22	6.6	2.72	0.18	6.7	4.35	0.26	6.0	3.49	0.27	7.8
5.0		11.4	4.3		7.0	2.7		18.7	2.4		10.0	4.0		15.0	3.3		9.3
5.0		13.6	4.4		6.5	2.8		19.8	2.4		9.1	4.0		15.2	3.4		8.1

[a]Each NPR mean is the average value for 10 rats.

[b]SD = standard deviation. The SEM (standard error of the mean) can be calculated by the following equation:

$$SEM = \frac{SD}{\sqrt{10}} = \frac{SD}{3.1623}$$

[c]CV = coefficient of variation = $\frac{SD}{mean} \times 100$

frequently lower by a factor of 2 or more for Laboratories 2 and 6, particularly for the 0-day and 4-day adaptations, respectively. Six CV values were about the same for both the 7- and 14-day data. Generally, the data indicated that extending the NPR test to 14 days decreased the SD and CV values, showing an increased precision. The level of variability (precision) is rather consistent in several laboratories for the 10% diets for all protein sources.

Six laboratories (Laboratories 1–4, 6, and 7) exhibited considerably higher variability for the 6% casein diet (Table 3) compared with the 10% casein diet (Table 2, 2-day adaptation, 14-day CV data). However, only 4 laboratories (Laboratories 1–4) showed greater variability for the 6% diet data of textured vegetable protein (Table 2 [2-day adaptation, 14-day CV data] and Table 3).

Analysis of Variance

An analysis of variance (ANOVA) was performed on both the 7- and 14-day NPR and R-NPR data over 5 protein diets, 3 adaptation periods, and 7 laboratories for the 10% diets and over 3 protein diets, one adaptation period (2-day), and 7 laboratories for the 6% diets (Table 4). When the 10% diets were fed, there were significant effects due to laboratories and diets and a significant interaction for laboratory-by-adaptation and laboratory-by-diet for the first week feeding. There was a large increase in the effect of diet in the second week which tended to overshadow (lower) the significance of the laboratory effect and the relatively small effect of adaptation time. Laboratory-by-diet was the most significant interaction. The R-NPR data for 10% protein diets also showed the highly significant effect of diet. The other individual effects and the laboratory-by-diet interaction were lowered by adjustment to the R-NPR.

The analysis of variance for the 6% protein diets showed significant effects due to laboratories and diets for the 7- and 14-day NPR values and a significant laboratory-by-diet interaction. Analysis of the R-NPR data indicated a highly significant diet effect and a significant laboratory-by-diet interaction. The significance of the laboratory-by-diet and laboratory-by-adaptation interaction terms means that the effects of diet and adaptation changed from laboratory to laboratory. This is quite evident from Table 2.

Two-Week Statistical Summary for Each Protein Source and Adaptation Period

Fourteen-day NPR intralaboratory variability data (mean, SD and CV) for each protein source and adaptation period for all laboratories are given in Table 5. Calculating R-NPR did not improve the intralaboratory variability of the NPR for protein source and/or individual adaptation periods as measured by the CV value (Table 5).

Interlaboratory variation or interlaboratory error (reproducibility) is defined by Youden and Steiner: "Reproducibility is determined by the sum of between-laboratory error, interaction, and within-laboratory error" (31). The interlaboratory variability data for each protein diet and adaptation period determined from analysis of data for all laboratories are also shown in Table 5.

The mean values are the same for calculating either intra- or interlaboratory variability. The lowest variability (interlaboratory CV of NPR) was found for the 4-day adaptation groups for casein, beef, and lactalbumin and in the 0-day adaptation groups for peanut flour and textured vegetable protein. Calculating the R-NPR value produced lower interlaboratory CV values for nearly all protein sources, adaptation periods, and the 2 levels of protein. The 2-day adaptation period probably would be the most practical for a minimum time–cost test. The analysis of variance indicated that variability due to laboratory or diet effects had higher significance than those of the adaptation period. The interlaboratory variation (CV) in this study for lactalbumin NPR was higher for 0- and 2-day adaptation data; about same for 4-day adaptation NPR data, but lower for all R-NPR data than that reported in a previous study (10). Lactalbumin was the only protein source common to both studies.

Table 3. Net protein ratio data for 6% protein diets from 7 laboratories[a]

	7 days			14 days		
Lab.	Mean	SD	CV, %	Mean	SD	CV, %
			Casein Diet			
1	5.90	0.39	6.6	4.80	0.66	13.8
2	4.32	0.33	7.6	3.20	0.35	10.9
3	5.79	1.32	22.8	4.49	0.65	14.5
4	3.90	0.48	12.3	3.94	0.45	11.4
5	4.94	0.44	8.9	4.08	0.23	5.6
6	3.04	0.99	32.6	2.93	0.80	27.3
7	5.98	0.50	8.4	4.80	0.34	7.1
Mean	4.8		14.2	4.0		12.9
			Wheat Flour Diet			
1	3.38	0.59	17.4	2.71	0.46	17.0
2	2.37	0.48	20.2	0.69	0.30	43.5
3	3.75	1.19	31.7	2.31	0.39	16.9
4	2.57	0.67	26.1	2.00	0.31	15.5
5	2.73	0.41	15.0	2.27	0.30	13.2
6	2.40	0.74	30.8	2.20	0.47	21.4
7	3.73	0.54	14.5	2.55	0.21	8.2
Mean	3.0		22.2	2.1		19.4
			Textured Vegetable Protein Diet			
1	4.77	0.56	11.7	4.07	0.33	8.1
2	3.84	0.38	9.9	2.69	0.40	14.9
3	3.78	0.98	25.9	3.53	0.41	11.6
4	3.61	0.81	22.4	3.62	0.30	8.3
5	4.47	0.44	9.8	3.95	0.21	5.3
6	3.14	0.57	18.2	3.26	0.39	12.0
7	4.82	0.41	8.5	4.09	0.26	6.4
Mean	4.1		15.2	3.6		9.5

[a]Each NPR mean is the average value for 10 rats.

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359	360	361	362	363	364	365	366	367	368	369	370	371	372	373	374
375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390
391	392	393	394	395	396	397	398	399	400						

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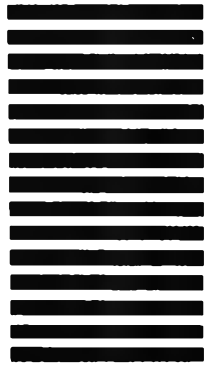

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Table 4. Analysis of variance on NPR and R-NPR data

Source[a]	Error term[b]	NPR 7 days Sum of sq.	df	F^c	NPR 14 days Sum of sq.	df	F^c	R-NPR 7 days Sum of sq.	df	F^c	R-NPR 14 days Sum of sq.	df	F^c
10% Protein Diet													
A	MS_{AL}	1.91	2	<1	7.98	2	3.1	477.90	2	<1	470.96	2	<1
L	MS_e	543.28	6	175.4**	119.60	6	225.2**	29564.01	6	16.5**	9707.21	6	31.0**
D	MS_{LD}	833.40	4	74.4**	558.65	4	281.2**	303717.77	3	163.6**	349878.60	3	303.8**
A × L	MS_e	54.92	12	8.9**	15.67	12	14.8**	7646.72	12	2.1*	3170.50	12	5.1**
A × D	MS_{ALD}	1.54	8	<1	0.96	8	1.2	911.11	6	<1	456.93	6	1.0
L × D	MS_e	67.24	24	5.4**	11.92	24	5.6**	11137.92	18	2.1**	6909.76	18	7.4**
A × L × D	MS_e	24.83	48	1.0	4.64	48	1.1	8600.89	36	<1	2646.40	36	1.4*
Error		487.80	945		83.63	945		225807.23	756		39440.80	756	
Total		2014.92	1049		803.05	1049		587863.56	839		412681.17	839	
6% Protein Diet													
L	MS_{LD}	99.30	6	8.1**	62.76	6	10.2**	8112.76	6	1.5	17794.33	6	4.1
D	MS_e	120.55	2	128.3**	143.67	2	409.2**	22371.40	1	128.5**	51649.15	1	590.9**
L × D	MS_e	24.58	12	4.4**	12.28	12	5.8**	5533.20	6	5.3**	4325.07	6	8.2**
Error		88.77	189		33.18	189		21933.58	126		11013.98	126	
Total		333.20	209		251.90	209		57950.94	139		84782.53	139	

[a]A = adaptation; L = laboratory; D = diet.
[b]Appropriate mean square (MS = ss/df) to calculate F (e.g., for adaptation, $F = MS_A/MS_{AL}$).
[c]* = ($P < 0.05$); ** = ($P < 0.01$).

Statistical Summary for Each Week and Adaptation Period Over 10 and 6% Diets

Data for each adaptation period and each week over the 5 protein diets fed at the 10% level (table not included) showed that: The NPR mean over all protein sources was lowered by extending the feeding period to 14 days; the 14-day NPR was less variable than the 7-day NPR; the 4-day adaptation 14-day NPR was the least variable and the 0-day adaptation was nearly as precise. The NPR following the 2-day adaptation had the highest variability, but the mean values for all adaptations had a maximum difference of only 0.21 NPR. Calculation of the R-NPR lowered the interlaboratory CV values in all but 1 adaptation period (7-day R-NPR, 0-day adaptation), which remained the same. The differences between the interlaboratory CV values for 14-day NPR and 14-day R-NPR are 4.2, 4.7, or 2.9% for the 0, 2, or 4-day adaptation groups, respectively.

Statistical Summary for Each Week Over All Factors (Adaptation Period, Protein Diets, and Laboratories for 10% and 6% Diets)

The intra- and interlaboratory variability over all factors for the five 10% and three 6% protein diets are given in Table 6. Again the calculation of R-NPR showed a lowering of the

Table 5. 14-day NPR and R-NPR data for 7 laboratories

Diet	Adapt., days	Intralaboratory NPR Mean[a]	NPR SD	R-NPR Mean[a]	R-NPR SD	CV,% NPR[b]	CV,% R-NPR[b]	Interlaboratory SD NPR[b]	SD R-NPR[b]	CV,% NPR[b]	CV,% R-NPR[b]
10% Protein											
Casein	0	4.06	0.39	100.00	10.25	9.7	10.3	0.60	10.49	14.8	10.5
	2	3.96	0.31	100.00	8.08	7.8	8.1	0.53	8.37	13.4	8.4
	4	3.88	0.23	100.00	6.18	5.9	6.2	0.44	6.32	11.3	6.3
Beef	0	4.43	0.30	109.52	7.66	6.7	7.0	0.57	9.49	12.9	8.7
	2	4.29	0.30	108.77	7.33	7.0	6.7	0.52	10.49	12.1	9.6
	4	4.19	0.25	108.35	6.57	5.9	6.1	0.39	8.94	9.3	8.3
Lactalbumin	0	4.54	0.33	112.19	8.56	7.3	7.6	0.57	8.94	12.6	8.0
	2	4.39	0.25	111.08	6.58	5.7	5.9	0.58	8.37	13.2	7.5
	4	4.32	0.32	111.50	8.58	7.4	7.7	0.51	10.00	11.8	9.0
Peanut flour	0	2.50	0.20	61.72	5.04	8.1	8.2	0.39	6.32	15.6	10.2
	2	2.35	0.22	59.03	5.77	9.2	9.8	0.48	7.75	20.4	13.1
	4	2.40	0.24	61.71	6.29	10.0	10.2	0.39	7.07	16.2	11.5
Textured vegetable protein	0	3.61	0.24	89.01	6.28	6.7	7.1	0.44	7.07	12.2	7.9
	2	3.42	0.30	86.65	7.78	8.6	9.0	0.52	10.49	15.2	12.1
	4	3.31	0.34	85.27	9.06	10.2	10.6	0.46	10.00	13.9	11.7
6% Protein[c]											
Casein	2	4.03	0.53	100.00	14.50	13.2	14.5	0.90	14.49	22.3	14.5
Wheat flour	2	2.10	0.36	51.98	9.63	17.1	18.5	0.75	18.17	35.7	35.0
Textured vegetable protein	2	3.60	0.34	90.40	9.06	9.3	10.0	0.60	13.78	16.7	15.2

[a]Means of 70 data points, 7 laboratories, 10 rats per laboratory.
[b]The means for intra- and interlaboratory variability of NPR for 1 adaptation period, diet, and 7 laboratories are the same. The R-NPR means are the same. Casein was given the value of 100 for the calculation of R-NPR.
[c]NPR determined following a 2-day adaptation period only.

Table 6. Intra- and interlaboratory variability for NPR data over all factors

Ratio calcd	Mean	SD		CV,%	
		Intralab.	Interlab.	Intralab.	Interlab.
		10% Protein Diets[a]			
NPR 7[b]	4.37	0.72	1.11	16.5	25.4
NPR 14[c]	3.71	0.29	0.49	7.7	13.2
R-NPR 7[b]	88.67	17.74	18.62	20.0	21.0
R-NPR 14[c]	93.65	7.46	8.66	8.0	9.2
		6% Protein Diets[d]			
NPR 7[b]	3.96	0.69	1.05	17.4	26.5
NPR 14[c]	3.25	0.42	0.76	12.9	23.4
R-NPR 7[b]	82.26	14.47	16.26	17.6	19.8
R-NPR 14[c]	80.79	11.33	15.44	14.0	19.1

[a]Five protein diets, 7 laboratories, 3-adaptation periods (0, 2, and 4 days), 10 rats/trial (1050 values).
[b]7-day data.
[c]14-day data.
[d]Three protein diets, 7 laboratories, 10 rats/trial, and 2-day adaptation period (210 values).

variability, indicating an increase in the precision of the interlaboratory data, for example, 4.4 and 4.0 percentage units for the 7-day and 14-day data, respectively, for the 10% diets.

The interlaboratory CV values for the 6% diets over the 3 protein sources (casein, wheat flour, and textured vegetable protein), 7 laboratories, and 2-day adaptation period (Table 6) were high. Calculating the R-NPR values for these diets lowered the interlaboratory CV values 4.3%.

Comparison of R-NPR and R-PER Data

R-NPR and R-PER data were compared (Table 7). The R-NPR, PER, and R-PER data from 7 laboratories, 10 and 6% protein diets, and 2-day adaptation period are based on 70 data points. The 7-laboratory R-NPR data over all 3 adaptation periods are based on 210 data points. All data are relative to casein which was given the value of 100. The R-PER data were calculated from the actual PER values obtained for the different test proteins and casein for the feeding and adaptation time periods specified, and not from PER values that were adjusted with the PER value of 2.5 assigned to casein. The latter procedure is commonly used to adjust PER values used for nutritional labeling. The use of either adjusted or unadjusted PER values will give the same R-PER values. The official method for calculating PER, although not used for nutritional labeling, specifies that the unadjusted PER of the test is expressed as a percentage of the unadjusted PER obtained for the reference protein, ANRC casein (9). Thus, the R-PER values shown in Table 7 have been calculated by the official method and the R-NPR values were calculated similarly. There are similarities and differences in the R-NPR and R-PER. The 14-day R-NPR and the 28-day R-PER values are similar for the high quality proteins, lactalbumin, beef, and casein. The data for textured vegetable protein indicate a higher R-NPR than either the 14-day or 28-day R-PER for both the 10 and 6% diets. Peanut flour had the greatest difference among the 10% diets, at least 13 or 14 NPR units higher than the 14- or 28-day R-PER, respectively. Wheat flour exhibited a dramatic difference between R-NPR and R-PER values.

Conclusions

The following conclusions can be drawn from the data in this collaborative study: *(1)* The analysis of variance for the NPR indicated significant ($P < 0.05$) effects due to the factors (laboratories, protein sources, and adaptation times of the animals for 0, 2, or 4 days) and interactions among the factors. The most significant effect identified was between protein diets, indicating that the NPR assay was finding a difference between proteins. The most significant interaction among the factors was laboratory-by-protein diet. *(2)* Inter- and intralaboratory precision were increased by feeding for 14 days compared with 7 days. Therefore, a 14-day NPR test would be advantageous. *(3)* Although results from the 4-day adaptation period were more precise than those from either the 0-

Table 7. Comparison of R-NPR and R-PER data

Diet	R-NPR 14 Days, Adapt., days: 2	R-NPR 14 Days, Adapt., days: Overall	PER[a], 2-Day adaptation: 14 Day	PER[a], 2-Day adaptation: 28 Day	R-PER[a], 2-Day adaptation: 14 Day	R-PER[a], 2-Day adaptation: 28 Day
			10% Protein Diet[b]			
Casein	100	100[c]	3.26	2.97	100	100
Beef	108	103[c]	3.66	3.14	112	106
Lactalbumin	111	111[c]	3.73	3.31	114	111
Peanut flour	59	61[c]	1.51	1.35	46	45
Text. veg. protein	86	87[c]	2.68	2.39	82	80
			6% Protein Diets[b]			
Casein		100	2.51	2.25	100	100
Wheat flour		52	−0.15	0.04	−6	2
Text. veg. protein		89	2.09	1.90	83	84

[a]Unadjusted to a PER value of 2.5 assigned to casein.
[b]Based on means of 70 data points (7 labs, 1 adaptation period, and 10 rats/trial).
[c]Based on means of 210 data points (7 labs, 3 adaptation periods, and 10 rats/trial).

or 2-day adaptation period, the differences were small and it would not be of practical significance to recommend a 4-day adaptation period. *(4)* A 2-day adaptation period for the rats to adjust to their environment and to recover from shipping stresses before initiation of the test would be more practical for a minimum time-cost NPR assay. *(5)* Adjustment of the NPR data to R-NPR did not improve the intralaboratory precision, but did increase the interlabortory precision of the data. *(6)* The NPR inter- and intralaboratory variation for the 10% diets over all factors were 13.2 and 7.7%, respectively, as measured by CV values, about the average for that of the 3 adaptation periods. The R-NPR inter- and intralaboratory variation over all factors, as measured by CV values were 9.2 and 8.0%, respectively. *(7)* Fourteen-day R-NPR and 28-day R-PER values agree closely for high quality protein sources, less closely for a medium quality protein source, and show large differences for the lower quality protein sources (peanut flour and wheat flour) in this study. *(8)* The NPR assay can be used as an alternative method to PER for the determination of protein quality. This conclusion is made despite knowledge that the R-NPR may overestimate the quality of lysine-deficient protein sources (4, 12, 15, 17, 18, 20). This is also the case for other rat bioassays (4, 19).

Reference Standard for NPR and R-NPR Determinations

Casein plus L-methionine was used by McLaughlan et al. (10) as the reference protein for NPR assay in a recent collaborative study and by Sarwar and McLaughlan (32) in a study of variables for the NPR method. Casein and purified L-methionine are each readily available to provide a mixture that can be used as a reference standard for the rat, with an NPR approaching that of the highest quality protein sources. High quality protein sources such as lactalbumin and egg white are not readily available as standardized products. Consideration could be given to using casein plus L-methionine as the reference protein in an official method for NPR and R-NPR. However, the greatest disadvantage is that there is little experience in feeding casein plus L-methionine to humans, and it becomes a contrived reference protein for the determination of protein quality or for ranking the quality of protein sources for human consumption and regulatory purposes. The average level of sulfur amino acids (s.a.a.) in the ANRC casein plus L-methionine used in the McLaughlan et al. (10) collaborative study probably was about 46 mg/g protein. Rama Rao et al. (33) found that the rat required about 50 mg s.a.a./g protein for maximum growth. The assumption that a mixture of casein and L-methionine reacts the same nutritionally in humans (infants and children) as in weanling rats is questionable.

The Food and Nutrition Board (FNB) estimated the total s.a.a. content of an ideal protein for humans as 26 mg/g protein (34). Pineda et al. (35) found that 27 mg methionine plus cystine/kg body weight (this translates to 27 mg s.a.a./g protein in a scoring pattern) when fed as milk or milk plus synthetic s.a.a. to children 21–27 months old, was similar to "recommended" or "safe levels" of intake rather than mean requirements. Torun et al. (36) used a level of 27 mg s.a.a./g protein (a value close to that of FNB) in an amino acid scoring pattern for 2-year-old children. The sample of ANRC casein [92.6% protein (N × 6.25)], the reference protein in this AACC–ASTM collaborative study, contained about 38 mg s.a.a./g protein (M. L. Happich (1979) unpublished data). Thus, ANRC casein without added methionine contained adequate s.a.a. and can be considered an appropriate reference protein for the evaluation of protein quality for human consumption, including young children. The use of casein plus L-methionine introduces a bias in relation to the evaluation of proteins for humans. It is appropriate that casein be the reference protein for NPR determinations (and that casein be continued as the reference protein for PER). Continuing with casein allows for direct comparisons with values already in the literature from past research and with values obtained for past production and regulatory control. A protein source that has higher protein quality than casein (or casein plus L-methionine), e.g., egg white, can still be evaluated.

Recommendation

From the results obtained in this study, the net protein ratio (NPR) method, following the general procedure used in this study but with the 5 specifications listed below, is recommended as an alternative method to the PER assay for determination of protein quality. The 5 specifications are as follows: (a) that the test diet contain 1.6% nitrogen originating solely from the test protein source; (b) that a 14-day NPR be determined by feeding the test protein for 14 days; (c) that there be a 2-day adaptation period; (d) that ANRC casein be used as the reference protein for the assay; and (e) that the NPR be expressed as R-NPR by calculating the ratio × 100 of the NPR for each assay group to the NPR for the reference ANRC casein group, and reported as R-NPR.

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Titrimetric Determination of Ascorbic Acid Using Chloranil

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Ascorbic acid is oxidized and quantitatively titrated with chloranii (2,3,5,6-tetrachloro-1,4-benzoquinone) in the presence of hexamethylenetetramine in acetone–water; the end point is determined visually by the appearance of a golden yellow color. Colored solutions are assayed by setting the initial absorbance at 451 nm to zero or the minimum, titrating with chloranil solution, and measuring absorbance after each increment of titrant. A plot of the volume of chloranil added against the absorbance gives a straight line with the volume intercept as the end point. Interference by the thiol group of cysteine, glutathione, etc., is avoided by masking with acrylamide; interference by iron(II) is masked with ammonium thiocyanate and sodium potassium tartrate. Hydrogen sulfite and thiourea (which do not interfere) are added as antioxidants during extraction of ascorbic acid from drugs and fruits.

The reducing property of ascorbic acid is due to the presence of the 1,2-enediol group. A number of oxidimetric titrants have been used for the quantitative determination of ascorbic acid in pure solutions (1–5), pharmaceutical preparations (6–8), and fruit juices (6, 7, 9). Titration with 2,6-dichlorophenolindophenol (10) is the most common method but its value is limited because of partial reaction of the reagent with cysteine, glutathione, and iron(II) which are often present in biological fluids and plant extracts, or with sulfite which is commonly added as a preservative to soft drinks. Titration in dilute acetic acid minimizes errors due to the participation of sulfhydryl substances and iron(II); however, the end point is very unstable, with the rate of fading depending on the concentration of interfering materials, and sulfite still affects the determination. *N*-Bromosuccinimide tolerates large amounts of iron(II) but only the freshly prepared reagent should be used. Cysteine, glutathione, and other thiols also react quantitatively with *N*-bromosuccinimide (11, 12). Masking the thiol group by cyanoethylation with acrylonitrile at pH 7 produces thio-ethers which do not interfere in the titration of ascorbic acid with chloramine-T (13) or *o*-iodosobenzoate (14).

Feigl and Anger (15) detected ascorbic acid by its reaction with chloranil: The discharge of the yellow color of the reagent is taken as a positive response. The same redox reaction has recently been used by Pandey (16) to determine ascorbic acid either by direct titration when the appearance of yellow color is taken as the end point, or indirectly when excess reagent is evaluated by its photometric measurement at 336 nm. The molar absorptivity is 532 l $mole^{-1}cm^{-1}$. Certain thiols were found to yield a permanent red color only when the total ascorbic acid has reacted, thus permitting analysis of ascorbic acid in combination with thiols, but iron(II) interferes badly and the method is not applicable to colored solutions. The formal redox potential of chloranil has been found to be 0.57 V by Belcher et al. (17).

We studied the oxidimetric determination of ascorbic acid with chloranil to find wider areas of application. This reaction is slow in water–acetone medium but considerably accelerated by EDTA which also acts as an indicator by giving a deep yellow color with excess chloranil at the end point (16). The reaction also proceeds much faster in hexamethylenetetramine than in EDTA (as evidenced by quicker fading of the yellow color of chloranil in direct titration). The greater stability of the iron(III)-EDTA complex raises the redox potential of iron(II) and its interference becomes severe. However, addition of a mixture of ammonium thiocyanate and sodium potassium tartrate together with hexamethylenetetramine eliminates this problem and the method can be used to determine ascorbic acid in drugs where iron(II) is one of the excipients. Colored solutions, such as pharmaceutical preparations of multivitamins, can be titrated photometrically with chloranil when the absorbance increases sharply and linearly at 451 nm. Chloranil does not react with thiourea and sulfite under the described conditions of titration. Therefore, addition of these substances is recommended to avoid aerial or enzymic oxidation during the extraction of ascorbic acid from drugs and fruits.

Experimental

Reagents

(a) *Chloranil (2,3,5,6-tetrachloro-1,4-benzoquinone).*—0.01M solution. Dissolve 0.6150 g chloranil (Fluka; recrystallized 3 times from ethanol) in ca 200 mL acetone in 250 mL volumetric flask and dilute to mark with ethanol. Standardize against high purity ascorbic acid by method described below. Strength thus determined should agree within 0.1% of value calculated on basis of weight of chloranil dissolved. Solution retains its strength for several months. Prepare less

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concentrated solutions by diluting stock solution with acetone.

(b) *Thiourea–sulfite solution.*—Dissolve 10 g thiourea and 5 g sodium hydrogen sulfite in 1 L deionized water.

(c) *Chemicals.*—Hexamethylenetetramine (HMT, urotropine), acrylamide, ammonium thiocyanate, and sodium potassium tartrate, all reagent grade.

(d) *Spectrophotometer*—Pye-Unicam SP 8-100.

Procedure

Extraction of ascorbic acid from pharmaceutical preparations.—Weigh a known number of tablets and grind together. Accurately weigh portion of powder containing ca 250 mg ascorbic acid, combine with 30 mL thiourea–sulfite solution, and stir ca 10 min. Filter insoluble portion through Whatman No. 41 paper and wash with 10 mL thiourea–sulfite solution. Combine filtrate and washings in 250 mL volumetric flask and dilute to volume with deionized water. Combine pharmaceutical injection preparations with 40 mL thiourea–sulfite solution and dilute.

Extraction of ascorbic acid from fruits.—Rapidly cut fresh fruit and squeeze juice in machine after adding 20 mL thiourea-sulfite solution and again extract any residual ascorbic acid. Mix combined juice with 5 mL 2% trichloroacetic acid and stir to coagulate protein. Suction-filter and dilute filtrate to known volume with deionized water.

Visual titration of ascorbic acid.—Mix a known aliquot (5–10 mL) of ascorbic acid solution (containing ca 1 mg ascorbic acid/mL) with 50 mg HMT and, after dissolution of solid, dilute with acetone to ca 30 mL. If turbidity appears, slowly add water with swirling until solution clears. Titrate ascorbic acid with 0.01M chloranil to sharp appearance of golden yellow color. End point is stable.

$$\text{Ascorbic acid, mg} = 176\ V \times M$$

where V = volume (mL) of chloranil (molarity M) used.

Photometric titration of ascorbic acid.—To a known aliquot of ascorbic acid solution (containing 10–100 μg ascorbic acid/mL) in 40 mL photometric cell, add 50 mg HMT and dilute with acetone to ca 20 mL. Adjust absorbance of solution to zero (or minimum) at 451 nm and titrate ascorbic acid with standard chloranil solution, measuring absorbance after each increment. Use 0.001M chloranil solution when test solution contains up to 50 μg ascorbic acid; use 0.005M titrant solution for greater amounts. Absorbance remains at zero (or practically minimum) before the equivalence point; thereafter it increases sharply and linearly. End point is determined graphically by plotting absorbance against volume of chloranil added, a straight line being obtained with intercept on volume axis showing the end point (Figure 1).

Determination of ascorbic acid in presence of iron(II).—Mix 10 mL portion of sample solution (containing ca 5 mg ascorbic acid) with 50 mg HMT and 0.5 g each of ammonium thiocyanate and sodium potassium tartrate. Shake contents and dilute with acetone to produce a little turbidity, shake again for 1 min, then clear by adding water in small portions. Titrate ascorbic acid with 0.01M chloranil by either method described before.

Determination of ascorbic acid in presence of thiols.—Mix 10 mL aliquot of solution (containing ca 5 mg ascorbic acid) with 0.1 g HMT and 0.5 g acrylamide; let stand ca 15 min. Add sufficient acetone to ca 25 mL and titrate with 0.01M chloranil as before.

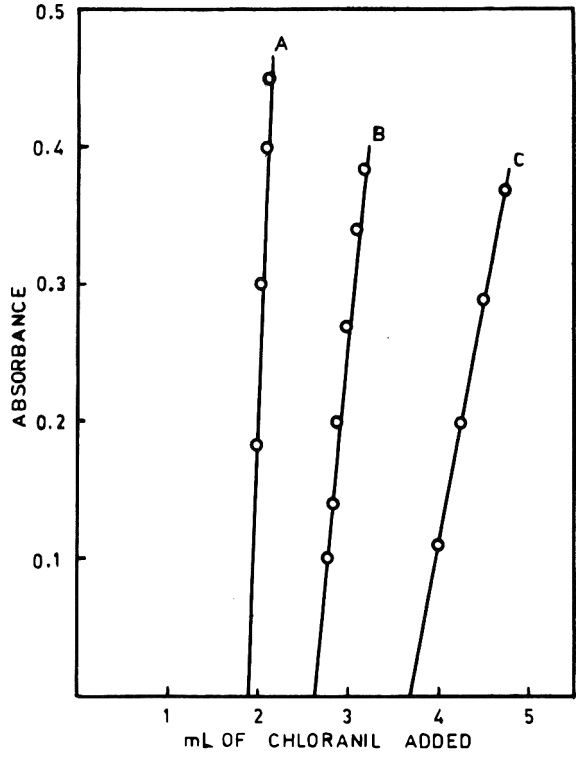
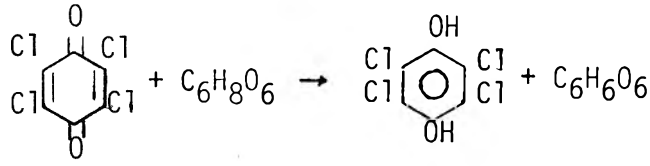


Figure 1. Photometric titration of ascorbic acid with chloranil: A, ascorbic acid, 167 μg/mL, 0.005M chloranil used; B, ascorbic acid, 46.4 μg/mL, 0.001M chloranil used; C, ascorbic acid, 32.6 μg/mL, 0.005M chloranil used.

Results and Discussion

The reaction of chloranil with ascorbic acid yields 2,3,5,6-tetrachlorohydroquinone and dehydroascorbic acid, respectively:



Hexamethylenetetramine accelerates the rate of reaction and participates to form a golden yellow color at the end point; the basic character of the reagent presumably is responsible for the color development since other bases behave similarly. Photometric titration of a known solution of chloranil with ascorbic acid at 451 nm (the λ_{max} of the color developed) gave an absorbance that increased initially, reaching a maximum when half of the chloranil was reduced, and then decreased until the total chloranil had reacted. Ostensibly, the 1:1 molar adduct of chloranil and 2,3,5,6-tetrachlorohydroquinone, held by hydrogen bonding (as in quinhydrone), is responsible for color development, and basic medium is favorable for its formation.

Results for the determination of ascorbic acid in pure solutions, synthetic combinations, drugs, and fruit juices are given in Tables 1–3, and are compared with those found by previously checked procedures. Substances in large amounts (about 10-fold molar excess to ascorbic acid) which do not affect the results include sulfite, thiocyanate, iodide, bromide, chloride, and thiosulfate. Iron(II) and ferrocyanide interfere severely but may be tolerated if iron(II) is masked by pretreatment with a mixture of ammonium thiocyanate and sodium potassium tartrate, and ferrocyanide is rendered nonreducing by reaction with zinc sulfate to yield insoluble zinc ferrocyanide. Iron(II) appears to form a ternary complex with ammonium thiocyanate and sodium potassium tartrate. Tryptophan, histidine, tyrosine, methionine, cystine, homocystine, glutamic

Table 1. Determination of ascorbic acid in pure solutions and synthetic mixtures

Ascorbic acid taken,[a] mg	Substance added (mg)	Ascorbic acid found,[b] mg			
		Visual titrn	CV, %	Photometric titrn	CV, %
1.82		1.80	0.2	1.81	0.2
2.38		2.36	0.1	2.39	0.2
3.81		3.79	0.1	3.80	0.1
3.69	cysteine (5)	3.66	0.2	3.68	0.2
4.55	glutathione (10)	4.58	0.2	4.56	0.2
6.29	*N*-acetylcysteine (5)	6.31	0.2	6.32	0.3
4.88	iron(II) sulfate (5)	4.85	0.3	4.86	0.2
5.24	sodium hydrogen sulfite (10)	5.26	0.1	5.25	0.1

[a]Solution standardized with chloramine-T (13).
[b]Average of 6 replicates; CV = coefficient of variation.

Table 2. Determination of ascorbic acid in drugs

Drug	Ascorbic acid, mg[a]				
	Specification	Present method	CV, %	DPIP titrn	CV, %
Sorvicin	500	492	0.2	487	0.3
Chewcee	500	510	0.2	504	0.2
Suckcee	500	489	0.3	493	0.2
Celin	500	501	0.2	496	0.1
Redoxon	500	490	0.1	493	0.2
Calcium-Sandoz	500	516	0.2	512	0.2
Chromostat[b]	150	138	0.2	143	0.3
Vidaylin-M[c]	50	46	0.2	—	—
Multi-Drugs[d]	30	36	0.1	38	0.2
Cilatin[e]	150	142	0.2	—	—
Becosules	300	310	0.2	308	0.3
Vibelan Forte[f]	300	308	0.3	—	—
Dristan[g]	20	16	0.1	18	0.2
Resteclin[h]	250	243	0.2	—	—
Micropyrin[i]	25	22	0.2	20	0.2
Ferro-Redoxen[j]	200	173	0.3	—	—
Fedicyte[k]	50	44	0.2	—	—
Basiton Forte	300	318	0.2	321	0.2
Beplex Forte	200	173	0.2	178	0.2
Rubragran-HP[l]	100	95	0.3	—	—
Neo-Ferilex[m]	75	68	0.3	—	—
Laboratory made					
Sample No. 1[n]	200	203	0.2	205	0.2
No. 2[o]	100	98	0.2	105	0.1
No. 3[p]	50	52	0.2	59	0.3

[a] Average of 6 replicates; CV = coefficient of variation; DPIP = 2,6-dichlorophenol-indophenol (10).
[b] Excipients include adrenochrome monosemicarbazide (0.5 mg), menaphtheone sodium hydrogen sulfite (10 mg), calcium dihydrogen phosphate (132 mg), calciferol (500 IU), and rutin (50 mg).
[c] Excipients include cysteine (5 mg).
[d] Excipients include *N*-acetylmethionine (30 mg).
[e] Excipients include sodium hydrogen sulfite (10 mg).
[f] Excipients include vitamin B_1 (50 mg), riboflavin (25 mg), nicotinamide (70 mg), calcium pantothenate (10 mg), vitamin B_6 (10 mg), vitamin B_{12} (10 μg), folic acid (5 mg); colored with Ponceau 4R, Sunset Yellow FCF, and Tartrazine.
[g] Excipients include phenylephrine hydrochloride (5 mg), phenindamine tartrate (10 mg), acetylsalicylic acid (230 mg), phenacetin (79 mg), and caffeine (16 mg).
[h] Excipients include tetracycline hydrochloride (500 mg).
[i] Excipients include acetylsalicylic acid (350 mg).
[j] Excipients include ferrous fumarate (350 mg), folic acid (2 mg), vitamin B_{12} (25 μg); colored with Tartrazine and Indigo Carmine.
[k] Excipients include thiamine mononitrate (2 mg), vitamin B_2 (2 mg), pyridoxine hydrochloride (1 mg), vitamin B_{12} (10 μg), and ferrous sulfate (0.3 g).
[l] Excipients include ferrous fumarate (300 mg), folic acid (2.5 mg), pyridoxine hydrochloride (10 mg), and cyanocobalamin (50 μg).
[m] Excipients include ferrous fumarate (150 mg), vitamin B_{12} (10 μg), folic acid (2 mg), thiamine hydrochloride (2 mg), riboflavin (1 mg), pyridoxine hydrochloride (2 mg), and nicotinamide (715 mg).
[n] Excipients include thiamine hydrochloride (50 mg), acetylsalicylic acid (20 mg), caffeine (5 mg), and folic acid (2 mg).
[o] Excipients include ferrous sulfate (20 mg), *N*-acetylmethionine (10 mg), and starch (100 mg).
[p] Excipients include lactose (100 mg), ferrous fumarate (20 mg), isoniazid (5 mg), and nicotinamide (5 mg).

acid, glucose, fructose, lactose, metol, hydroquinone, phloroglucinol, 2-mercaptobenzothiazole, 2-mercaptobenzimidazole, and 2-mercaptobenzoxazole also do not interfere. Ethyldithiocarbonate, diethyldithiocarbamate, thiobarbituric acid, and hydrazine interfere severely. Cysteine, glutathione, and other thiols, if present with ascorbic acid, produce a red color during titration which fades more or less rapidly, obscuring the true end point. Masking the thiol group with acrylamide is recommended, according to the following reaction:

$$RSH + CH_2=CH-CONH_2 \rightarrow RSCH_2CH_2CONH_2$$

Hexamethylenetetramine acts as a basic catalyst for the above reaction involving nucleophilic addition of thiol to the deactivated π bond.

The present method of determining ascorbic acid has many advantages over that using 2,6-dichlorophenol-indophenol. The standard solutions of chloranil are stable for long periods even when stored at room temperature. Unlike indophenol, chloranil has a lesser tendency to react with substances (as

Table 3. Determination of ascorbic acid in fruits

Fruit	Ascorbic acid, mg/100 g: Present method[a]	CV, %	DPIP titrn[b]	CV, %
Orange	82	0.4	75	0.6
Amla	596	0.3	589	1.2
Lemon	106	0.3	97	0.6
Grape	113	0.3	106	0.5
Papaya	41	0.4	38	0.6

[a]Average of 5 replicates; CV = coefficient of variation.
[b]DPIP = 2,6-dichlorophenol-indophenol. Average of 5 replicates; results obtained without using antioxidant (10).

listed before) that are known to interfere in redox reactions. This selective oxidation makes the end point sharp and stable, and enables the use of pretreatment methods to mask the substances that otherwise interfere. The addition of thiourea and sulfite prevents the air or enzymic oxidation of ascorbic acid during its extraction (Table 3) yielding higher recovery values in chloranil method. These additives react with indophenol and could not be used as antioxidants.

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Composition and Protein Efficiency Ratio of Meat Samples Partially Defatted with Petroleum Ether, Acetone, or Ethyl Ether

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Freeze-dried beef samples were partially defatted with either petroleum ether, acetone, or ethyl ether before determination of protein efficiency ratio (PER) to study the extraction effects on the composition and protein nutritional quality of the extracted beef. Defatting a protein source, such as meat or a meat product, may often be necessary to produce a test diet that contains 10% protein and 8% fat. Amino acid, carnosine, anserine, creatine, creatinine, inosine, and proximate compositions were determined on the extracted samples. Resulting data were compared to the composition and PER data of the beef that had no solvent treatment. Although the chemical analysis data from the study showed some variation between the proteins and other nitrogenous components of the unextracted and the extracted beef, these variations were too small to affect the protein nutritional quality of the beef as measured by PER.

AOAC method **43.212** (1) for determination of protein efficiency ratio (PER) states that the test diet fed to rats shall consist of 10% protein originating from the test protein sample, and 8% fat. In many meats, meat products, and meat food products, the fat content exceeds the protein content, necessitating an alteration in the official method to obtain the required protein–fat ratio in the test protein and ANRC casein diets. Possible solutions to the problem were reviewed and a procedure for removing excess fat by partial extraction with petroleum ether was used by the authors (2). Before partial extraction of the fat, the water content in a coarsely ground meat sample was reduced to about 1% by freeze-drying. To prevent protein denaturation and oxidative changes, extractions were conducted at a low temperature with a nonpolar solvent. Because extraction of fat with a solvent might nevertheless affect the nutritional quality of the meat proteins, we investigated and compared the effects of 3 different solvents, acetone, ethyl ether, and petroleum ether, on extraction of lyophilized beef. PER, amino acid composition, and composition of nonprotein nitrogenous constituents of extracted beef samples were compared to those of unextracted beef to determine whether changes in composition or protein nutritional value had occurred.

Experimental

Reagents

(a) *Solvents.*—Nanograde ethyl ether, petroleum ether (30–60°C), and acetone (Mallinckrodt).

(b) *Perchloric acid.*—1M and 0.6N perchloric acid.

(c) *Alkaline picrate reagent.*—10 mL saturated picric acid + 2 mL 10% NaOH.

(d) *Diacetyl-α-naphthol reagent.*—Equal volumes of diluted diacetyl solution (stock solution of 1% diacetyl diluted 1:20 before use) and 1% α-naphthol in 2N NaOH. Prepare stock solution of diacetyl by heating 1.6 g dimethyl glyoxime with 200 mL 5N H_2SO_4 in an all-glass distilling apparatus and collecting first 50 mL of distillate. Dilute distillate to 100 mL with water. Amount of dimethyl glyoxime taken yields about 1 g diacetyl. Stock solution is stable at least one month (3).

(e) *Phosphate buffer, 0.1M.*—pH 7.0.

(f) *Potassium hydroxide.*—30% (w/w).

(g) *6N HCl.*

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Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.
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(h) *3N Mercaptoethanesulfonic acid.*

(i) *15% Sodium thiosulfate.*

(j) *Sodium citrate buffer solutions for amino acid analyzer.—(1)* 0.2N Na^+, pH 2.90 for 126 min; *(2)* 0.2N Na^+, pH 4.15 for 26 min; *(3)* 1.0N Na^+, pH 5.90 for 100 min.

Apparatus

(a) *Meat grinder.*—Butcher Boy, B-52, Lasar Manufacturing Co., Los Angeles, CA.

(b) *Buffalo ribbon blender.*—John E. Smith's Sons, Buffalo, NY.

(c) *Freezer-dryer.*—Stokes Model 338-F shelf dryer (Sharples-Stokes Div., Pennwalt Corp., Warminster, PA).

(d) *Stainless steel trays.*—26 × 9.5 × 88.9 cm.

(e) *Wiley mill.*—Model No. 1, Arthur H. Thomas Co., Philadelphia, PA.

(f) *Soxhlet extractor.*

(g) *Blender.*—Waring, semi-micro, stainless steel jar.

(h) *Spectrophotometer.*—Perkin-Elmer what model, 650–40.

(i) *Amino acid analyzer.*—Beckman Model 119B (Beckman Instruments, Inc., 1117 California Ave, Palo Alto, CA 94304).

Beef Samples

Beef sample was 44 kg of 90% lean cow chuck obtained from a local slaughter house. Proximate composition of the beef as determined by AOAC methods **24.003(a)** (moisture), **24.005(a)** (petroleum ether extract), **24.027** (Kjeldahl nitrogen), and **24.062** (ash) (1) was moisture, 70.86%; fat, 7.4%; nitrogen, 3.32%; protein (N × 6.25), 20.74%; and ash, 1.0%.

Freeze-Drying

Grind 44.0 kg beef in meat grinder through ¾ in. plate. Spread ca 2 kg ground beef per stainless steel tray and freeze at −35°C. Place trays of frozen meat in a Stokes shelf dryer and dry 18–24 h at shelf temperature of 41.6–43.3°C under vacuum of 0.5 mm mercury. Tray of meat was considered dry when bottom of tray was warm to the touch. Freeze-dried meat weighed 13.4 kg, about 30.5% of original weight of beef.

Partial Extraction of Fat

Thoroughly mix freeze-dried beef and divide into 4 equal portions. Remove substantial proportion of fat from each of 3 portions by extraction with petroleum ether, ethyl ether, or acetone as described: Place 2 kg beef in double-layer cheesecloth bag and suspend for 3 h at 25°C in 20 L battery jar containing 10 L solvent. Raise bag occasionally, let it drain briefly, and return bag to the solvent. After 3 h, briefly drain solvent from bag and continue extraction for 1 h in battery jar containing 10 L fresh solvent. Then drain solvent from bag of beef and evaporate remaining solvent in flow of air inside fume hood at room temperature. Place meat in large vacuum chamber and remove last remnants of solvent. Thoroughly intersperse partially defatted meat with finely ground Dry Ice and grind mixture through Wiley Mill to pass 2 mm screen. Let CO_2 evaporate, thoroughly mix ground sample, and store in tightly closed air-impermeable plastic food-freezer bag or other air-tight container in refrigerator at 1°C.

Chemical Analyses

Determine moisture, fat, ash, and nitrogen by AOAC methods **24.003(a)**, **24.005(a)** petroleum ether, **24.062 (31.012)**, and **24.027** (1), respectively, on 3 partially defatted samples and on the unextracted beef sample (control). Calculate percent protein as percent nitrogen × 6.25.

Amino acid analysis of protein hydrolysates.—Weigh 10 g each of lyophilized control and the partially defatted beef samples into individual extraction thimbles and completely defat with petroleum ether (30–60°C, reagent grade) in Soxhlet extractor, using lowest setting on heater to keep solvent boiling. Continue extraction for 4 h, then evaporate solvent in air with stream of nitrogen flowing around thimble containing extracted beef, and finally, in a vacuum. Mix each sample well and store in glass jar with tight fitting plastic-lined screw cap at about 1°C. Determine nitrogen, protein (%N × 6.25), and moisture content.

Hydrolyze 0.1–0.15 g samples of defatted beef (0.09–0.135 g protein) by refluxing under nitrogen for 24 h with 75 mL 6N HCl, adding 0.75 mL 5% phenol solution to protect the sulfur amino acids. After hydrolysis, remove HCl and water under vacuum by using rotary evaporation with the hydrolysis flask rotating in 40°C water bath. Wash dried residue 3 times with 25 mL deionized water, evaporating the water after each wash. Dissolve residue and dilute to 50 mL with sample dilution buffer (0.2N Na^+, pH 2.2). Add 0.1 or 0.2 mL diluted solution to sample holder on amino acid analyzer. Use sodium citrate, 3-buffer system (0.2N Na^+, pH 2.90 for 126 min; 0.2N Na^+, pH 4.15 for 26 min; and 1.0N Na^+, pH 5.90 for 100 min) to elute 19 amino acids including hydroxylysine and hydroxyproline (4). Conditions: buffer flow, 70 mL/h; ninhydrin flow, 35 mL/h; column, 0.9 cm diameter, containing 31.5 cm Beckman cation exchange resin AA-20. Use computer system to integrate area of amino acid peaks and to calculate concentration of each amino acid in solution applied to column.

Determine tryptophan on separate sample as follows: Hydrolyze 4 mg lyophilized, defatted beef with 2 mL 3N mercaptoethanesulfonic acid (5) under vacuum in sealed tube for 24 h at 110°C. Neutralize mercaptoethanesulfonic acid with 4 mL 1N NaOH, dilute solution to 10 mL with sample dilution buffer, pH 4.25, and quantitate tryptophan by using a short column (0.9 cm diameter containing 5.75 cm Beckman cation exchange resin AA-20) and a sodium citrate buffer (0.2N Na^+, pH 5.4) (6, 7).

Calculate each amino acid as g amino acid residue (molecular weight of amino acid minus molecular weight of 1 molecule of water) or as g amino acid (molecular weight) per 100 g protein or per g nitrogen, whichever is more appropriate for the experiment conducted. (We calculated results as g amino acid residue/100 g protein. Results also can be calculated to g of either amino acid residue or amino acid per weight of partially defatted beef or of the original beef if the proximate analysis of these stages is determined.)

Preparation of sample and analysis of free amino acids, anserine, carnosine, creatine, and creatinine.—Hydrate 10 g freeze-dried meat control and solvent-extracted beef samples individually by adding 20 mL deionized water to each. Mix well, cover tightly, and store in refrigerator overnight. The next morning, homogenize each hydrated sample with 50 mL 0.6N perchloric acid (8) for 30 min in semi-micro stainless steel jar of Waring blender (Cenco 17246B). Filter through Whatman No. 1 paper. Solution from control sample may filter more slowly if much fat is present. Extract remaining solids and filter paper in blender with second 50 mL aliquot of 0.6N perchloric acid, filter, and wash the residue with two 10 mL portions of deionized water. Combine the filtrates and washes, neutralize with 30% (w/w) potassium hydroxide, using phenolphthalein indicator and spot plate for testing near the end point. Let solution stand overnight to complete precipitation. Filter potassium perchlorate precipitate from solution,

wash residue with deionized water, combine filtrate and washes, and dilute to 200 mL with deionized water. Note: Potassium perchlorate is a highly explosive compound especially when dry. Therefore, immediately react the wet potassium perchlorate precipitate with a solution of 15% sodium thiosulfate to prevent explosion before discarding.

Creatinine determination.—Determine creatinine content by using a method described by Hawk et al. (9), and modified by Strange and Benedict (10). Combine 5, 2, and 1 mL aliquots of the potassium perchlorate filtrate with 0, 3, and 4 mL deionized water, respectively, and mix with 2.5 mL freshly prepared alkaline picrate reagent. After 15 min, read absorbance at 540 nm in Perkin Elmer spectrophotometer, using a 1-cm pathlength cuvet. Prepare blank for each run. Determine concentration of creatinine from prepared standard curve. Report results as mg creatinine/100 g moisture-free, fat-free beef.

Creatine determination.—Determine creatine content on potassium perchlorate filtrate by modified method of Eggleton et al. (3). Prepare diacetyl–naphthol reagent, immediately before use, by mixing equal volumes of diluted diacetyl solution (stock solution of 1% diacetyl diluted 1:20 before use) prepared by the method of Eggleton (3) and 1% α-naphthol in 2N NaOH (11). Mix 2 mL of 1:99 or 1:49 dilutions of potassium perchlorate filtrate with 2 mL of the reagent, and heat reaction mixture in boiling water for 10 min. Read absorbance at 550 nm, and determine absorbance of reagent blank for each run. Prepare standard curve for creatine; Beer's Law applies in range of concentration used. Give results as mg creatine/100 g moisture-free, fat-free beef.

Determination of free amino-acids.—Determine free amino acids in potassium perchlorate filtrate by ion exchange chromatography using amino acid analyzer and same buffer system as for acid hydrolysates. Inject 0.25 mL filtered extract per analysis onto column. The dipeptides carnosine and anserine were assumed to be in the extract, although individual peaks did not show on chromatogram for either one. Apparently, anserine co-elutes with lysine and carnosine with histidine under conditions and buffer system used. Peak in lysine position was calculated as mg lysine/100 g moisture-free, fat-free beef, and not as anserine (reasons discussed later). Calculate peak in histidine position as mg carnosine/100 g moisture-free, fat-free beef.

Determination of nucleotides.—Determine nucleotides by method of Honikel and Fisher (12) after pretreatment to hydrate sample and remove water solubles as follows: Weigh 2 g sample of each of freeze-dried control, acetone, ethyl ether, or petroleum ether-extracted beef into centrifuge tubes. Add 14 mL deionized water to each tube, mix thoroughly, cover with plastic wrap, and place in refrigerator overnight. Next day, centrifuge at 19 000 rpm for 30 min at 0–4°C, and filter supernatant through glass wool to remove fat accumulated on top of solution. There is 6 or 7 mL of filtrate from each solution. To each sample, add 10 mL 1M perchloric acid, stir well, and filter out precipitate through glass wool. Dilute 0.3 mL portion of filtrate to 5 mL with 0.1M phosphate buffer, pH 7.0. Determine absorbance at 250 nm from peak maxima and concentration of nucleotides as inosine 5′-monobasic phosphoric acid, by reference to standard curve prepared with this nucleotide. Calculate inosine 5′-monobasic phosphoric acid as mg/100 g moisture-free, fat-free beef. (This method does have an error inherent from absorption at 250 nm of other low molecular components, such as peptides and amino acids.)

Protein Efficiency Ratio

Determine PER by AOAC method **43.212–43.216** (1) (5 rats per assay, 2 replicate determinations, or by 10 rats per assay) for the unextracted beef sample, for the 3 samples of solvent-extracted beef, and for the reference protein casein. Prepare each diet to contain 10% protein (1.60 × 100/%N of test sample) supplied by test protein source and 8% fat supplied by test protein source and supplemented with corn oil. Use rats of Sprague-Dawley strain. Feed rats ad libitum for 28 days. Calculate PER (wt gain, g ÷ protein intake, g) for beef samples and for ANRC casein reference group. Also calculate by correcting to PER of casein at 2.5 (13).

$$\text{Corrected PER, test protein} = \frac{\text{actual PER, test protein}}{\text{actual PER, ANRC casein control}} \times 2.5$$

Apparent Nitrogen Digestibility Determination

Determine apparent nitrogen digestibility by using modified method of Mitchell (14, 15) during second week of PER test. Collect feces from each rat (5 rats per assay, 2 replicate determinations, or 10 rats per assay) on days 7–14 for the unextracted beef sample, for each of the 3 samples of solvent-extracted beef, and for the reference protein casein during PER test. Carefully separate feces from any spilled food or other extraneous matter. Composite feces from rats on an individual diet and dry feces overnight in 100°C oven. Equilibrate composited, dried feces sample(s) at room temperature and humidity, weigh each composite sample, grind in an Omni blender in metal cup immersed in ice water (or use other similar method). Mix thoroughly and determine nitrogen by the Kjeldahl method [1 (**24.027**)]. Determine feed intake by monitoring uneaten food. Carefully collect scattered food and uneaten food in feed cups, separate all extraneous matter, weigh, and determine Kjeldahl nitrogen (1). Weight of food offererd to animals minus weight of uneaten food equals weight of food eaten. Calculate apparent nitrogen digestibility using following equation (15–18):

$$\text{Apparent nitrogen digestibility} = \frac{\text{N intake} - \text{fecal N} \times 100}{\text{N intake}}$$

Results and Discussion

The beef samples that were partially defatted with petroleum ether, acetone, or ethyl ether had less than half the fat and correspondingly higher moisture and protein than the control sample (Table 1). Ethyl ether was the most efficient solvent and petroleum ether was the least efficient in extracting fat at ambient temperature. The amount of protein lost with the fat extracted from 2000 g freeze-dried beef was small: 1.2 g (0.31%), 0.9 g (0.25%), or 2.0 g (0.48%) for the petroleum ether, acetone, or ethyl ether extraction, respectively.

Table 1. Proximate analysis of freeze-dried beef control and beef partially defatted with solvents

Solvent	Ash, %	Fat, %	Moisture, %	Protein, N × 6.25	Total, %
None	3.1	30.2	3.9	63.8	101.0
Petroleum ether	3.6	14.7	8.9	73.0	100.2
Acetone	3.9	10.2	8.9	79.1	102.1
Ethyl ether	3.9	9.3	9.3	79.0	101.5

Amino Acid Analysis

Comparison of the amino acid analysis data from the beef and from the beef samples partially defatted with any 1 of 3 solvents (Table 2) indicates that the beef extracted with acetone had the highest values for each amino acid quantified, except for ½ cystine, tryptophan, and hydroxylysine (Table 2), and the highest value for the total indispensable amino acids and their sparing amino acids, histidine through valine (38.33, 38.11, 39.69, and 37.50 g/100 g protein for the control), and for beef extracted with petroleum ether, acetone, or ethyl ether, respectively [mean = 38.41 g; SD 0.92 g; CV 2.40%]. The F-value by an analysis of variance, 4.33, was significant for $P < 0.05$ but not for $P < 0.01$). The individual residue values were less than 0.30 g higher/100 g protein, with exceptions for glutamic acid, proline, and aspartic acid, which had higher differences. However, a one-way analysis of variance over the replicate amino acid residue values obtained for the 4 beef samples showed no statistically significant ($P < 0.05$) difference between the individual amino acid residue content of beef and of the beef samples extracted with any 1 of the 3 solvents (Table 2, F-values) except for phenylalanine and hydroxylysine. The value for phenylalanine in the acetone-extracted beef is significantly higher than for phenylalanine in the beef extracted with ethyl ether or petroleum ether or in the unextracted beef. The value for hydroxylysine is significantly higher for the unextracted beef sample. The acetone-extracted beef had the highest total amino acid residues (86.66 g/100 g protein). Data for beef extracted with either petroleum ether or ethyl ether were in closest agreement with data for the unextracted beef. An analysis of variance over the total amino acid residue values obtained for the 4 beef samples showed no statistically significant difference between the total amino acid residue content of the 4 samples of beef ($F_{0.05} = 1.43$).

Table 2. Amino acid content (g residue/100 g protein) of freeze-dried beef control and partially defatted beef samples

Amino acid	Solvent treatment: None[a]	Petroleum ether[b]	Acetone[a]	Ethyl ether[c]	F-value[d]
His	2.95	2.75	2.96	2.69	1.56
Ile	3.61	3.67	3.72	3.59	0.20
Leu	6.54	6.57	6.75	6.43	0.45
Lys	7.10	7.00	7.31	6.85	0.90
Met	2.39	2.35	2.52	2.38	0.57
½ Cys	1.09	0.97	1.05	0.99	0.15
Phe	3.50	3.59	3.79	3.54	4.62**
Tyr	3.09	3.09	3.20	3.05	0.71
Thr	3.29	3.47	3.58	3.44	1.27
Trp	0.88	0.81	0.79	0.84	—[e]
Val	3.89	3.85	4.02	3.70	0.46
Ala	4.80	4.89	5.03	4.83	0.54
Arg	5.68	5.66	5.98	5.58	1.08
Asp	7.23	7.44	7.70	7.39	0.84
Glu	12.69	12.89	13.40	12.64	1.66
Gly	4.83	4.78	5.07	4.93	1.27
Hyl	0.46	0.39	0.39	0.39	3.50**
Hyp	1.38	1.34	1.57	1.48	3.11
Pro	3.77	4.26	4.48	4.31	1.98
Ser	3.06	3.09	3.31	3.10	1.24
Total[f]	82.24	82.86	86.66	82.15	

[a]Mean of 5 replicates.
[b]Mean of 4 replicates.
[c]Mean of 3 replicates.
[d]**Variance, ratio significantly different at the 5% level. Reference value for significance is 3.41 where F_1 is 13 and F_2 is 3.
[e]F-value was indeterminate because the within-treatment variation was zero (i.e., perfect agreement between replicates). Mean of tryptophan values = 0.83, SD = 0.039, CV = 4.7%.
[f]Mean = 83.48, SD = 2.14, CV = 2.6%.

Free Amino-Acids, Dipetides, and Other Nitrogenous Components

In addition to the total amino acids, free amino acids and several other nitrogenous components were determined in the control and solvent-extracted samples of beef. These were 2 dipeptides found in meat, anserine and carnosine; creatine; creatinine; and nucleotides reported as inosine 5′-monophosphoric acid. The results are shown in Table 3. These components account for 4.2–5.5% of the nitrogen in the samples of partially defatted, freeze-dried beef (Table 4). About 4.9% was found in the undefatted freeze-dried beef. Seventeen free amino acids were found in measurable amounts, including taurine, an oxidation product of cysteine metabolism (19) (Table 3). The free amino acids serine and alanine were found in the highest quantities. A small amount of free histidine was present but could not be quantified. Free hydroxyproline, hydroxylysine, and tryptophan were not measurable and possibly were completely lacking. There was considerable evidence that the 2 dipeptides anserine (β-alanyl-1-methylhistidine) and carnosine (β-alanylhistidine) (20) were present and co-eluting with other amino acids under the chromatographic separation conditions used in these experiments, i.e., anserine with lysine and carnosine with histidine. The 440/570 nm peak height ratios of the substances eluting at the time of lysine and histidine supported this conclusion (Table 5). The 440/570 nm peak height ratio for anserine was 4.9 times that for lysine, and the ratio for carnosine was about 3 times that for histidine (Table 5). Data obtained from the chromatogram and the peak height ratios from the filtrate of the potassium perchlorate precipitate of the control and of the 3 partially defatted beef samples, indicated that the peak eluted at 191.5–192 min, in the lysine position, was produced mainly by free lysine (size of peak plus 440/570 peak height ratio), but undoubtedly had a small amount of the dipeptide, anserine (Table 5). However, the anserine/carnosine ratio in beef is low, 0.06–0.2 (21, 22), and the peak was calculated as lysine. Similarly, the data obtained (440/570 peak height ratio) (Table 5) on the peak in the histidine position (195 min) was expected to be nearly all carnosine and was calculated as carnosine. This value may be high because there may be a small amount of free histidine present. Nevertheless, the lysine–anserine and histidine–carnosine content of the beef samples, control or solvent-extracted, were similar.

Nitrogen Recovery

A comparison of the nitrogen recovery data for the total amino acid residues in the unextracted beef (control sample), the petroleum ether- and ethyl ether-extracted beef (Table 4) is similar to that found by Happich et al. (2) in a sample of lean beef.

Data for the total recovery of nitrogen by all analyses from each of the 4 beef samples (Table 4) indicated that nitrogen recovery was highest from acetone-extracted beef. Nitrogen recovery data from analyses of petroleum ether- and ethyl ether-extracted beef, although lower than recovery data from the control beef, agreed more closely with it.

Protein Efficiency Ratio (PER)

An analysis of variance (ANOVA) indicated that the PER values of the individual rats (5 per test) by 5 protein sources, by 2 experiments, or by interactions of protein source and experiment were not significantly different. Estimation of within-diet variability was performed (ANOVA) on PER data for each replicate and there was no evidence of any significance between the error variances. Thus the data from the 2 replicates were combined and analyzed statistically.

Table 3. Nonprotein nitrogenous components of freeze-dried beef and partially defatted beef (mg/100 g fat-free, moisture-free beef)

Chemical component	Solvent treatment			
	None	Petroleum ether	Acetone	Ethyl ether
Inosine	388	430	405	360
Creatine	1968	1574	2212	1636
Creatinine	105.2	98.4	116.3	137.0
Free dipeptides:				
Anserine[a]	UQ	UQ	UQ	UQ
Carnosine	1182	1045	1096	1102
Free amino acids:				
Taurine	170.0	145.0	160.0	155.0
Aspartic acid	8.9	5.1	4.9	4.9
Threonine	17.3	23.8	17.7	22.5
Serine	219.0	298.6	305.4	248.3
Glutamic acid	95.3	46.9	45.2	57.8
Proline	9.3	7.0	7.3	7.9
Glycine	37.4	45.0	42.8	40.8
Alanine	216.0	189.2	154.8	178.3
Valine	22.3	25.1	26.9	24.9
½ Cystine	34.3	19.3	24.4	23.7
Methionine	32.8	14.8	26.9	22.5
Isoleucine	32.0	19.9	31.0	24.9
Leucine	48.4	41.2	52.5	42.0
Tyrosine	43.2	46.9	45.8	39.5
Phenylalanine	32.8	38.6	39.1	31.6
Lysine	81.9	65.0	64.1	61.4
Histidine[b]	UQ	UQ	UQ	UQ
Arginine	49.2	44.4	51.3	40.8
Total free amino acids	1150.1	1075.8	1100.1	1026.8
Ammonia	90.9	45.7	53.1	60.8

[a]UQ = unable to quantify completely in the system used. Lysine and anserine eluted together.
[b]UQ = unable to quantify completely in the system used. Histidine and carnosine eluted together.

Table 4. Nitrogen recovered by analysis of beef samples partially defatted with petroleum ether, acetone, or ethyl ether[a]

Component	Solvent extraction			
	None	Petroleum ether	Acetone	Ethyl ether
Total nitrogen	15254.2	15239.9	15247.6	15245.9
Recovered amino acid residue N	12476.1	12492.7	13088.1	12393.2
% of total nitrogen	81.8	82.0	85.8	81.3
Recovered ammonia N	1192	1115	1125	1164
Inosine 5' monobasic phosphoric acid (I)	81.0	89.9	84.6	75.2
Creatine (C)	630.5	504.3	708.7	524.2
Creatinine (Cr)	39.0	36.6	43.2	50.9
Total I + C + Cr	750.5	630.8	836.5	650.3
% of total N	4.93	4.20	5.52	4.30
Total N recovered from 5 categories	14141.9	14238.5	15049.6	14207.5
% of total N	94.6	93.4	98.7	93.2
% N unaccounted for	5.4	6.6	1.3	6.8

[a]Nitrogen (N), mg/100 g fat-free, moisture-free beef.

Table 5. Elution time and peak height ratios of selected amino acids and dipeptides, and elution data from analysis of beef samples

Std soln	Elution time, min	Peak height ratio, 440/570 nm	Beef sample	Peak height ratio, 440/570 nm	
				191.5–192.0 min	195.0 min
Lysine	191.5–192.2	0.28–0.30	Control	0.46	0.64
Histidine	194.0–195.3	0.21 and 0.22	Acetone ext	0.45–0.47	0.65–0.66
1-Methyl His	197.2	0.26	Ethyl ether ext	0.46–0.48	0.63–0.65
3-Methyl-L-His	195.8	0.18	Pet. ether ext	0.46–0.49	0.65–0.67
Anserine	191.7	1.41			
Carnosine	195.9–196.2	0.68 and 0.69			

There were no significant differences between the actual PER (10 rats) determined by bioassay for the control beef and those determined for the beef samples partially extracted with any 1 of 3 solvents (Table 6). An ANOVA (10 rats, 5 diets) showed no significant differences between diets by an F test (F = 1.367) at 95 or 99% levels of probability. A comparison of the actual PER value for the untreated beef with that for each solvent-extracted beef sample shows them to have a range of differences no larger than 0.11 PER, values within experimental limits. Adjusting the PER to that of casein at 2.5, decreased the range of differences, but only slightly to 0.08 PER.

Table 6. PER[a] and apparent nitrogen digestibility of freeze-dried beef control and partially defatted beef samples

Dietary source of protein[b]	Final body weight, g ± SE[c,d]	Total feed consumption, g ± SE[c]	PER[e]		Apparent nitrogen digestibility,[g] %
			Actual ± SE[c]	Adjusted[f]	
ANRC casein	162 ± 4.7^{bB}	314 ± 11^{bB}	3.41 ± 0.06^{aA}	2.50	94.2 ± 0.45^{aA}
Beef	185 ± 7.4^{aA}	391 ± 18^{aA}	3.34 ± 0.07^{aA}	2.45	90.4 ± 0.10^{bB}
Beef, pet. ether extd	180 ± 4.4^{aAB}	388 ± 14^{aA}	3.23 ± 0.04^{aA}	2.37	91.5 ± 0.70^{bAB}
Beef, acetone extd	194 ± 4.5^{aA}	411 ± 13^{aA}	3.41 ± 0.03^{aA}	2.50	91.5 ± 0.95^{bAB}
Beef, ethyl ether extd	195 ± 7.1^{aA}	418 ± 13^{aA}	3.34 ± 0.09^{aA}	2.45	90.8 ± 0.30^{bB}

[a]28-day feeding tests; mean of 2 assays, 5 rats/assay.
[b]Diets contained 10% protein (N × 6.25).
[c]Mean ± SE. Duncan's multiple range test: Means without a superscript letter in common are significantly different. Lower case, $P < 0.05$; upper case, $P < 0.01$. $N = 10$.
[d]Average initial body weight of the rats was 54.5 g.
[e]PER = protein efficiency ratio = wt gain (g)/protein intake (g).
[f]Adjusted to PER of casein, assumed to be 2.5.
[g]Apparent nitrogen digestibility = N intake − fecal N/N intake × 100. Feces collected on days 7–14 during each 28-day PER feeding test; mean of 2 assays, 5 rats/assay.

Comparing the amino acid content (Table 2) and the actual PER values (Table 6) for each beef sample, the beef extracted with acetone had the highest total value (39.69 g/100 g protein) for the 11 indispensable and their sparing amino acids, histidine through valine, a value only 1.4 g higher than for the control. The acetone-extracted beef also had the highest adjusted PER value, although not significantly higher than the other values. The differences in PER values are considered within experimental error.

Apparent nitrogen digestibility data (means for 10 rats) for all beef samples were similar, with casein exhibiting slightly higher nitrogen digestibility than the beef.

Conclusions

Although the chemical analysis data obtained in this study showed some variations between the proteins and other nitrogenous components of the unextracted beef and the beef extracted with petroleum ether, acetone, or ethyl ether, these variations were too small to significantly affect the protein nutritional quality of the extracted beef as measured by PER. The PER data suggest that any one of these 3 solvents could be used to extract excess fat from samples of meat before determination of PER.

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Liquid Chromatographic Cleanup and Determination of Low Levels of Vitamin D_3 in Sheep Plasma

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A liquid chromatographic (LC) method is described for the determination of vitamin D_3 in sheep plasma. Samples are extracted by one of 2 different methods, depending on the concentration of vitamin D_3. The samples are purified by using either a Sep-Pak silica cartridge or a small alumina column, followed by additional cleanup on a Metalsorb LC column. Final analysis was carried out on a 5 μm C_{18} column using a radial compression separation system with an acetonitrile–methanol solvent system. Vitamin D_3 was completely resolved from any interfering compounds in the plasma; total run time was less than 15 min, using a variable wavelength detector set at 264 nm. The method was successfully applied to samples at levels of 1–10 ng added vitamin D_3/mL sheep plasma, with recoveries in the range 90–97%.

Vitamin D_3 is an important factor in calcium (Ca) and phosphorus (P) metabolism (1, 2). Active transport of Ca and P in the intestinal mucosa and Ca and P resorption from bone depend on vitamin D_3 (3). Lamb raised in total confinement are susceptible to vitamin D deficiency disease. It has been shown that sheep kept under total confinement develop a high incidence of an osteodystriphic condition, a vitamin D responsive disease (4). The importance of vitamin D_3 for the prevention and cure of rickets in livestock (5) is apparent from the great number of studies and analytical methods published (6) using liquid chromatography (LC). Some cleanup methods have specified use of either solvent partitioning (7, 8), a silica column (9–12), or Lipidex (13, 14).

In this paper, 2 different methods of extraction from sheep plasma are described; the choice depends on the level of vitamin D_3 to be determined.

Experimental

Apparatus

(a) *Liquid chromatograph.*—Waters Associates, equipped with M6000 pump and U6K septumless injector (Waters Associates Inc., Milford, MA).

(b) *Schoeffel Spectroflow monitor.*—SF 770, ultraviolet (UV) variable wavelength detector set at 264 nm (Schoeffel Instruments, Westwood, NJ).

(c) *Liquid chromatographic column.*—10 cm × 5 mm. Radial-Pak, 5 μm C_{18} cartridge used in conjunction with radial compression system (Waters Associates, Inc.)

(d) *Cleanup column.*—Stainless steel LC column, 25 cm × 4 mm id, 10 μm HgAC (Technical Marketing Associates, Ottawa, Ontario, Canada).

(e) *Liquid scintillation counter.*—Beckman LS235 (Beckman Co., Palo Alto, CA).

(f) *Filter paper.*—Whatman No. 54 (Fisher Scientific Co.).

(g) *Recorder.*—Linear Instruments Model No. 260, 10 mV (Irvine, CA).

Reagents

(a) *Solvents.*—Distilled in glass (Caledon, Georgetown, Ontario, Canada).

(b) *Pyrogallol solution.*—1 mg pyrogallol (Fisher Scientific Co.)/100 mL ethanol.

(c) *Mobile phases.—Sep-Pak:* hexane–isopropyl (95 + 5). *Alumina:* hexane–isopropyl alcohol (99 + 1). *Metalsorb HgAC column:* cyclohexane–hexane–isopropyl alcohol (50 + 50 + 1). *Radial-Pak C_{18}:* acetonitrile–methanol (9 + 1).

(d) *Adsorbents.*—Sep-Pak™ silica cartridge (Waters Associates, Inc.); neutral alumina (Fisher Scientific Co.); Metalsorb HgAC (Technical Marketing Associates); Radial-Pak C_{18} (Waters Associates, Inc.).

(e) *Vitamin D_3.*—Aldrich Chemical Co. (Canada) Ltd, Montreal, Quebec. ^{1,2,3}H vitamin D_3 (12.3 Ci/mm) (Amersham Corp., Arlington Heights, IL).

Procedures

(a) *For vitamin D_3 concentrations greater than 5 ng/mL.*—Carry out all experiments under subdued lighting. Transfer 1 or 2 mL plasma into 500 mL Erlenmeyer flask, add 200 mL methanol–dichloromethane (2 + 1), and stir mixture magnetically in cold room (−4°C) for 90 min. Filter solution through Whatman No. 54 paper previously washed with 50 mL methanol–dichloromethane extracting solvent. Evaporate filtered extract to dryness in 500 mL round-bottom flask by using Buchi Rotavapor evaporator under reduced pressure. Attach Sep-Pak silica gel cartridge to 50 mL syringe and wash with 25 mL hexane–isopropyl alcohol (95 + 5) followed by 25 mL benzene. Transfer plasma residue to syringe with three 2 mL portions of benzene and let flow into cartridge. Discard any eluate. Rinse flask 3 times with 2 mL hexane-isopropyl alcohol (95 + 5), add rinses to syringe, and elute into cartridge. Let an additional 30 mL hexane-isopropyl alcohol (95 + 5) elute through cartridge. Retain entire hexane-isopropyl alcohol eluate for vitamin D_3 analysis. Evaporate this fraction to near dryness on Rotavapor evaporator, and add 200 μL LC mobile phase (cyclohexane–hexane–isopropyl alcohol (50 + 50 + 1)) before additional purification on Metalsorb LC column.

(b) *For vitamin D_3 concentrations between 1 and 5 ng/mL (15).*—Into 125 mL separatory funnel, sequentially add 5 mL plasma, 10 mL water, and 15 mL 1% pyrogallol in ethanol. Place this mixture in ice bath, add 6 g KOH and, after initial reaction has cooled, transfer funnel to horizontal shaker and shake overnight at room temperature. Add 15 mL ethanol and 15 mL water to flask, followed by 45 mL hexane. Shake mixture vigorously for 1 min, and then let phases separate. When necessary, add a few drops of ethanol to induce separation. Transfer hexane layer to 250 mL separatory funnel and re-extract residue twice with 45 mL hexane. Add 20 mL water to pooled hexane extracts and drain water. Wash hexane layer with two 100 mL portions of 5% KOH, two 100 mL portions of water, and two 100 mL portions of 55% ethanol. Evaporate hexane solution under reduced pressure at 50°C. Add 5 mL ethanol and re-evaporate to remove traces of water. Dissolve residue in 2.0 mL hexane–isopropyl alcohol

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(99 + 1). Prepare 5.0 cm column of 100–200 mesh neutral alumina in Pasteur pipet. Condition column with 5 mL hexane–isopropyl alcohol (99 + 1) and discard washings. Apply residue to column and elute with five 2 mL portions of hexane–isopropyl alcohol (99 + 1). Collect eluate containing vitamin D_3 portion in 15 mL graduated centrifuge tube and evaporate to dryness under nitrogen. Dissolve residue in hexane-cyclohexane–isopropyl alcohol (50 + 50 + 1) for LC analysis.

Liquid Chromatographic Cleanup

Inject vitamin D_3 standard (100 ng in 100 μL cyclohexane–hexane–isopropyl alcohol (50 + 50 + 1)) onto Metalsorb column and record elution time. With flow rate of 1.5 mL/min, vitamin D_3 elutes between 13 and 17 min.

Fractions from Sep-Pak cartridge or alumina column are further purified by using Metalsorb system. Collect vitamin D_3 fraction from 13 to 17 min. Check this fraction daily before start of any analysis. Use detector setting of 0.02 AUFS for both standard and sample collection. Evaporate collected fraction to dryness under stream of nitrogen and dissolve residue in 100 μL acetonitrile–methanol (9 + 1).

Liquid Chromatographic Analysis

Final analysis is done on 5 μm Radial-Pak C_{18} reverse phase column. Evaporate eluate from Metalsorb column to dryness under nitrogen. Dissolve residue in 100 μL acetonitrile–methanol (9 + 1) and inject 80 μL aliquot onto reverse phase column. Calculate vitamin D_3 concentration from peak height of vitamin D_3 in sample and peak height of 100 μL of vitamin D_3 standard (1 ng vitamin D_3)/μL acetonitrile–methanol (9 + 1). Both standard and sample elute on reverse phase column at flow rate of 1 mL/min.

Results and Discussion

Cleanup Procedures

For extraction using methanol–dichloromethane (2 + 1), vitamin D_3 was extracted along with plasma lipid. The silica cartridge provided quick and easy filtration. Elution with benzene removed most lipid content and the hexane–isopropyl alcohol system quantitatively recovered vitamin D_3. Recovery of vitamin D_3 at levels greater than 5 ng/mL was in excess of 95%, using a silica cartridge for cleanup.

The second extraction, for vitamin D_3 levels below 5 ng/mL, used saponification and eliminated the fatty acids present in the plasma, which interfere with the analysis of vitamin D_3 by LC. At this stage, the vitamin D_3 was unsuitable for LC analysis.

Recoveries using alumina column cleanup were in excess of 95%.

Liquid Chromatographic Analysis

The Metalsorb HgAC column was introduced to further clean up the extract to permit LC analysis down to 1 ng/mL plasma in sheep.

The use of such a column to fractionate the extract, removed almost all of the contaminants that interfere with vitamin D_3 analysis. This column proved to be the most efficient and convenient method of cleanup. The vitamin D_3 was collected in approximately 6 mL within 13–17 min. After the sample had been collected, the flow rate was increased and allowed to run for 10–15 min. The Metalsorb column was now ready to process another sample.

The use of the RCM reverse phase column reduced the required operating pressure. Baseline separation was achieved using acetonitrile–methanol (9 + 1), giving a retention time for vitamin D_3 of 12 min. The chromatograph shown in Figure 1 illustrates the reverse phase separation of 100 ng vitamin D_3, using RCM equipped with a (5 μm, 10 cm × 5 mm) C_{18} column. Identification of vitamin D_3 in samples was achieved by reference to the standard.

Liquid Scintillation Counting Study

In the early stages of method development, after establishing the elution profiles for tritiated vitamin D_3 on the Sep-Pak cartridge, the alumina column, and the Metalsorb column, plasma lipid extracts containing 2000–3000 CPM tritiated vitamin D_3 were chromatographed as described in *Procedures*. Tracer recovery was determined by liquid scintillation counting of radioactivity. Recoveries averaged above 90%.

Recovery studies were performed on 3 different samples from different sheep. Overall recoveries averaged above 90 ± 10%. Figure 2 is a chromatogram of an extract of plasma to which 1 ng/mL of vitamin D_3 was added for recovery purposes; Figure 3 is a chromatogram of 10 ng added vitamin D_3/mL plasma extract.

Figure 4 shows the separation of a sample placed on the Metalsorb column for cleanup before reverse phase LC analysis.

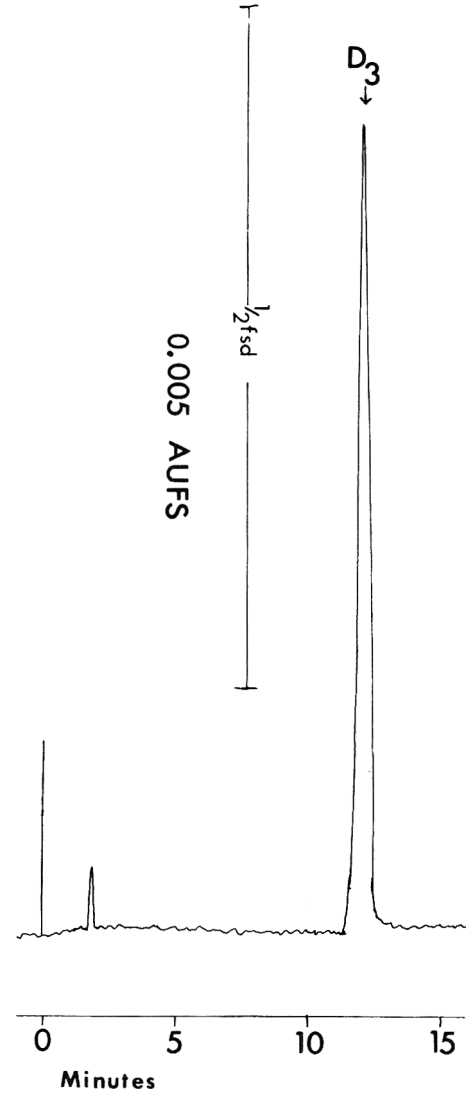

Figure 1. Chromatogram of 100 ng vitamin D_3. See text for LC conditions.

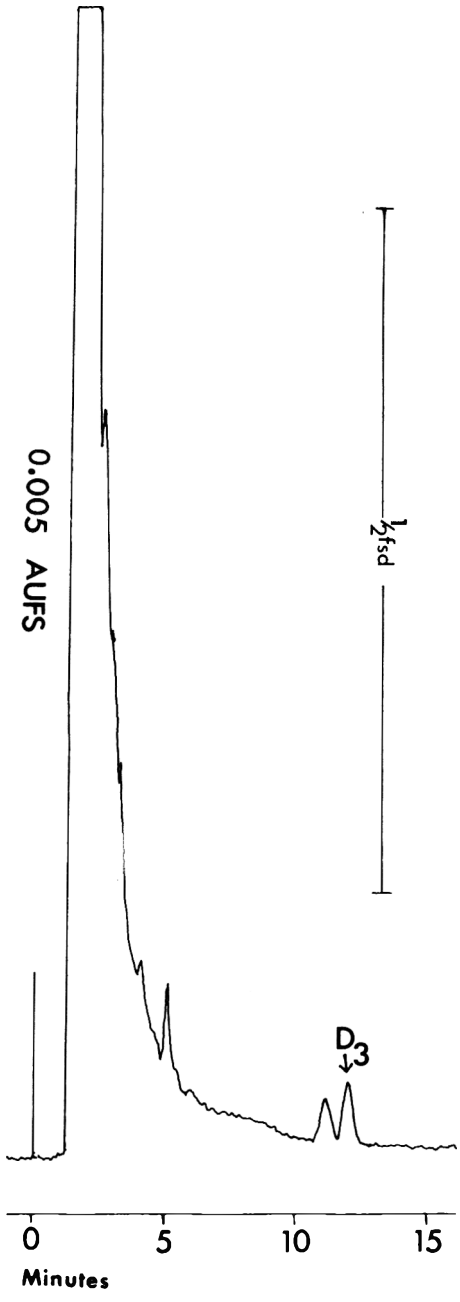

Figure 2. Chromatogram of extract of plasma showing 1 ng/mL of added vitamin D_3. See text for LC conditions.

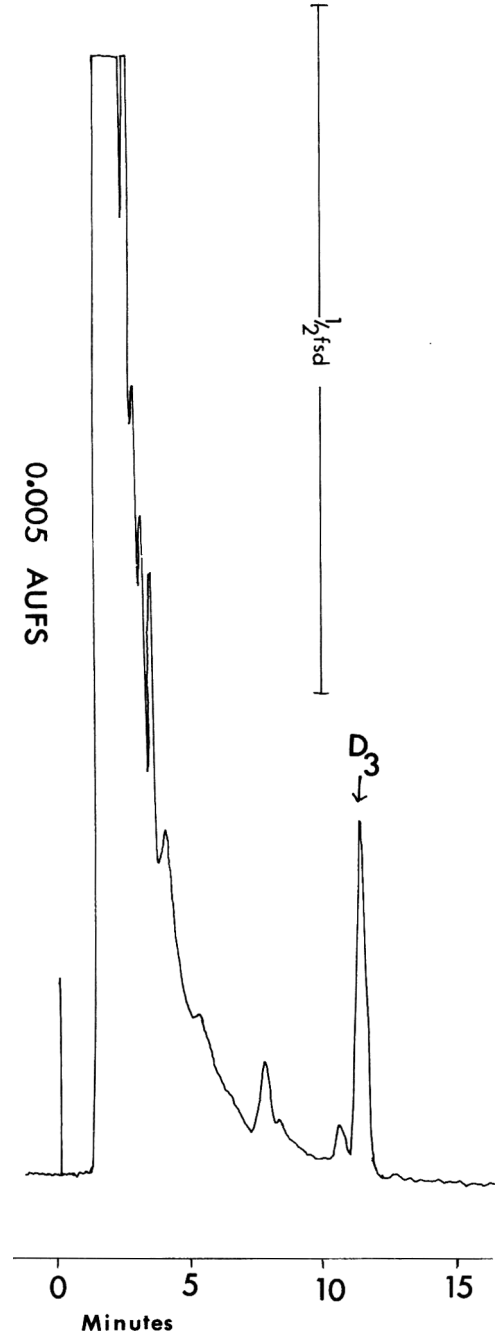

Figure 3. Chromatogram of extract of plasma showing 10 ng/mL of added vitamin D_3. See text for LC conditions.

Recoveries of vitamin D_3 from spiked sheep plasma are summarized in Table 1. The minimum detectable amount was 1 ng/mL. Table 2 indicates the amount of vitamin D_3 (ng/mL) found in different sheep over a period of 22 days after a single injection (IV) of vitamin D_3 (5×10^6 IU). Figure 5 is a chromatogram showing the presence of 41 ng/mL of vitamin D_3 in a sheep plasma sample. Figures 1, 2, 3, and 5 were all run under identical conditions. The results in Table 2 suggest that the relatively low concentration of vitamin D_3 found may be the result of metabolism of vitamin D_3 in the liver, which is in agreement with previous findings by Jones (12).

Conclusions

The cleanup and LC analysis described are efficient techniques for the determination of vitamin D_3 in sheep plasma. In this laboratory, 4 samples could be processed and analyzed per day. Due to the complexity of the substrate and the low levels required for this analysis, we felt that a multi-step purification was needed to accurately determine vitamin D_3 in sheep plasma.

Table 1. Recovery of vitamin D_3 from sheep plasma

Level,[a] ng/mL	% Recovery[b]
1	90.6 ± 10.0
5	90.8 ± 4.1
10	97.7 ± 2.3

[a]Vitamin D_3 was added to sheep plasma to give indicated concentrations.
[b]All samples equivalent to 5 mL plasma. Results are an average of triplicate analyses (mean ± RSD).

Table 2. Plasma vitamin D_3 levels in live ewes[a] administered intravenously with 5×10^6 IU vitamin D_3

Time after injection	Vitamin D_3,[b] ng/mL plasma	RSD,[b] %
0[c]	0.83 ± 0.15	18.0
1 h	7064 ± 1108	19.0
5 h	4462 ± 603	13.5
8 h	3548 ± 237	6.7
24 h	2331 ± 124	5.3
48 h	1442 ± 130	9.0
7 days	483 ± 58	12.0
10 days	326 ± 14	4.3
15 days	185 ± 20	10.8
22 days	121 ± 23	19.0

[a]The ewes in this experiment were born and raised in confinement.
[b]Mean values ± standard deviation of triplicate analyses. RSD = relative standard deviation.
[c]Saponification used before LC analysis on time 0 samples.

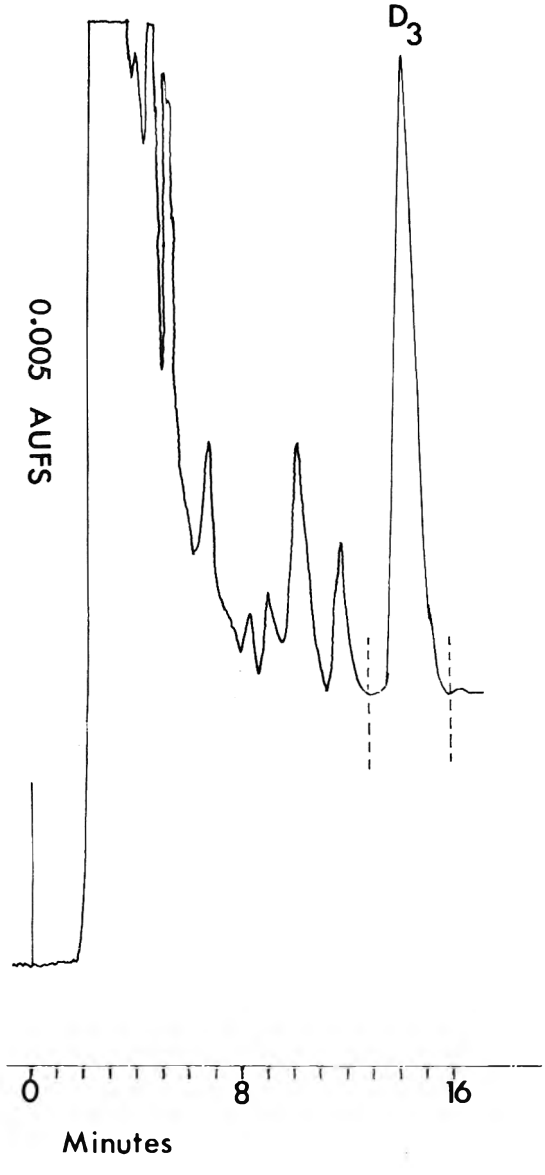

Figure 4. Chromatogram showing separation of sample injected onto Metalsorb column. See text for LC conditions.

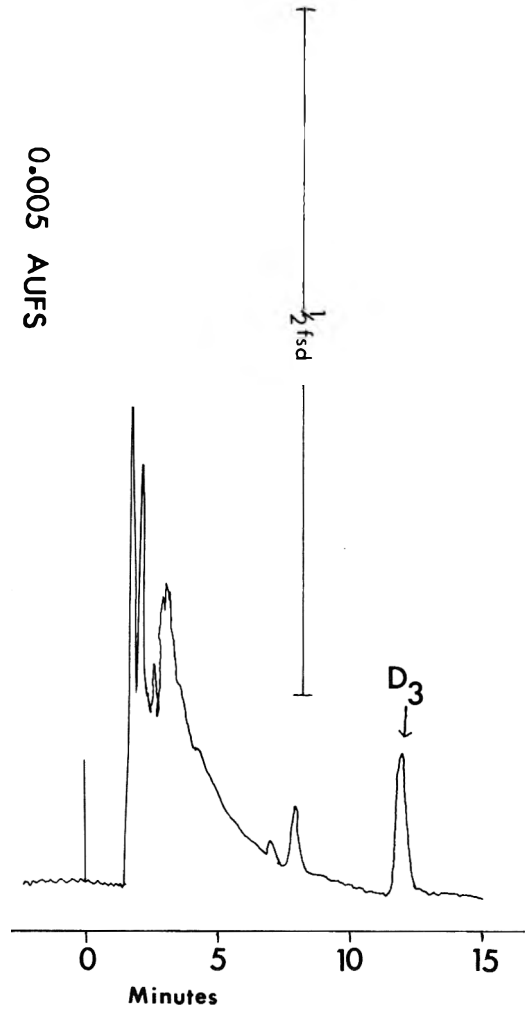

Figure 5. Chromatogram showing presence of 41 ng/mL of vitamin D_3 in sheep plasma. See text for LC conditions.

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PESTICIDE AND INDUSTRIAL CHEMICAL RESIDUES

Gas Chromatographic Determination of Pentachlorophenol in Gelatin

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A method is described for the determination of pentachlorophenol (PCP) in gelatin. The method employs acid and heat to hydrolyze the gelatin matrix, a base partition and wash for separation and cleanup, and a reacidification and extraction with hexane for direct determination of PCP, without preparation of a derivative, using gas chromatography (GC) with a 1% SP-1240DA liquid phase and a ^{63}Ni electron capture detector. Recoveries averaged 106% for fortifications between 0.02 and 1.0 ppm. The limit of quantitation is 20 ppb. The limit of detection is 4–6 ppb. The method, which has undergone a successful intralaboratory trial, is simple and rapid, and requires only general laboratory reagents and equipment. GC of the acetate derivative of PCP is used for confirmation of identity.

Pentachlorophenol (PCP) is extensively used as a fungicide, bactericide, insecticide, and herbicide. One use is its application to animal hides for protection against mold and fungal growth. If PCP-treated hides are subsequently diverted to gelatin manufacturing, PCP residues may be present. Since the major use of gelatin in the United States is in food products (1), a rapid, reliable analytical method is needed for use in the Food and Drug Administration's (FDA's) food surveillance program.

The method described is an extension of a procedure originally developed for the determination of bioincurred residues of PCP in milk and blood of dairy cattle (2). The procedure makes use of the chemical characteristics of PCP in acidic and basic media to effect separation and cleanup. PCP is determined with gas chromatography (GC) without the necessity of preparing a derivative. This approach provides an advantage in simplicity and speed over most previous GC methods, which utilize a derivative for quantitation (3). Although rapid liquid chromatographic methods are available (4) the procedure already established (2) was, in this instance, more convenient for application to gelatin.

METHOD

Apparatus

(a) *Test tubes.*—25 × 150 mm with Teflon-lined screw caps (Arthur H. Thomas Co., Philadelphia, PA).

(b) *Disposable pipets.*—Pasteur type, 23 cm long (Arthur H. Thomas Co.).

(c) *Centrifuge.*—IEC Model UV with head for spinning 25 × 150 mm test tubes at 1000 × g (International Equipment Co., Needham Heights, MA).

(d) *Shaker.*—Shaker-in-the-Round Model S-500 (Kraft Apparatus Co., Mineola, NY).

(e) *Gas chromatograph.*—Hewlett-Packard Model 5730A equipped with ^{63}Ni electron capture (EC) detector and Model 5709A linear EC control, operated under following conditions: column oven 180°C; injector 250°C; detector 350°C; carrier gas 5% methane in argon, flow rate 60 mL/min. Under these conditions ca 0.1 ng PCP gives one-half full-scale deflection (attenuation ×8).

(f) *Chromatographic column.*—1.8 m × 4 mm id glass column containing 1% SP-1240DA on 100–120 mesh Supelcoport (Supelco Inc., Bellefonte, PA). Place small plug (2–3 mm) acid-washed glass wool in detector end of column (silanized glass wool may be substituted; however, peaks will be slightly broader). Install column packed for on-column injection. Condition by purging with carrier gas at ambient temperature for 10–15 min. Program from 70 to 190°C at 1°/min, holding at 190°C for 6–8 h. Lower temperature to 180°C for PCP determination.

Reagents

Store all reagents in ground glass-stoppered or Teflon-lined screw-cap containers.

(a) *Buffer solution.*—Dissolve 13.8 g K_2CO_3 and 116.8 g NaCl in 1 L water.

(b) *Acetic anhydride.*—Reagent grade, freshly opened or distilled in all-glass apparatus (Fisher Scientific Co., Pittsburgh, PA).

(c) *PCP standard solutions.*—Dissolve 2.5 mg PCP reference standard (Standard No. 5260, Pesticide Reference Standards Section, Environmental Protection Agency, Research Triangle Park, NC) in 100 mL benzene (pesticide quality). Make appropriate dilutions with hexane.

(d) *Extraction solvent mixture.*—Hexane–isopropanol (pesticide quality) (4 + 1).

(e) *Acid-washed glass wool.*—Phosphoric acid-treated (Supelco).

Extraction and Cleanup

Weigh 2.0 g gelatin into 25 × 150 mm screw-cap test tube and add 10 mL 12N H_2SO_4. Tightly cap and heat 1 h in 100°C water bath in fume hood. Remove tube twice during hydrolysis, wrap in cloth towel, and mix sample by carefully shaking.

After 1 h, remove tube, let cool, add 10 mL hexane–isopropanol (4 + 1), and shake 2 min. Centrifuge 2 min at 1000 × g and transfer upper hexane layer to second test tube with Pasteur pipet. Repeat extraction and centrifugation 2 additional times, combining all hexane extracts in second test tube. To combined extracts, add 5 mL 1.0N KOH, cap, shake 2 min, centrifuge as before, remove upper layer with Pasteur pipet, and discard. Add 10 mL hexane wash, cap, shake, and centrifuge as before, remove hexane wash with Pasteur pipet, and discard. Add 5 mL 12N H_2SO_4, cap, and mix by carefully swirling tube. Extract 3 times by shaking 2 min each with 5, 2, and 2 mL hexane. After each extraction, centrifuge as before and transfer extracts with Pasteur pipet to 10 mL volumetric flask. Adjust to volume for GC determination.

GC Determination

Before each injection, rinse syringe by pumping 3–5 times with solution to be injected. Inject 5 μL sample solution (equivalent to 1.0 mg sample) into gas chromatograph. Measure area or height of PCP peak and determine amount of residue by comparison to peak area or height obtained from injection of known amount of PCP reference standard. To ensure valid measurement of PCP residue, size of PCP peak

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from sample and standard should be within ±10%. Make dilutions as needed. Following each injection, rinse syringe by pumping 5–10 times with hexane.

Confirmation of Identity

The identity of PCP residues may be confirmed by preparation and GC of the acetate derivative as follows: To final hexane solution of sample (or portion) add 5 mL buffer solution, shake 5 min, and then centrifuge 2 min at 1000 × g. Remove upper layer with Pasteur pipet and discard. (Remove as much of this layer as possible, since residual hexane will lower conversion of PCP to acetate.) Add 0.20 mL acetic anhydride, cap, and shake 5 min. Add 5 mL hexane, cap, shake 5 min, and centrifuge as before. Transfer upper layer with Pasteur pipet to 5 mL volumetric flask for GC. Prepare PCP acetate reference standard by treating PCP reference standard solution following same procedure. Chromatograph acetate derivative using conditions and column described in *Apparatus*. Retention time is ca 2–4 min. An OV-101 column may also be used (5). Retention time of acetate derivative prepared from PCP detected in sample should coincide with retention time of derivative prepared from PCP reference standard.

Results and Discussion

This method is based on a procedure for milk and blood of dairy cattle (2), which has been adapted to provide rapid and reliable determination of PCP in gelatin. It consists of an acid hydrolysis, base partition and wash, reacidification, and GC determination of underivatized PCP, using ^{63}Ni EC detection.

The acid hydrolysis breaks down the gelatin (collagen) matrix and provides a uniform hydrolysate from which PCP is readily extracted. No emulsions are formed. For gelatin the centrifugations are not necessary to force separation of the layers and are retained to maintain uniformity with the original procedure (2). Gelatin, in residue analytical terms, is a relatively "clean" matrix. Consequently, the base partitioning step, which was designed to separate acidic components (including PCP), also provides cleanup. Generally this cleanup is adequate. On occasion, determinations near the quantitation limit show some interferences from coextractives which may affect quantitation of PCP. These substances, which have not been identified, may be removed by shaking the final hexane solution 5–10 min with 3–5 mL concentrated sulfuric acid.

PCP is determined as the free phenol, using the commercially available 1% SP-1240DA GC liquid phase. This approach eliminates the need for derivatization and decreases overall analysis time. This liquid phase, whose composition is proprietary, is also useful for chromatography of other chlorophenols as well as nitrogen-, phosphorus-, and sulfur-containing pesticides and industrial chemicals (Borsetti, A. P., Roach, J. A. G., and Thurston, L. S., unpublished data, 1983).

Limits of determination and quantitation, based on published guidelines (6), were determined by injecting an appropriate quantity of reference standard using peak to peak instrument noise (σ) as the gross blank signal. The limit of detection (ca 3σ) is 4–6 ppb. The limit of quantitation (ca 10σ) is 20 ppb.

Some variations in detector response have been noted when PCP is determined underivatized. Cleaning the syringe by pumping with sample before injection and thoroughly rinsing the syringe with hexane after injection gives reproducible responses. With time, the GC column may show peak broadening, and on occasion, variations in reproducibility of PCP response. This condition is remedied by replacing the glass wool and a portion of packing at the injector end of the column. Detector response to PCP under the conditions noted is linear in the range of 0.02–0.10 ng PCP injected. Injections of PCP outside this range have resulted in deviations from linearity. For quantitation, closely matched sample and standard peak heights or areas should be compared. If it is necessary to confirm the identity of PCP residues, the acetate derivative may also be chromatographed on the SP-1240DA column under the conditions described for PCP. Confirmation using this approach is easy and rapid, although others may also be used (3).

Recovery of PCP through the method was evaluated by fortifying separate control samples of gelatin in triplicate at each level just before analysis. Samples fortified with PCP at 0.02, 0.05, 0.10, and 1.0 ppm gave mean recoveries (mean ± standard deviation) of 105.3 ± 3.2, 111.0 ± 5.6, 107.7 ± 7.8, and 98.7 ± 2.9, respectively. The overall mean recovery was 105.7 ± 6.5. Representative chromatograms are shown in Figure 1. The level of fortification shown is 20 ppb and represents the limit of quantitation. The additional GC responses were not identified. They occur occasionally at this level and may be removed with the additional cleanup noted above. The method has been subjected to an intralaboratory trial. Trial results (single analyst) were acceptable with mean recoveries of 77 and 104% from samples fortified in duplicate at 0.05 and 1.0 ppm PCP, respectively.

Because of the widespread use of PCP, analysts must exercise caution when examining samples for residues of PCP. All samples should be collected and stored in all-glass containers sealed with caps lined with Teflon or solvent-rinsed aluminum foil. Cleanliness of reagents and apparatus should be checked frequently by analysis of reagent blanks. Control gelatin should be analyzed before use in fortification experiments. Finally, identity of PCP residues should be confirmed using the procedure described above, or another suitable technique.

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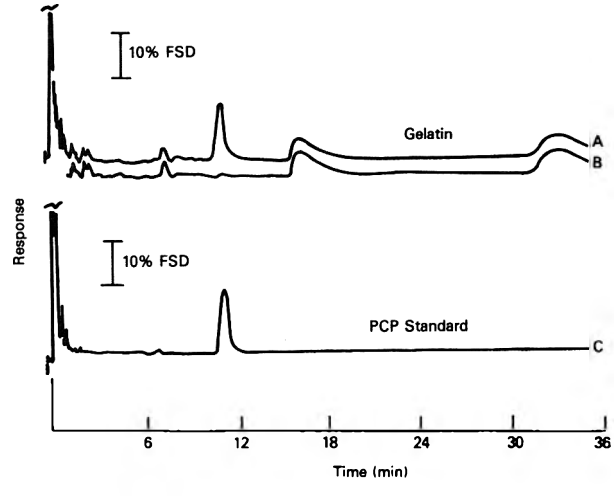

Figure 1. Gas chromatograms of (A) gelatin fortified with 20 ppb PCP, (B) gelatin control, and (C) PCP reference standard, 0.02 ng. Chromatograms A and B represent 1.0 mg equivalent sample injected.

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Atomic Absorption Spectrometric Determination of Arsenite in Water Samples by Graphite Furnace After Extraction with Ammonium *sec*-Butyldithiophosphate

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Arsenite—but not arsenate, methylarsonic acid, or dimethylarsinic acid—is extracted from water samples by 5 mL of a 0.01M hexane solution of *sec*-dibutylthiophosphate. A 10 μL aliquot of the extract is injected into an atomic absorption spectrometer with a graphic furnace for the determination of arsenic. The calibration curve is linear to 1500 ng As. Quantities of arsenic as low as 100 ng As (0.2 ppb As when contained in 500 mL) can be confidently determined. The method was tested with natural river water and synthetic river water. The relative standard deviations associated with the recoveries of arsenite from natural water and synthetic river water spiked with arsenite range from 4.8 to 8.0%. Ten to 15 arsenite determinations can be completed in 1 h.

Considerable concern exists about the effects of arsenic compounds on human health. Water is one of the important media through which humans are exposed to arsenic. Arsenite and arsenate are the most common arsenic compounds in natural waters not polluted by human activities. Because arsenite behaves in biological systems quite differently (1) and is more toxic than arsenate (1, 2), the concentration of arsenite in water samples is an important parameter for the evaluation of water quality. A routine method for the determination of arsenite in the presence of other arsenic compounds must be selective, rapid, and sensitive. The legal limit for total arsenic in potable water has been set in the United States at 0.05 mg/L (3).

Several methods are available for the determination of total arsenic in water samples, but only a few techniques are specific to arsenite (1, 4–7). Colorimetric methods suffer from interferences and lack of sensitivity (8, 9). The pH-controlled reduction of arsenite to arsine and the subsequent detection of arsenic by atomic absorption or emission spectrometry (6, 7, 10, 11) require special equipment for hydride generation. The method of separating arsenite from other arsenic compounds by liquid chromatography and determining arsenic with a graphite furnace atomic absorption (12, 13) or an inductively coupled argon plasma emission spectrometer (14) as an arsenic-specific detector have detection limits for arsenic higher than the legal limit of 0.05 mg/L. Most of these methods including polarographic procedures (5, 15, 16) are too time-consuming for the routine analysis of arsenite in a large number of samples. The concentration of arsenite by extraction with organic solutions of dithiocarbamates (17) requires rather large volumes of reagent solutions. Extraction with *sec*-butyldithiophosphate as reported in the literature (18) suffers from lengthy re-extraction procedures.

This paper describes the extraction of arsenite from aqueous solutions containing other arsenic compounds by small volumes of ammonium *sec*-butyldithiophosphate and the subsequent direct determination of arsenic in the extract by graphite furnace atomic absorption (GFAA) spectrometry. Ten to 15 determinations can be performed per hour.

METHOD

Instrumentation and Reagents

Solutions of arsenite and arsenate in distilled, deionized water were prepared from reagent grade $NaAsO_2$ and $Na_2HAsO_4 \cdot 7H_2O$. These compounds and purified dimethylarsinic acid were purchased from Fisher Scientific Co. Methylarsonic acid (99%) was obtained from Vineland Chemical Co. Reagent grade hexane was used to prepare 0.01M solutions of ammonium *sec*-butyldithiophosphate, which was synthesized as described previously (18).

All arsenic determinations were performed at 193.7 nm on a Hitachi Model 170-70 graphite furnace Zeeman atomic absorption spectrometer. The graphite cups can hold 50 μL. The nitrogen flow was adjusted to 4 L/min and 100 mL/min for the sheath gas and the internal carrier gas, respectively. The carrier gas stream was stopped during atomization. The Instrument Laboratory arsenic hollow cathode lamp was operated at 10 mA. The slit was set at 2, expansion at ×3, and response at 3. The following furnace parameters were used: drying, ramp 6 (26 A, 120°C); ashing, 20 s (120 A, 900°C); atomization, 5 s (290 A, 2500°C). The signals were recorded on a Fisher Recordall Series 5000 recorder set at 10 mV full scale (250 mm). A Glenco-Houston microsyringe was used to transfer 10 μL of the extract to the graphite cup.

Extraction and Determination of Arsenite

Pass water sample (50 to 500 mL) containing not more than 1500 ng As in form of arsenite through Nucleopore 0.47 μm filter into separatory funnel. Then add 6.0M HCl (1 mL per 100 mL sample) and 5 mL 0.01M solution of *sec*-butyldithiophosphate in hexane. Shake mixture 3 min. Discard aqueous layer. Wash hexane layer with 20 mL distilled, deionized water and then transfer to test tube. When upper portion of hexane in stoppered test tube becomes clear, withdraw 10 μL aliquot of clear solution and inject into graphite cup.

Calibration Curves

Prepare calibration curves for determination of arsenite before analysis of samples. Acidify solutions of reagent grade $NaAsO_2$ in water (50–500 mL) with 6M HCl (1 mL per 100 mL sample) and extract with 5.0 mL 0.01M solution of *sec*-butyldithiophosphate in hexane as described above. Inject 10 μL hexane extract into graphite cup. Volumes of solutions

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extracted for establishing calibration curve should be equal to volumes used in analysis of samples.

Ashing Curves

Extract 500 mL solutions of arsenite in distilled, deionized water with arsenic concentration of 200 or 400 ng/mL as described above. Inject 10 μL aliquots of extracts into graphite cup. Keep conditions for drying and atomization cycles constant. Vary temperature of ashing cycle.

Results and Discussion

The sensitivity of a method for determination of arsenite can be increased several hundred-fold when arsenite is extracted preferentially by a suitable reagent from a large volume of an aqueous sample into a small volume of an organic solvent. Ammonium *sec*-butyldithiophosphate (ABDP) in hexane has been shown to extract arsenite efficiently and selectively from aqueous solutions containing arsenite and arsenate (18) and was, therefore, chosen as the reagent for the development of a rapid, sensitive, and arsenite-specific method applicable to routine determination of arsenite in water samples.

Arsenite probably reacts with ABDP to form $[(RO)_2PS_2]_nAs(OH)_{3-n}$ (n = 1, 2, 3). Arsenic compounds containing organic groups or sulfur atoms are rather volatile and may be partially lost during the ashing cycle. When arsenic was determined by GFAA in 10 μL aliquots of the extract at various ashing temperatures, the arsenic signals were independent of temperature in the range 800 to 1000°C (Figure 1).

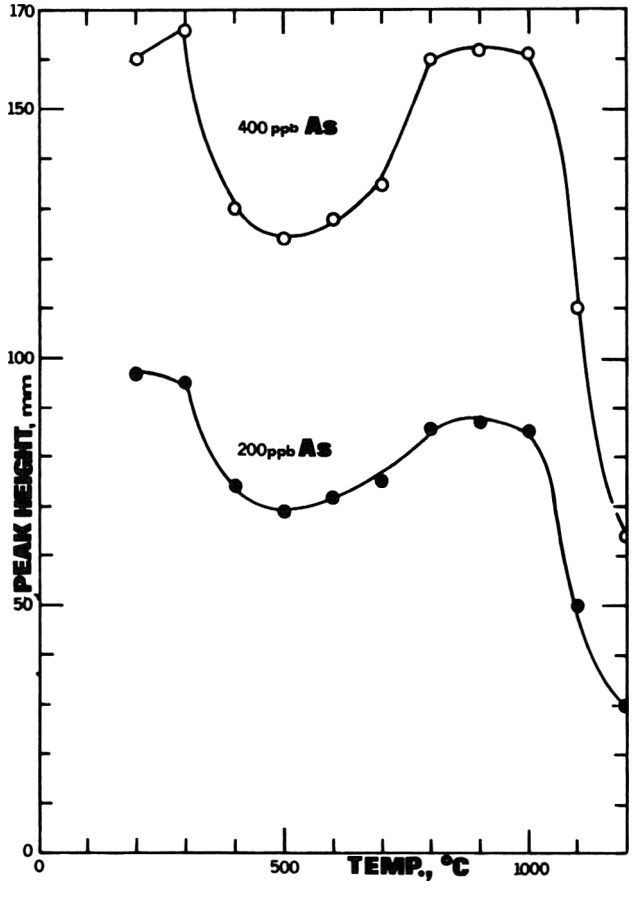

Figure 1. Influence of ashing temperature on 193.7 nm arsenic signal from solutions of sodium arsenite in water.

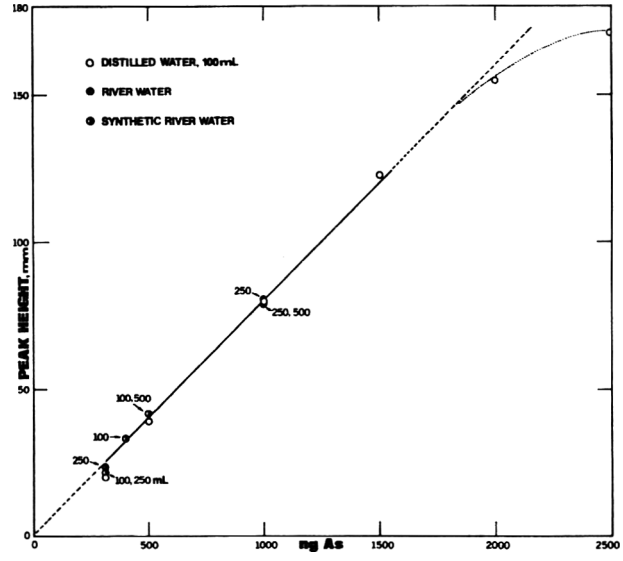

Figure 2. Calibration curve for determination of arsenite. Numbers in graph identify volumes of water samples which were extracted.

The minimum in the peak height/temperature curve is probably caused by volatilization of the extracted compound or products of its partial decomposition. The flow rate of the carrier gas also influences the signal intensity, which decreases with increasing flow rate. A flow rate of 100 mL/min was suitable. An investigation of the dependence of the signal intensity on the volume of extract injected into the graphite cup showed that the response is linear for volumes ranging from 5 to 15 μL. The signal intensities were lower than those extrapolated from the values obtained in the 5 to 10 μL experiments when more than 15 μL was injected. Acidification of the water to be extracted with HCl resulting in 0.01–4.0 molar solutions did not affect the results. Similarly, nitric acid at concentrations used for preservation of water samples or ascorbic acid (1 mg/mL) added to prevent the oxidation of arsenite (5, 19) did not influence the extraction and determination of arsenite. All further experiments were, therefore, carried out with samples 0.06M with respect to HCl, with 10 μL injections, a 900°C ashing temperature, and a carrier gas flow of 100 mL/min.

A calibration curve (Figure 2) was obtained by extracting standard solutions of arsenite in distilled, deionized water ranging in volume from 50 to 500 mL with 5 mL of the reagent solution, and analyzing 10 μL extract by GFAA. As documented in Table 1, the recovery of arsenite is quantitative (96 ± 3%) and within experimental error, independent of the sample volume from which arsenite is extracted. To make the calibration curve applicable to various sample volumes, the quantity of arsenic in the water sample expressed in nanograms was plotted vs the peak height of the GFAA signal (Figure 2). The calibration curve is linear at least to 1500 ng As. The responses at 2000 ng and 2500 ng As are considerably lower than expected from the extended linear calibration curve. Quantities of arsenic as low as 100 ng can still be determined. If the availability of a water sample allows 500 mL to be used for the analysis, arsenite can be determined at a concentration as low as 0.2 ppb As. Figure 2 also contains results of determinations of arsenic in a Texas river water sample and a synthetic river water sample spiked with arsenite. The average peak height of the signal generated by 1 ng As is 0.082 ± 0.002 nm when all the signals except those corresponding to 2000 ng and 2500 ng As are averaged. The least squares slope of the line defined by the points corre-

Table 1. Recovery of arsenite from distilled, deionized water solutions by extractions with ABDP/hexane followed by determination of arsenic by GFAA

Sample vol., mL	As (arsenite) in sample, ng	As found, ng	Rec., %	RSD[a]
50	250	221	88.4	6.5
	500	470	94.0	5.8
	1000	960	96.0	5.2
100	250	245	98.0	5.2
	400	390	97.5	5.1
	500	501	100.2	4.8
250	250	245	98.0	5.5
	500	492	98.5	5.2
	750	735	98.0	5.4
500	250	233	93.0	8.2
	500	475	95.0	6.5
	1000	950	95.0	6.7

[a]Obtained from 3 experiments each.

Table 2. Recovery of arsenite from Texas river water sample and synthetic river water sample[a]

Sample vol., mL	As (arsenite) added to Sample, ng	As found, ng	Rec., %	RSD[b]
250[b,c]	0	0	—	—
	250	255	102.0	4.8
	500	527	105.5	5.1
	750	775	103.3	6.2
100[a]	250	261	104.4	4.9
	400	415	103.7	5.5
	500	512	102.4	5.7
250[a]	250	252	101.0	6.0
	500	537	107.5	6.8
	750	777	103.7	5.1
500[a]	250	260	105.5	7.3
	500	540	108.0	8.0
	1000	1055	105.5	6.3

[a]Synthetic river water containing in mg/L: Na 85, K 5.0, NH_4 2.0, Mg 17, Ca 80, Cl^- 277, SO_4^{-2} 69, phosphate 5.4.
[b]Obtained from 3 experiments each.
[c]Texas river water containing in mg/L: Al 0.8, Ca 5.0, Fe 0.3, K 3.7, Mg 5.3, Na 42, Cl^- 55, F^- 0.4, NO_3^- 2.1, phosphate 0.9, SO_4^{-2} 11.

sponding to 250, 500, 1000, and 1500 ng As is 0.0825, the intercept is −1.6, and the correlation coefficient is 0.999. When the data for the spiked river water and synthetic river water samples were evaluated similarly, the least squares slope was 0.0767, the intercept was 2.7, and the correlation coefficient was 0.998.

To establish the suitability of this method for determination of arsenite in natural water samples, a Texas river water and a synthetic river water, to which known quantities of arsenite had been added, were examined. The composition of these water samples is given in footnotes to Table 2. The synthetic river water was formulated to examine the influence of higher concentrations of Na, Mg, Ca, Cl, sulfate, and phosphate on the extraction and determination of arsenite. The results are summarized in Table 2. The recovery of arsenite is quantitative with an average recovery of 104 ± 2%. Arsenite is stable in the hexane extract at least 24 h. The extraction and determination of arsenite is not hindered by the cations and anions commonly found in fresh waters. When experiments were performed with tap water, no arsenite was found even after sodium arsenite was added. Because recovery of arsenite added to boiled tap water was quantitative, residual chlorine from the chlorination of the water supply is very likely responsible for the oxidation of arsenite to arsenate.

To ascertain that only arsenite is extracted by ABDP, 2 different 250 mL samples of synthetic river water containing (quantities for second sample in parentheses) 500 ng (1.0 μg) arsenite, 100 μg (150 μg) arsenate, 1.0 μg (2.5 μg) methylarsonic acid, and 1.0 μg (2.5 μg) dimethylarsinic acid were analyzed. In both cases arsenite was recovered quantitatively (RSD 4.8%).

This method was applied to an arsenic-containing well water sample. The results obtained for arsenite by the extraction procedure agree well with those achieved with the hydride generation technique (Table 3). These experiments were carried out to check the usefulness of this method for the determination of arsenite in preserved and unpreserved naturally arsenic-containing water samples.

Conclusion

The extraction of arsenite from samples of fresh water ranging in volume from 50 to 500 mL by 5 mL of a 0.01M solution of ammonium *sec*-butyldithiophosphate and injection of 10 μL of the extract into the graphite cup of a graphite furnace atomic absorption spectrometer constitutes an accurate, precise, rapid, and selective method for the determination of arsenite. Arsenate, methylarsonic acid, dimethylarsinic acid, and the cations and anions commonly found in fresh water do not interfere with the determination. Arsenite at concentrations as low as 0.2 μg/L can be determined reliably when 500 mL sample is extracted. Ten to 15 determinations can be carried out per hour.

Acknowledgments

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Table 3. Comparison of results of arsenic determinations (± SD) in preserved and unpreserved well water sample

Sample	Arsenic concns, μg/L			
	Arsenite/ABDP[a]	Arsenite HG[a]	Total As HG[a]	Total As GFAA[a]
Preserved with concd HNO_3 (7 mL/L)	181 ± 6	168 ± 5	222 ± 6	232 ± 4
Preserved with ascorbic acid (1% by wt)	218 ± 7	210 ± 10	218 ± 5	229 ± 6
Unpreserved	5.0 ± 0.2	6.0 ± 0.5	194 ± 2	201 ± 4

[a]ABDP: extraction method described in this paper.
HG: reduction of arsenite (or arsenite and arsenate) to arsine and determination of arsenic by DC helium emission spectrometry (20).
GFAA: determination by direct injection into graphite cup.

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Gas Chromatographic Determination of Ametryn and Its Metabolites in Tropical Root Crops

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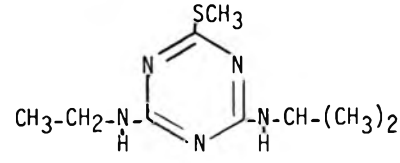
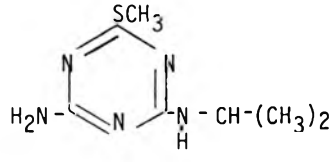
Residue analysis of the herbicide ametryn (2-methylthio-4-ethylamino-6-isopropylamino-*s*-triazine) is widely known but an analytical method for determining its metabolites has not yet been reported in the literature. A method has been developed for the extraction and determination of ametryn and 3 metabolites, 2-methylthio-4-amino-6-isopropylamino-*s*-triazine (GS-11354), 2-methylthio-4,6-diamino-*s*-triazine (GS-26831), and 2-methylthio-4-amino-6-ethylamino-*s*-triazine (GS-11355) in taniers, yams, cassava. Residues were extracted from crops with ethyl acetate–toluene (3 + 1 v/v), using a Polytron homogenizer and anhydrous sodium sulfate added for drying. The extracts were cleaned up by automated gel permeation chromatography on Bio-Beads SX-3 gel in the same solvent system. Quantitative determination was performed by gas chromatographic (GC) analysis on a column packed with 5% DEGS-PS on 100–120 mesh Supelcoport using either an N-P detector or a flame photometric detector (FPD) in the sulfur mode. Minimum detection by the flame photometric detector is 10 ng each for ametryn, GS-11354, and GS-11355 and 21 ng for GS-26831; by the N-P detector, 0.3 ng of each component gives easily quantitatable peaks. On a parts per million basis, starting with 25 g sample, the FPD detected a minimum level of 0.04 μg/g each for ametryn, GS-11354, and GS-11355, and 0.08 μg/g for GS-26831. The N-P detector could detect 0.0024 μg/g for all 4 compounds. In addition to superior sensitivity, instrumental conditions allowed the complete separation of components in 10 min, for the N-P detector; more than 30 min was required for the FPD. Recoveries from fortified crops ranged from 67 to 111% at levels of 0.1–1.0 μg/g.

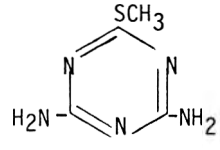
Ametryn (2-methylthio-4-ethylamino-6-isopropylamino-*s*-triazine) (I) is a pre- and post-emergence herbicide (1) for general and selective use. It has been used as an effective herbicide in croplands for growing pineapple, banana, sugarcane, etc. The potential use for its selective application has wide interest to crop growers. Although residue analysis of the parent herbicide is widely known (2, 3), an analytical method for determining its metabolites has not yet been reported in the literature. In addition, there is great interest in ametryn for weed control in fields of taniers, yams, and cassava, the tropical root crops grown in Puerto Rico and elsewhere, and a need exists to gather residue data for submission to regulatory agencies for tolerance and registration purposes. This report presents a facile method developed for the extraction and determination of ametryn and 3 metabolites, 2-methylthio-4-amino-6-isopropylamino-*s*-triazine (GS-11354) (II), 2-methylthio-4,6-diamino-*s*-triazine (GS-26831) (III), and 2-methylthio-4-amino-6-ethylamino-*s*-triazine (GS-11355) (IV) in taniers, yams, and cassava.



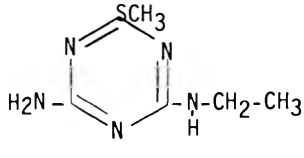
I. AMETRYN



II. GS-11354



III. GS-26831



IV. GS-11355

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Florida Agriculture Experiment Station Journal Series No. 5191.

METHOD

Reagents

Pesticide-grade solvents were used throughout.

(a) *Standards.*—Ametryn, obtained from U.S. Environmental Protection Agency; GS-11354, GS-26831, and GS-11355, provided by Ciba-Geigy Corp., Box 18300, Greensboro, NC 27409.

(b) *Standard solutions.*—12 mg ametryn/25 mL ethyl acetate–toluene (3 + 1 v/v); 9 mg GS-11354/25 mL ethyl alcohol; 5.9 mg GS-11355/25 mL ethyl alcohol; 4.4 mg GS-26831/25 mL ethyl alcohol. *Working solutions.*—Appropriate dilutions were made as needed in ethyl acetate–toluene (3 + 1).

Apparatus

(a) *Gas chromatograph.—(1)* Hewlett-Packard Model 5880A gas chromatograph equipped with nitrogen-phosphorus detector. Parameters: glass column 60 cm × 2 mm id packed with 5% DEGS-PS on 100–120 mesh Supelcoport (Supelco, Inc., Bellefonte, PA 16823); gas flows (mL/min): carrier nitrogen 30, air 50, hydrogen 5; temperatures (°C): column oven 185, detector 300. Voltage to detector was adjusted for ca 10% offset at attenuation 2^2. *(2)* Hewlett-Packard Model 5730 gas chromatograph equipped with flame photometric detector (sulfur mode). Parameters: glass column 83 cm × 4 mm id packed with 5% DEGS-PS on 100–120 mesh Supelcoport; gas flows (mL/min): carrier nitrogen 40, hydrogen 125, air 100, and oxygen 25; temperatures (°C): column oven 200, detector 200.

(b) *Gel permeation apparatus.*—Autoprep Model 1001 GPC (Analytical Biochemistry Laboratories, Inc., Columbia, MO), equipped with 60 cm × 2.5 cm id glass column packed with 200–400 mesh Bio-Beads S-X3 resin (Bio-Rad Laboratories, Richmond, CA) compressed to bed length of 56 cm. Eluting solvent was ethyl acetate–toluene (3 + 1) pumped at constant flow rate of 2.5 mL/min. Five mL standard solution was applied to Autoprep 1001 GPC apparatus and twenty-three 10 mL fractions were collected to determine elution profile.

Preparation of Sample

Spike 12.5 or 25 g representative sample of chopped crop with ametryn and/or II, and/or III, and/or IV, and then extract, after addition of ca 10 g anhydrous sodium sulfate, with 100 mL ethyl acetate–toluene (3 + 1), using Polytron homogenizer 1 min at medium speed. Decant supernate and save. Extract residue twice more with 100 mL same solvent and filter with suction into 500 mL suction flask. Rinse filter cake with ca 50 mL same solvent. Pool extracts and concentrate by rotary evaporation at 35–40°C. Finally dilute to 10 ml with ethyl acetate–toluene (3 + 1).

For cleanup, process samples with gel permeation system using Autoprep 1001 GPC system, precalibrated with standards. (We detected these compounds in the 171–200 mL fractions.)

Concentrate collected fractions almost to dryness and then adjust to desired volume by reconstituting in ethyl acetate–toluene (3 + 1) for GC analysis.

Results and Discussion

Ametryn (I) may be metabolized in vivo to compounds II, III, and IV. Possibilities of other metabolites may also exist. As of now, for regulatory action in the United States, EPA requires residue data for the parent herbicide and these 3 metabolites.

The procedure presented here consists of an extraction using a mixed solvent, ethyl acetate–toluene (3 + 1), followed by cleanup with gel permeation chromatography in the same solvent. The method takes advantage of a miscible pair comprising a polar and a nonpolar solvent, which solubilizes and extracts the chemicals present at the residue levels in a single step. Furthermore, the cleanup technique allows unattended processing of 23 samples. Quantitative analysis can be performed on a gas chromatograph equipped with a FPD detector (sulfur mode) or an N-P detector. The procedure was successfully applied to the residue analysis of the chemicals in taniers, yams, and cassava.

Figure 1 shows the chromatogram of standards, and Figures

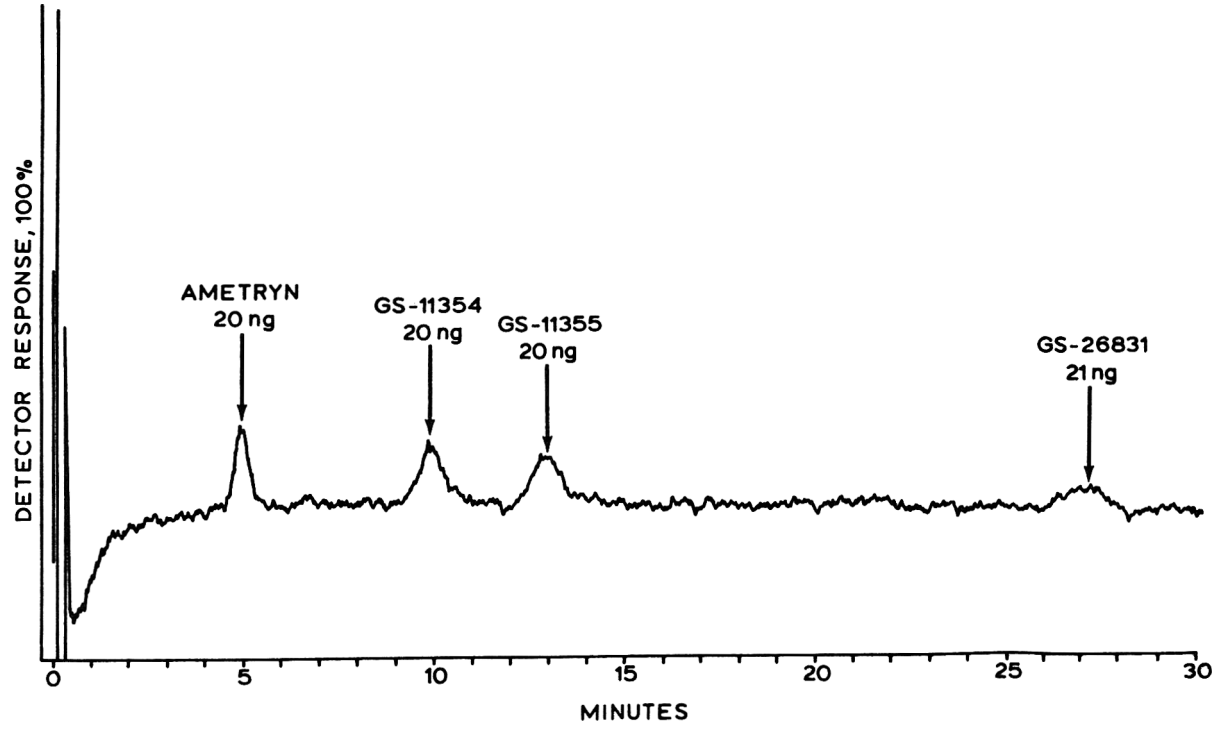

Figure 1. Chromatogram of mixture of standards, FPD detector-sulfur mode.

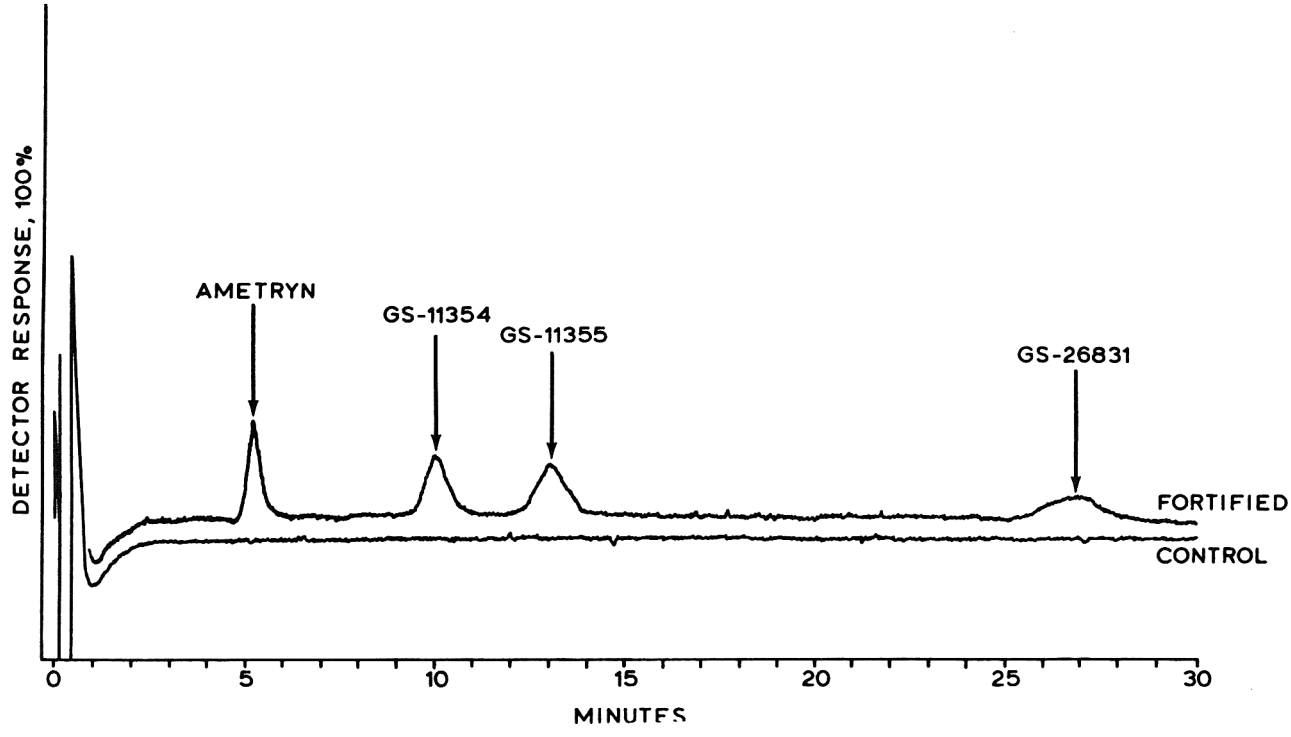

Figure 2. Chromatograms of control and fortified (0.2 μg/g) taniers; 25 g crop, 5 μL/mL injection; FPD detector-sulfur mode.

2, 3, and 4 represent the same for taniers, yams, and cassava respectively, fortified with ametryn and the metabolites, on the FPD (sulfur mode) detector. The chromatograms obtained on the N-P detector are shown in Figures 5–8.

The limits of detection on the FPD were 10 ng each for ametryn, GS-11354, and GS-11355, and 21 ng for GS-26831. On the N-P detector, 0.3 ng of each of the components gives easily quantitable peaks at attenuation 2^2. On a parts per million basis, starting with a 25 g sample, and finally injecting 10 μL/1.0 mL total sample volume, the FPD detected a minimum level of 0.04 μg/g each for ametryn, GS-11354, and GS-11355, and 0.08 μg/g for GS-26831. Under these conditions, the N-P detector could detect 0.0024 μg/g for all 4 components. In addition to superior sensitivity, instrumental conditions allowed the complete separation of components in 10 min, for the N-P detector; more than 30 min was required for the FPD. Recoveries from fortified taniers, yams, and cassava ranged from 67 to 111% at levels of 0.1–1.0 μg/g.

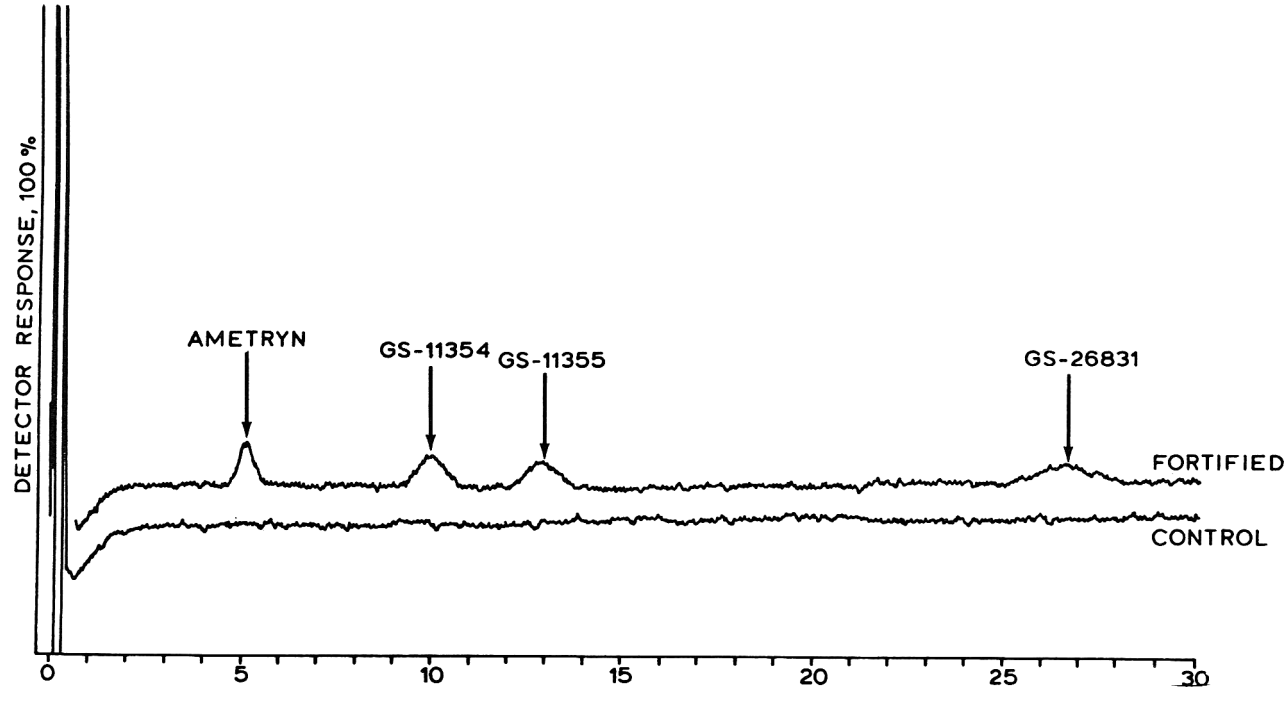

Figure 3. Chromatograms of control and fortified yams; 0.12 μg/g ametryn, GS-11354, and GS-11355, and 0.2 μg/g GS-26831; 25 g crop; 5 μL/mL injection; FPD detector-sulfur mode.

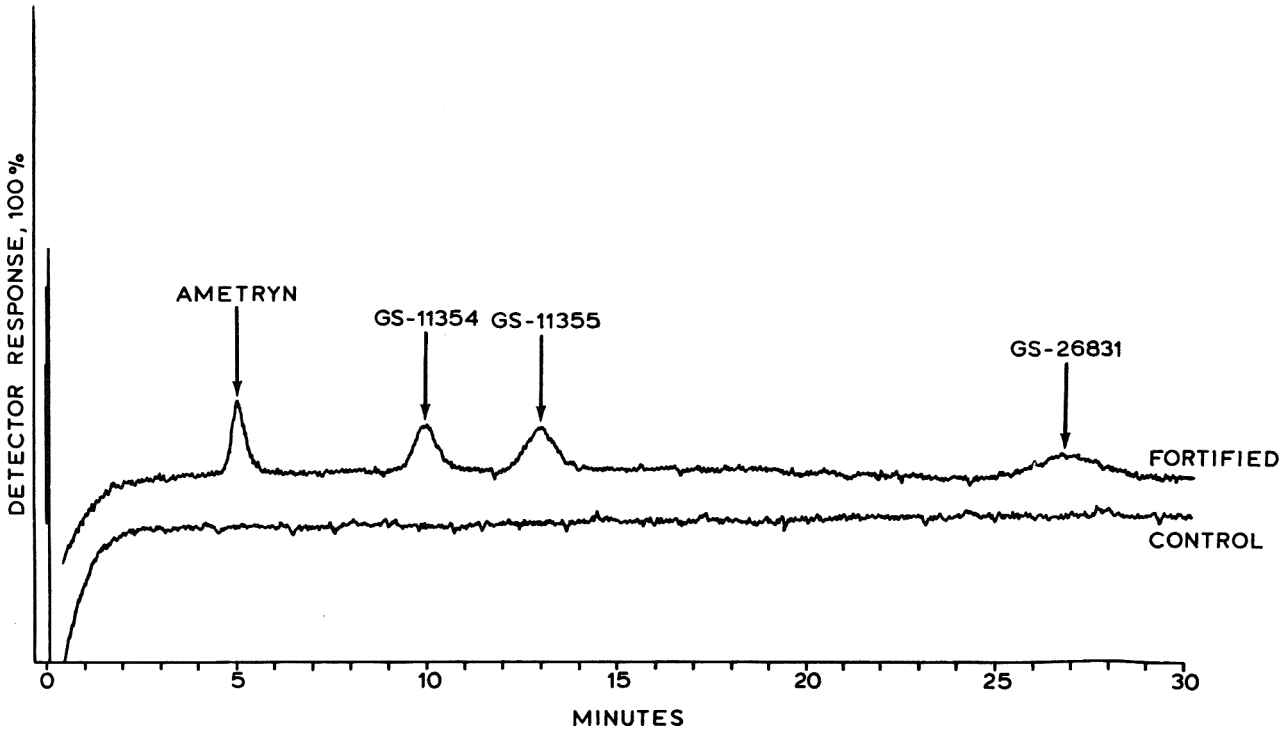

Figure 4. Chromatograms of control and fortified (0.2 μg/g) cassava; 25 g crop; 5 μL/mL injection; FPD detector-sulfur mode.

Conclusion

A method is presented for a one solvent-pair extraction followed by a single step gel permeation cleanup and GC analysis of ametryn and its dealkylated products in the tropical root crops, taniers, yams, and cassava.

Acknowledgments

The authors thank Ciba-Geigy Corp. for the dealkylated ametryn standards, and Rafael Montalvo-Zapata of the University of Puerto Rico for the tropical root crops. This research was conducted to gather residue data for IR-4 project Nos. 473, 489, and 490.

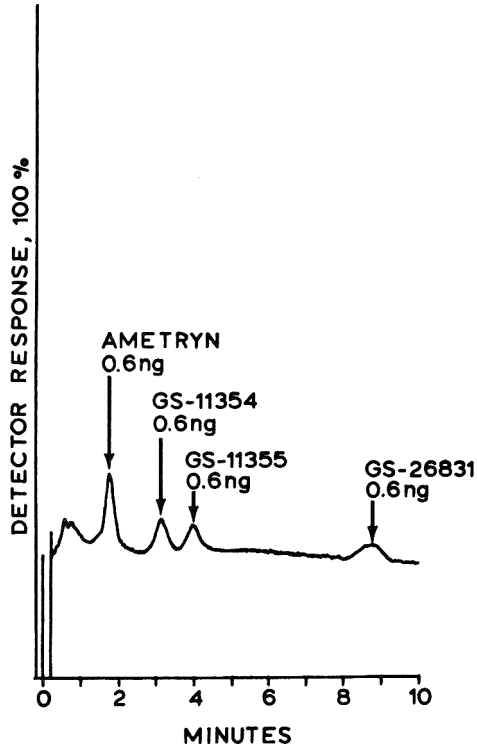

Figure 5. Chromatogram of a mixture of standards, N-P detector.

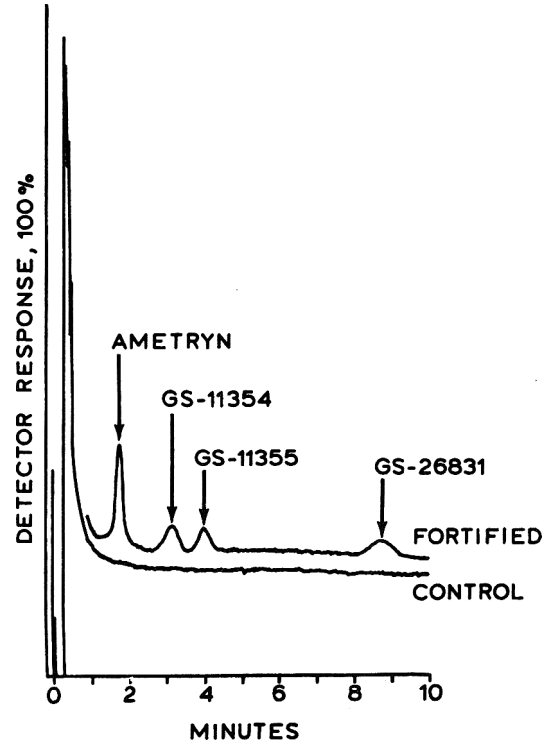

Figure 6. Chromatograms of control and fortified (0.37 μg/g) taniers; 12.5 g crop, 10 μL/30 mL injection; N-P detector.

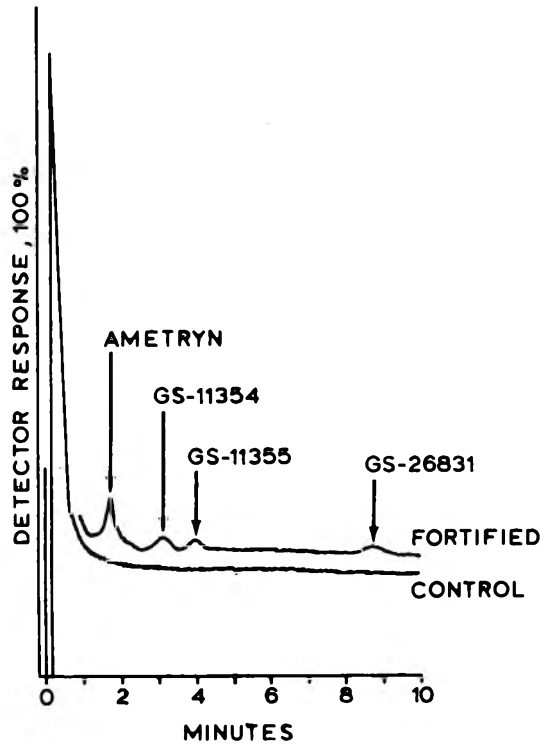

Figure 7. Chromatograms of control and fortified (0.1 μg/g) yams; 12.5 g crop, 10 μL/30 mL injection; N-P detector.

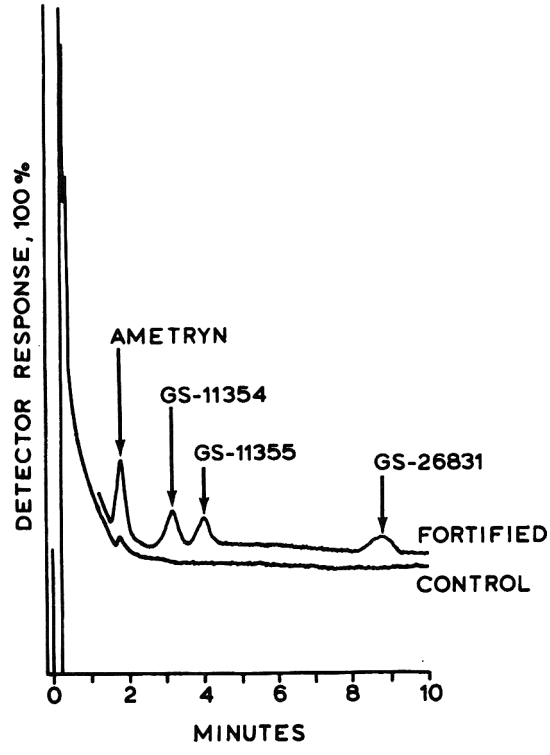

Figure 8. Chromatograms of control and fortified (0.12 μg/g) cassava; 12.5 g crop; 10 μL/30 mL injection; N-P detector.

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(3) *Analytical Methods for Pesticides and Plant Growth Regulators* (1972) Vol. VI, Academic Press Inc., New York, NY, p. 587

Multiresidue Gas Chromatographic Method for Determining Organochlorine Pesticides in Poultry Fat: Collaborative Study

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A gas chromatographic electron capture detector method is described for the quantitative determination of organochlorine pesticide residues in poultry fat. The samples are rendered and cleaned up using automated gel permeation chromatography. The collaborative samples consisted of 10 fortified samples and one incurred residue sample, all in duplicate. Fortification levels ranged from 0.15 to 1.0 ppm for α-BHC, lindane, *cis*- and *trans*-chlordane, octachlor epoxide, *o,p'* and *p,p'*-DDT, *p,p'*-DDE, *p,p'*-TDE, hexachlorobenzene, heptachlor epoxide, dieldrin, endrin, methoxychlor, mirex, and toxaphene. The average recovery was 91.9% with a range of 81–102%. The ranges of coefficients of variation were: CV_o = 3.39–14.79%; CV_L = 0–16.6%; and CV_x = 5.82–19.0%. The results indicate accuracy and precision comparable to other official methodology. The method has been adopted official first action.

This report of the Associate Referee, J. A. Ault, was presented at the 97th Annual International Meeting of the AOAC, Oct. 3–6, 1983, at Washington, DC.

The recommendation of the Associate Referee was approved by the General Referee and Committee E and was adopted by the Association. See the General Referee and Committee reports, *J. Assoc. Off. Anal. Chem.* (1984) **67,** March issue.

Received July 12, 1983.

There are currently no official methods for analyzing poultry fat for nonionic organochlorine residues other than polychlorinated biphenyls (PCBs) (1). Current pesticide multiresidue methodology used in government and industry laboratories for screening poultry fat includes Florisil column (1), microalumina column (unpublished), and gel permeation chromatographic (GPC) (2–5) cleanup, with action-level residues from the alumina or GPC procedures confirmed by AOAC method **29.001–29.018** (1). This duplication of effort could be eliminated through collaborative study and official recognition of one of the screening methods.

Gel permeation chromatography uses size exclusion to separate residues from high molecular weight lipids on the basis of molecular size. A molecular size separation is generally appropriate when the molecular weight of the pesticide ranges between 200 and 400, and lipid molecular weights are ≧600. Most chlorinated pesticides with high molecular weights are compact molecules containing a high percentage of chlorine and are thus efficiently separated from lipids on the gel permeation column.

Numerous combinations of elution solvents and gels have

been reported in the literature (2–5). For the specific separation of chlorinated pesticides from animal fats, the most effective system was found to be BioBeads® SX-3 (3% crosslinked, styrene-divinylbenzene copolymers) eluted with an equal mixture (v/v) of methylene chloride and cyclohexane.

Organochlorine Pesticide Residues in Poultry Fat Gel Permeation Chromatographic Method First Action

Principle

Liq. poultry fat is dissolved in CH_2Cl_2–cyclohexane (1 + 1). Residues are sepd from lipid by gel permeation chromatgy (GPC), and identified and measured in concd eluates by GC-EC detection.

Reagents and Apparatus

(a) *Solvents.*—CH_2Cl_2, cyclohexane (C_6H_{12}), isooctane. Must meet criteria in **29.002.**

(b) *Gel permeation chromatographic system (GPC).*—AutoPrep gel permeation chromatograph (Analytical Bio-Chemistry Laboratories, Inc., PO Box 1097, Columbia, MO 65205) or equiv. with 60 g BioBeads SX-3 resin, 200–400 mesh, in 60 × 2.5 cm id chromatgc tube, ca 48 cm bed length, elution solv. CH_2Cl_2–C_6H_{12} (1 + 1). Flow rate calibrated to 5.0 mL/min, operating pressure 7–10 psig.

(c) *Flash evaporator.*—Rotary evapn system (Calab, Emeryville, CA, or equiv.) with 30° H_2O bath.

(d) *Gas chromatograph.*—EC detector (^{63}Ni) operated as in **29.008.** 1.85 m × 4 mm id column packed with 1.5% SP2250/1.95% SP-2401 on 100–120 mesh Supelcoport (Supelco, Inc., Bellefonte, PA 16823). Operating conditions: injector 250°, column 200°, detector 350°, N flow 60–80 mL/min. Condition column 2 days at 250°.

GPC Calibration Procedure

Chromatographic system will fractionate effluent from column into 23 equal fractions for elution calibration. (It is necessary to det. correct "dump" and "collect" times for desired residues, as function of pump flow rate.) Before fractionating, check flow rate with SX-3 gel column connected and adjust flow to 5.0 ± 0.2 mL/min (start pump ≥ 5 min before measuring flow to let flow equilibrate and improve accuracy). Fractionate vol. of eluant from 150 to 320 mL to ensure residue collection. Evap. fractions, resuspend in isooctane, and analyze to det. collection vol. for samples (fractionation procedure is described in instrument manual). Check calibration for quant. recovery with 2.0 g corn oil fortified with relevant compds.

Preparation of Sample

Place ca 40 g representative poultry fat sample in glass funnel (8.0 cm) with glass wool plug. Place funnel in flint glass bottle or 250 mL beaker on hot plate at ≤110° until fat ceases to drip. Mix thoroly.

Determination

Weigh 2.0 g liq. fat into 10 mL vol. flask. (Fortifications may be made here with stds dild in CH_2Cl_2–C_6H_{12} (1 + 1).) Dil. to 10 mL with CH_2Cl_2–C_6H_{12} (1 + 1) and mix thoroughly. Centrf. or filter if particulate matter is visible. Use ca 7 mL sample to load sample loops on precalibrated GPC (5 mL aliquot (1.0 g equiv. of sample) is accepted into sample loop). Process thru GPC system using dump/collect times from fractionation procedure and collect eluate in 250 mL boiling flask. Rotary-evap. to just dry at ≤30°. Transfer quant. with 10 mL isooctane or equiv. GC-EC compatible solv. into precalibrated culture tube. Adjust vol. under gentle, dry N stream to 5.0 mL.

Gas Chromatography

Inject 3–6 μL aliquots into a gas chromatograph operated as in **29.008** with ^{63}Ni ECD. Measure peaks (ht or area). If necessary, dil. sample to give residue concn approx. that of std soln. Inject aliquot of pesticide std soln (in same solv. as sample) and again measure peaks.

Each residue, ppm = concn std (μg/mL)
× (peak size sample/peak size std) × (μL std/μL sample)
× (diln vol/1.0 g sample)

(Note: Since only 5 mL of original 10 mL vol. contg 2.0 g fat is injected into GPC sample loop, only 1.0 g fat is analyzed.)

Collaborative Study

Ten poultry fat samples were prepared and fortified with combinations of the 16 nonionic chlorinated hydrocarbons studied. An incurred residue sample was also obtained for inclusion in the study. Control tissue interferences were avoided wherever possible when fortifications were made. For those interferences that were impossible to avoid, fortifications were 20–100 times higher than background. Background residues found in the control tissues were as follows: dieldrin 4–50 ppb, *p,p'*-DDE 2–10 ppb, α-BHC 0.6–6 ppb, and heptachlor epoxide 1.4–1.7 ppb. All samples were submitted as blind duplicates to the 12 participating laboratories. Analytical standards were also supplied to the collaborators. All participating laboratories used automated GPC equipment to clean up the samples. Nine laboratories submitted results to the Associate Referee.

Results and Discussion

Of the 12 sets of samples mailed to collaborators, 9 laboratories reported results. The 3 collaborators not reporting data experienced increased workloads and, in one instance, a hospitalization that prevented analysis of the samples.

Elution profiles for the organochlorine pesticides studied are shown in Table 1. Two of the collaborators deviated from the specified method. Laboratory 1 did not maximize the GC performance, and thus missed many of the low-level fortifications. Laboratory 1 standard peak heights were on the order of 1–5 cm and resulted in a nonlinear interpretation of the sample residues, especially at higher concentrations, because many of the sample peak heights were in the 10–20 cm range. Laboratory 2 used a 50 g SX-3 GPC column eluted with a 15 + 85 (v/v) mixture of methylene chloride–cyclo-

Table 1. Organochlorine pesticide elution profiles through 60 g SX-3 gel, ca 48 cm bed length × 2.5 cm id column

Organochlorine pesticide	Elution vol. (mL) with CH_2Cl_2–C_6H_{12} (1 + 1)
Aldrin	160–240
α-BHC	180–240
Lindane	170–240
cis-Chlordane	160–250
trans-Chlordane	160–230
Dieldrin	160–230
p,p'-TDE	160–230
p,p'-DDE	160–220
o,p'-DDT	160–220
p,p'-DDT	160–230
Endrin	160–220
Heptachlor epoxide	160–230
Hexachlorobenzene	190–240
Methoxychlor	160–230
Mirex	160–230
Octachlor epoxide	150–200
Toxaphene	160–230

hexane, which, in the Associate Referee's experience, allows more fat carryover into the collected residue fraction. The majority of results reported by Laboratory 2 were low, but with high values consisting of late GC-eluting compounds, which indicates that fat carryover may have been the cause. Data from Laboratories 1 and 2 are reported, but were not used for the statistical analysis.

Laboratory 11 inadvertently received 2 unspiked poultry fat samples instead of the toxaphene fortification duplicates.

All other reported results were analyzed statistically according to Steiner's analysis of variance (6). Single determinations were averaged and analyzed for within-laboratory (repeatability) standard deviation (S_o) and coefficient of variation (CV_o), between-laboratory standard deviation (S_L) and coefficient of variation (CV_L), and reproducibility standard deviation (S_x) and coefficient of variation (CV_x). Data were next subjected to Dixon's test for outlying individual values and laboratory averages. If an outlying laboratory was identified from the laboratory average Dixon test, it was eliminated and the entire statistical analysis was recalculated using the remaining data. Cochran's test was used to test the homogeneity of the data at the 5% significance level. No laboratory data were omitted on the basis of the Cochran test alone. In one instance, the Cochran test was used in conjunction with Dixon's test of the individual results to eliminate a reported data set. The overriding factors in this decision were (a) a range of variability that approached the average level of residue reported by all laboratories; (b) identification of the high value as a Dixon outlier at the 98% confidence interval (α = 0.02); and (c) occurrence of the Cochran outlier in the incurred residue sample where the true level of residue was uncertain.

Laboratory 9 presented a problem in data interpretation because 12 of 33 reported averages were identified as Dixon outliers in the statistical analysis. The collaborator reported having problems with the automated GC equipment. A first run produced chromatograms, but did not print data. The second run with a new printer was successful, but some of the rerun chromatograms contained contamination peaks not observed in the initial analysis, one at a retention time matching α-chlordane and another late-eluting peak which interfered on subsequent GC injections. Because of this, those laboratory average reported values identified as Dixon outliers at the 99, 98, or 95% confidence interval (α = 0.01, 0.02, or 0.05) where eliminated from the statistical analysis.

The reported results from all collaborators are given in Tables 2–10. The average percent recovery for all fortified samples was 91.8% with a range of 81.3 to 102%. The following means and ranges were calculated from the statistical data: repeatability, $\%CV_o$: $\bar{x}$ = 9.17%, range = 3.39–14.79%; between-laboratories, $\%CV_L$: $\bar{x}$ = 6.56%, range = 0–16.6%, reproducibility, $\%CV_x$: $\bar{x}$ = 12.33%, range = 5.82–19.0%.

Recommendation

On the basis of the collaborative results, it is concluded that the accuracy and precision of the method are sufficient to warrant consideration for official status for the following residues in poultry fat samples: α-BHC, lindane, *cis*- and *trans*-chlordane, dieldrin, *p,p'*-TDE, *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDT, endrin, heptachlor epoxide, hexachlorobenzene, methoxychlor, mirex, and octachlor epoxide. Toxaphene is not recommended due to lack of suitable techniques for determining altered toxaphene residues. It should be noted that samples found to contain nonionic chlorinated hydrocarbons and PCBs will have to be analyzed by another method that allows separation of PCBs from the nonionic chlorinated hydrocarbons.

Acknowledgments

The Associate Referee thanks Richard Albert, Food and Drug Administration, Washington, DC, for statistical consultations, and Marie Brady, U.S. Department of Agriculture, Washington, DC, for her time to enter data into the computer.

The following collaborators deserve a special thanks from the Associate Referee for their time, patience, and cooperation:

Marvin M. Anderson, Cargill, Inc., Springdale, AR

Ray Bowers and Vicram Patel, General Mills, Indianapolis, MN

Ellis Brunton, Holly Farms Poultry Industries, Wilkesboro, NC

Table 2. Collaborative results (ppm) for recovery of *p,p'*-TDE and *p,p'*-DDT in poultry fat

	p,p'-TDE				*p,p'*-DDT			
Coll.	Level 1		Level 2		Level 1		Level 2	
1[a]	—	0.14	0.51	—	0.31	0.29	0.21	0.21
2[a]	0.11	0.12	0.24	0.40	0.22	0.23	0.10	0.16
3	0.17	0.19	0.54	0.59	0.36	0.46	0.19	0.27
4	0.15	0.17	0.59	0.57	0.34	0.38	0.20	0.19
5	0.20	0.16	0.68	0.52	0.46	0.36	0.24	0.22
8	0.17	0.16	0.54	0.51	0.41	0.42	0.23	0.21
9	0.23[b]	0.26[b]	0.70	0.62	0.44[b]	0.54[b]	0.25[b]	0.33[b]
11	0.15	0.17	0.50	0.52	0.39	0.37	0.19	0.19
12	0.18	0.17	0.56	0.62	0.42	0.37	0.22	0.19
Added, ppm	0.18		0.60		0.42		0.21	
Av. rec., ppm	0.171		0.58		0.395		0.212	
Av. rec., %	95.0		96.7		94.0		100.8	
Data sets used	6		7		6		6	
S_o	0.016		0.053		0.045		0.026	
S_L	0		0.033		0		0	
S_x	0.016		0.063		0.045		0.026	
CV_o, %	9.30		9.26		11.46		12.35	
CV_L, %	0		5.67		0		0	
CV_x, %	9.30		10.86		11.46		12.35	

[a]Laboratory data were not used because of method deviations.
[b]Laboratory data were not used because statistical analysis of average identified it as Dixon outlier.
[c]Cochran outlier (in succeeding tables).
Subscript notation: o = repeatability; L = between laboratories; x = reproducibility.
[d]False negative

Table 3. Collaborative results (ppm) for recovery of mirex and toxaphene in poultry fat

	Mirex				Toxaphene			
Coll.	Level 1		Level 2		Level 1		Level 2	
1[a]	0.43	0.49	0.73	—	0.49	1.06	0.53	0.57
2[a]	0.34	0.44	0.82	0.83	0.66	0.63	0.61	0.60
3	0.45	0.51	0.8	1.0	0.37	0.39	0.93	0.78
4	0.40	0.43	0.78	0.82	0.54	0.55	0.97	1.03
5	0.46	0.46	1.06	0.80	0.58	0.50	1.04	1.02
8	0.49	0.49	1.00	0.97	0.42	0.36	0.79	0.67
9	0.56[b,c]	0.8[b,c]	1.06	1.23	0.45	0.39	0.88	0.79
11	0.39	0.45	0.74	0.79	—	0.49	—	0.85
12	0.49	0.48	0.88	0.91	0.54	0.6	0.99	1.06
Added, ppm	0.51		1.02		0.49		0.98	
Av. rec., ppm	0.458		0.917		0.475		0.908	
Av. rec., %	89.8		89.9		96.9		92.6	
Data sets used	6		7		7		7	
S_o	0.026		0.101		0.038		0.067	
S_L	0.028		0.103		0.077		0.108	
S_x	0.038		0.144		0.086		0.127	
CV_o, %	5.70		11.00		8.08		7.38	
CV_L, %	6.03		11.25		16.26		11.94	
CV_x, %	8.30		15.73		18.16		14.04	

Footnotes and subscripts, see Table 2.

Table 4. Collaborative results (ppm) for recovery of hexachlorobenzene and methoxychlor in poultry fat

	Hexachlorobenzene				Methoxychlor			
Coll.	Level 1		Level 2		Level 1		Level 2	
1[a]	0.22	0.21	0.18	0.22	0.42	1.04	0.35	0.46
2[a]	0.13	0.12	0.08	0.15	0.99	0.96	0.64	0.58
3	0.39	0.36	0.17	0.19	1.3[b]	1.2[b]	0.46[b]	0.61[b]
4	0.28	0.27	0.16	0.16	0.64	0.84	0.31	0.40
5	0.43[c]	0.29[c]	0.22	0.14	1.02	0.88	0.50	0.38
8	0.32	0.34	0.17	0.18	0.95	1.01	0.50	0.50
9	0.46[b]	0.50[b]	0.21[b]	0.33[b]	1.02[b]	1.0[b]	0.59[b]	0.75[b]
11	0.24	0.26	0.14	0.14	0.82	0.92	0.40	0.42
12	0.29	0.33	0.15	0.14	0.89	0.97	0.48	0.47
Added, ppm	0.39		0.19		1.04		0.52	
Av. rec., ppm	0.317		0.163		0.894		0.436	
Av. rec., %	81.3		85.8		86.0		83.9	
Data sets used	6		6		5		5	
S_o	0.044		0.024		0.089		0.048	
S_L	0.037		0.005		0.071		0.045	
S_x	0.057		0.025		0.114		0.066	
CV_o, %	13.83		14.79		9.98		11.00	
CV_L, %	11.72		3.06		7.96		10.43	
CV_x, %	18.13		15.10		12.76		15.16	

Footnotes and subscripts, see Table 2.

Table 5. Collaborative results (ppm) for recovery of dieldrin and octachlor epoxide in poultry fat

	Dieldrin				Octachlor epoxide			
Coll.	Level 1		Level 2		Level 1		Level 2	
1[a]	0.30	0.19	0.65	0.83	0.37	0.28	0.26	0.44
2[a]	0.14	0.20	0.27	0.35	0.29	0.25	0.22	0.24
3	0.29	0.34	0.57[c]	0.66[c]	0.38	0.38	0.32	0.41
4	0.26	0.29	0.52	0.53	0.22	0.30	0.25	0.26
5	0.33	0.27	0.62	0.66	0.36	0.32	0.40	0.28
8	0.27	0.26	0.57	0.57	0.30	0.29	0.36	0.35
9	0.43[b]	0.31[b]	0.76[b]	0.79[b]	0.44	0.33	0.47[b]	0.51[b]
11	0.29	0.30	0.53	0.54	0.28	0.28	0.23	0.27
12	0.31	0.29	0.56	0.55	0.34	0.33	0.34	0.34
Added, ppm	0.29		0.59		0.38		0.38	
Av. rec., ppm	0.291		0.573		0.325		0.318	
Av. rec., %	100		97.1		85.5		83.7	
Data sets used	6		6		7		6	
S_o	0.026		0.029		0.038		0.045	
S_L	0.002		0.041		0.040		0.040	
S_x	0.026		0.050		0.055		0.060	
CV_o, %	8.82		5.04		11.72		14.17	
CV_L, %	0.77		7.08		12.40		12.66	
CV_x, %	8.86		8.69		17.06		19.00	

Footnotes and subscripts, see Table 2.

Table 6. Collaborative results (ppm) for recovery of *p,p'*-DDE and *o,p'*-DDT in poultry fat

	p,p'-DDE				*o,p'*-DDT			
Coll.	Level 1		Level 2		Level 1		Level 2	
1[a]	0.24	0.17	0.55	0.68	0.34	0.27	0.77	0.84
2[a]	0.14	0.10	0.16	0.19	0.20	0.21	0.33	0.45
3	0.26	0.30	0.50[c]	0.59[c]	0.37	0.41	0.71[c]	0.87[c]
4	0.22	0.25	0.44	0.44	0.28	0.34	0.60	0.58
5	0.28	0.24	0.54	0.58	0.41	0.34	0.83	0.82
8	0.24	0.23	0.51	0.51	0.38	0.39	0.74	0.74
9	0.31	0.24	0.53	0.55	0.57[b]	0.43[b]	0.98	1.0
11	0.24	0.26	0.45	0.45	0.31	0.35	0.65	0.64
12	0.27	0.25	0.50	0.51	0.40	0.36	0.76	0.69
Added, ppm	0.27		0.61		0.39		0.78	
Av. rec., ppm	0.256		0.507		0.362		0.758	
Av. rec., %	94.8		83.1		92.8		97.2	
Data sets used	7		7		6		7	
S_o	0.027		0.027		0.033		0.047	
S_L	0		0.043		0.023		0.126	
S_x	0.027		0.050		0.041		0.135	
CV_o, %	10.37		5.32		9.24		6.26	
CV_L, %	0		8.41		6.47		16.60	
CV_x, %	10.37		9.95		11.28		17.74	

Footnotes and subscripts, see Table 2.

Table 7. Collaborative results (ppm) for recovery of α-BHC and lindane in poultry fat

	α-BHC				Lindane			
Coll.	Level 1		Level 2		Level 1		Level 2	
1[a]	0.30	0.10	0.45	0.45	0.18	0.20	0.49	0.50
2[a]	0.10	0.10	0.17	0.13	0.16	0.14	0.16	0.18
3	0.30	0.33	0.49	0.56	0.23	0.22	0.39	0.46
4	0.30	0.30	0.44	0.45	0.20	0.19	0.40	0.42
5	0.36	0.28	0.54	0.58	0.22	0.20	0.44	0.39
8	0.32	0.26	0.42	0.40	0.20	0.21	0.38	0.38
9	0.21	0.24	0.31	0.37	0.16	0.14	0.29	0.35
11	0.29	0.28	0.53	0.50	0.19	0.22	0.36	0.43
12	0.25	0.27	0.42	0.43	0.19	0.21	0.36	0.34
Added, ppm	0.31		0.50		0.21		0.42	
Av. rec., ppm	0.285		0.460		0.199		0.385	
Av. rec., %	91.9		92.0		94.8		91.7	
Data sets used	7		7		7		7	
S_o	0.030		0.029		0.013		0.034	
S_L	0.026		0.074		0.021		0.029	
S_x	0.039		0.080		0.025		0.045	
CV_o, %	10.4		6.26		6.59		8.98	
CV_L, %	9.02		16.15		10.82		7.59	
CV_x, %	13.77		17.32		12.67		11.75	

Footnotes and subscripts, see Table 2.

Table 8. Collaborative results (ppm) for recovery of *cis*-chlordane and *trans*-chlordane in poultry fat

	cis-Chlordane				*trans*-Chlordane			
Coll.	Level 1		Level 2		Level 1		Level 2	
1[a]	0.24	0.20	0.42	—	0.16	0.11	0.64	0.79
2[a]	0.22	0.22	0.31	0.33	0.12	—	0.22	0.24
3	0.29	0.27	0.45	0.54	0.17	0.19	0.51	0.60
4	0.24	0.24	0.50	0.50	0.15	0.16	0.44	0.44
5	0.26	0.24	0.57	0.42	0.18	0.16	0.53	0.59
8	0.25	0.25	0.50	0.49	0.15	0.15	0.49	0.48
9	0.22[b,c]	0.14[b,c]	0.35	0.49	0.16	0.21	0.63	0.62
11	0.25	0.26	0.43	0.44	0.14	0.18	0.45	0.46
12	0.26	0.26	0.51	0.50	0.17	0.17	0.49	0.47
Added, ppm	0.28		0.56		0.18		0.54	
Av. rec., ppm	0.256		0.478		0.167		0.514	
Av. rec., %	91.4		85.4		92.8		95.2	
Data sets used	6		7		7		7	
S_o	0.009		0.060		0.189		0.030	
S_L	0.012		0		0		0.064	
S_x	0.015		0.060		0.189		0.070	
CV_o, %	3.39		12.57		11.31		5.79	
CV_L, %	4.73		0		0		12.40	
CV_x, %	5.82		12.57		11.31		13.69	

Footnotes and subscripts, see Table 2.

Table 9. Collaborative results (ppm) for recovery of endrin and heptachlor epoxide in poultry fat

	Endrin				Heptachlor epoxide			
Coll.	Level 1		Level 2		Level 1		Level 2	
1[a]	—	0.1	0.36	—	0.37	0.29	1.16	1.17
2[a]	0.1	0.12	0.17	0.30	0.20	0.20	0.23	0.39
3	0.15	0.17	0.40	0.45	0.39	0.41	1.0	1.1
4	0.14	0.15	0.38	0.38	0.33	0.39	0.82	0.88
5	0.17	0.14	0.46	0.36	0.45	0.36	1.12	0.96
8	0.15	0.15	0.40	0.39	0.36	0.36	0.94	0.84
9	0.22[b]	0.20[b]	0.43[b,c]	0.66[b,c]	0.46	0.33	0.80	0.89
11	0.14	0.16	0.36	0.39	0.38	0.38	0.93	0.98
12	0.16	0.15	0.36	0.39	0.39	0.38	0.91	0.96
Added, ppm	0.15		0.42		0.42		1.04	
Av. rec., ppm	0.153		0.393		0.384		0.938	
Av. rec., %	102		93.6		91.4		90.2	
Data sets used	6		6		7		7	
S_o	0.013		0.035		0.046		0.067	
S_L	0		0		0		0.069	
S_x	0.013		0.035		0.046		0.096	
CV_o, %	8.25		8.81		11.89		7.11	
CV_L, %	0		0		0		7.36	
CV_x, %	8.25		8.81		11.89		10.24	

Footnotes and subscripts, see Table 2.

Table 10. Collaborative results (ppm) for analysis of incurred residues in poultry fat

	Incurred residues reported					
Coll.	Dieldrin		*p,p'*-DDE		Heptachlor epoxide	
1[a]	1.91	1.88	0.00[d]	0.03	0.09	0.09
2[a]	0.53	0.46	0.00[d]	0.00[d]	0.11	0.11
3	0.92	0.95	0.14	0.15	0.1	0.12
4	0.86	0.82	0.15	0.13	0.1	0.09
5	0.94	0.74	0.14	0.12	0.09	0.09
8	0.93	0.98	0.14	0.15	0.1	0.11
9	0.88	1.07	—	—	0.17[c]	0.09[c]
11	0.75	0.81	0.14	0.15	0.1	0.09
12	0.91	0.88	0.16	0.14	0.12	0.11
Av. ppm detd	0.889		0.143		0.102	
Data sets used	7		6		6	
S_o	0.078		0.011		0.008	
S_L	0.045		0		0.008	
S_x	0.090		0.011		0.114	
CV_o, %	8.80		7.85		8.03	
CV_L, %	5.08		0		7.83	
CV_x, %	10.16		7.85		11.21	

Footnotes and subscripts, see Table 2.

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Alumina Blending Technique for Separation of Pesticides from Lipids

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An evaluation of an extraction technique for elimination of lipids during pesticide residue analysis was undertaken, which included a study of the parameters involved in an attempt to optimize the separation and recovery of selected pesticides from lipid samples. The choice of alumina, the degree of water deactivation of alumina, the amount of alumina relative to the weight of sample taken, the volume and composition of extraction solvent, the blending time, and the filtration procedure were all significant factors. Optimization of these factors resulted in >90% recoveries of selected chlorinated pesticides from fortified fatty samples. In addition, the amount of co-extracted lipid material was about one-third of that obtained with the official method (AOAC 29.014), and less analytical time was required for the extraction by the proposed technique. The formation of troublesome emulsions sometimes encountered with the official method was also eliminated.

An extraction technique proposed in a Food and Drug Administration communication (1) offered some potential advantages over the official AOAC method (2). These included better cleanup with less analytical time, and elimination of emulsion formation which can occur in the official method (as cautioned in section **29.014**). This proposal involved the use of alumina as an initial step to disperse and adsorb the fat or oil while extracting the pesticides with an acetonitrile–water mixture in a high-speed blender.

Numerous applications of alumina to the removal of fats and other interfering substances from solvent extracts of fatty samples have been reported. Several of these are of particular interest to the present study. Holden and Marsden (3) and Lea (4) used partially deactivated alumina to effectively separate pesticide residues from lipids. Claeys and Inman (5) found that alumina had a larger capacity per unit volume for lipids and oils than did Florisil and silicic acid. They also reported better separation of lipids from chlorinated hydrocarbons as a result of deactivation of the adsorbent. Greve and Grevenstuk (6) reported that more effective removal of fats and better yields of β-hexachlorocyclohexane were achieved with basic than with neutral alumina. Duinker and Hillebrand (7) recommended the use of basic alumina for easier control of activity and recommended solvent pre-washing of the alumina to remove substances interfering in the gas chromatographic (GC) determination of chlorinated hydrocarbons.

In a recent report, Kuwabara and co-workers (8) compared a Florisil dry column chromatographic extraction procedure with the acetonitrile partitioning procedure reported by Johnson (9) for the recovery of pesticides from soybean oil and butter fat. (The latter procedure is the basis of the current official method (2).) They found equivalent or better recoveries for the pesticides tested using the dry Florisil as a lipid adsorbent. However, no comparison of the extent of lipid removal was presented in that report.

In the present study, we have undertaken an evaluation of each of the several parameters involved in the proposed procedure (1) in an attempt to achieve good pesticide recoveries, while at the same time effecting the maximum lipid removal possible.

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Experimental

Apparatus and Reagents

The apparatus and reagents used were generally as specified in the official method for pesticide residues in fat-containing foods (2) with the following exceptions:

(a) *Blender.*—Sorvall Omni-Mixer homogenizer with stainless steel chamber assembly (Dupont Co., Biomedical Products Div., Newton, CT 06470), or equiv.

(b) *Mechanical shaker.*—Burrell wrist-action shaker (Burrell Corp., Pittsburgh, PA).

(c) *Alumina.*—Fisher adsorption alumina, 80–200 mesh (Fisher Scientific Co., Fair Lawn, NJ 07410, Cat. No. A-540), or equivalent. Wash alumina to rid GC interferences as follows: Soak in methylene chloride ≥ 1 h. Decant and discard the methylene chloride, and soak alumina 1 h in *n*-hexane. Decant and discard *n*-hexane, and dry alumina over steam bath.

Prepare deactivated alumina as follows: Place washed and dried alumina in muffle furnace at 260°C for 4 h. Transfer to tightly closed containers and withdraw convenient amounts (e.g., 500 g each) for deactivation. Add water incrementally (*Caution:* Heat will be produced.) to final concentration > 16% (w/w) but not > 19% (w/w), shaking briefly after each addition. Then shake ≥ 4 h on mechanical shaker. Store in tightly closed containers. Alumina, thusly prepared, has been found to be stable at least 4 months.

(d) *Extraction solvent.*—Acetonitrile–water (80 + 20), free from substances interfering in GC analysis.

(e) *Filter paper.*—Whatman No. 40, 15 cm or larger diameter, or equivalent. Wash paper by soaking in acetonitrile–water (80 + 20) to eliminate substances interfering in GC analysis. Dry and store in closed container.

(f) *Pesticide standards.*—All standard solutions used for gas chromatographic quantitation were prepared in isooctane, using reference standards obtained from the Environmental Protection Agency Pesticide Repository. Aliquots of these same solutions were also used to fortify samples used for recovery studies, thereby minimizing errors which could arise due to the use of different solutions for this purpose.

Procedure

Weigh 2 g fat into 150 ml beaker containing 50 g deactivated alumina and mix well. Transfer to blender jar and add 350 mL extraction solvent. Blend 2–4 min at high speed. Transfer most of contents (without rinsing) to a funnel, fitted with single paper, and filter without suction. (Alternatively, centrifuge blended contents 5 min at 1500 rpm and decant supernatant liquid through funnel plugged with glass wool.) Record mL solvent recovered (*V*) and calculate g sample (*W*) in *V* by *W* = g sample taken × (*V*/350 mL). Transfer to 1 L separatory funnel containing 100 mL petroleum ether and shake vigorously 30 s. Add 10 m L satd NaCl soln and 500 mL water. Proceed as in section **29.014,** beginning with "Hold separator in horizontal position and mix thoroly 30–45 sec."

Preliminary Evaluation

As a first step in this study, recovery studies of selected pesticides added to several fatty samples were performed using the procedure exactly as originally proposed (1). This

was done to determine if this procedure warranted more extensive investigation because only limited test results were provided in that paper. The original procedure was essentially the same as presented in this report except that 20 g activated alumina was used and filtration was done with suction through an S & S sharkskin filter.

For whole milk samples, the procedure specified mixing 50 g milk (equivalent to ca 2 g fat) with 20 g Fisher A-540 alumina, then adding 25 mL water and 280 mL acetonitrile (to give a final extract consisting of about 20 parts water and 80 parts acetonitrile) prior to blending and proceeding with the filtration step and partitioning as for fats and oils. This approach was suggested as an alternative for the analysis of pesticide residues (on the whole-milk basis) without first extracting the fat as required by the official method.

Samples of milk, butter, lard, and unrefined soybean oil were fortified with various pesticides and analyzed by this procedure. Butter and lard samples were warmed and filtered as specified in section **29.012(b)** of the official method to obtain the fat before weighing and fortifying. Recovery results for these fortified samples are shown in Table 1. The generally adequate recoveries obtained indicated that the procedure merited further consideration.

Subsequently, 2 cheese samples were obtained which had been recently analyzed by the official method in another laboratory and found to contain incurred residues of pirimiphos methyl. These samples were analyzed by the proposed procedure (the fat was first isolated by section **29.012(d)** of the official method) for comparison. Results of 0.24 ppm and 0.31 ppm for the 2 samples by the proposed procedure compared favorably with results of 0.19 ppm and 0.30 ppm, respectively, by the official method.

Investigation of Parameters

Each of the critical parameters of the proposed blending procedure was subsequently investigated in an attempt to ascertain the optimal conditions for both lipid removal and pesticide recovery. Unless otherwise specifically stated, the conditions used throughout these studies were as specified in the original proposal except that filtering was done without suction through a Whatman No. 40 paper. Recovery studies were done using fortification levels in the general range of regulatory concern for the pesticides used.

(a) *Alumina.*—Several types of alumina were selected from materials available in the authors' laboratory and compared with regard to recovery of selected pesticides. Each of these materials was treated as recommended (1) which included heating at 600°C overnight, storing in tightly closed containers, then washing a 20 g portion with 100 mL ethanol (95%) and 200 mL acetone, and drying at ca 100°C just before use. Duplicate determinations were made on portions of a pesticide-free lard sample, each fortified with heptachlor epoxide and dieldrin.

Results are shown in Table 2. Heptachlor epoxide recoveries did not vary significantly. However, dieldrin recoveries were definitely affected by the pH of the alumina. The basic Fisher A-540 alumina appeared to be appropriate for further study. An equivalent alumina such as Alcoa F-20 would be expected to yield similar results. This material is relatively inexpensive but was considered suitable since the intended use in this study was not for effecting a separation of compound classes, but rather to simply adsorb lipids.

(b) *Filtering technique.*—In the course of the previous experiments, it became apparent that the filtering technique originally proposed (1) to separate the extraction solvent from the blended sample-alumina substrate could be of significance. It was surmised that the application of suction to this substrate (contained on a filter paper in a Buchner funnel placed on a vacuum flask) resulted in some degree of physical withdrawal of adsorbed lipids from the alumina. Therefore, a comparison was made between filtration with suction and by gravity flow with regard to the amount of lipid material retained by the alumina. Filtered extracts of portions of a butter fat sample blended with alumina were partitioned into petroleum ether (see *Procedure*) and the lipid residues were weighed after evaporation to dryness. Duplicate determinations thus made by each filtering method demonstrated that about 50% more fat was retained on the alumina when the extraction solvent was filtered by gravity. Therefore, gravity flow filtration was deemed to be preferable though it required as much as 30 min more time than suction to collect ca 320 mL filtrate. However, this was not applied analytical time since it required no analyst attention. It may be possible to apply a slight, carefully controlled vacuum to decrease the filtration time but this would require much greater attention and additional study.

It was also observed that a single S & S sharkskin filter paper did not effectively retain alumina fines which resulted in a cloudy suspension in the filtrate. A single Whatman No. 40 paper yielded a clear filtrate in a reasonable time and was therefore used throughout the remainder of this investigation. The filter paper was also a source of minor peaks in the gas chromatograms; these peaks were potential interferences in the quantitation of some pesticides. Thorough soaking of the paper with 20% water in acetonitrile effectively removed these contaminants.

Subsequent to completing all of the various studies described in this report, it was determined that centrifuging the blended

Table 1. Preliminary study of pesticide recoveries from fortified samples, using proposed procedure

Pesticide	Product	Fortification level, ppm	No. of trials	Mean recovery, %
Hept. epox.	milk	0.4	2	106.4
Hept. epox.	butter	0.15	3	104.3
Hept. epox.	lard	0.3	3	93.0
Hept. epox.	soybean oil	0.3	3	93.7
Diazinon	milk	0.4	3	93.9
Parathion	soybean oil	0.2	3	90.3
α-BHC	lard	0.3	3	84.6
α-BHC	soybean oil	0.3	3	97.8
Trans-chlordane	lard	0.15	3	104.5
Trans-chlordane	soybean oil	0.3	2	82.7
Dieldrin	milk	0.4	3	101.0
Dieldrin	butter	0.3	3	100.9
Dieldrin	lard	0.3	3	103.7
Dieldrin	soybean oil	0.2	2	86.8
PCNB	soybean oil	0.3	3	83.1

Table 2: Comparison of pesticide recoveries from fortified lard, using different types of alumina

Alumina	pH	Recovery, %			
		Heptachlor epoxide[a]		Dieldrin[b]	
		Mean[d]	Range	Mean[d]	Range
Bio-Rad AG4	2.5	91.2	88.5–94.0	82.6	79.1–86.1
Bio-Rad AG7	6.9	97.5	95.8–99.2	58.0	53.1–62.9
Fisher A-950	6.9	87.8	81.8–93.7	64.6	63.4–65.7
Bio-Rad AG10	10.4	95.8	89.7–102.1	85.1	84.5–85.6
Fisher A-540	9.6	99.0	97.9–100.6	96.4	90.2–102.6

[a]0.3 ppm added.
[b]0.4 ppm added.
[c]pH was determined by slurrying 1 g alumina with 20 mL deionized water and measuring on pH meter.
[d]Duplicate determinations.

contents in the blender jar and decanting the supernatant solvent was a suitable alternative to transferring the entire contents of the jar onto a filter paper. This approach reduces the total analysis time considerably and precludes the need for washing and testing the filter paper for GC interferences.

(c) *Alumina deactivation.*—Previous reports (3–5) indicated that the alumina activity (as controlled by the amount of water present) is a factor in its capacity to retain lipids. It has been determined that water deactivation generally results in a substantial increase in the sample capacity of the adsorbent in liquid-solid chromatography (10). The effect of alumina activity on lipid cleanup was therefore evaluated.

Fisher A-540 alumina was first activated by heating at 400°F (≥ 4 h) as specified in the Fisher Scientific Co. catalog and was then stored in a tightly stoppered container. Portions were removed and water was added at various concentrations from 5% to 24% (w/w). Each batch was thoroughly mixed in a tightly stoppered container on the mechanical shaker for 4 h at maximum speed, and each was used within 1 week. Duplicate portions of each alumina preparation were each mixed with a portion of prepared butter fat, blended with extraction solvent, and filtered. The filtrates (about 320 mL was recovered in each case) were each partitioned into petroleum ether and evaporated to dryness. The residues were weighed and the percent of lipid material extracted was calculated for each.

As shown by the plot in Figure 1, a nearly 3-fold increase in lipid cleanup was achieved for butter fat as a result of deactivation of the alumina. The fat retention capacity of the alumina began to plateau at the 16% water level. As the water level was increased above 19%, the alumina became lumpy and somewhat unmanageable for practical use. This would be expected since the maximum adsorptive capacity of activated alumina for water is ca 19% (11). Therefore, it was concluded that deactivation with 16–19% water was optimal for lipid removal.

A comparison of lipid removal from portions of a prepared lard sample using duplicate portions of both activated and 16% water-deactivated alumina was subsequently done using the same procedure as for butter fat. The average lipid residue in the petroleum ether extract was only 0.68% of the sample weight when using the deactivated alumina as compared with 5.0% when using activated alumina.

Having thus established an optimal level of alumina deactivation for use with the proposed procedure, a comparison was made with the official method as to the extent of lipid removal achieved prior to the Florisil elution step. Duplicate 2.0 g portions of the prepared butter fat and lard samples were extracted by the procedure in section **29.014** of the official method, and the residues were weighed after evaporating the final petroleum ether extracts to dryness. The official method extracted an average of 3.1% of the lard sample and 13.6% of the butter fat sample compared with only 0.68% and 5.0%, respectively, extracted by the proposed procedure (using 16% water-deactivated alumina). Therefore, significantly less lipid material would subsequently be placed on the Florisil column when using the proposed procedure. This is important since it has been shown (12) that virtually all of the lipid material placed on the Florisil column is ultimately eluted in the polar solvent fractions used in the official method. The GC system is subsequently often rapidly contaminated by injections of these polar fractions. The 16% water-deactivated alumina was therefore used in all of the further studies.

In the course of the studies presented in this report, several different lots of Fisher A-540 alumina were used and were found to give equivalent and consistent results when treated in the manner described.

(d) *Water content of extraction solvent.*—The addition of water to the extraction solvent would logically result in less coextraction of lipids from the alumina-adsorbed sample. However, a lower recovery of pesticides could be concurrently anticipated. Therefore, the effect of varying the water content (10%, 20% and 30%) of the acetonitrile–water extraction solvent on the recoveries of three selected pesticides from fortified lard was evaluated. Duplicate extractions were

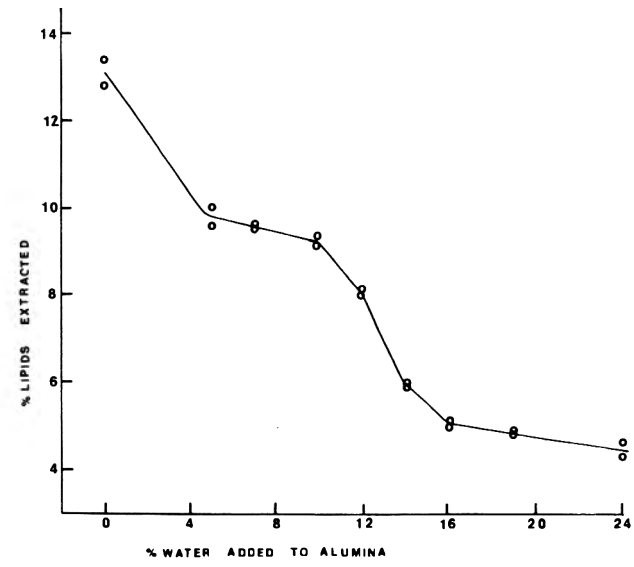

Figure 1. Effect of water deactivation of alumina on percentage of lipids extracted from 2 g butter fat.

made with each solvent on portions of prepared lard each fortified with heptachlor epoxide, dieldrin and *p,p'*-DDE. The extracts were taken through the entire proposed procedure (including partitioning into petroleum ether and Florisil elution) for GC quantitation. While heptachlor epoxide was satisfactorily recovered in each case, dieldrin and *p,p'*-DDE recoveries decreased below acceptable levels (<70%) using 30% water in the extraction solvent.

Although no appreciable differences in recoveries were found between 20% and 10% water in this limited study, reduction of the water content would generally be expected to provide better solubility of pesticides in the solvent with a subsequent improvement in recoveries. A study was therefore done to determine if the use of less water would seriously affect lipid cleanup. Extraction solvents varying in water content from 0% to 20% were prepared. Duplicate extractions of portions of a butter fat sample were made with each of these solvents by the proposed procedure. After filtered extracts were partitioned into petroleum ether and evaporated to dryness, the lipid residues were weighed. The amount of lipids extracted increased significantly as the water content of the solvent was decreased (i.e., 5% extracted with 20% water, 8% with 16% water, 17% with 10% water, and 58% with no water added). It was concluded that the use of less than 20% water would negate the improved cleanup offerred by the proposed procedure.

(e) *Volume of extraction solvent.*—The effects of varying the volume of extraction solvent (80% acetonitrile–20% water) on the recoveries of heptachlor epoxide and *p,p'*-DDE was next evaluated. Using 100, 350, and 450 mL volumes, duplicate extractions with each volume were made of fortified portions of prepared butter fat by the proposed procedure. The extracts were partitioned and eluted through Florisil as before (**d**) for GC quantitation.

Results are given in Table 3. As anticipated, recoveries were dependent on solvent volume. A very acceptable recovery (>90%) of heptachlor epoxide was achieved with 350 mL, but a volume of 450 mL was required for a similar recovery of *p,p'*-DDE. Subsequently, the amount of lipid material extracted from butter fat by 450 mL solvent was determined and compared with the amount extracted by 350 mL. The percent of lipid material extracted and partitioned into petroleum ether from the 450 mL volume was about 27% higher than from the 350 mL volume (6.5% vs 5.1%). Therefore, some sacrifice in lipid cleanup would be made in using the larger volume.

(f) *Weight of alumina.*—The next study undertaken involved varying the weight of deactivated alumina from 3 to 50 g and determining the relative effect on lipid removal. Each weight of alumina was mixed in duplicate with a portion of butter fat and extracted per the procedure. The amount of lipid material carried through petroleum ether partitioning was calculated in each case. Virtually no difference in lipid cleanup was found as a result of varying the weight of alumina.

Subsequently, recovery studies of heptachlor epoxide and *p,p'*-DDE from portions of butter fat were done by the proposed procedure using different weights of alumina ranging from 5 to 50 g. Results are shown in Table 4. While heptachlor epoxide recoveries showed little variation, the *p,p'*-DDE recoveries showed a definite improvement with greater weight of alumina. Recoveries of both pesticides were very acceptable (>90%) when 50 g alumina was used.

(g) *Blending time.*—The final parameter studied was the length of time used for the extraction in the high-speed blender. The blending time was increased to 4 min and 10 min for comparison. Duplicate recovery studies of heptachlor epoxide and *p,p'*-DDE from portions of fortified butter fat were done using these blending times, and the results were compared with those obtained previously using a 2 min time. Some improvement in recovery (from 80% to 86%) as a result of increasing the blending time to 4 min was found for *p,p'*-DDE only. The 10 min time showed no significant advantage with regard to recovery of either pesticide. Heptachlor epoxide recovery was acceptable (ca 90% or higher) at all 3 blending times.

The effect of increased blending times on lipid extraction from portions of butter fat was also studied. The amount of lipids extracted was undesirably increased from 5.0% at 2 min to 6.4% at 10 min.

Summary and Conclusions

Each of the critical parameters involved in the proposed alumina blending procedure for the extraction of pesticides from lipids has been evaluated with respect to both the removal of lipid material prior to Florisil elution and the recovery of pesticides from fortified samples. Based on the findings, the optimal conditions allowing acceptable pesticide recoveries will concurrently result in the coextraction of about 5% (100 mg) of a 2 g butter fat sample and about 0.7% (14 mg) of a 2 g lard sample. The amount of fatty material extracted by the proposed procedure and subsequently placed on a Florisil column is reduced by at least 2.5 times when compared with the official method, sec. **29.014.**

The proposed procedure generally requires less analytical time than the official method since a single extraction is required in the former as compared with the 4 extractions specified in the latter. A significant advantage of the proposed procedure in this regard is that troublesome emulsions do not form as often occurs in the official method. Furthermore, the need for additional cleanup steps sometimes required by the official method should be largely precluded as a result of the significant improvement in lipid removal achieved with the

Table 3. Effect of varying extraction solvent volume on recoveries of pesticides from fortified butter fat by proposed procedure

Solv. vol., mL	Recovery, %			
	Heptachlor epoxide[a]		*p,p'*-DDE[b]	
	Mean[c]	Range	Mean[c]	Range
100	75.4	74.1–76.6	65.4	63.1–67.6
350	95.9	94.4–97.4	80.1	78.1–82.1
450	92.1	90.4–93.8	92.2	90.6–93.9

[a]0.3 ppm added.
[b]0.25 ppm added.
[c]Duplicate determinations.

Table 4. Effect of varying weight of deactivated alumina on recoveries of pesticides from fortified butter fat

Wt Al, g	Recovery, %			
	Heptachlor epoxide[a]		*p,p'*-DDE[b]	
	Mean[c]	Range	Mean[c]	Range
5	89.2	88.5–89.8	75.6	74.8–76.5
20	95.9	94.4–97.4	80.1	78.1–82.1
35	90.4	86.7–94.1	85.0	81.3–88.6
50	96.1	94.4–97.7	94.6	93.0–96.3

[a]0.3 ppm added.
[b]0.25 ppm added.
[c]Duplicate determinations.

proposed procedure. Therefore, a substantial saving in analytical time could be expected in many instances with the use of the proposed procedure.

Heptachlor epoxide and dieldrin were used in the collaborative study (9) which preceded the official method, and were therefore initially selected for use in the present investigation also. However, in comparative trials with *p,p'*-DDE, it became apparent that the recovery of the latter compound was somewhat more sensitive to changes in parameters and tended to be somewhat lower than dieldrin under the same conditions. Therefore, *p,p'*-DDE was substituted for dieldrin in subsequent studies since it was felt that it served as a better indicator for the optimization of parameters.

It was observed throughout this investigation that the 3 index pesticides used in the recovery studies were affected to different degrees by changes in the parameters. With the exception of the extraction solvent volume study (Table 3), heptachlor epoxide recoveries were nearly 90% or better regardless of the conditions. Dieldrin and *p,p'*-DDE recoveries showed much greater variation as the conditions were changed. The recovery of *p,p'*-DDE approached the same high level as heptachlor epoxide (>90%) only in the instances where a 450 mL volume of extraction solvent was used (Table 3) and where a 50 g weight of alumina was used (Table 4). A 10 min blending time also resulted in an acceptable (nearly 90%) recovery of *p,p'*-DDE. Of these 3 parameter changes, the larger weight of alumina would be considered the best since the other 2 parameter changes result in greater extraction of lipid material. An improvement in recovery is predictable with an increase in the amount of alumina used since the fat sample would be dispersed over a larger surface area, thereby improving the efficiency of partitioning of the pesticides into the extraction solvent. However, the additional alumina expense (ca 1.3¢/g) must be considered relative to the gain in recovery desired. Reducing the amount of fat sample taken should permit a proportionate reduction in the amount of alumina required, but must be evaluated relative to the need for an adequate representative sample.

It was noted in reviewing the recovery data obtained by Kuwabara and co-workers (8) that their recovery of *p,p'*-DDE from fortified butter fat, using the acetonitrile partitioning procedure as in section **29.014** of the official method, was about 80%. A comparison of that data with the data presented herein (Table 4) suggests that an equivalent recovery could be achieved using 20 g alumina in the proposed procedure. However, the increase in recovery to >90% achieved using 50 g alumina would appear to be desirable.

The individual results obtained for the duplicate studies performed are presented in some cases to illustrate the precision attained. Since the differences in mean results are sometimes fairly small, the ranges of individual results are of some value in estimating whether or not these differences are significant.

On the basis of the studies and results presented, a method is proposed for the extraction of pesticides from fat-containing foods. It is recommended that this method be studied further to include the application to additional commodities and pesticides. As has been the practice over the years in the development of residue methods, the parameters of the method must first be developed using a limited selection of products and pesticides as a practical matter. The method can then be evaluated and expanded to a wider variety of applications. Following a successful intra- or inter-laboratory trial on a limited scale, a collaborative study of this method will be recommended.

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Recent Advances in Cleanup of Fats By Sweep Co-Distillation. Part 1: Organochlorine Residues

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A redesigned fractionation tube has resulted in development of a new, commercial sweep co-distillation unit. It is compact, reliable, cost-efficient, and permits cleanup of pesticides in up to 10 fat samples simultaneously. Recovery of several organochlorine residues (HCB, α-BHC, lindane, heptachlor epoxide, dieldrin, *p,p'*-DDE, *p,p'*-DDD, *p,p'*-DDT) at ppm and ppb levels in meat (beef) fat are 83–105%, with coefficients of variation between 4 and 6%. Cleaned up extracts are quantitated by gas chromatography using an electron capture detector with a vitreous silica capillary column containing a medium polarity bonded phase.

Storherr and Watts (1) reported sweep co-distillation (SCD) methods for fats in 1965. Dingle (2) used sweep co-distillation equipment designed and produced in Australia to monitor meat fats for organochlorine and organophosphorus residues. Using similar equipment, Heath and Black (3) simplified the technique by showing that regular injections of solvent during the distillation period were completely unnecessary, and McDougall (4) reported on the use of Florisil traps for isolation of the volatilized pesticides.

The analysis of Australian export primary produce, including fats (meat, butter, and cheese), is a vital part of a national quality assurance program to ensure that our products comply with the pesticide maximum residue limits (MRLs) set by many countries. Between 1975 and 1981, five different laboratories throughout Australia analyzed, annually, a total of approximately 30 000 fat samples for organochlorine and organophosphorus residues. Equipment and procedures used were similar to those reported by Heath and Black (3).

One of the major problems in gaining more widespread acceptance of SCD for fats has been the lack of suitable commercial equipment. Apart from other difficulties in successfully operating the Kontes system, the capacity to handle only 4 samples at one time is a major disadvantage which prohibits proficient application of the technique. The Unitrex system is the result of investigations into previous problems in SCD, and a re-evaluation of the critical parameters which determine the reliability of the technique.

METHOD

Reagents and Apparatus

(a) *Unitrex.*—Universal Trace Residue Extractor (supplied by Scientific Glass Engineering (SGE) Pty Ltd, Australia). The unit consists of an insulated, cylindrical, aluminum block containing a cartridge heater which stands atop a square base incorporating both the heater controls and the pneumatics for the carrier gas system (Figure 1).

The block is drilled to provide 10 stations for simultaneous treatment of up to 10 samples. The temperature of the unit is sensed by means of a chromel-alumel thermocouple mounted in the base of the heater block. The temperature is controlled to ± 1°C by means of a temperature controller on the front panel of the instrument.

Carrier gas (nitrogen) enters from the rear of the instrument and is pressure-controlled from the front panel across a 10-port manifold which supplies gas to each station through stainless steel delivery tubes. The flow through these tubes has been balanced to ensure that each sample is subjected to similar carrier gas flows. The head pressure on the manifold is indicated by the pressure gauge on the front of the unit. The carrier gas is led into the fractionation tube through a septum head which is attached by means of a nut and graphite ferrule. The fluid fat sample is injected through the septum using a specially designed syringe.

The fractionation tube (Figure 2), which is all glass, is designed so that the sample passes down a central tube, with the carrier gas, then out and up onto the silanized glass beads which are packed in the annular space between the central tube and the glass walls. The volatized pesticides are collected in a trap (Figure 2) containing sodium sulfate and partially deactivated Florisil. Elution of this trap with a solvent mixture facilitates isolation of an extract which needs no further cleanup prior to injection into the gas chromatograph.

(b) *Florisil.*—60–100 mesh (Floridin Co., Pittsburgh, PA). Material is washed several times with water and decanted to remove fines and soluble salts. After drying, Florisil is ignited at 600°C for 2 h, and stored in sealed glass jars. Partial deactivation is carried out by careful addition of a measured amount of water, followed by intermittent hand shaking for 15 min, and then allowed to equilibrate overnight. Because of batch-to-batch variation in bulk Florisil the optimum level of deactivation is determined experimentally, in 0.5% increments, between 0.5 and 2.0%. (See further details in *Results and Discussion.*) Deactivated Florisil is suitable for use up to 1 week. We used 1% deactivated Florisil for our work.

(c) *Sodium sulfate.*—Granular, anhydrous AR sodium sulfate is Soxhlet-extracted with dichloromethane for 2 h, air-dried, then heated in an oven at 130°C for 2 h.

(d) *Trap preparation.*—The traps must be prepared just before use; the composition is shown in Figure 2. Packing is best carried out by plugging the cone of the B5 joint with a small wad of silanized glass wool, then connecting this same end to a vacuum line and dipping the other end of the trap into the container of prepared sodium sulfate. With the vacuum still applied, the trap is then dipped into the prepared Florisil. After disconnecting the vacuum line, the outlet end of the trap is packed with a plug of silanized glass wool. The trap must be completely packed such that, when connected to the fractionation tube and placed in the Unitrex, there is no horizontal settling of the trap media.

(e) *Solvents.*—Hexane, dichloromethane (Mallinckrodt Nanograde) and ethyl ether (redistilled, peroxide-free).

(f) *Keeper solution.*—0.2% paraffin oil (British Pharmacopoeia grade) in hexane. Elute 1 g through activated (600°C for 2 h). Florisil column with 100 mL hexane, prior to dilution.

(g) *Nitrogen gas.*—High purity (for Unitrex and GC).

(h) *Gas purifier.*—Alltech Associates, containing indicating Drierite and Molecular Sieve 5A (for Unitrex and GC).

(i) *Gas chromatograph.*—Varian 3700 fitted with ^{63}Ni electron capture detector, 12 m × 0.33 mm id vitreous silica column BP10 (SGE), Unijector (SGE) operating in the direct injection mode, and Varian 8000 Autosampler. Operating conditions: temperatures (°C), column 210, injector 230,

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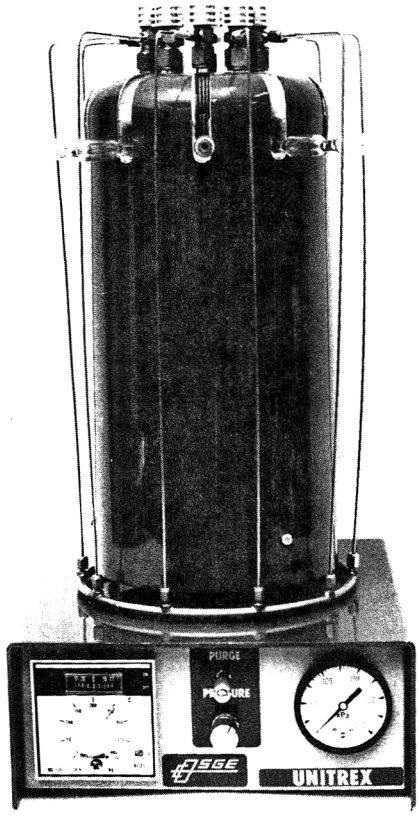

Figure 1. Assembled Unitrex equipment showing fractionation tubes and traps (unpacked).

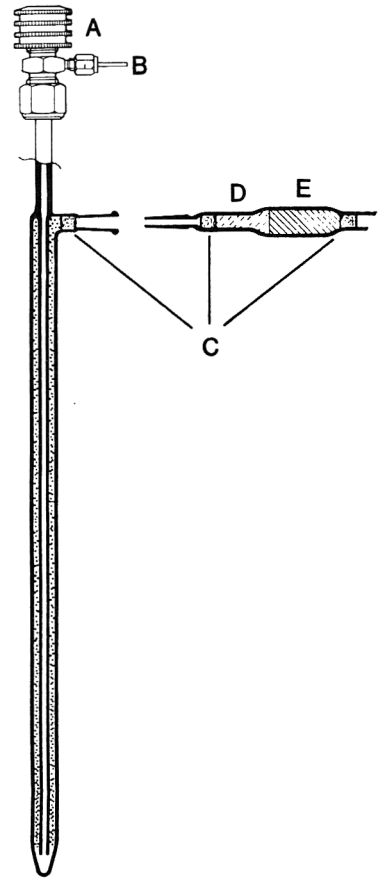

Figure 2. Fractionation tube and trap design: A, injection septum head; B, N_2 carrier gas inlet; C, silanized glass wool plug; D, anhydrous sodium sulfate; E, 1% deactivated Florisil.

detector 350; carrier gas (He) 90 cm/s; detector make-up gas (N_2) 40 mL/min; range 10; attenuation 256; injection volume 2 μL.

Preparation of Fortified Fat

Beef fat (200 g), previously analyzed to confirm that no organochlorine or organophosphorus residues above 0.01 mg/kg were present, was fortified with 2.0 mL of a mixed pesticide standard in hexane to produce a fat containing HCB (0.02 mg/kg), α-BHC (0.02 mg/kg), lindane (0.02 mg/kg), heptachlor epoxide (0.04 mg/kg), dieldrin (0.05 mg/kg), *p,p'*-DDE (0.05 mg/kg), *p,p'*-DDD (0.08 mg/kg), *p,p'*-DDT (0.12 mg/kg). The fat was then mixed, at 60°C, using a magnetic stirrer/hot plate for 20 min.

Procedure

Connect power and turn on heater unit. Let block temperature stabilize at 233–235°C (ca 35 min). Place assembled fractionation tubes and traps into unit and connect nitrogen gas lines to each.

Adjust nitrogen carrier gas to give a flow of 230 mL/min to each fractionation tube. Wait 5–10 min for temperature equilibration. Draw up into barrel of syringe 1.14 mL (≡1.00 ± 0.02 g) of rendered fat which has been heated previously to liquid state. Loosen injection nut, insert needle of syringe through pre-punched septum, and inject. Withdraw needle from injection port, and re-tighten septum nut. Insert insulation wedge in slot above fractionation tube side arm. Rinse syringe and needle with hexane and discard washings. Small quantities of hexane remaining in syringe are removed by drawing up and expressing air several times. Proceed to next injection, rinsing syringe with ca 1 mL sample to ensure that no cross-contamination occurs. Repeat injection procedure for all samples.

At end of 30 min fractionation period (timed from injection of first sample), disconnect carrier gas by pulling small piece of plastic tubing off stainless steel tubing of septum head, leaving it attached to long stainless steel carrier gas delivery tube. Remove insulation wedge. Lift fractionation tube, with trap connected, out of unit. Detach trap, and set hot fractionation tube aside for cleaning. Immediately attach trap to small solvent reservoir (Figure 3). When the 10 traps have been connected to their respective reservoirs, add 12 mL eluting mixture consisting of hexane–ethyl ether (90 + 10) to

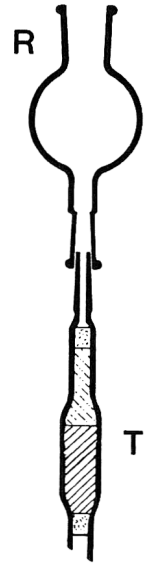

Figure 3. Trap (T) and solvent reservoir (R) design.

each, and collect eluate (ca 10 mL). Depending on concentration of pesticides in sample, this extract may be used directly for injection into gas chromatograph, or concentrated, either by use of nitrogen gas stream or Kuderna-Danish (KD) technique. In our case, KD concentration and adjustment to 2.0 mL preceded transfer of extract to vials for automatic injection into gas chromatograph.

Results and Discussion

Initial calibration of the temperature controller is carried out by inserting a thermocouple, with digital readout facility, midway down the inner tube of a packed fractionation tube, which has been able to equilibrate in the unit for at least 15 min. It is not necessary to have nitrogen carrier gas flow during this calibration. Temperature stability of a particular core should be ± 1°C over a 30 min period, and temperature uniformity between all 10 cores should be within ± 2°C at any particular time.

The stainless steel carrier gas delivery tube to each port has been crimped so that a flow rate of approximately 230 mL/min is obtained when the front panel pressure gauge is set at 30 kPa. For the results in Table 1, we used a flow rate of 230 mL/min when measured at the outlet of the Florisil trap with 1.14 mL beef fat injected. Occasional testing, by bubbleometer, is recommended to ensure correct nitrogen flow. One of the principal aims of gas flow is to obtain efficient dispersion of the fat onto the glass beads. Ideally, the fat should be spread to within 5–8 cm of the side arm. When removing each column after the 30 min fractionation period, check to see that sufficient fat dispersion has occurred; if this has not occurred, then low results can be expected.

The accuracy and precision of sample weight obtained by syringe injection technique was verified by weighing the fat (from 10 samples) expressed from the syringe under experimental conditions. With minimal practice, precision of 1.00 ± 0.02 g was readily achieved.

To eliminate errors in the volume of the final extract, all 2 mL collecting tubes were calibrated and the resulting factors were applied in recovery calculations. Such precautions are probably not necessary in most routine applications of this technique, but were included in our work to demonstrate that coefficients of variation of no greater than 6% can be obtained for a range of pesticides (Table 1).

Anhydrous sodium sulfate is packed in the front of the trap to remove traces of moisture resulting from the sample which might otherwise cause further deactivation of the Florisil, and subsequent loss of pesticides.

The method described has been validated using beef fat. However, the system has been applied in our laboratory to other meat fats, such as sheep, goat, chicken, and pork, as well as to butter and cheese fats, with equal success. Table 1 shows that all pesticides are recovered in acceptably high yield, and Figure 4 depicts a chromatogram of the cleaned up recovery extract and the blank beef fat used for fortification. In selecting field samples suitable for use in fortification trials, it was almost impossible to find samples that were completely free of *p,p'*-DDE. Accordingly, at these low levels it was sometimes necessary to subtract the peak due to *p,p'*-DDE in the blank fat from that of the recovery extract.

Table 1. Recoveries of organochlorine pesticides in spiked meat (beef) fat, using Unitrex

Pesticide	Spike level, mg/kg	Mean rec.,[a] %	SD	CV, %
HCB	0.02	96	5.8	6.0
α-BHC	0.02	87	3.7	4.2
Lindane	0.02	83	5.3	6.4
Hept. expox.	0.04	96	4.3	4.5
Dieldrin	0.05	93	5.3	6.2
p,p'-DDE	0.05	90	4.3	4.8
p,p'-DDD	0.08	105	5.1	5.4
p,p'-DDT	0.12	87	4.8	5.5

[a]Results derived from 26 recoveries.

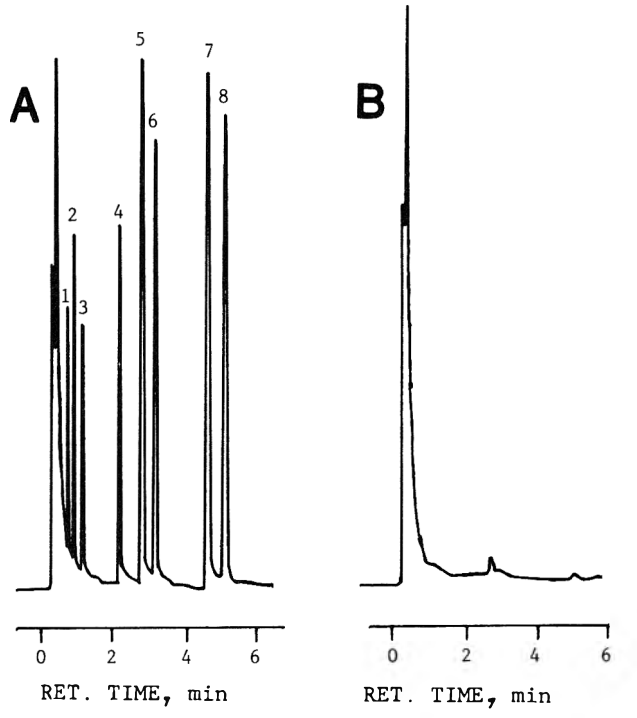

Figure 4. Gas chromatograms of extracts from Unitrex system: A, Beef fat fortified with organochlorine pesticides at levels given in Table 1. (1) HCB, (2) α-BHC, (3) lindane, (4) heptachlor epoxide, (5) *p,p'*-DDE, (6) dieldrin, (7) *p,p'*-DDD, (8) *p,p'*-DDT. B, Unfortified beef fat (blank).

The new design of the fractionation tube has several advantageous features. It permits construction of a very compact unit that offers the capacity for high sample through-put.

The introduction of a combined injection and nitrogen inlet system together with the inner glass tube facilitates downward insertion of the syringe needle rather than the inverted needle injection technique necessary with most previously reported units. The latter is awkward, particularly for inexperienced operators.

Almost complete elimination of glass wool in the fractionation tube means that the potential for contamination between samples, and degradation of pesticides due to incomplete removal of fat from previous injections, is significantly reduced. Also, on repeated use of conventional fractionation tubes containing relatively large amounts of glass wool, it is not unusual for the glass wool to be displaced from its original position. In some cases this can result in significant adverse variation in the actual glass bead area available for fat distribution. In the system described, the effective area for fat dispersion remains the same from one injection to the next.

Use of 1.5 mm diameter glass beads rather than the more commonly reported 3 mm diameter beads give a significant increase in glass bead surface area. The system is not unlike a preparative gas chromatograph, with the fat acting as the stationary phase. Accordingly, the increased surface area produces thinner films of fat, and this leads to more efficient partitioning of the pesticides out of the fat into the gas phase.

Better dispersion of fats has another major advantage. Whereas optimum nitrogen flows of approximately 600 mL/min are recommended when 3 mm diameter beads and 7 mm id fractionation tubes are used, we have found with Unitrex,

similar high recoveries of pesticides are achieved with nitrogen flows of 230 mL/min. This flow rate is equivalent to a gas velocity of 51 cm/s which is very similar to carrier gas velocities used in capillary gas chromatography. Apart from the obvious advantage of saving nitrogen, the most significant effect is that pesticides are trapped more efficiently on Florisil traps at these lower gas flow rates.

To determine the total weight of fat volatiles retained by the traps, we eluted the traps, after the normal 30 min fractionation period, with polar solvent mixtures. Although it is expected that some variation will result from one meat fat to another, 6 of 10 samples tested had less than 1 mg in their respective traps, while the remaining 4 traps collected only 1–2 mg of fat-related components. The absence of some coextract can in fact lead to problems if KD concentrations are subsequently carried out. It has been shown by us (and others) that in the absence of a fixative, losses of up to 15% of the more volatile pesticides (e.g., HCB, α-BHC, lindane) can occur during concentration. For this reason we routinely add 2–3 drops of a keeper solution to all solutions concentrated by KD technique. This practice has not adversely affected the efficiency or performance of our gas chromatographs in any discernable way over several years of operation.

Cleaning of the fractionation tubes is carried out with the aid of a vacuum line to flush approximately 25 mL hexane in through the stainless steel tube of the septum head, through the fractionation tube, and out the side arm to waste. This is best carried out when the fractionation tubes are warm. Fractionation tubes cleaned in this way, between each sample, are suitable for about 50 sample analyses before a more rigorous cleaning is necessary. The latter involves soaking the packed tubes overnight in an alkaline detergent solution, followed by thorough rinsing, drying, and resilanizing with 5–10% HMDS in toluene. The latter treatment is similar to that often used when preparing packed glass columns for gas chromatography, including removal of HMDS by washing thoroughly with toluene, methanol, and hexane.

In our experience, removal of glass beads from the fractionation column, cleaning, and re-assembly has not been necessary, but if required, could easily be carried out in the laboratory.

Periodic replacement of the small silanized glass wool plug at the top of the fractionation tube is necessary, taking care not to get any glass wool between the ground glass surfaces of the B5 joint.

One of the most important advantages of the Unitrex system is that it offers a very-cost effective technique for single or multi-sample, multi-residue cleanup. One operator can cleanup 30 samples per day, and on a per sample basis the method utilizes only 12 mL solvent, 6.9 L nitrogen, and less than 1 g Florisil, the latter being recovered and re-used.

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OILS AND FATS

Karl Fischer Determination of Water in Oils and Fats: International Collaborative Study

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Four samples of soybean oil refining process—a crude oil, a refined oil, an acid oil, and a deodorizer distillate—representing water contents from 0.02 to 3% were submitted to 16 laboratories for analysis by the Karl Fischer method ISO/TC 34/SC 11/N99 recommended by the International Organization for Standardization. The laboratories were asked to perform 2 sets of replicate analyses within 2 weeks. Data population was homogeneous at the 95% confidence level except for the acid oil sample where a sampling problem caused wider than expected variation between laboratories. However, overall results indicated interlaboratory variations of the order of 15% at the 0.3% moisture level, in line with the 10% coefficient of variation suggested by the American Oil Chemists' Society at the 0.5% level. The experience of several participants in practicing the method leads to the recommendation that the draft method be modified to include special care in handling heterogeneous and semisolid or solid samples, use of test sample sizes of 5–25 g, adoption of alternative weighed water standardization, use of chloroform–methanol (1 + 1) or (2 + 1) premixed solvent, and use of reagent dilution for low water samples. The method has been adopted official first action by AOAC.

The Karl Fischer method of water determination has been studied in depth (1) and need not be reviewed here. The importance of both the determination of water (moisture) and the Karl Fischer method for oils and fats has also been stressed (2). The AOAC *Official Methods of Analysis* (3) does not list a Karl Fischer method for the actual water content of oils and fats, and method Ca 2e-55 of the American Oil Chemists' Society (4) has not kept pace with several modern developments, including the wide availability of stabilized one-solution Karl Fischer reagent.

The present work was undertaken in 1980–1981 in support of the International Organization for Standardization (ISO) and on the behalf of the U.S. Technical Advisory Group of ISO, which is sponsored by AOAC. An international collaborative study of proposed method ISO/TC 34/SC 11/N99 (ISO-N99)[1] provided an opportunity to try and update U.S. official methodology.

The ISO-N99 method was first evaluated at our laboratory. The evaluation was primarily carried out on soybean oil process samples, because of the importance of soybean oil in international trade. Karl Fischer reagent stabilized by use of methoxyethanol solvent was used. At water levels as low as 0.04% (refined vegetable oils) and as high as 2.5% (soybean acid oil), good repeatabilities were found in replicate sampling, with relative standard deviations below 10% for low moisture samples and below 2% for the higher moisture samples. These results (Table 1) were in line with the precision cited in AOCS method Ca 2e-55 (4). Repeatabilities were excellent when only 2 significant figures were taken in each determination. Some samples used in these studies, i.e., crude soybean oil, vegetable oil deodorizer distillate, and soybean acid oil, contained variable amounts of sediment, but could be made homogeneous by shaking and were stable for several hours.

Recommended standardization using sodium tartrate dihydrate was cumbersome because the salt is only moderately soluble in methanol. A weighed water standard seemed more practical and quite reproducible; it was included in the collaborative plan as an alternative approach.

In dissolving the samples in chloroform–methanol (1 + 1), we found that some did not dissolve completely unless the ratio was increased to 2 + 1, yet 2-phase separation in the titration vessel did not affect the result. With repeated titrations of 2-phase systems, however, an oil film tended to coat the potentiometer electrodes, with ensuing electrical "noise."

Absence of method bias was tested by matching ISO-N99 and an acetyl chloride method by Kaufmann and Funke (5) on replicate samples of water-saturated corn oil; 0.11% water was obtained by either method.

The laboratories participating in this study were asked to follow all directions of the ISO-N99 method, and to give an opinion, if possible, about weighed water vis-a-vis sodium tartrate dihydrate standardization. A report form was distributed including questions about equipment, conditions, and raw data.

Moisture in Oils and Fats
Karl Fischer Method
First Action
ISO/TC 34/SC 11/N99 Method

(Applicable to oils and fats except for alk. or oxidized samples)

Apparatus and Reagents

(a) *Karl Fischer titration assembly.*—Manual or automated, with stirrer.

(b) *Karl Fischer reagent.*—Stabilized single soln. Available com. or prep. as in **31.069(a).** Stdze daily with Na tartrate · $2H_2O$. 1 mg Na tartrate · $2H_2O$ = 0.1566 mg H_2O. Alternatively, stdze with weighed H_2O in MeOH as follows: Transfer accurately weighed amt (50 mg) H_2O to titrn vessel and titr. to electrometric end point. Calc. C = mg H_2O/mL reagent.

(c) *Karl Fischer reagent diluent.*—2 Methoxyethanol–pyridine (4 + 1).

(d) *Sample solv.*—Anhyd. $CHCl_3$–MeOH (1 + 1 or 2 + 1).

Determination

Weigh, to nearest 0.01 g, 5–25 g prepd sample, contg. ≤100 mg H_2O, into titrn vessel, dissolve in anhyd. $CHCl_3$–MeOH. Titr. with undild or dild (1 + 1) Karl Fischer reagent to

[1]The initials stand for ISO Technical Committee 34 (Agricultural Food Products), Sub-Committee 11 (Oils and Fats), document No. 99. ISO/TC34/SC11/N99 is a proposal by the German Member Body of ISO, based on ISO Recommendation R760.

This report of the Associate Referee, R. Bernetti, was presented at the 97th Annual International Meeting of AOAC, Oct. 3–6, 1983, at Washington, D.C.

The recommendation of the Associate Referee was approved by the General Referee and Committee C and was adopted by the Association. See the General Referee and Committee reports, *J. Assoc. Off. Anal. Chem.* (1984) 67, March issue.

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Table 1. Moisture content of vegetable oils by Karl Fischer method

Sample type	H_2O, %	No. of analyses	SD ± % H_2O	Rel. SD, %
Corn oil satd with water	0.107	4	0.0019	1.8
Refined and bleached soybean oil satd with water	0.068	7	0.0069	10.1
Refined and bleached soybean oil	0.042	3	0.0006	1.4
Crude soybean oil	0.112	3	0.0020	1.8
Vegetable oil deodorizer distillate	0.309	4	0.0036	1.2
Soybean acid oil	2.58	3	0.006	0.2
AOCS Method Ca 2e-55 as reported (4)	0.55	—	0.015	2.7

electrometric end point. Carry out blank test using same amts of reagent, diluent, and solvs. Subtract blank titer.

$$\% H_2O = (\text{mL reagent} \times C)/(\text{g sample} \times 10)$$

Collaborative Study

Four samples of commercial process oils were sent to 16 collaborators, including the Associate Referee's laboratory. Sample I was a refined and bleached soybean oil of less than 0.1% moisture; Sample II was a crude (unrefined) soybean oil at 0.1% moisture; Sample III was a vegetable oil deodorizer distillate at 0.3% moisture; Sample IV was a soybean acid oil at 2.5–3.0% moisture. The samples were packed in 12 oz. steel cans tightly sealed with screw caps lined with aluminum foil. To obtain some measure of the stabilities of the samples, collaborators were asked to perform 2 sets of replicate analyses, respectively, on receipt of samples (time 1) and approximately 2 weeks later (time 2). The report form included a questionnaire on Karl Fischer apparatus used, type of Karl Fischer reagent used (commercial or laboratory-prepared), sample weight, solvent, and preferred standardization. (AOAC *Official Methods of Analysis* is equally divided between standardizations using sodium tartrate dihydrate or weighed water. See sections **2.016, 13.004, 31.069, 32.048.**)

Table 2. Collaborative results of ISO/TC 34/SC 11/N99 method for moisture determination by Karl Fischer titration

	Time 1 Sample				Time 2 Sample			
Lab.	I	II	III	IV	I	II	III	IV
A	0.02	0.16	0.24	3.18	0.02	0.15	0.23	3.32
	0.02	0.17	0.24	3.14	0.02	0.15	0.22	3.28
B	0.02	0.15[a]	—	3.22[a]	0.02	0.15[b]	0.22[b]	3.37[b]
	0.03	0.06[a]	—	3.79[a]	0.02	0.14[b]	0.22[b]	3.49[b]
C	0.03[b]	0.13	0.23	2.8	0.02[a]	0.10	0.14	1.3
	0.02[b]	0.14	0.24	3.0	0.04[a]	0.10	0.10	1.3
D	0.04	0.11	0.22	3.43	0.04	0.12	0.27	3.27
	0.04	0.11	0.23	3.31	0.04	0.12	0.25	3.30
E	0.03	0.11	0.22	2.97	0.02	0.10	0.19	3.01
	0.04	0.10	0.23	3.04	0.02	0.10	0.22	2.96
F	0.03	0.09	0.21	2.89	0.02	0.09	0.21	2.88
	0.03	0.09	0.20	2.90	0.02	0.09	0.21	2.87
G	0.01[a]	0.09	0.24	2.85[b]	0.04[b]	0.08	0.24	3.01[a]
	0.03[a]	0.08	0.20	2.75[b]	0.04[b]	0.10	0.25	2.69[a]
H	0.04	0.07	0.22	4.25[a]	0.02	0.09	0.19	4.05[b]
	0.04	0.11	0.19	4.15[a]	0.03	0.08	0.19	4.10[b]
I	0.02	0.10	0.14[a]	2.66	0.02	0.09	0.22[b]	3.01
	0.02	0.10	0.15[a]	2.64	0.03	0.07	0.22[b]	2.96
J	0.02[b]	0.09[b]	0.26[b]	3.40	—	—	—	2.26
	0.02[b]	0.10[b]	0.27[b]	3.34				2.26
K	0.03[b]	0.15	0.27[a]	3.16	—	0.14	0.27[b]	3.32
	0.03[b]	0.16	0.20[a]	2.97		0.14	0.23[b]	3.49
L	0.03	0.12	0.23	3.03	0.03	0.13	0.24	2.77
	0.02	0.13	0.23	2.89	0.04	0.13	0.24	2.82
M	0.03	0.17	0.32[a]	4.16	0.03	0.15	0.27[b]	4.00
	0.03	0.18	0.32[a]	4.17	0.03	0.17	0.27[b]	4.10
N	—	0.06	0.17	2.31	—	0.05	0.18	2.07
		0.08	0.18	2.25		0.07	0.18	2.11
O	0.13[a]	0.16[b]	0.25[b]	3.36[b]	—	—	—	—
	0.10[a]	0.15[b]	0.23[b]	3.33[b]				
P	0.04	0.12	0.22	3.17	0.04	0.13	0.23	3.19
	0.04	0.11	0.22	3.22	0.04	0.13	0.22	3.28
Mean	0.029	0.118	0.223	3.083	0.029	0.113	0.218	3.005
Reprod.	0.008	0.033	0.023	0.435	0.009	0.031	0.039	0.743
CV, %	27.66	28.01	10.70	14.10	31.67	27.42	17.94	24.72
Repeat.	0.004	0.010	0.012	0.070	0.004	0.009	0.014	0.052
CV, %	13.42	8.47	5.40	2.28	12.9	7.10	5.99	1.73

[a]Data eliminated as outliers.
[b]Data eliminated to balance outliers at the other time, or to balance missing data at the other time.

All collaborators followed the recommended procedure except for the suggested sample weights, which were deemed excessive relative to the sizes of titration vessels available. Nearly all collaborators used purchased automatic titrators and Karl Fischer single-solution reagent (containing methoxyethanol). Seven collaborators reported testing both tartrate · $2H_2O$ and weighed water standardization and indicated their preference for the latter.

Results and Recommendations

All collaborative results are listed in Table 2. The statistical evaluation done according to Steiner (6) showed no outlying laboratories, but several Dixon and Cochran outliers. To obtain a balanced view of times of analysis, it was decided to calculate means, reproducibilities, and repeatabilities, and the corresponding coefficients of variation individually for each sample time which corresponded to outliers and to missing (unreported) results. An overall statistical summary, covering all data in Table 2, did not seem meaningful. In fact, a 2-way analysis of variance for each sample, and a model consisting of laboratory, time, and laboratory × time interaction gave significant ($P = 0.05$) interaction terms, which advised against combining time 1 and 2 data. However, the reduced set showed reproducibilities and repeatabilities which were nearly the same at time 1 and 2 and quite acceptable. For instance, the mean of 0.22% moisture for Sample III, and its interlaboratory coefficient of variation of 11–12% compares well with corresponding AOCS-reported (4) data of 0.55% moisture level and a CV of 10.8%.

It is probable that data for sample type IV could have been improved with greater care in sampling.

The collaborated method is recommended for adoption as official first action by AOAC, with a change, however, of test sample size to read "5–25 g" instead of "20–40 g" as originally stated to the collaborators. This change should also be considered by ISO.

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PESTICIDE FORMULATIONS

Liquid Chromatographic Determination of Benomyl in Wettable Powders

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Pesticide formulations containing benomyl are extracted with acetonitrile containing 3% *n*-butyl isocyanate (BIC) and chromatographed on a reverse phase C-18 column. The equilibrium of benomyl degradation to methyl 2-benzimidazole carbamate (MBC) and BIC is driven toward benomyl. Thus, the method offers quantitation of benomyl in its intact form, using a mobile phase of acetonitrile–2% acetic acid (80 + 20) and detection at 290 nm. This assay was evaluated for linearity, precision, recovery, stability, and matrix effects. Linearity checks of standard solutions and spiked samples yielded coefficients of determination, r^2, of 0.9996 and 0.9998, respectively. Quadruplicate analysis of 5 benomyl formulations gave an average relative standard deviation of 1.95%. A multiple standard addition plot revealed the absence of matrix effects. Mean recovery of standard from the sample pool was 95% with a standard deviation of 3.5%. Samples chromatographed 2 weeks after extraction indicated excellent analyte stability.

Benomyl (methyl 1-(butylcarbamoyl)-2-benzimidazole carbamate) is a systemic fungicide marketed as a 50% wettable powder. The analysis of benomyl is complicated by its degradation to methyl 2-benzimidazole carbamate (MBC) and *n*-butyl isocyanate (BIC) in water and organic solvents at a rate rapid enough to preclude chromatographic separation as a single compound. Published chromatographic methods of benomyl analysis are based on the determination of the acid hydrolysis product MBC (1–8) rather than of the intact pesticide. The reports that MBC is at least as toxic to most organisms as benomyl (9, 10) and that the breakdown is slow following foliar spraying (10) would suggest that this strategy is valid. Acid hydrolysis requires upward of 1 h and has not been applied to formulation samples.

The work of Chiba (11–13) shows that the breakdown of benomyl is reversible and that addition of BIC will cause the reformation of benomyl from MBC. This work culminates with the report (13) of a simple spectrophotometric analysis of intact benomyl reformed from MBC. Coupling this technique with liquid chromatography (LC) should result in a specific method for analyzing benomyl which is not complicated by its degradation during chromatography and handling. Following the development of this method, the authors discovered an unpublished method used by the California Department of Food and Agriculture which is essentially the same as the one reported here.

METHOD

See reference 14.

Method Evaluation

The method was evaluated as previously described (14). Precision was tested by assaying 5 formulations in quadruplicate. Linearity was evaluated by chromatographing several dilutions of the standard. Benomyl stability was tested in the extractant by comparing peak heights of the same standard over a one week period and by rechromatographing the precision extracts after 2 weeks.

The method was tested for recovery and matrix effects by performing a multiple standard addition in triplicate, at 3 levels, to a pool of formulation samples. Recovery was determined by calculating the amount of analyte found due to standard as a percentage of that added. The matrix effects were tested by plotting % found vs amount added. The x-intercept corresponds to the amount that would have to be subtracted from the matrix to yield a 0% determination. Absence of a matrix effect is indicated when this intercept corresponds to the amount of analyte in the sample. These extracts were rechromatographed after 2 weeks as a further test of analyte stability.

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Results

Figure 1 represents a typical chromatogram of a 50% benomyl wettable powder. Retention time of the analyte peak was about 5.5 min. No interfering peaks were ever observed.

Benomyl showed excellent stability for the length of time evaluated. No deterioration of diluted standard was observed over one week's time. Sample extracts gave slightly higher results after 2 weeks (Table 1). The linearity of standard injections was very good (coefficient of determination, $r^2 > 0.9996$) as was that of the spiked sample extracts ($r^2 > 0.9998$).

The results of the precision study are shown in Table 1. The samples used in this study varied in age from fresh to 5 years old. Quadruplicate analysis of five 50% wettable powder formulations gave an average relative standard deviation (RSD = standard deviation/mean × 100) of 1.95% for the initial analysis and 0.69% for the 2 week analysis. The largest RSD for any one sample was 3.70%.

Mean recovery of standard from the formulation pool was 95% with a standard deviation of 3.5%. This standard deviation is inflated by the variation in analysis of analyte from the sample pool. The plot of % found vs amount of standard added gave an x-intercept of −10.98 mg which corresponds well to an expected 10.87 mg. This indicates that the assay is free from matrix effects. Re-injection of these extracts after 2 weeks revealed no changes in these findings.

Discussion

The results presented here show this method of benomyl analysis to possess good precision, linearity, and recovery and to be free of matrix interferences. These results also show benomyl to be very stable in the extractant used.

The authors have found this method to be straightforward and productive in routine use. It is simpler and quicker than other available chromatographic methods and has the advantage of specificity over spectrophotometric methods. The concept of adding a breakdown product to the extractant to inhibit analyte degradation could be applied to many other compounds. This concept must, however, be applied with care. The analyte benomyl is likely recreated from degradation products present in the sample. In most cases, this would preclude the use of the technique but the facts that MBC is as effective as benomyl (9, 10) and that benomyl is applied in aqueous suspension where breakdown naturally occurs (10) support the use of this method.

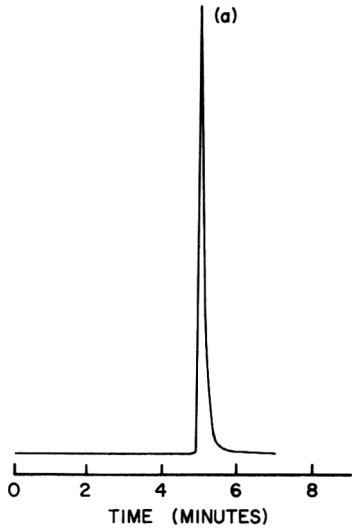

Figure 1. Chromatogram of extracted 50% wettable powder benomyl (a) formulation (5 μg benomyl injected).

Table 1. Results of quadruplicate analysis of 5 benomyl formulations (% benomyl ± SD (% RSD)[a])

Sample	Day 0	Day 14
83–333	52.8 ± 1.27 (2.41)	53.4 ± 0.67 (1.25)
82–562	52.1 ± 1.93 (3.70)	54.5 ± 0.38 (0.70)
80–143	52.0 ± 1.21 (2.33)	54.6 ± 0.39 (0.72)
79–324	52.9 ± 0.37 (0.70)	54.5 ± 0.08 (0.15)
78–195	52.1 ± 0.33 (0.63)	54.4 ± 0.34 (0.63)
	Av. RSD = 1.95%	Av. RSD = 0.69%

[a] ± Standard deviation (% relative standard deviation).

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Liquid Chromatographic Determination of Benomyl in Wettable Powders: Collaborative Study

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A simple and rapid determination of benomyl in wettable powders was tested by 10 collaborators. Formulations of benomyl are extracted into acetonitrile containing 3% *n*-butyl isocyanate, filtered, and chromatographed on a reverse phase (C-18) column with a minimum of 1000 plates. Benomyl is detected at 290 nm and quantitated by peak height. Three closely matched pairs of benomyl formulations were assayed along with a duplicate pair of 98% technical benomyl. The coefficients of variation were 1.20% for the 50% wettable powder, 5.89% for the 10% benomyl formulation with 50% captan, and 2.49% for a formulation of 6% benomyl with 10% captan and 50% lindane. Analysis of the technical benomyl gave a coefficient of variation of 1.52%. Collaborators stated the method was simple, rapid, and reproducible, and the statistical analysis showed it to be precise and free from expected interferences. The method has been adopted official first action.

Benomyl (methyl 1-(butylcarbamoyl)-2-benzimidazole carbamate) is a systemic fungicide whose analysis is complicated by its spontaneous degradation to methyl 2-benzimidazole carbamate (MBC) and *n*-butyl isocyanate (BIC) in water and organic solvents. Published chromatographic methods of analysis (1–9) are based on the determination of MBC following hydrolysis. These methods are time consuming and have not been well tested on formulation samples.

Chiba (10) reported a simple spectrophotometric analysis of benomyl including benomyl reformed from MBC by the addition of BIC. Stringham and Teubert (11) adapted this concept to liquid chromatography (LC) which resulted in a simple, rapid, and specific assay. The collaborative study of this method is the subject of this report.

Benomyl in Pesticide Formulations
Liquid Chromatographic Method
First Action

Method Performance

6–98% (S_x = 0.158–1.48, S_o = 0.158–0.767)

Principle

Benomyl is extd from inerts with CH_3CN contg 3% (v/v) *n*-butylisocyanate (BIC). Equilibrium of benomyl spontaneous decomposition is driven toward benomyl and no significant degradation occurs. Ext is filtered, chromatographed on reverse phase (C_{18}) column, using CH_3CN–2% HOAc mobile phase, and quantitated by comparing peak hts of sample exts and std from UV detector set at 280 or 290 nm. *Caution:* BIC IS A SEVERE LACHRYMATOR!

This report of the Associate Referee, W. E. Teubert, was presented at the 97th Annual International Meeting of the AOAC, Oct. 3–6, 1983, at Washington, DC.

The recommendation of the Associate Referee was approved by the General Referee and Committee A and was adopted by the Association. See the General Referee and Committee reports, *J. Assoc. Off. Anal. Chem.* (1984) 67, March issue.

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Reagents

(a) *Extractant.*—3% (v/v) *n*-butyl isocyanate (Aldrich Chemical Co.) in CH_3CN (LC grade).

(b) *Mobile phase.*—CH_3CN (LC grade)–2% HOAc (80 + 20), or as adjusted to give $k' > 2$ for analyte when delivered at 1.0 mL/min, and retention time for benomyl of 4–6 min.

(c) *Reference std.*—Benomyl (E. I. DuPont de Nemours & Co., Inc.).

Apparatus

(a) *Liquid chromatograph.*—Able to generate > 1000 psi and measure UV absorbance at 290 or 280 nm. Also must be capable of reproducibly injecting 10 μL.

(b) *Chromatographic column.*—10 μm reverse phase C_{18} column which produces ≥ 1000 theoretical plates for benomyl (defined as 5.5 $(t/w)^2$, where t = retention time and w = width at half ht.

(c) *Filtration.*—13 mm glass fiber disc (Gelman Sciences, Ann Arbor, MI 48106) inserted into 5 mL disposable syringe (Pharmaseal Labs, Glendale, CA 91201), or equiv.

Procedure

Accurately weigh stds and samples to contain ca 25 mg benomyl, add 50.0 mL extractant, and shake 30 min. Filter thru glass fiber pad and inject 10 μL aliquot. Bracket each 2 sample injections with std injections and av. std response for calcn of sample concn.

Calculation

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

where R and R' = peak ht of sample and std, resp.; W and W' = wt of sample and std; and P = purity of std.

Results and Discussion

The validation and limitations of the methods collaborated here have previously been discussed (11). Each collaborator received a copy of the method, a practice sample, a vial of standard benomyl (99%), a vial of *n*-butyl isocyanate, and 8 samples. Two different lots of 50% benomyl formulations (Samples 1 & 7), 2 lots of 10% benomyl with 50% captan (Samples 2 & 6), and 2 lots of 6% benomyl formulated with 10% captan and 50% lindane (Samples 5 & 8) were sent with duplicate vials of 98% technical benomyl (Samples 3 & 4). Only one determination per sample was asked of each collaborator. The collaborative design was taken directly from Youden and Steiner (12).

An actual chromatogram from the study is presented in Figure 1. Two collaborators observed that a small but well separated peak eluted after the benomyl. Its identity has not been determined. Results from the study are presented in Tables 1 and 2. Collaborator 1 did not achieve the required chromatographic performance (Table 1) so this set of data was eliminated from further consideration. One sample pair each from Collaborator 4 and Collaborator 5 were identified as outliers by the Dixon test and therefore eliminated. Values derived for SD, S_r, S_b, and CV (Table 2) were quite acceptable with the exception of those for pairs 2–6. These samples were labeled as 10% benomyl with 50% captan. When we checked with the supplier, we found that these samples were intentionally over-formulated and contained "a few lots of captan-benomyl mixed together." The authors suspect that incomplete sample mixing may have contributed to the problems encountered with these samples. This suspicion is supported by the relatively large systematic error (S_b) shown in Table 2.

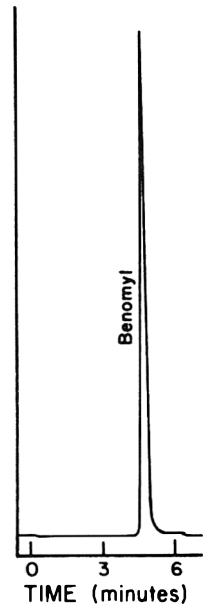

Figure 1. Typical sample chromatogram from collaborative study.

The authors have found that for those with fixed wavelength detectors, 280 nm is a satisfactory wavelength to monitor benomyl. The concept of adding a breakdown product to the extractant to inhibit analyte degradation could be applied to many other compounds. This concept must however be applied with care. The analyte benomyl is likely recreated from degradation products present in the sample. In most cases this would preclude the use of the technique but the facts that MBC is as effective as benomyl (13, 14) and that benomyl is applied in aqueous suspension where breakdown to an equilibrium naturally occurs (14) support the use of this method.

Table 1. Collaborative results (%) for analysis of benomyl formulations

	Collaborator									
Sample	1	2	3	4	5	6	7	8	9	10
1	46.76	51.24	52.20	52.44	50.52	51.58	51.25	50.92	51.33	52.92
2	13.42	12.42	13.30	13.12	11.47	12.90	11.82	11.40	13.08	13.56
3	100.87	95.58	98.00	97.22[a]	101.04	96.50	98.17	97.60	96.23	98.46
4	89.62	95.79	98.30	102.73[a]	99.07	96.54	97.75	96.85	97.88	99.33
5	5.88	6.36	6.04	6.39	6.31[a]	6.30	6.37	6.42	6.40	6.38
6	12.76	12.05	12.50	12.37	11.38	13.32	11.74	11.61	12.71	12.82
7	49.68	51.08	51.50	50.34	50.91	50.59	51.18	51.36	51.22	51.54
8	6.45	6.28	6.60	6.47	7.05[a]	6.32	6.24	6.31	6.40	6.54
N^b	484	1111	2555	1936	3249	3600	3170	2304	2209	2774

[a]Data pairs omitted by Dixon test.
[b]Theoretical plates for benomyl.

Table 2. Statistical summary of collaborative results

Statistic	Sample pair and label claim 1–7 (50%)	2–6 (10%)	3–4 (98%)	5–8 (6%)
SD	0.606	0.732	1.483	0.158
S_r	0.599	0.309	0.767	0.158
S_b	0.145	0.664	1.269	—
CV, %	1.20	5.89	1.52	2.49
n (pairs)	9	9	8	8

A large majority of collaborators who commented have expressed their satisfaction with this method, calling it "simple, rapid, and reproducible" and the statistical analysis showed it to be precise and free from expected interferences. Therefore, it is recommended that this method be adopted official first action.

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Diane E. Bradway, NEIC, Denver, CO

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MYCOTOXINS

Effect of Solvent Composition on Aflatoxin Fluorescence

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The influence of the chloroform–methanol solvent system on the fluorescence of aflatoxins B_1, B_2, G_1, and G_2 was investigated. Fluorescence intensity is markedly affected by solvent composition. The formation of excited state dimers and ionization are proposed to explain the effects observed.

The influence of solvent type on the fluorescence of molecules is a well recognized phenomenon (1, 2). The ultraviolet absorption spectra of aflatoxins have also been noted to differ significantly in different solvents (3, 4). Because the 363 nm absorption band is the excitation band responsible for aflatoxin fluorescence, any variation in its absorption coefficient will affect the fluorescence intensity (3). Excitation and emission maxima of aflatoxins have been reported for chloroform (4, 5) and methanol (4, 6) solutions. Marked differences in the fluorescence intensities in solvents of different polarity were observed (4, 6) but no explanation for these differences was offered. The present investigation into the effects of chloroform–methanol solvent composition on aflatoxin fluorescence attempts to explain the differences observed.

Experimental

Reagents and Apparatus

(a) *Aflatoxins.*—Aflatoxins B_1, B_2, G_1, and G_2 were purchased in the dry state from Sigma Chemical Co. (St. Louis, MO).

(b) *Solvents.*—Analytical grade chloroform and methanol (British Drug Houses, Poole, UK) were used to make up all solutions.

(c) *Spectrophotometer.*—Perkin Elmer double beam fluorescence spectrophotometer, Model 512, with digital readout. Instrumental settings for both excitation and emission scans—mode: energy; scan: single; scan speed: fast; response: medium; slit widths: 10 nm for excitation and emission slits.

Preparation of Aflatoxin Solutions

A stock solution (40 μg/mL) of each aflatoxin was prepared in chloroform in amber-colored volumetric flasks, covered with aluminum foil to exclude light. Solutions were stored at 5°C. Each stock solution was diluted 100-fold with pure chloroform. Aliquots (2.45 mL) of the diluted (400 ng/mL) solutions were pipetted into separate 10 mL flasks and the solvent was removed rapidly with a stream of nitrogen at room temperature. To the flasks containing 0.98 μg of an aflatoxin, solvents were added of varying composition, ranging from pure chloroform through various chloroform–methanol mixtures [(75 + 25), (50 + 50), (25 + 75), (10 + 90)] to pure methanol. The new aflatoxin solutions were purged for about 1 min with nitrogen to remove dissolved oxygen. Solutions were subjected immediately to fluorescence analysis.

Fluorometric Analysis

The excitation spectrum of each sample was determined first. Emission was monitored at 400 nm at sensitivity setting 10. For emission scans, the excitation wavelength was set at the excitation maximum obtained, at sensitivity setting 3. The digital readings at the excitation and emission maxima were recorded.

Results and Discussion

There is a marked effect of solvent polarity on the fluorescence intensities of 3 of the 4 aflatoxins studied. Plots of these intensities for different chloroform–methanol solutions are recorded in Figure 1. The plots of excitation and emission intensities for aflatoxin B_1 show distinct maxima at 50% methanol in chloroform. On the other hand, the fluorescence inten-

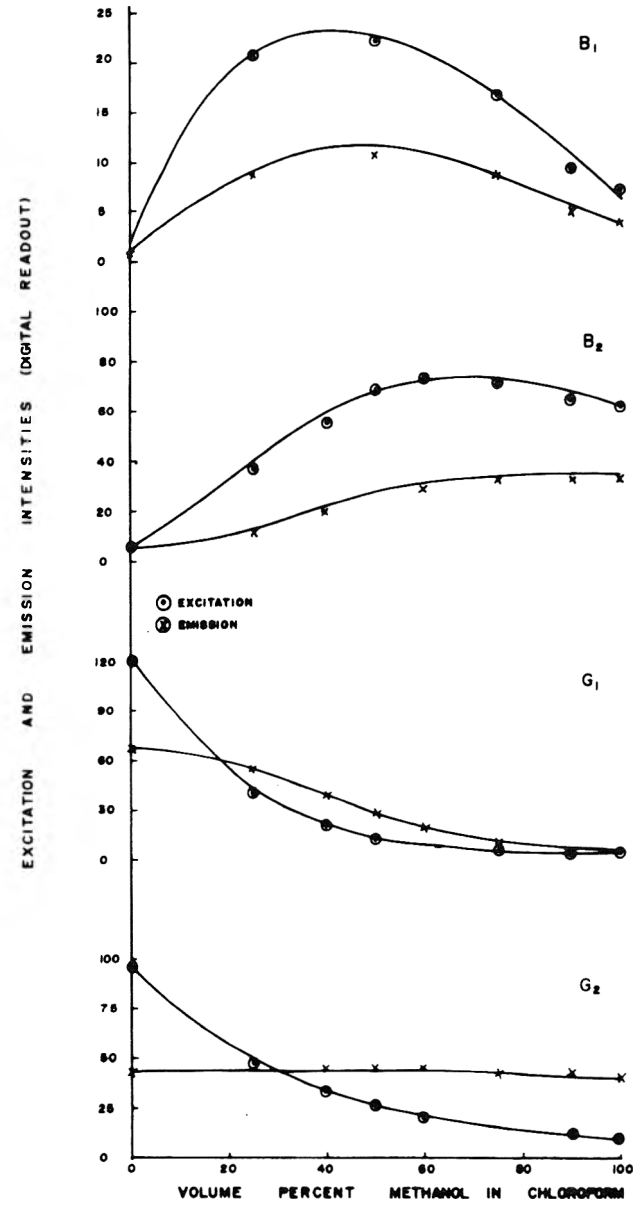

Figure 1. Effect of solvent composition on emission and excitation intensities of aflatoxins.

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sity of B_2 increased steadily, reaching a plateau value beyond 60% methanol in chloroform. The G_1 compound exhibits the opposite effect from B_2: Its intensity decreases steadily to its lowest value in pure methanol. The fluorescence intensity of the G_2 compound appears to be unaffected by solvent polarity, yet, curiously, the intensity of excitation decreases steadily as the methanol content of the solvent is increased.

The aflatoxins B_1 and G_1 differ from their analog B_2 and G_2 only in the presence of the C-1 double bond (Figure 2). This double bond has been shown to be the site of photochemically induced attack by hydrolytic solvents (7–10), as well as the site of facile electrophilic addition reactions with various reagents used to confirm aflatoxin identity (8, 11).

The B and G aflatoxin series differ from each other only in so far as the cyclopentenone ring (with labile C-8 hydrogens) of the B series is replaced by a lactone ring in the G compounds.

It is conceivable that it is these differences among the 4 compounds which account for the different emission patterns observed.

In the B_1 and G_1 compounds, containing both the C-1 double bond and carbonyl groups, it may be possible to have some solute association involving the excited state. This association could be strengthened by at least the partial transfer of charge (12, 13a) between the carbon-carbon double bond of one molecule and the carbonyl group of another (Figure 3). Since a molecule in its excited state is both a better electron donor and a better electron acceptor than in its ground state, excited state complexation is expected to be a common phenomenon (13a). Charge redistribution on complexation can lead to a species that is more polar than the excited state and its formation is promoted, therefore, in solvents with large dielectric constants. It is suggested that increasing solvent polarity may be enhancing an association of this type, leading to the subsequent quenching of fluorescence intensity as the 2 processes (emission and association) compete for deactivation of the S_1 excited state (Figure 3). After excitation of the ground state (S_o) via k_E, the excited state may decay by several competitive pathways (12). Two of the pathways, fluorescence (k_f) and association of S_1 with S_o to give the $[S_o - S_1]$ complex, are illustrated in Figure 3.

This argument is sufficient to interpret the results observed with G_1. The steady decrease in emission intensity can be correlated with the steady increase in complex formation with increasing solvent polarity. However, for B_1, the maximum observed in the emission intensity indicates that, in this case, a second effect may be operating simultaneously with association.

Ionization (Figure 4) of labile C-8 hydrogens is expected to increase with solvent polarity. If the resulting anion is a more efficient fluorescing agent, then the result of this effect would be an increase in fluorescence intensity. It is possible that this is operating in the aflatoxin system. Overall therefore, it is proposed that both dimerization and ionization effects are minimal in solutions involving pure chloroform as the solvent. As the methanol concentration increases, the increasing but opposing effects of the 2 mechanisms on fluorescence could result in the maximum observed with B_1. Significantly, the curve is almost symmetrical about the maximum.

It is further proposed that the effect of solvent polarity on intermolecular association is more marked than on ionization. Even though they may both increase with the percentage of methanol, association becomes ultimately the predominant factor, resulting in a net decrease in the fluorescence intensity beyond 50% methanol. The observed emission intensities of the other aflatoxins are also consistent with this proposal. B_2

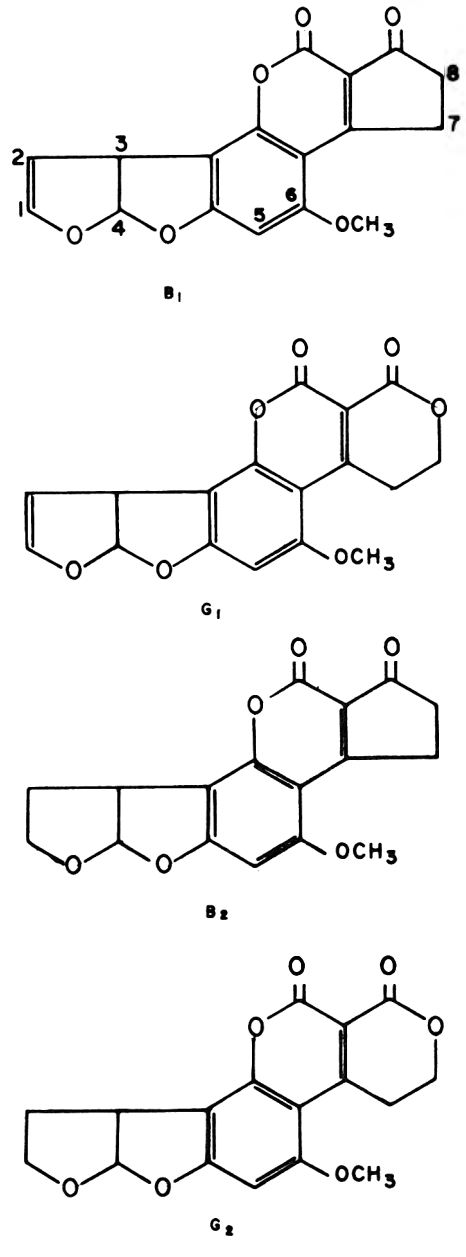

Figure 2. Chemical structures of aflatoxins B_1, G_1, B_2, G_2.

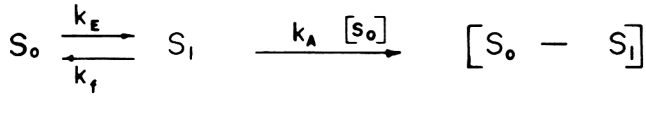

Figure 3. Decay of S_1 excited state by competitive fluorescence (k_f) and association ($k_A[S_o]$) pathways.

has no C-1 double bond, perhaps a necessary requirement for measurable deactivation of the S_1 state via complex formation with resultant fluorescence quenching. It should, however, undergo ionization as easily as B_1 and the steady increase in fluorescence with solvent polarity supports the argument that ionization alone may be operating in B_2. It is interesting to note that the increase in B_2 fluorescence is not as large as the decrease in G_1 fluorescence, again supporting the argument that solvent polarity affects complexing of the excited state to a greater extent than ionization.

Aflatoxin G_2 lacks both the C-1 double bond and the C-8 hydrogens and it is not expected to be affected by solvent

Table 1. Effects of solvent composition on excitation and emission maxima of aflatoxins

Solvent	Scan mode	Wavelength maxima, nm			
		B_1	B_2	G_1	G_2
Chloroform	excitation	358	365	363	366
	emission	424	412	428	424
Chloroform–methanol (75 + 25)	excitation	360	365	363	366
	emission	424	421	438	435
Chloroform–methanol (50 + 50)	excitation	358	365	363	366
	emission	424	420	438	438
Chloroform–methanol (25 + 75)	excitation	362	365	363	366
	emission	424	423	445	442
Chloroform–methanol (10 + 90)	excitation	360	365	363	366
	emission	431	424	443	442
Methanol	excitation	358	365	363	366
	emission	431	423	445	442

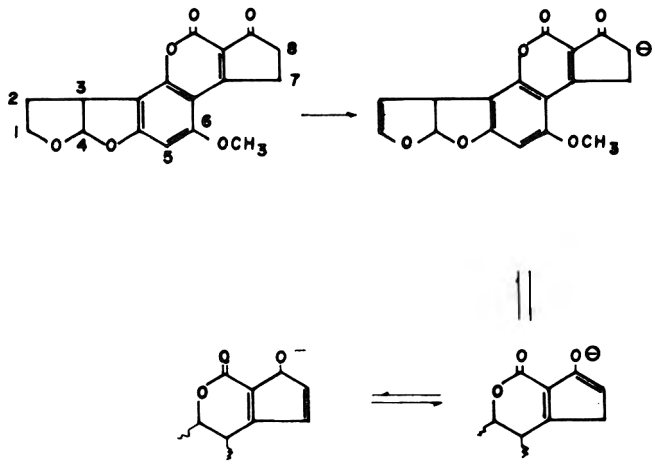

Figure 4. Proposed ionization of aflatoxin B_1. Anion at C-8 is stabilized by delocalization of charge onto neighboring oxygen and C-7 atoms.

changes. This is confirmed by the constant fluorescence intensity observed throughout the range of solvent mixtures. Its excitation intensity however decreased rapidly as solvent polarity was increased. The reason for the latter observation is not clear.

It was also noted (Table 1) that, for all 4 aflatoxins studied, the fluorescence maximum shifted to longer wavelengths with increasing solvent polarity, although the excitation maxima remained nearly constant. This shift has been suggested to be due to the interaction between the excited aflatoxin molecules and the more polar solvent (2, 4). The connection between shifts of excitation and emission maxima and the nature of the excited state is a difficult area to interpret because both solute-solvent interactions and the consequences of the Franck-Condon principle must be considered. It has been pointed out (13b) that electronic excitation is so rapid a process that the excited state may be formed before the solvent cage of the ground state can rearrange itself. If the lifetime of the excited state is long enough so that rearrangement of the solvent cage takes place before emission, solvent stabilization of the excited state will affect the wavelength of emission but may not necessarily affect that of excitation. Increasingly polar solvents will stabilize a polar excited state more (but sometimes only after excitation) and lead to longer wavelengths for fluorescence maxima.

Any variation in the purity of solvents that changes their polarity significantly may affect both the fluorescence wavelength maxima and intensities of these compounds. In addition, maximum fluorescence intensity is observed for B_1, B_2, and G_1 in different solvent mixtures. Sensitivity of analysis may be improved, therefore, by use of different solvent systems giving the best sensitivity for a specific aflatoxin. This may be used, for example, in testing the purity of aflatoxin standards, as suggested by Jones (14). For example, the fluorescence intensity of B_1 in a 50% methanol in chloroform solution is more than 12 times that in pure chloroform and more than 6 times that in pure methanol. B_2 is over 6 times more fluorescent in pure methanol than in chloroform. On the other hand, the G_1 fluorescence intensity in chloroform is over 27 times that in methanol. The effect of solvent polarity on fluorescence thus explains the previous reports by Chelkowski (4) and by Carnaghan and co-workers (6) of marked differences in fluorescence output by the 4 aflatoxins in methanolic solution. Finally, since the problem of photodecomposition may be enhanced for solutions showing maximum excitation intensities, the use of solvents in which the excitation intensities are minimal could result in longer storage times for standard stock solutions.

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Aflatoxin Levels in Airborne Dust Generated from Contaminated Corn During Harvest and at an Elevator in 1980

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Bulk corn samples and airborne samples generated therefrom were collected on a Georgia farm during the 1980 harvest and while the 1980 corn was being ground and fed to swine. The bulk corn harvested had 1640 ng total aflatoxin/g. Airborne dust samples collected at the front of the combine had an average of 3850 ng total aflatoxin/g; at the side of the combine, the average aflatoxin level was 2550 ng/g of dust. At one time, the operator was exposed to airborne dust containing 1360 ng/g while inside the combine cab. An airborne dust sample taken with a personal sampler during transfer of the corn to a truck had 52 000 ng/g. Airborne dusts collected while corn containing aflatoxin (1240–1850 ng/g) was being ground and fed had total aflatoxin levels ranging from nondetected to 43 700 ng/g. Airborne dust, bulk corn, and settled dust samples were collected at a grain elevator. Airborne dust samples collected during the delivery of corn in farm trucks to the elevator had an average of 1240 ng total aflatoxin/g. The airborne dust samples taken while corn was being unloaded from the elevator into trucks and railroad cars had lower average aflatoxin levels than those in dusts collected during delivery. Settled dusts in the elevator had 173–669 ng total aflatoxin/g. The highest aflatoxin level in the air in the elevator was 13 000 ng/cu. m near the conveyor belt. Airborne dust samples collected by personal samplers on elevator workers contained aflatoxin levels of 429–1300 ng/g; aflatoxin levels in the air were 9–1120 ng/cu. m.

The potential hazard to agricultural workers through inhalation of airborne dusts from aflatoxin-containing corn continues to be a major concern. Various aspects of the possible problem have been reviewed (1). Previous studies have established the presence of aflatoxin in airborne dusts from contaminated corn, but not the extent of the hazard to farmers, elevator operators, and other agricultural workers (2, 3). The International Agency for Research on Cancer (IARC) classifies aflatoxin as 2A, which means it is probably carcinogenic to humans, with a high degree of evidence (4). A recent report of the Department of Health and Human Services lists aflatoxin as one of 88 compounds believed to be a human carcinogen (5). The strongest evidence that aflatoxins may be an inhalation hazard is a study conducted in The Netherlands (6). A group of workers exposed to aflatoxins in processing peanuts for oil was studied. The exposed workers had 3 times more cancers than a group of control workers, but the number of workers (60–70) was too small for the results to be statistically significant.

The present investigation describes the aflatoxin content in airborne dust samples collected while corn was being harvested in 1980; the corn had 1640 ng total aflatoxin/g. A farm was selected for this study before the severity of its aflatoxin problem was known. Total airborne dust samples were collected to include all particles that might be deposited at any site within the respiratory tract. Major deposition sites vary markedly in structure, size, function, and response to deposited particles (7). Even particles generally considered to be nonrespirable (>7–10 μm) have been shown to be deposited in the trachea and bronchi. Factors other than the diameter of dust particles have an effect on deposition and retention times, such as differences in the regions or sites of the respiratory tract, individuals, and pattern and rate of the air stream. Samples of airborne dusts were taken on the same farm as corn was being ground and fed. Airborne dust samples were also collected at an elevator during delivery of corn from the surrounding areas and during loading of railroad cars and trucks from the elevator. Settled dusts were collected in the elevator. The results of the aflatoxin analyses on these samples are reported in this paper.

Experimental

Collecting and Handling Samples

Total dust samples were collected on 20 × 25 cm type "A" glass fiber filters (free of organic binders), using high-volume samplers with a sampling rate of approximately 0.029 cu. m/s, which was determined for each pump with a Magnehelic gauge. After field sampling, filters were folded and placed in 7.5 × 2.5 cm round seamless tin cans for storage and transportation. Filters containing dusts were dried the day of collection at 65°C. Airborne dust samples, including personal samples, were also taken with battery-driven, low-volume (ca 2 L/min) pumps. Efforts were made to collect bulk corn samples that corresponded to airborne samples, but this was not always possible either on the farm or at the elevator. Bulk corn samples were placed in flat, open trays for overnight drying at 85°C.

Weighing Airborne Dust Samples

Before use, the filters and a control filter were allowed to stand at least 24 h in an air-conditioned room for moisture equilibration and then were accurately weighed and placed in individual envelopes. After sample collection and drying, the filters containing dusts were placed in the same air-conditioned room for 24 h for a second equilibration before weighing. Changes in the weight of the control filter were used to correct for moisture variations.

Aflatoxin Analysis

Aflatoxin levels in the bulk corn samples were determined by the CB method approved for corn by AOAC (8) and the American Association of Cereal Chemists (9). Airborne dust samples were analyzed by a modification of the CB method for application to the small dust samples (1.5–488 mg) (10). The identity of aflatoxin in extracts was confirmed by the formation of the water adducts on thin layer chromatographic plates with trifluoroacetic acid (8, 11).

Results and Discussion

The results of the aflatoxin analysis of dust samples collected during harvest on the farm where total aflatoxin level in the harvested corn was 1640 ng/g are summarized in Table 1. Airborne dust samples collected in front of the combine ranged from 1150 to 5260 ng/g, with an average of 3850 ng/g. At the side of the combine, the range of aflatoxin levels in airborne dusts was 281–4640 ng/g, averaging 2550 ng/g. The 2 dust samples collected while the corn was being transferred to the truck had 1330 and 3000 ng/g. The one personal airborne dust sample taken while the operator was in the combine cab had 1360 ng/g. The personal dust sample taken while the operator was standing outside the cab and downwind during transfer of corn to a truck had 52 000 ng/g. The high aflatoxin

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Table 1. Aflatoxin in airborne dusts generated during harvest of contaminated corn (1640 ng/g)

Sample No. and kind[a]	Location	Dust wt, mg	Dust concn, mg/cu. m	Total aflatoxin in dust, ng/g	Airborne total aflatoxin, ng/cu. m
1/H	front of combine	1033	127	3370	427
2/H	front of combine	1300	53.2	4860	258
3/H	front of combine	1077	52.8	1150	60.5
4/H	front of combine	2482	305	5260	1600
5/B	front of combine	10.4	126	4620	581
6/H	side of combine	1283	153	4640	712
7/H	side of combine	852	33.9	3960	134
8/H	side of combine	862	41.1	1310	53.8
9/B	side of combine	3.7	44.8	281	12.5
10/H	transfer to truck[b]	1694	118	1330	111
11/H	transfer to truck[b]	1233	83.5	3000	29.1
12/B	operator	1.6	19.6	52 200	1020
13/B	inside cab	1.5	17.8	1360	24.0

[a]H represents high-volume sampler; B represents battery-operated low-volume sampler.
[b]Samples 10 and 11 were collected simultaneously, approximately 2 ft apart.

levels in airborne dusts during harvest might be explained by the toxin content of the conidia (spores) of producing fungi. From studies on farmers' lungs, it has been established that most of the airborne dusts collected during harvest of grain were fungal conidia (12). A study of the aflatoxin content of conidia of 5 strains of *Aspergillus flavus* and 4 strains of *A. parasiticus* that produce aflatoxins showed an average of 153 000 ng/g and 27 600 ng/g total toxin, respectively (13).

Levels of aflatoxin were high in the bulk corn (1240 and 1850 ng/g) being ground and fed, as well as in airborne dusts generated during grinding and feeding operations (Table 2). Only one dust sample did not have detectable aflatoxin: levels in the others ranged from 1100 to 43 200 ng/g. The farmer lost more than 60 of 300 young pigs that ate the bulk corn, due to aflatoxicosis; the cause of death was determined by the Veterinary Diagnostic and Investigation Laboratory, Tifton, GA.

Airborne dust, bulk corn, and settled dust samples were collected at a grain elevator (Tables 3, 4, and 5). Airborne dusts collected while farm trucks delivered and unloaded corn at the elevator contained from nondetectable to 3270 ng aflatoxin/g with an average of 1240 ng/g (Table 3). The bulk corn being delivered had an average total aflatoxin of 120 ng/g. Attempts were made to collect the bulk corn samples to correspond to the airborne samples being taken. Ratios of aflatoxin in the airborne dusts to that in the bulk corn being delivered ranged from 0 to 40.6.

Airborne dust samples also were collected as corn was being unloaded from the elevator onto trucks and railroad cars (Table 4). Although airborne dusts usually contain higher aflatoxin levels than the bulk corn from which they are generated, 2 airborne dust samples collected showed no detectable toxin when the corresponding bulk corn samples had 154 and 138 ng/g. It is difficult to collect bulk corn samples exactly representative of the airborne dust samples being taken. Filters in the dust samplers have to be changed at intervals to prevent overloading, sometimes not convenient for collecting bulk corn samples. At best, it is difficult to obtain a corn sample representative of a lot, much less part of a lot, being unloaded as the airborne dust sample is taken.

One of the more important parts of this study was the determination of aflatoxin in airborne dusts and of toxin levels at several locations in a grain elevator (Table 5). Settled dusts in grain elevators located in geographical regions where aflatoxin tends to be a problem in corn have been found to be contaminated (3, 14). We detected aflatoxin (173–669 ng/g) in settled dusts again in 1980. Settled dusts are swept up from time to time as part of the cleaning process, to prevent the dust accumulation that causes elevator explosions. Airborne dusts were collected in 4 work areas of the elevator (Table 5). The highest aflatoxin concentration in the air was near the conveyor belt—13 000 ng/cu. m. Workers wore personal samplers while completing operations around the elevator. Airborne dusts collected by personal samplers on the workers contained aflatoxin levels of 429–1300 ng/g. Aflatoxin concentrations in the air ranged from 9 to 1120 ng/cu. m.

Conclusions

Although we know aflatoxin occurs in airborne dusts generated from contaminated corn, the effect on agricultural workers by inhalation is not known. It is estimated that the

Table 2. Aflatoxin in airborne dusts generated while grinding corn and feeding

Sample No. and kind[a]	Location	Dust wt, mg	Dust concn, mg/cu. m	Total aflatoxin in dust, ng/g	Airborne total aflatoxin, ng/cu. m	Total aflatoxin in bulk corn, ng/g
14/H	isle between feeding pens	1694	118	1170	183	1240
15/H	isle between feeding pens	1233	83.5	1330	111	1240
16/B + 20/B[b]	operator of grinding and feeding	3.25	123	3740	210	1240–1850
17/B + 21/B[c]	midpoint of feeding pens and back of grinding building	2.28	121	43 200	1760	1240–1850
18/H	side of grinding building	488	9.7	3000	29.1	1850
19/H	side of grinding building	1104	23.3	ND[d]	ND	1850

[a]H represents high-volume sampler; B represents battery-operated low-volume sampler.
[b]Samples 16/B and 20/B were combined before aflatoxin analysis.
[c]Samples 17/B and 21/B were combined before aflatoxin analysis.
[d]ND = nondetected.

Table 3. Aflatoxin in airborne dusts generated from corn while unloading farm trucks at elevator

Location	Dust wt, mg	Dust concn, mg/cu. m	Total aflatoxin in dust, ng/g	Airborne total aflatoxin, ng/cu. m	Total aflatoxin in bulk corn, ng/g
2 m Behind truck	2317	265	2850	755	152
~0.75 m High	2727	391	841	328	—
~0.75 m High	2064	227	1380	313	35.0
~0.75 m High	2879	18.3	2560	46.7	53.0
~0.75 m High	1325	100	419	41.8	143
~0.75 m High	6988	1750	766	1340	238
<0.75 m High	5882	562	3270	1840	128
<0.75 m High	1645	147	1190	176	180
<0.75 m High	4317	317	1900	601	139
<0.75 m High	3719	595	607	361	179
<0.75 m High	1507	148	210	31.1	9
<0.75 m High	5060	693	70.0	48.5	44
<0.75 m High	11 850	3490	22.0	76.6	151
<0.75 m High	5061	497	ND[a]	ND	ND
~0.75 m High	2612	256	ND	ND	119
~0.75 m High	1857	144	ND	ND	49

[a]ND = nondetected.

Table 4. Aflatoxin in airborne dusts from corn during loading railroad cars and trucks from elevator

Sample No. and kind[a]	Location	Dust wt, mg	Dust concn, mg/cu. m	Total aflatoxin in dust, ng/g	Airborne total aflatoxin, ng/cu. m	Total aflatoxin in bulk corn, ng/g
Loading RR car						
18/H	edge RR car hatch	7811	1240	924	1150	—
19/H	edge RR car hatch	6225	557	907	506	35
20/H	edge RR car hatch	5135	2680	1060	2820	—
21/H	edge RR car hatch	13 280	1530	1860	2850	229
22/H	near loading spout	6979	685	2430	1670	140
23/H	near loading spout	8259	772	710	548	216
24/B	near worker[b]	6.0	1240	<200	<247.6	—
25/B	beside RR car	1.1	4.3	578	2.5	—
Loading trucks						
31/H	hand held 0.75 m above box	10 440	6640	551	3660	191
32/H	hand held 0.75 m above box	12 090	11 500	696	8030	273
33/H	above grain box	2985	732	ND[c]	ND	154
34/H	above grain box	4408	618	ND	ND	138

[a]H represents high-volume sampler; B represents low-volume sampler; RR = railroad.
[b]Three small similar samples combined for assay.
[c]ND = nondetected.

Table 5. Aflatoxin in dusts from corn at several locations in a grain elevator

Location	Dust wt, mg	Dust concn, mg/cu. m	Total aflatoxin in dust, ng/g	Airborne total aflatoxin, ng/cu. m
Airborne dusts collected with a battery-driven, low-volume sampler				
Control room	2.8	31.1	ND[a]	<12.4
Control room	2.2	9.3	NA[b]	—
Control room, work area	867	709	1480	1050
Work area near conveyor	7790	11 100	1170	13 000
Worker, scale tower	87.3	285.4	621	414
Worker, general	1.8	5.5	1160	1120
Worker, general	6.1	7.5	1140	1110
Worker, general	15.6	118	1300	151
Worker, general	1.9	11.6	782	9
Worker, unload 2 trucks	2.2	26.2	429	10.8
Settled dust				
Beside auto scale	—	—	296	—
Beside auto scale	—	—	173	—
Ledge, pit 1	—	—	669	—
Ledge, pit 2	—	—	203	—

[a]ND = nondetected.
[b]NA = not assayed.

average worker inhales over 10 cu. m of air in an 8 h work shift. Our results show that exposure to aflatoxin could be high, but the data we obtained were during certain operations that usually do not last the entire 8 h. Aflatoxin levels in the corn being handled can vary markedly during a work day. Epidemiological studies and animal inhalation experiments are being planned. Farmers do have short-term exposures to aflatoxin-containing dusts during harvest, but grinding and feeding take place all year. The farm in Georgia where we collected airborne dust samples in 1980 had an acute problem,

with the harvested corn containing 1640 ng aflatoxin/g. During that year, aflatoxin contamination of corn was severe in Georgia (15). Aflatoxin occurred at levels of over 1000 ng/g in 4 of 67 samples collected at random, which were considered to be representative of the corn harvested in Georgia. Only 17 of the 67 samples, or 25%, had less than 20 ng/g, the guidelines set by the Food and Drug Administration. Exposure of elevator workers is likely to extend over a period of time, but many are seasonal workers. They are issued protective face masks that would prevent inhalation of dusts, but such masks are very uncomfortable during the summer months and usage tends to be intermittent. It would be desirable if agricultural workers exposed to dusts generated from aflatoxin-contaminated corn would wear protective masks.

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Determination of Aflatoxins in Peanut Butter, Using Two Liquid Chromatographic Methods: Collaborative Study

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Two methods for determining aflatoxins in peanut butter, one using normal phase and the other reverse phase liquid chromatography (LC), were studied by 8 and 10 collaborators, respectively. Fluorescence detection was used for the determinative step in both methods. For reverse phase LC, aflatoxins B_1 and G_1 were converted to B_{2a} and G_{2a}; for normal phase LC, a silica gel-packed flow cell was placed in the irradiating light path of the detector. The samples included spiked and naturally contaminated peanut butter with total aflatoxin levels from about 5 to 20 ng/g and controls in a balanced pair design. For the normal phase LC method, recoveries of B_1, B_2, G_1, and G_2 from spiked samples averaged 79, 92, 74, and 88%, respectively; for the reverse phase method, the recoveries were 103, 104, 89, and 163%. For the normal phase LC method, pooled repeatabilities were 20, 23, 28, and 17% for B_1, B_2, G_1, and G_2, respectively; for the reverse phase method, the repeatabilities were 19, 22, 38, and 31%. For the normal phase method, pooled reproducibilities were 34, 33, 39, and 34% for B_1, B_2, G_1, and G_2, respectively; for the reverse phase method, the reproducibilities were 32, 46, 51, and 52%. Both methods show an improved limit of detection and better within-laboratory precision over current AOAC methods; however, between-laboratory precision is no better, and the reverse phase method shows evidence of interferences being measured. For these reasons and because of no benefits of present value, neither method was submitted for adoption as official first action.

The study reported here was initiated to evaluate 2 liquid chromatographic (LC) methods for the determination of aflatoxins in peanut butter. One method, developed by Beebe (1), uses a reverse phase system; the other, developed by Francis et al. (2), uses a normal phase system. The Francis et al. method (2) combines 2 procedures developed by Pons for the determination of aflatoxins in corn (3) and peanut products (4).

It was anticipated that the use of LC instrumentation for the determinative step would provide improved precision over current AOAC official methods that use thin layer chromatography (TLC).

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Collaborative Study

Description of Samples

In an effort to find an aflatoxin-free peanut butter for use as a blank or control and for spiking, several samples in which no aflatoxins were detectable by the CB method (5, secs. **26.026–26.031**) were analyzed by both LC methods used in this study. All of the samples were found to contain amounts of aflatoxins below the detection level of the CB method, which uses TLC for the determinative step. Therefore, the sample that contained the lowest level of naturally incurred aflatoxins was used as the control and for spiking.

Description of Study

Ten and 8 collaborators used the reverse and normal phase LC methods, respectively; only 2 collaborators used both methods. Each collaborator was sent one practice sample and blind pairs of the following peanut butter samples: unspiked control, naturally contaminated peanut butter at 2 levels of total aflatoxins (9 and 17 ng/g), and peanut butter spiked to total aflatoxin levels of 5, 10, 15, and 20 ng/g. All samples were supplied in individual glass jars, and collaborators were instructed to use the entire contents of the jar (50 g) for each analysis.

Aflatoxin Reference Standard

A mixture of standards dissolved in benzene–acetonitrile (98 + 2) was supplied in sealed ampules; the concentrations of B_1 and G_1 in the mixture were each 1 ng/μL, and the concentrations of B_2 and G_2 were each 0.2 ng/μL. Collaborators were instructed to report any deviation in ampule weight from that recorded on the ampule. Collaborators were instructed to transfer the ampule contents without rinsing to a small (2–5 mL) volumetric flask or vial, stopper the container tightly, store in a freezer, bring to room temperature before use, and shake 2 min with a Vortex mixer to ensure solution of the aflatoxins after thawing.

Data Requested

Each collaborator was provided a report sheet which requested the reporting of method, instrument, detector, column packing, column dimensions, mobile phase, flow rate, pressure, chart speed, standard and sample dilutions, amounts of standard and sample injected, injecting solvent, sample numbers, and level of each aflatoxin found.

METHODS

Reverse Phase LC

The reverse phase method was to be used as published (1) with the following modifications:

Apparatus

(a) *Liquid chromatograph.*—Change ". . . and 500 μL loop injector" to read ". . . and use injection loop with minimum volume of 100 μL; 500 μL loop injector is preferred."

Reagents

(a) *Solvents.*—Add "Anhydrous ethyl ether, Mallinckrodt No. 0848 (or equivalent, sold in cans without preservatives)."

(d) *Silica gel.*—See ref. 5, sec. **26.027(a)**; use 0.063–0.2 mm.

Sample Preparation

See ref. 5, sec. **26.028.**

Extraction

Weigh 50 g prepared sample into 600 mL beaker. Add 25 mL water and mix well with spatula. Add 20 g diatomaceous earth, mix well, and transfer to blender jar or 500 mL glass-stopper Erlenmeyer flask. Add 1 mL water and 5 g diatomaceous earth to beaker, mix well to clean bottom and sides, and add to jar or flask. Add 250 mL methylene chloride and secure top or stopper. Blend 3 min or shake flask 30 min on wrist-action shaker. Filter through fluted paper, collecting ≥15 mL filtrate.

Column Chromatography

Place 2 g silica gel in 20 mL beaker and slurry with 10 mL methylene chloride–hexane (1 + 1). Transfer to column with 5 mL solvent, let settle, and layer with ca 2 cm anhydrous granular Na_2SO_4. Drain solvent to top of column.

Transfer 11 mL filtrate (equivalent to 1 mL oil and 10 mL methylene chloride representing 2 g product) to 50 mL beaker and add 11 mL hexane. Pour mixture onto column and let drain at maximum flow rate. When level of liquid reaches top of Na_2SO_4, rinse beaker and walls of column with 3–5 mL methylene chloride–hexane (1 + 1), followed successively by 10 mL hexane and 10 mL anhydrous ethyl ether. Discard rinses and place 100 mL beaker under column. Elute aflatoxins into beaker with 30 mL methanol–methylene chloride (5 + 95). Evaporate just to dryness over low heat with nitrogen stream. Dissolve in 10.0 mL methanol–methylene chloride (5 + 95) and transfer 5.0 mL to 3 dram glass vial. Using same solvent, transfer remaining half to second 3 dram vial, rinsing pipet and beaker with transfer solvent. Evaporate contents of both vials just to dryness.

Trifluoroacetic Acid-Catalyzed Reaction

Add 200 μL hexane to each vial and swirl to wet residue. To first vial, add 50 μL trifluoroacetic acid (TFA) (use disposable tip micropipet), cap, and mix well. To both vials, add injection solvent equal to 10 times volume of injection loop (1–5 mL) and gently shake to dissolve remaining residue and complete hexane extraction. (Vigorous shaking may cause emulsions.) After separation, aqueous layer may be slightly cloudy. Using disposable glass Pasteur pipets, transfer lower layers to clean glass vials, avoiding transfer of droplets of hexane. If particles of residue are suspended in aqueous layer, filter through syringe-mounted Millipore filter or through small plug of glass wool packed in Pasteur pipet. Inject samples same day as derivatized.

Preparation of Derivatized Aflatoxin Standards

Prepare individual aflatoxin stock solutions in benzene–acetonitrile (98 + 2) and determine concentration by ultraviolet absorbance (5, secs. **26.008–26.009**). Prepare standard mixture of aflatoxins by combining individual standards of B_1, G_1, B_2, G_2 (5 + 5 + 1 + 1). Dilute to obtain 1 ng B_1 and G_1/μL and 0.2 ng B_2 and G_2/μL, respectively. Transfer 10 μL mixed standard to 3 dram glass vial containing 200 μL hexane. Add 50 μL TFA, stopper, and mix well. Add 5.0 mL injecting solvent and mix well. Let phases separate and transfer lower layer with Pasteur pipet to clean glass vial. (Standards allowed to remain dry will adsorb on glass and may not completely redissolve.) Store derivatized standard in refrigerator and prepare fresh when detector response indicates decomposition.

Determination

Successively inject 500 μL (or volume to accommodate loop of <500 μL) of following solutions: derivatized standard

Table 1. Collaborative results (ng/g) for aflatoxins in peanut butter determined by reverse phase and normal phase LC methods[a]

Reverse phase LC					Normal phase LC				
Coll.	B_1	B_2	G_1	G_2	Coll.	B_1	B_2	G_1	G_2
				A (Control)					
1	0.3, 0.3	0.4, ND[b]	ND, ND	ND, ND	11	ND, ND	ND, ND	ND, ND	ND, ND
2	1.6, 0.7	ND, ND	0.2, ND	0.6, 0.2	12	1.5, 1.8	ND, ND	ND, ND	ND, ND
3	0.4, 0.4	0.9, 0.8	ND, ND	1.6, 1.6	5	1.3,[c] 0.7[c]	0.9,[c] ND	1.0,[c] ND	ND, 0.3[c]
4	1.2, 1.1	0.5, 0.8	ND, ND	ND, 0.7	13	0.5, 0.4	T,[d] ND	ND, ND	ND, ND
5	0.2, 0.1	1.2, 0.7	0.2, ND	0.2, 0.6	14	ND, 0.5	ND, ND	ND, ND	ND, ND
6	0.4, 0.5	1.6, ND	ND, ND	0.4, ND	15	0.5, 1.7	ND, 0.7	ND, 1.9	ND, 0.4
7[e]	ND, —	ND, —	4.4, —	ND, —	16	0.4, 0.6	ND, ND	0.3, 0.4	0.1, 0.1
8	0.5, 0.5	ND, 0.3	0.1, ND	0.5, ND	8	0.8, 1.0	ND, 1.1′	0.5, 0.4	0.3, 0.1
9	2.1, 5.7′	ND, 1.2	ND, 7.1′	ND, 1.0					
10	1.1, 1.4	0.3, 0.5	ND, 3.0	0.3, 0.7					
				B (Spiked)					
	4.0	1.0	0	0		4.0	1.0	0	0
1	3.3, 4.0	1.2, 0.9	ND, ND	ND, ND	11	ND, 3.5	ND, 0.5	ND, ND	ND, ND
2	4.1, 4.1	ND, ND	5.8,′ 1.1	0.1, 0.4	12	4.3, 4.8	1.1, 1.2	ND, ND	ND, ND
3	3.7, 3.9	0.6, 0.6	ND, ND	1.5, 1.6	5	4.6, 3.9	1.4, 0.9	0.3,[c] 0.2	0.1,[c] ND
4	5.0, 5.6	1.4, 1.5	ND, ND	0.7, 0.8	13	2.4, 2.4	0.7, 0.5	ND, ND	ND, ND
5	3.7, 8.1	1.0, 2.2	0.7, 0.5	0.6, 0.5	14	3.9, 3.3	1.1, ND	0.4, ND	ND, ND
6	5.3, 8.1	0.7, 0.4	ND, 0.9	ND, ND	15	1.2, 2.0	0.5, 0.7	ND, ND	ND, ND
7[e]	—, 1.9	—, ND	—, 2.2	—, ND	16	3.5, 3.5	1.3, 1.0	0.4, 0.4	0.1, 0.1
8	4.2, 3.9	1.4, 1.2	ND, ND	2.2, ND	8	2.3, 2.6	0.9, 0.7	0.3, 0.6	0.1, 0.7
9	5.1, 6.1	1.6, 1.3	2.1, ND	ND, ND					
10	3.6, 4.2	0.9, 0.5	2.2, 3.4	0.6, 1.3					
				C (Spiked)					
	4.0	0.8	4.0	0.8		4.0	0.8	4.0	0.8
1	4.0, 3.8	1.1, 1.0	3.5, 3.5	0.8, 0.8	11	3.0, 1.6	0.6, 0.5	3.0, 1.6	T, ND
2	4.5, 4.8	0.7, 0.9	3.4, 3.5	0.8, 1.0	12	4.0, 4.7	0.7, 0.8	3.0, 4.4	0.7, 0.8
3	3.5, 3.6	0.5, 0.4	2.8, 2.8	2.1, 0.8	5	4.3, 4.3	1.1, 1.4	2.8, 3.9	0.9, 0.8
4	5.4, 6.5	1.1, 1.3	4.2, 4.8	1.3, 1.4	13	2.7, 2.7	0.7, 0.6	1.0, 2.0	0.5, 0.1
5	3.9, 4.0	1.4, 2.4	5.2, 5.4	1.1, 1.1	14	3.7, 3.8	0.8, 0.8	3.2, 3.5	0.7, 0.8
6	3.7, 3.4	0.4, 0.3	1.4, 1.0	0.3, 0.2	15	1.4, 0.7	4.8,′ ND′	2.4, ND	0.7, 0.7
7[e]	4.5, 5.3	ND, ND	3.8, 4.7	ND, ND	16	1.6, 3.2	0.7, 0.8	1.0, 2.9	0.5, 0.6
8	3.8, 4.3	1.1, 0.9	3.7, 4.3	0.8, 1.0	8	4.1, 3.1	0.9, 0.8	3.6, 3.1	0.7, 0.4
9	5.5, 6.7	1.1, 1.5	3.6, 3.3	1.8, 1.8					
10	1.8, 1.2	0.6, 0.3	2.3, 3.0	0.5, 0.5					
				D (Spiked)					
	10.0	2.0	2.0	1.0		10.0	2.0	2.0	1.0
1	8.9, 8.9	2.6, 2.2	1.8, 1.7	1.9, 1.3	11	8.8, 6.1	1.3, 1.2	ND, 1.1	ND, 0.1
2	14.4, 9.5	1.6, 1.1	2.9, 2.2	1.6, 1.8	12	11.2, 11.2	2.3, 2.2	2.5, 2.1	1.2, 1.3
3	8.9, 9.2	1.7, 1.6	1.1, 1.2	1.1, 1.2	5	9.3, 9.5	1.9, 2.5	2.1, 2.9	0.8, 1.4
4	12.8, 11.3	2.7, 2.1	2.4, 1.7	1.5, 1.7	13	6.1, 6.0	1.5, 1.4	1.3, 1.3	T, T
5	10.0, 11.2	3.5, 3.1	2.2, 4.1	1.1, 2.0	14	8.6, 9.6	2.0, 2.1	1.7, 2.1	0.9, 1.0
6	6.3, 8.6	0.8, 0.9	0.9, 0.6	0.3, 0.2	15	4.6, 4.6	1.7, 1.0	0.8, 1.2	0.7, 0.6
7[e]	1.7, 3.8	ND, 2.1	ND, 3.8	ND, ND	16	7.6, 6.4	1.7, 1.5	1.8, 1.5	0.9, 0.8
8	8.9, 8.8	1.8, 2.0	1.4, 1.4	0.8, 1.0	8	6.0, 5.2	1.3, 1.3	1.7, 1.3	0.6, 0.5
9	13.2, 10.4	2.8, 3.0	2.5, ND	2.3, 2.3					
10	5.8, 6.4	1.3, 1.3	2.8, 3.5	0.9, 1.0					
				E (Spiked)					
	13.0	2.5	3.0	0.5		13.0	2.5	3.0	0.5
1	12.2, 11.5	2.5, 2.6	2.7, 2.7	0.4, 1.0	11	20.7, 10.8	3.8, 1.4	0.7, 1.7	ND, ND
2	10.9, 11.9	ND, 0.4	1.8, 2.8	ND, 2.8	12	13.1, 13.7	2.8, 2.8	3.0, 3.2	0.6, 0.7
3	11.5, 11.9	2.0, 1.8	1.8, 2.1	0.6, 2.1	5	11.1, 11.3	3.8, 2.3	2.5, 3.3	0.5, 0.6
4	14.3, 14.3	3.1, 2.8	2.1, 2.4	1.5, 1.1	13	7.4, 8.9	1.9, 1.9	1.8, 2.0	0.5, 0.4
5	10.6, 12.8	3.9, 3.1	1.7, 5.5	1.1, 2.0	14	10.1, 11.5	2.2, 2.6	0.6, 2.3	ND, 0.4
6	9.6, 10.0	1.1, 1.4	0.9, ND	0.2, 0.2	15	6.1, 16.6	1.5, 1.8	1.3, 1.4	2.8,′ 0.3
7[e]	3.4, —	2.1, —	4.2, —	ND, —	16	9.3, 5.2	2.5, 1.5	2.7, ND	0.6, ND
8	12.6, 9.4	2.3, 2.4	1.8, 2.4	0.5, 0.8	8	8.8, 6.7	1.8, 1.9	2.1, 1.5	0.4, 0.5
9	13.3, 14.4	3.1, 3.0	ND, ND	ND, ND					
10	5.5, 3.5	1.7, 0.4	2.3, 6.0	0.7, 1.0					
				F (Naturally Incurred)					
	13.7	3.1	0	0		13.7	3.1	0	0
1	16.4, 15.8	3.8, 3.9	ND, ND	ND, ND	11	14.6, 7.2	2.6, 1.4	ND, ND	ND, ND
2	17.3, 17.0	ND, ND	3.8, 3.6	0.6, 5.3′	12	16.9, 17.8	3.8, 4.1	ND, ND	ND, ND
3	14.8, 16.4	3.9, 2.5	ND, ND	1.6, 1.4	5	13.7, 13.7	3.4, 3.8	0.6, ND	0.1, ND
4	14.6, —	4.2, —	ND, —	1.0, —	13	8.6, 9.7	1.3, 2.3	ND, ND	ND, ND
5	7.9, 2.8	1.9, 2.1	1.4, ND	0.5, 0.1	14	14.1, 14.0	3.4, 3.4	0.2, ND	ND, ND
6	24.4, 10.7	2.7, 1.8	ND, ND	0.3, 0.1	15	5.1, 9.0	2.0, 5.1	ND, ND	ND, ND
7[e]	5.5, —	2.1, —	2.2, —	2.6, —	16	15.8, 13.8	4.2, 3.3	ND, ND	ND, ND
8	14.3, 14.9	3.3, 3.1	ND, ND	ND, ND	8	9.4, 11.7	2.2, 3.1	0.3, ND	0.1, 0.1
9	21.7, 22.0	5.1, 5.0	ND, ND	ND, ND					
10	10.0, 10.0	2.5, 2.3	2.5, ND	0.3, 0.4					

Table 1. (cont'd)

Reverse phase LC					Normal phase LC				
Coll.	B_1	B_2	G_1	G_2	Coll.	B_1	B_2	G_1	G_2
	G (Naturally Incurred)								
	6.0	1.2	1.7	0.4		6.0	1.2	1.7	0.4
1	7.4, 7.9	1.7, 1.8	1.5, 2.3	0.5, 0.6	11	7.3, 6.6	0.7, 1.0	1.7, 1.2	ND, ND
2	8.8, 7.6	0.4, 0.3	1.4, 1.6	1.0, 1.1	12	7.2, 7.6	1.3, 1.4	1.8, 1.6	0.5, 0.5
3	7.5, 6.8	1.4, 1.6	2.0, 1.2	0.7, 0.7	5	6.6, 6.9	1.4, 2.2	1.7, 3.4	0.3, 1.0
4	10.1, 11.8	2.0, 1.8	1.4, 1.7	0.9, 0.7	13	4.3, 4.4	1.0, 0.9	1.4, 1.2	0.3, 0.3
5	2.9, 0.2	1.5, ND	1.7, 0.6	0.8, 0.7	14	6.5, 6.6	1.4, 1.3	1.6, 1.6	0.4, 0.4
6	2.1, 5.0	0.7, 0.8	1.3, 1.0	0.4, ND	15	3.2, 3.1	2.0, 2.0	0.6, 0.9	0.2, ND
7[e]	6.4, —	2.1, —	2.7, —	ND, —	16	6.0, 6.4	1.2, 1.6	1.8, 2.1	0.4, 0.5
8	6.2, 6.1	1.4, 1.3	2.1, 1.6	0.5, 0.5	8	4.3, 2.6	1.0, 0.8	1.7, 1.0	0.2, 0.1
9	10.0, 7.2	2.4, 2.2	5.1, ND	0.9, 0.8					
10	4.4, 5.7	1.0, 1.0	2.1, 0.9	0.6, 0.9					

[a]Samples B, C, D, and E = peanut butter samples spiked to contain 5.0, 9.6, 15.0, and 19.0 ng total aflatoxins/g, respectively. Samples F and G = peanut butter samples naturally contaminated at 16.8 and 9.3 ng total aflatoxins/g, respectively. Values represent duplicate determinations.
[b]Not detectable.
[c]Results were not included in calculations; collaborator reported trouble distinguishing signal from noise.
[d]Trace; approximating detection level.
[e]Detector had one-fifth the specified sensitivity; results were not used in statistical evaluation.
[f]Outlier; not used in statistical evaluation.

Table 2. Repeatability (CV_o) and reproducibility (CV_x) for each sample set and each aflatoxin determined by reverse phase and normal phase LC methods

	Reverse phase LC				Normal phase LC			
Sample set	Mean	CV_o	CV_x	Rec., %	Mean	CV_o	CV_x	Rec., %
				B_1				
A[a] (blank)	0.75	32	77	—	0.69	53	89	—
B (spiked)	4.8	27	30	120	3.2	11	33	80
C (spiked)	4.1	11	34	103	3.1	21	40	78
D (spiked)	9.6	16	24	96	7.6	11	30	76
E (spiked)	12.0	9	14	92	10.7	36	37	82
F (incurred)	14.8	25	37	—	12.2	18	31	—
G (incurred)	6.5	20	45	—	5.6	9	31	—
Pooled		19	32	Av. 103		20	34	Av. 79
				B_2				
A[a] (blank)	0.77	29	54	—	0.7	—	—	—
B (spiked)	1.1	32	45	110	0.89	22	35	89
C (spiked)	0.94	30	56	118	0.8	13	29	100
D (spiked)	2.0	12	41	100	1.7	18	34	85
E (spiked)	2.2	18	45	88	2.3	33	33	92
F (incurred)	3.2	14	34	—	3.1	30	34	—
G (incurred)	1.4	7	45	—	1.3	18	34	—
Pooled		22	46	Av. 104		23	33	Av. 92
				G_1				
A[a] (blank)	2.5	—[b]	—[b]	—	0.8	—[b]	—[b]	—
B (spiked)	2.1	—[b]	—[b]	—	0.4	—[b]	—[b]	—
C (spiked)	3.4	8	34	85	2.8	31	37	70
D (spiked)	1.9	42	54	95	1.7	18	34	85
E (spiked)	2.6	56	56	87	2.0	30	43	67
F (incurred)	2.7	—[b]	—[b]	—	0.4	—[b]	—[b]	—
G (incurred)	1.7	30	58	—	1.6	31	40	—
Pooled		38	51	Av. 89		28	39	Av. 74
				G_2				
A[a] (blank)	0.7	—[b]	—[b]	—	0.2	—[b]	—[b]	—
B (spiked)	0.9	—[b]	—[b]	—	0.2	—[b]	—[b]	—
C (spiked)	1.0	31	52	138	0.68	15	21	85
D (spiked)	1.3	20	46	130	0.82	23	44	82
E (spiked)	1.1	49	71	220	0.49	14	25	98
F (incurred)	1.2	—[b]	—[b]	—	0.1	—[b]	—[b]	—
G (incurred)	0.7	14	28	—	0.35	13	41	—
Pooled		31	52	Av. 163		17	34	Av. 88

[a]Not included in pooled data; error term obviously influenced by measurement close to limit of determination.
[b]Samples A, B, and F contained no aflatoxins G_1 or G_2; submitted results eliminated because recovery data indicate noise approximated limit of determination.

solution, TFA-treated sample solution, and non-TFA-treated sample solution. If G_{2a} or B_{2a} peak appears in chromatogram of TFA-treated sample solution, examine nontreated sample chromatogram for absence of that peak. Absence of B_{2a} or G_{2a} peak in non-TFA chromatogram confirms presence of B_1 or G_1 in sample. Peak with retention time of B_{2a} or G_{2a} in non-TFA-treated sample chromatogram denotes presence of artifact, which must be subtracted from B_{2a}/G_{2a} in chromatogram of treated portion. Carefully evaluate such chromatograms. If sample chromatogram peaks are off scale, dilute 1.0 mL TFA-treated sample solution to suitable volume with injecting solvent.

Calculate B_1 as follows:

$$B_1, \text{ ng/g} = (H_x \times C_s \times V_s)/(H_s \times C_x \times V_x)$$

where H_x = height sample B_{2a} peak; C_s = concentration of B_1 in injection standard (ng/mL); V_s = volume of standard injected; H_s = height of standard B_{2a} peak; C_x = concentration of TFA sample solution (g/mL); and V_x = volume of sample injected. Area integration may be substituted for peak heights. Repeat calculation for other aflatoxins, using appropriate peak heights and standard concentrations.

Normal Phase LC

The normal phase LC method was to be used as published (2), without modification.

Results and Discussion

The results reported by each collaborator for the aflatoxin determinations using reverse phase and normal phase LC are presented in Table 1. Results obtained by one collaborator for the reverse phase method were not used in the statistical evaluation because the collaborator reported that the detector had one-fifth the specified sensitivity. Some of the results for the normal phase method obtained by one collaborator were eliminated because of reported difficulties in distinguishing signal from noise. Measures of precision were determined by an analysis of variance (6) after elimination of outliers by the Dixon test (6).

Several collaborators who used the reverse phase method commented and showed chromatograms which indicated that obtaining chromatographically good recoveries would be difficult using the cleanup method described. The chromatographically poor extracts probably contributed to the variability obtained in the determinations by this method. As a result, the repeatabilities and reproducibilities shown in Table 2 were generally higher by this method, compared with the normal phase results also shown in Table 2, tending to make results look worse than they might be. The consensus was that the column wash volumes of hexane and ether should be increased to 30 mL each to help eliminate problems attributed to dirty extracts. The authors concur; data (not reported) indicate that incorporation of the recommended changes does improve recoveries.

Both LC methods showed considerable improvement in limit of detection and determination over current AOAC official methods that use TLC. However, there seems to be no improvement over current official methods in between-laboratory precision. The results showing improvement over TLC methods in limit of detection and determination were expected because of the analytical results from peanut butter samples found to be aflatoxin-free by the CB method.

The results of this study show that, for current regulatory purposes, no real analytical advantage is gained by using LC in place of TLC, that is, there is no improvement in speed, cost, or between-laboratory precision. An advantage of these LC methods is their amenability to automated determination. These methods are also viable complements to TLC methods when TLC plates are difficult or impossible to interpret.

Although both methods show an improved limit of detection and better within-laboratory precision over current AOAC official methods, the between-laboratory precision is no better and the recovery of aflatoxin G_2 by the reverse phase method shows evidence of interferences being measured. For these adverse reasons and because of no compensating benefits of present value, neither method was submitted for adoption as official first action.

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Determination of Aflatoxicol and Aflatoxins B_1 and M_1 in Eggs

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Procedures from 2 methods, one for aflatoxins B_1 and M_1 in eggs and one for aflatoxicol in milk, blood, and liver, have been combined to determine the 3 toxins in eggs. The sample is blended with sodium chloride-saturated water and this mixture is then blended with acetone. After separation from the solid residue, the aqueous acetone extract is defatted with petroleum ether. The toxins are next partitioned into chloroform and separated from interferences on a silica gel column. Aflatoxicol is determined by fluorescence measurement after separation on a C_{18} reverse phase liquid chromatographic column, and aflatoxins B_1 and M_1 are determined by fluorescence densitometry after separation on a silica gel thin layer chromatographic plate. In a recovery study with eggs, mean recoveries of aflatoxicol added at levels of 0.1, 0.05, and 0.025 ng/g were 87, 77, and 78%, respectively. Mean recoveries of aflatoxins B_1 and M_1 added at a level of 0.1 ng/g were 75 and 87%, respectively, and at an added level of 0.05 ng/g were 86 and 75%. The within-laboratory precision (repeatability) ranged from 2 to 13%.

The most significant mycotoxins, on the basis of concern engendered and research stimulated, are the aflatoxins, which are produced by the ubiquitous molds *Aspergillus flavus* and *A. parasiticus*. The most potent of the aflatoxins, aflatoxin B_1 (B_1), is a hepatocarcinogen in some animal species (1). B_1 is rapidly metabolized to form carcinogens and other products involved in its elimination (2). Aflatoxicol (R_0) and aflatoxin M_1 (M_1) are the only 2 isolated B_1 metabolites that have been identified as being toxic, carcinogenic, and mutagenic (3–5), R_0 being the more potent of the 2 compounds. The presence of B_1 and its metabolites in food and feed poses potential health problems to humans and animals (6). Humans can be exposed to B_1 by direct consumption of the toxin-contaminated food, and by consumption of milk, meat, or eggs from animals which have ingested enough toxin-contaminated feed to leave a residue of B_1 or its metabolites in the tissue (7). A great deal of work has been directed to the determination of B_1 and M_1 in grains, milk, eggs (8–10), and liver (11). Recently, a method for determining R_0, B_1, and M_1 in milk, blood, and tissue was reported (12, 13). However, there is no quantitative method for measuring R_0 in eggs. The objective of this study was to develop an analytical method for the simultaneous determination of R_0, B_1, and M_1 in eggs.

In a preliminary study, a method for determining R_0, B_1, and M_1 in milk, blood, and tissue (12, 13) was used. With this method the recovery of R_0 added to eggs was only 30%. Subsequently, the AOAC official method for B_1 in eggs was tried (8). The recovery of added R_0 was about 70%, but the R_0 recovered was not well resolved from interferences. When selected procedures of the 2 methods were combined, recoveries of added R_0, B_1, and M_1 were each about 80% and the toxins were completely resolved from background interferences. The final method consisted of the extraction step according to the AOAC method for B_1 in eggs (8) and the chromatographic procedure and quantitation according to the method for R_0, B_1, and M_1 in milk, blood, and tissue (12, 13).

[1]Retired.

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METHOD

Apparatus

(a) *Blender.*—Waring, with 1 L jar and cover.

(b) *Chromatographic column.*—10 mm id × 200 mm glass equipped with Teflon stopcock and 200 mL reservoir (Kontes K420-280).

(c) *Solvent delivery system.*—Altex Model 110A (Altex Scientific Inc., Berkeley, CA 94710).

(d) *Sample injection system.*—Syringe loading sample injection valve with 25 μL loop (Valco Instruments Co., Houston, TX 77024).

(e) *Liquid chromatographic (LC) column.*—3.9 mm × 30 cm μBondapak C_{18} (Waters Associates, Milford, MA 01757).

(f) *Fluorescence detector.*—DuPont Model 836 with excitation filter, transmission range 325–385 nm (Corning CS 7-60), and emission filter, cutoff below 408 nm (Corning CS 3-74) (E. I. du Pont de Nemours & Co.).

(g) *Sample vial.*—2 dram, with foil-lined screw cap.

(h) *Thin layer chromatographic (TLC) plates.*—20 × 20 cm (E. Merck 5763) silica gel 60, precoated.

(i) *Viewing cabinet.*—Chromato-Vue Model C-6 (Ultra-Violet Products, Inc., San Gabriel, CA 91778), fitted with 15 W longwave ultraviolet (UV) lamp.

(j) *Fluorodensitometer.*—Schoeffel SD 3000.

(k) *Electronic integrater.*—Infotronics CRS 204, Columbia Scientific Industries, Austin, TX 78766.

Reagents

(a) *Solvents.*—Distilled in glass $CHCl_3$ (with 1% ethanol), methanol, and acetonitrile; ACS grade acetone, petroleum ether, anhydrous ethyl ether, and methylene chloride; LC grade tetrahydrofuran (THF); and Millipore (0.2 μm diameter pore size)-filtered distilled water.

(b) *Silica gel 60.*—0.063–0.200 mm (E. Merck No. 7734, Brinkmann Instruments, Inc.). Wash 200 g silica gel with 1 L methanol; filter and wash with 1 L $CHCl_3$. Spread in tray in hood to let solvent evaporate; activate by drying 1 h at 105°C. Add 1 mL water/100 g silica gel; seal in jar. Shake contents well to disperse moisture and let stand overnight before use.

(c) *Saturated NaCl solution.*—Dissolve 36 g NaCl in 100 mL distilled water.

(d) *LC Mobile phase.*—Water–methanol–acetonitrile–THF (70 + 15 + 20 + 3); degas in ultrasonic bath.

(e) *Aflatoxicol standard.—Stock solution.*—1 μg/mL methanol. *Working standard solutions.*—0.01, 0.02, and 0.04 μg/mL; prepare by diluting stock solution to appropriate volumes with methanol. Keep all solutions in freezer when not in use and make new working standard solutions weekly.

(f) *Aflatoxin B_1 standard.*—Prepare in benzene–acetonitrile (98 + 2) as in AOAC method **26.006–26.011** (8) to contain 0.5 μg B_1/mL. Store diluted standards in refrigerator in vapor-proof container.

(g) *Aflatoxin M_1 standard.*—Prepare in benzene–acetonitrile (90 + 10) as in AOAC method **26.006–26.011** (8) to contain 0.5 μg M_1/mL. Store in refrigerator.

Extraction and Partition

Weigh 2 shelled eggs (ca 100 g) in blender jar. Add 40 mL saturated NaCl solution and blend 1 min at medium speed. Add 300 mL acetone and blend additional 3 min. Filter through folded medium speed paper.

Transfer 250 mL filtrate to 500 mL Erlenmeyer flask. Rinse graduate with 200 mL water and add rinse to flask. Stir; then let stand ca 5 min. Filter through paper.

Transfer 400 mL filtrate to 500 mL separatory funnel; add 100 mL petroleum ether (bp 30–60°C), and shake ca 1 min. Let phases separate, and drain lower phase into second 500 mL separatory funnel. Discard upper phase and repeat extraction with 50 mL petroleum ether. Drain lower phase into first separatory funnel and discard upper phase. Extract aflatoxins from aqueous acetone phase with two 25 mL portions of $CHCl_3$; shake 1 min each time. Combine $CHCl_3$ fractions in 250 mL round-bottom flask. Add 15 mL acetone to azeotrope residual water and evaporate extract to dryness under vacuum at ca 27°C. Retain dry extract for silica gel column chromatography. Store extract in freezer if necessary to stop at this point.

Silica Gel Column Chromatography

Place wad of glass wool at bottom of chromatographic tube. Add 2 g anhydrous Na_2SO_4. Add anhydrous ethyl ether–hexane (2 + 3) to half-fill tube; add 2 g silica gel and then ca 4 mL ether–hexane. Disperse silica gel with long rod. Wash sides of column with ether-hexane and let silica gel settle. Drain ether–hexane to 2 cm above silica gel. Add 4 g Na_2SO_4 to form layer on top of silica gel.

Dissolve sample residue in 5 mL dichloromethane–acetone (98 + 2), and quantitatively transfer to silica gel column; use additional solvent (2–5 mL), as required, for transfer. Elute column with following solvents, adding each in sequence as preceding one reaches top of Na_2SO_4 layer: 50 mL ether–hexane (2 + 3), 50 mL dichloromethane–acetone (98 + 2) and discard; then elute R_0 and B_1 with 50 mL $CHCl_3$–acetone (98 + 2). Immediately after adding eluting solvent, collect eluate in 200 mL round-bottom flask and evaporate to dryness under vacuum at ca 27°C. After elution of B_1, elute M_1 from column with 50 mL $CHCl_3$–acetone (4 + 1). Collect eluate in 125 mL Erlenmeyer flask and evaporate to near dryness on steam bath. Quantitatively transfer residues to separate 2 dram vials, using $CHCl_3$ for transfer, and evaporate to dryness on steam bath under nitrogen stream. Save residues for TLC and LC.

Liquid Chromatography of Aflatoxicol

Stabilize instrument with mobile phase at flow rate of 1.5 mL/min ca 30 min. Adjust sensitivity, using nanoampere range of fluorescence detector to give 35–40% of full-scale recorder deflection for 1 ng R_0 injected in loop volume. Record chromatogram at speed of 0.2 in./min until R_0 elutes at ca 12 min.

Load sample loop, using 30 μL of each standard solution for injection onto liquid chromatograph. (Note: The 25 μL loop provides constant volume injection.) Flush loop with 30 μL mobile phase after each injection. Prepare standard curve by plotting peak heights vs quantity of R_0. Prepare new standard curve daily.

Dissolve R_0 and B_1 eluate residue in 100 μL methanol and use 30 μL to load sample loop. Flush loop with mobile phase after each injection. Identify R_0 peak from sample extract by comparing retention time with that of reference standard. If sample peak height is higher than that of highest standard peak, dilute sample quantitatively with methanol to bring within range of standard curve. On completion of LC of R_0, immediately evaporate remaining extract solution to dryness with stream of nitrogen at room temperature and save for TLC. Determine quantity of R_0 in sample extract from standard curve, and calculate concentration of R_0 in eggs using the following formulas:

$$W = A \times [250/(A \times (74/100) + 40 + 300)] \times [400/(250 + 200)] = 100\,000A/(333A + 153\,000)$$

$$\text{ng/g} = R_0V/25W$$

where W = weight of sample represented by extract, g; A = original egg weight; 74 = usual percentage of water in egg; V = final sample extract volume, μL; and 25 = extract injected, μL. If dilution was required, correct sample weight by proportionate volume of extract removed for initial injection.

Thin Layer Chromatography of Aflatoxins B_1 and M_1

Aflatoxin B_1.—Redissolve sample extract remaining from above LC R_0 analysis in 70 μL $CHCl_3$. Apply 20 μL spot of $CHCl_3$ solution alongside 0.5, 1.0, 2.0, and 4.0 ng B_1 standard on TLC plate. Develop plate with hexane–THF–ethanol (70 + 20 + 10). Evaporate solvent in air control hood for 2 min, and then at 50°C in forced draft oven for additional 1 min to ensure that solvent is completely removed. Examine plate under longwave UV light to confirm presence or absence of B_1 in sample. If B_1 is present, use densitometer (transmission mode, excitation 365 nm and emission cutoff 430 nm) to scan sample and B_1 reference spots. Calculate concentration of B_1 in eggs, using the following formula:

$$\text{ng/g} = 100\,Ru/20\,R_sW$$

where Ru = densitometer response for B_1 in sample aliquot; R_s = calculated average densitometer response per ng for the 4 B_1 standard spots; W = weight of sample represented by extract, g; 100 = sample extract volume, μL (70 μL + 30 μL used for LC); and 20 = extract applied to plate, μL.

Aflatoxin M_1.—Dissolve M_1 eluate residue in 100 μL $CHCl_3$. Apply 20 μL spot of $CHCl_3$ solution alongside 0.5, 1.0, 2.0, and 4.0 ng M_1 reference spots on TLC plate. Develop plate with ether–methanol–water (95 + 4 + 1); then proceed as for B_1.

Confirmation of Aflatoxin Identity

Add measured amount of standard R_0 to sample extract containing presumptive R_0. Inject extract onto LC column. Increase in R_0 peak height related to amount of R_0 added provides further evidence of identity of R_0.

After TLC quantitation, confirm identity of B_1 on same TLC plate used for original analysis. Under UV light, locate B_1 spots originating from both sample and standard spots. With pencil, make 2 small marks on silica gel beside each toxin spot. Use mark as guide to apply 2 μL trifluoroacetic acid (TFA) to each toxin spot. Heat plate at 40°C 10 min. Cool, and develop plate in $CHCl_3$–acetone–isopropanol (85 + 10 + 7). Compare R_f values of sample B_1 derivative with that of standard B_1. Coincidence of R_f values is confirmation of identity of B_1 in sample.

The identity of M_1 can be confirmed in a similar manner (14). Overspot M_1 sample spot and M_1 standard spot with TFA. Cover spot plate with clean glass plate; then heat in 70°C oven 8 min. Remove cover plate and develop with $CHCl_3$–acetone–isopropanol (85 + 10 + 7).

Results and Discussion

The recovery data for R_0, B_1, and M_1 in eggs spiked with the 3 toxins are given in Table 1. Mean recoveries of R_0 at

Table 1. Recovery of aflatoxicol (R_0) and aflatoxins B_1 and M_1 added to eggs

Toxin	Added, ng/g	Mean rec., % of added	SD	CV, %
R_0	0	0 (0,0,0)[a]		
	0.1	87 (86, 86, 87, 88, 90)	1.7	2
	0.05	77 (83, 76, 76, 74)	4.0	5
	0.025	78 (82, 60, 82, 80, 80, 68, 84, 88)	9.3	12
B_1	0	0 (0.0)		
	0.1	75 (80, 70, 70, 87, 69)	8.0	11
	0.05	86 (80, 80, 84, 94, 94)	7.1	8
M_1	0	0 (0.0)		
	0.1	87 (85, 91, 77, 95)	7.8	9
	0.05	75 (86, 70, 86, 65, 70)	9.9	13

[a]Individual values are in parentheses.

added levels of 0.1, 0.05, and 0.025 ng/g were 87, 77, and 78%, respectively. Mean recoveries of B_1 and M_1, each at an added level of 0.1 ng/g, were 75 and 87%, respectively, and at an added level of 0.05 ng/g were 86 and 75%. The repeatabilities calculated from this study for the determination of R_0, B_1, and M_1 at levels of 0.05 and 0.1 ng/g ranged from 2 to 13%; for R_0 at 0.025 ng/g, the repeatability was 12%. The approximate limits of determination for R_0, B_1, and M_1 in eggs were 0.01, 0.02, and 0.04 ng/g, respectively.

The LC chromatograms of all unspiked samples showed no fluorescent peaks near the R_0 retention time (Figure 1). A few samples exhibited a single noninterfering peak eluting about 5 min after the R_0 peak.

The TLC chromatograms showed no interferences from other fluorescent spots in the extracts. B_1 migrated behind other fluorescent spots while M_1 migrated between them (Figure 2).

To evaluate the applicability of the method to naturally contaiminated samples, eggs from laying hens given aflatoxin-contaminated feed were analyzed for R_0, B_1, and M_1. R_0 and B_1 were detected in all samples; no M_1 was found in any of the eggs (15).

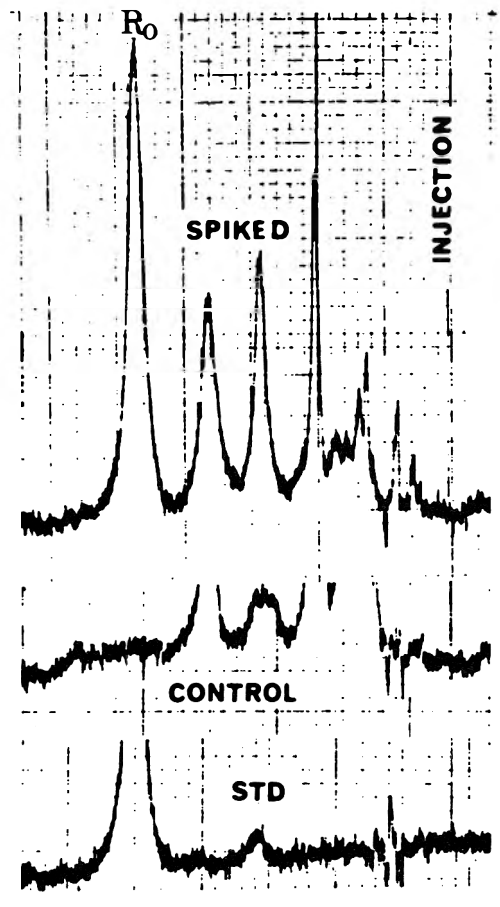

Figure 1. LC chromatogram of aflatoxical (R_0) in eggs and of standard R_0 solution. Spiked: egg spiked with 0.05 ng R_0/g; control: egg to which no R_0 was added; STD: standard R_0.

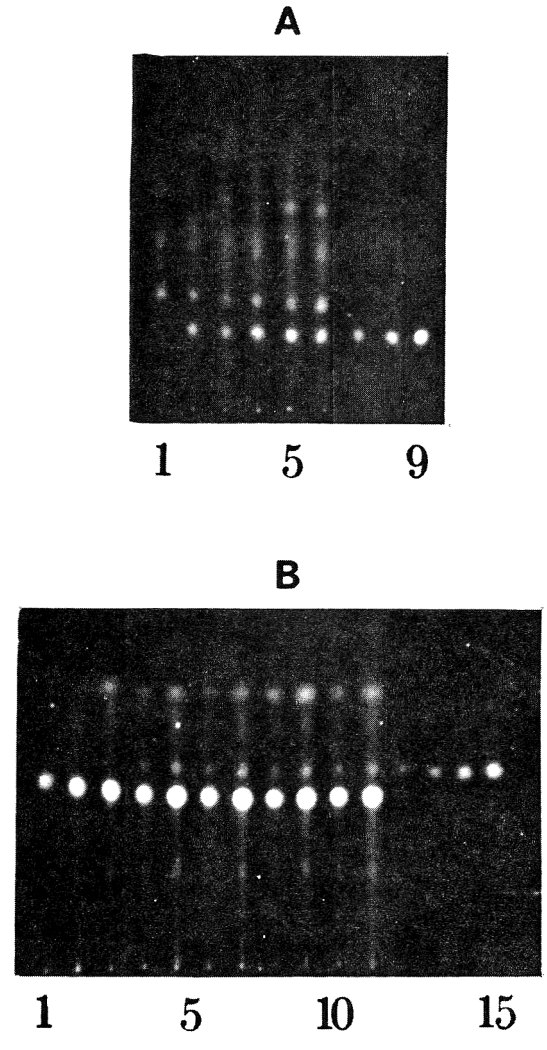

Figure 2. TLC chromatograms of aflatoxins B_1 and M_1 in eggs on silica gel 60. A. mobile phase: hexane-tetrahydrofuran-ethanol (70 + 20 + 10), unequilibrated tank. 1, control egg; 2, 3, egg spiked with 0.05 ng B_1/g, extract equivalent to 5.4 g egg applied; 4, 5, 6, egg spiked with 0.05 ng B_1/g, extract equivalent to 10.7 g egg applied; 7, 8, 9, B_1 standards of 0.5, 1.0, 2.0 ng. B. mobile phase: ether-methanol-water (95 + 4 + 1), unequilibrated tank, 1, 2, 3, control egg; 4, 6, 8, 10, egg spiked with 0.1 ng M_1/g, extract equivalent to 5.4 g egg applied; 5, 7, 9, 11, egg spiked with 0.1 ng M_1/g, extract equivalent to 10.7 g egg applied; 12, 13, 14, 15, M_1 standard 0.5, 1.0, 2.0, 4.0 ng.

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DRUGS IN FEEDS

Liquid Chromatographic and Ultraviolet Spectrophotometric Determination of Bevantolol and Hydrochlorothiazide in Feeds

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Separate assay methods have been developed for the 2 components of an 80 + 20 drug blend of bevantolol and hydrochlorothiazide (HCT) in admixtures with animal feed. Drug/diet admixtures are extracted with methanol for reverse phase ion-pair liquid chromatographic (LC) assay of bevantolol, and with acetonitrile for ultraviolet spectrophotometric assay of HCT. Bevantolol, a cardioselective beta blocker, is separated from soluble feed components with an RP-18 column, using methanol–water–acetic acid (60 + 40 + 1) containing 0.005M octanesulfonic acid, sodium salt, as ion-pairing reagent. HCT is determined spectrophotometrically in acetonitrile extracts, using a suitable blank extract as reference. Average recovery of HCT from an admixture of 0.5 mg blend/g diet is 94.5% ± 4.3 RSD and at 2.0 mg/g, 101.5% ± 3.5 RSD. Bevantolol recovery from the same admixtures is 101.8% ± 2.7 RSD and 99.0% ± 3.5 RSD, respectively, using the method as described.

Admixture of drug with animal feeds provides the simplest and most economical route for dosing animals in long-term toxicological studies. To validate such studies, confirmation of drug levels in the diets is necessary. In addition, homogeneity of mixing and stability of the drug in the feed are required to satisfy Good Laboratory Practices guidelines recommended by the Food and Drug Administration. Liquid chromatography (LC) is well adapted to the assay of drugs in diet admixtures; chromatographic systems give good separation of analytes from feed extractibles. Many approaches have been used in the development of LC assay methods for drugs in medicated feeds. Such approaches usually depend on dose levels; if drug concentration is low, a cleanup procedure is required to isolate detectable amounts of drug from the complex feed extract background. Extensive cleanup was necessary in the normal phase LC procedures reported by Fink (1) in a collaborative study with arprinocid, and by King et al. (2) who used 3 parallel methods, including LC, for trace analysis of diethylstilbestrol. Cohen and LaPointe (3) developed an assay for vitamin E which included saponification and filtration, followed by evaporation to dryness before redissolution and chromatography. Reverse phase LC was used after extensive cleanup for the determination of melengestrol acetate in feed supplements (4), and Stringham et al. (5) used postcolumn derivatization of clarified extracts of sulfamethazine and sulfathiazole for assay of the drugs in feeds and feed premixes. Methods with minimum cleanup have also been reported (6–8); this usually involves only filtration through a column packed with alumina.

Injection of diet extracts is, of course, the most direct way to handle such assays. Bowker et al. (9), Shaikh and Hallmark (10), Townley and Ross (11), and Cieri (12) have all reported the results from such procedures. We have been fortunate that medicated diets used for toxicological studies done in our laboratories have concentration levels that are high enough to allow direct injection of diet extracts into the LC system. We have found that by using guard and precolumns, and by flushing the LC system daily with acetonitrile–water, the performance of our analytical columns has not been affected.

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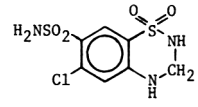

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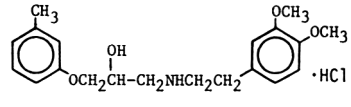

II

Hydrochlorothiazide, HCT (I), a well known diuretic, and bevantolol, a recently developed cardioselective β-blocker (II), had been assayed separately in feed admixtures for toxicological animal studies, using ultraviolet spectrophotometric methods to determine concentrations of drug in acetonitrile extracts of both diet admixtures. The decision to market a combination product for control of hypertension resulted in a bevantolol–HCT (80 + 20) blend for animal tests. Studies were subsequently required for diet admixtures with the drug blend, together with an assay method to confirm concentrations of each component of the blend in the diets.

Experimental

Apparatus and Reagents

(a) *Liquid chromatograph.*—Waters 6000A solvent delivery system, Micrometrics Model 725 autoinjector equipped with a 50 μL loop, Waters Model 450 variable wavelength detector set at 278 nm, Houston Omniscribe recorder with chart speed at 0.25 cm/min. A 4.6 × 250 mm stainless steel column packed with EM-60 silica was used as a precolumn between the solvent reservoir and the injector, and a ¼ in. × 10 cm column packed with 30–38 μm Whatman Co:Pell ODS was inserted as a guard column between the injector and the analytical column.

(b) *Analytical column.*—4.6 mm id × 250 mm E. Merck 10 μm Lichrosorb RP-18. Column was flushed with acetonitrile–water (50 + 50) after each use.

(c) *Spectrophotometer.*—Cary Model 219 UV-visible recording spectrophotometer (Varian Instruments, Serial 0321808).

(d) *Solvents.*—Distilled-in-glass methanol (Burdick & Jackson Laboratories, Inc., Muskegon, MI) and laboratory-distilled deionized water were used for mobile phase preparation. Spectrophotometric grade methanol and acetonitrile (Fisher Scientific) were used to extract the blend from drug/diet admixtures.

(e) *Mobile phase.*—Methanol–water–acetic acid (60 + 40 + 1), containing 0.005M octanesulfonic acid, sodium salt, as ion-pairing reagent, was used at a flow rate of 2 mL/min. Mobile phase was filtered before use through a Gelman Metricel membrane 5 μm Millipore filter and then degassed for ca 5 min.

(f) *Bulk drug.*—The blend was assayed for relative concentrations of the 2 components, using an LC method to quantitate both. Peak heights in millimeters of the chromatograms of a solution of the blend at known concentration were compared with peak heights of chromatograms of each component at a known concentration. Replicate assay confirmed that the blend was 80% bevantolol, 20% HCT.

(g) *Standard solution.*—Absorptivity, a, for HCT under the extraction conditions was determined as follows: A solution of the blend at a concentration equivalent to 0.004% HCT was prepared in acetonitrile. Thirty mL of this solution was mixed for 1 h in replicate ($n = 3$) with 3.0 g blank Ralston Purina 5002 feed, then centrifuged to obtain a sample of clear supernate. Absorptivity, a, was calculated from $a = A/bc$, where A is the absorbance of HCT read from the recorder chart; b is the cell path length, 1 cm; c is HCT concentration expressed in mg/mL. Mean absorptivity value determined was 10.0 ± 0.1 ($n = 3$). An acetonitrile extract of blank feed at 3.0 g/30.0 mL was used in the reference cell.

For bevantolol, a stock solution of the blend in methanol was prepared at a concentration corresponding to 40 mg/mL. The stock solution was suitably diluted with methanol to prepare working solutions for the construction of calibration curves for study of dose levels. Five such solutions were made for each level at a range bracketing concentrations of the blend in the test diets.

(h) *Matrix.*—Ralston Purina certified 5002 feed was used throughout.

(i) *Diets.*—Diets were prepared at 0.5 mg blend/g and at 2.0 mg blend/g.

Preparation of Sample and Extraction Procedure

HCT: Weigh exactly 5 g or 2.0 g samples of blank or medicated feed into separate 50 mL screw-cap round-bottom centrifuge tubes for assay of blend at 0.5 mg and 2.0 mg blend/g diet dose levels, respectively. Add 20.0 mL acetonitrile to each tube by Brinkmann Dispensette. Prepare samples for study of precision and accuracy of method by adding aliquots of freshly made stock solutions of blend to tubes containing blank feed plus acetonitrile ($n = 5$ for each dose level). Include tube containing blank feed with appropriate volume (20.0 or 21.0 mL) of acetonitrile in each dosage series for use as UV reference. Cap tubes, extract 1 h on wheel rotating at 20 rpm, then centrifuge 15 min at ca 1300 rpm. Remove portions of extracts for determination of UV spectrum.

Bevantolol: Weigh 2.0 g samples of blank or medicated feed into 50 mL tubes as for HCT. Add 20.0 mL methanol to each tube with Brinkmann Dispensette. Prepare extracts for calibration curves by adding additional 1 mL aliquot of appropriate working solution containing blend to each tube. Determine precision and accuracy of method by adding 1 mL aliquots ($n = 5$) of freshly made spiking solutions at concentrations of 1.0 mg and 4.0 mg blend/mL, respectively, for each of the 2 dose levels. Cap tubes, extract, and centrifuge. Remove portions of extracts for chromatography.

Calculations

HCT: Concentration of HCT in diet is calculated from absorbance, A, of extract vs acetonitrile extract of blank feed at 316 nm and absorptivity, a, equal to 10.0:

$$\text{mg HCT/g diet} = A \text{ at } 316 \text{ nm} \times \text{wt diet taken}/10.0 \times \text{mL extg solv.}$$

where weight of diet taken is 2.0 or 5.0 g and mL extracting solvent is 20.0 or 21.0.

Bevantolol: Plot of chromatographic response of bevantolol measured in mm height vs concentration of bevantolol expressed in mg/g diet is linear. Calibration curves are determined for both dosage levels. Concentration of bevantolol in diet is found by $(Y - A)/B$, where Y = peak height in mm, bevantolol response; A = Y intercept of standard curve; B = slope of standard curve.

Stability

Stability of the blend in the diets was determined for admixtures prepared using Hobart mixers and standard procedures. After initial test for homogeneity, portions of diet were transferred to open jars and maintained at room temperature for 2 week test period. Blank feed was stored under same conditions. Diet was stirred daily with spatula to simulate aerating effects of feeding animals. Replicate ($n = 4$) samples were removed on each test day for individual stability assay of HCT and of bevantolol.

Stability of bevantolol alone in diets was established in our earlier laboratory studies. HCT has been reported unstable in aqueous acid and base (13), the products being formaldehyde and 6-chloro-2,4-disulfamoyl aniline. It was necessary, therefore, to establish stability of HCT in acetonitrile, in acetonitrile extract of diet, and in diet admixtures. In practice, diets do not age more than 2 to 3 days before assay, and diet extracts are injected within an hour or so after centrifugation. However, longer-term stability is required in case of unexpected delays, and for this study, a total time period of 8 days was used for stability determinations.

The LC system used to separate HCT from 6-chloro-2,4-disulfamoyl aniline was as follows: E. Merck RP-8 column, 4.6 mm id × 250 mm; mobile phase 0.05M $(NH_4)_xH_yPO_4$, adjusted to pH 3 with H_3PO_4, at 1.7 mL/min flow rate; detection at 267 nm, where HCT and product have approximately equal UV absorbance. All extracts were diluted 2× with mobile phase before injection; retention of the breakdown product is about 4.5 min compared with ca 6.5 min for HCT.

Thirty mL of a solution of HCT at 0.04 mg/mL CH_3CN was used to extract 3.0 g blank 5002 feed. Portions of the solution as prepared (without feed), and of the diet extract containing HCT were chromatographed immediately after preparation and again after 1, 2, and 8 days. No breakdown product was detected in either solution. A diet admixture of 0.4 mg HCT/g prepared using standard mixing procedures was assayed initially for homogeneity and again after 2 and 8 days in open jars at room temperature. Again, no breakdown product was detectable. After one week, recovery of HCT from diet was 95.8% ± 1.5 RSD of theory.

Results and Discussion

Figure 1 shows the ultraviolet spectra of HCT, bevantolol, and the 20 + 80 blend of the 2 compounds, all at a concentration of 0.03 mg/mL methanol. Ideally, both compounds would be assayed from a single extract of the diet using a suitable LC system and standard calibration curves determined at a detection wavelength of 270 nm. In practice, however, a mobile phase and column which gave reasonable retention times for one of the components of the blend could not be used for the other component. Figure 2a shows the chromatogram of HCT, using an RP-2 column and a mobile phase consisting of 5 + 95 methanol–water. In this system, bevantolol does not elute even after 30 min. Figure 2b shows a chromatogram of bevantolol using an RP-18 column and a mobile phase consisting of methanol–water–acetic acid (60 + 40 + 1) with 0.005M octanesulfonic acid, sodium salt (PIC-B-8) as an ion-pairing reagent. In this system, HCT elutes with the solvent front and bevantolol is retained. LC, therefore, can be used for the assay of both components, but under quite different conditions. Although an LC system might have been found that would separate HCT from bevantolol in a reasonable time, inspection of the ultraviolet spectra of the 2 compounds and of the blend suggested an alternative procedure. HCT has maxima at 316 and 270 nm, and bevantolol absorbs at 278 nm with no interference at 316 nm, so an

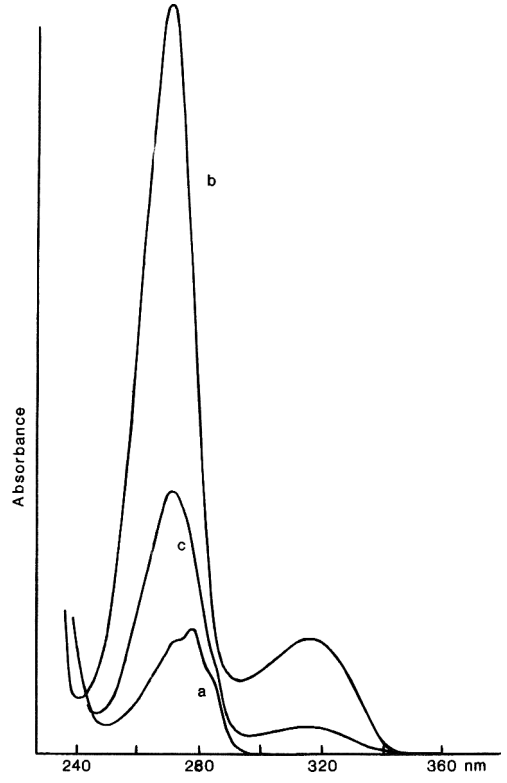

Figure 1. Ultraviolet spectra of a, bevantolol; b, hydrochlorothiazide; c, 80 + 20 blend. Concentration for all 3 is ca 0.03 mg/mL absolute methanol.

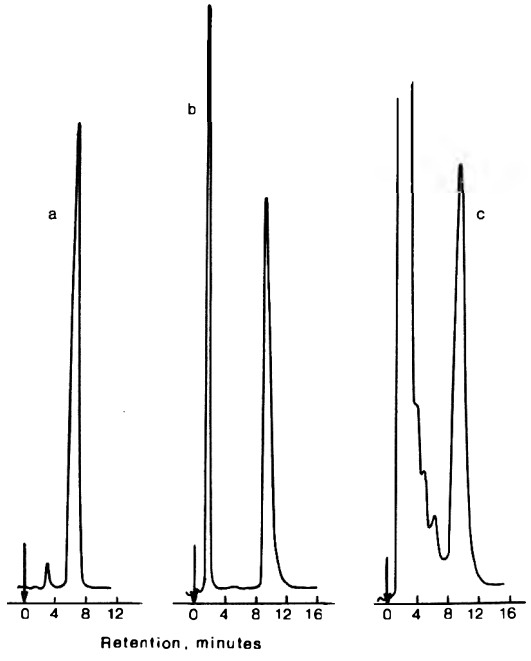

Figure 2. Chromatograms of a, HCT in methanol, RP-2 column; b, 80 + 20 blend in methanol, RP-18 column; c, 80 + 20 blend in methanol, containing feed extractibles, RP-18 column.

ultraviolet assay method can be used for HCT in the presence of bevantolol. Bevantolol, then, can be assayed chromatographically by LC, which separates it from the HCT and from the feed matrix. Figures 2b and 2c show the chromatograms of the blend, without and with feed extract background, respectively.

Acetonitrile is preferable to methanol as an extracting solvent for diet assays when the detection method is ultraviolet spectrophotometry because it removes a lesser quantity of UV-absorbing feed constituents. Spectra of acetonitrile extracts at 1 g diet/10 mL solvent can be read without cleanup to 250 nm, using a suitable blank extract in the reference cell and adjusting instrument sensitivity. A similar extract of diet with methanol cuts off at about 320 nm. If drug concentration in the diet is sufficient for at least a 5-fold dilution of the extract, methanol can be used to about 270 nm. The dose levels in the blend under study were not high enough for dilution; therefore, in a preliminary investigation, assay of each of the 2 components was attempted from a single extraction with acetonitrile using UV for assay of HCT and LC for bevantolol. Although the UV assay for HCT was satisfactory, there was a persistent artifact in the bevantolol chromatograms, a response not traceable to chow extract or to the compound itself. A methanol extract of the diet, chromatographed in the same system, did not have the interfering peak. It was necessary, then, to extract the diets twice, once with acetonitrile for UV assay of HCT and then, using a fresh diet sample, with methanol for LC assay of bevantolol.

For the study of the precision and accuracy of the HCT ultraviolet assay procedure, 5 replicate extracts were prepared at each dose level on each of 2 days by spiking either 5.0 or 2.0 g feed in 20.0 mL acetonitrile with 1.0 mL aliquots of solutions of HCT at concentrations of 2.5 and 4.0 mg blend/mL, respectively. Recovery at the low level was 101.3% ± 2.7 RSD; at the high concentration, 100.0% ± 0.9 RSD. The UV assay method is sensitive to changes that occur in the blank 5002 feed as it ages in open jars. UV-absorbing characteristics of the chow change with time, the absorbance of extracts increasing noticeably at lower wavelengths. Assay sensitivity is maintained by adjusting instrument gain to keep slit widths within a constant range. It is important that the blank feed to be used in preparing UV reference extracts be kept under the same conditions as the medicated feed, including daily stirring. As expected, standard deviation is greater in the assay results of the low-dose diets where UV absorbance is about 0.100. At this concentration, careful techniques in weighing and adding solvent are a factor in obtaining good results.

Diets for the study of the method as applied to diet admixtures were prepared using standard mixing procedures (Hobart mixers). The entire mix was spread out on a large sheet of glassine paper, and 6 random samples were taken for initial assay of homogeneity and concentration. A portion of the bulk mix was then transferred to open jars for stability at room temperature and 4 samples were taken for re-assay at intervals over a total of 8 days. There was no detectable change in appearance of the UV spectra and both recovery and precision were good (see Table 1).

For the study of method suitability of bevantolol assay in the blend/diet samples, 2.0 g blank feed in 20.0 mL methanol was spiked with 1.0 mL aliquots of solutions of the blend at 1.0 and at 4.0 mg/mL for the low and high dose levels, respectively. The tubes for calibration curve assays were spiked with 1.0 mL of working solutions at 0.8, 0.9, 1.0, 1.1, and 1.2 mg blend/mL for the low dose set and with 1.0 mL of working solutions at 3.2, 3.6, 4.0, 4.4, and 4.8 mg/mL for the high dose set. Recovery and precision of assay from the wet-spiked diets is excellent; 101.6% ± 1.6 RSD at the low level, and 100.4% ± 1.8 RSD at the high level. Samples for homogeneity of mixing and for stability were taken from the same diet admixtures as the HCT samples, above, and assayed over the same 8-day period. Calibration curves establish that

Table 1. Results of stability assay of HCT in the HCT/bevantolol blend in diets

	HCT recovery, % of theory ± RSD	
Day of assay	0.5 mg/g	2.0 mg/g
0	94.5 ± 4.3	101.5 ± 3.5
2	95.3 ± 4.2	97.8 ± 1.9
4	96.0 ± 4.7	98.8 ± 2.6
8	94.5 ± 4.9	95.3 ± 1.0

Table 2. Results of stability assay of bevantolol in HCT/bevantolol blend in diets

	Recovery, % of theory ± RSD	
Day of assay	0.5 mg/g	2.0 mg/g
0	101.8 ± 2.7	99.0 ± 3.5
2	102.8 ± 4.2	100.3 ± 1.9
4	99.5 ± 2.6	102.5 ± 1.0
8	96.5 ± 2.5	92.8 ± 2.7

bevantolol response is linear at both dosage levels. The correlation coefficient for the lower dose is 0.9998, that for the higher dose is 0.9982. Table 2 reports the recoveries of bevantolol from the diets at the 2 dosing concentrations. There is no indication, from earlier chromatographic results, that bevantolol, as part of the blend, is unstable in diets.

The LC method described can detect bevantolol to about 50 μg/g diet. With some loss in precision, HCT could also be detected at 50 μg/g diet using the UV assay procedure. By applying an LC method for HCT, additional sensitivity can be achieved by detection at 265 nm, where HCT has absorbance about 8 times that at 316 nm. For the current studies, lower limits of detection were not explored further.

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CHEMICAL CONTAMINANTS MONITORING

Residues of Organochlorine Insecticides, Polychlorinated Biphenyls, and Heavy Metals in Biota from Apalachicola River, Florida, 1978

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Seventy-seven composite samples composed of largemouth bass (*Micropterus salmoides*), channel catfish (*Ictaluras punctatus*), threadfin shad (*Dorosoma petenense*), Asiatic clam (*Corbicula fluminea*), burrowing mayfly (*Hexagenia* sp.), water snake (*Natrix* spp.), and little green heron (*Butorides virescens*) were collected from upper and lower reaches of the Apalachicola River, Florida, in 1978 for residue analysis of organochlorine insecticides, PCBs (polychlorinated biphenyls), and metals. Compared with data from the National Pesticide Monitoring Program and criteria recommended for the protection of aquatic life, residue concentrations were moderately high in the Apalachicola River. Biota from the upper river generally had higher organic and lower metal residues than those from the lower river. Highest residues in the biota were total DDT, total PCBs, and toxaphene. Although individual mean concentrations were below 2 μg/g and total organic contaminant residues never exceeded 5 μg/g, residue concentrations of DDT, PCBs, and toxaphene (particularly from the upper river) exceeded recommended permissible levels for the protection of aquatic life. Metal residues were generally below 1 μg/g. Exceptions were arsenic residues in threadfin shad (1.07 μg/g) and Asiatic clams (1.75 μg/g), and selenium in eggs of channel catfish (1.39 μg/g). The residues observed in the biota, particularly from the upper station, indicated moderate contamination of the Apalachicola River system at the time samples were collected.

The Apalachicola River flows 172 km, from the Jim Woodruff Lock and Dam at the Florida-Georgia border to Apalachicola Bay (Figure 1). The dam, located below the confluence of the Chattahoochee and Flint Rivers, forms Lake Seminole, which serves as the headwaters for the Apalachicola River. The Chipola River, the only major tributary, enters the Apalachicola from the northwest about 70 km upstream from the mouth. The free-flowing Apalachicola River drains 613 827 ha of relatively undeveloped land of the Florida panhandle (1). About 9% (55 466 ha) of the drainage is floodplain consisting of forested wetlands dominated by mixed hardwood forests and cypress-tupelo swamps (2). Much of this area is subjected to periodic overflow from the Apalachicola River which contributes to the highly productive river-dominated wetlands that terminate in and influence the productivity of Apalachicola Bay (2–4). The sparsely populated Apalachicola drainage system has little agricultural or industrial development, but timber harvesting represents the major economic base, with about 30% of the forested wetlands owned by private forestry interests (5). In contrast, the Chattahoochee and Flint drainage systems have major population centers (Albany, Atlanta, Bainbridge, and Columbus) in addition to extensive agricultural and industrial developments (1). The Apalachicola River and its associated wetlands are relatively undisturbed; however, the installation of Jim Woodruff Dam in 1954 and the maintenance of a navigation channel 2.7 m deep and 30 m wide has resulted in an overall reduction in the quality of fish habitat (6). Projected plans to improve navigation on the Apalachicola River include channel modification, installation of 2 to 4 dams, and channel-diking (1). Associated with these proposed water projects is the increased potential for development of lands adjacent to the river for industrial, municipal, and agricultural uses (7).

Contaminants associated with such potential land development pose a serious threat to the highly productive Apalachicola River and associated estuary (3). Little information is available on contaminants in the Apalachicola River system. Lawrence (8) determined some trace metal concentrations for the river, and Livingston et al. (3) reported on water quality and DDT and PCB (polychlorinated biphenyl) contaminants in sediments and biota of Apalachicola Bay. Dieldrin (0.016 μg/kg) was the only contaminant detected in the Apalachicola River in a survey of chlorinated hydrocarbon

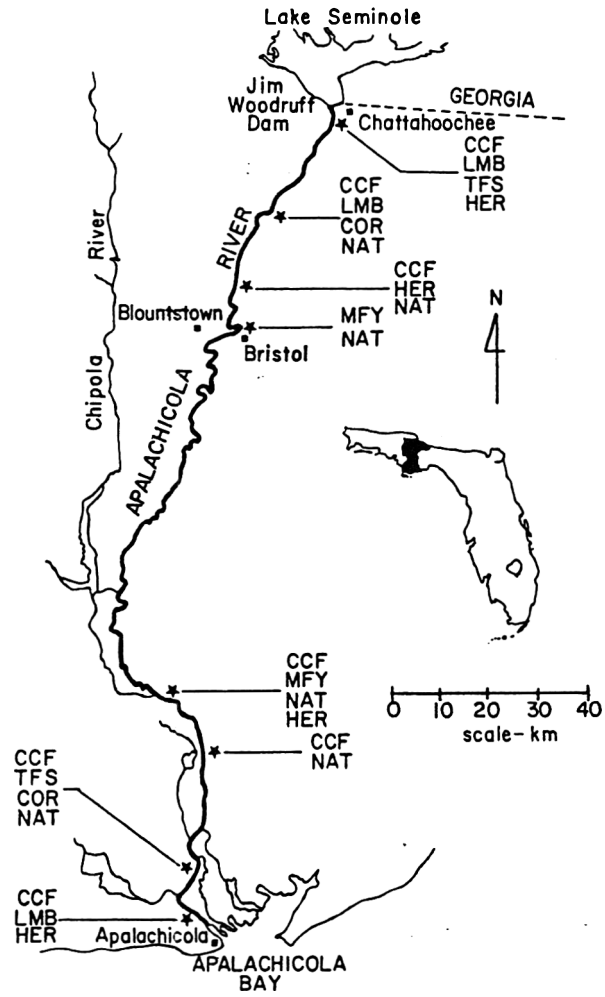

Figure 1. Sampling locations along the upper and lower reaches of the Apalachicola River, Florida.
CCF = channel catfish; LMB = largemouth bass; TFS = threadfin shad; COR = Asiatic clam; MFY = burrowing mayfly; NAT = water snake; HER = little green heron

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pesticides in U.S. river basins in 1965 (9). Residue data from fish collected below Jim Woodruff Dam as part of the National Pesticide Monitoring Program (NPMP)-Freshwater Fish from 1969 to 1977 indicated that levels were moderately high compared with those at other southeastern and Gulf Coastal monitoring stations. Some compounds followed the nation-wide trend and decreased from 1969 to 1977. For example, total DDT decreased from 1.37 to 1.04 μg/g in channel catfish *(Ictalurus punctatus)* and from 2.69 to 0.82 μg/g in largemouth bass *(Micropterus salmoides)*. Dieldrin decreased from 0.36 to 0.02 μg/g in channel catfish and from 1.59 to 0.045 μg/g in largemouth bass. However, PCBs increased from 0.69 to 0.80 μg/g in channel catfish and from 0.10 to 0.80 μg/g in largemouth bass. Toxaphene was not found in 1971, but largemouth bass contained 3.5 μg/g in 1974 (10, 11; U.S. Fish and Wildlife Service, Columbia National Fishery Research Laboratory, Columbia, MO, unpublished NPMP data).

Metal residues in fish from the NPMP station on the Apalachicola River were reported by Henderson et al. (12) and Walsh et al. (13). Mean mercury residues for all fish sampled ranged from 0.16 μg/g in 1969 (12) to 0.09 μg/g in 1972 (13). Lead and cadmium were not detectable. Arsenic residues ranged from 0.07 to 0.12 μg/g, and selenium ranged from 0.47 μg/g in 1972 to 0.29 μg/g in 1973 (13).

Protection and management of the Apalachicola River system are contingent on the availability of baseline information. The objectives of the present study were to document the residue levels of organochlorine pesticides, PCBs, and heavy metals in the aquatic biota from the upper and lower portions of the Apalachicola River. This baseline information can be used to compare and assess future changes in contaminant residues that may be associated with land-use modifications.

Methods and Materials

Sample Collection

Representative species of the aquatic biota of the Apalachicola River were collected in 1978 for residue analysis of selected organochlorine pesticides, PCBs, and metals (Table 1). Methods of field sample collection varied with species. Largemouth bass and threadfin shad *(Dorosoma petenense)* were collected exclusively by electrofishing. Adult channel catfish were collected by electrofishing, gill nets, or slat traps; however, because too few were collected, we purchased some from local commercial fisherman to fill our needs for composite samples. Juvenile catfish were obtained with an otter trawl. Asiatic clams *(Corbicula fluminea)* were collected from shallow shoal and bank areas by hand and seine. Mayflies (*Hexagenia* sp.) were collected with a sweep net around lights at night. Water snakes (*Natrix* spp.) were collected with gigs, mechanical grabs, dip nets, wire loops, and by hand. Little green heron *(Butorides virescens)* in post-fledging stage were collected with a 12 gauge shotgun and steel shot.

Field samples of the biota were collected from upper and lower reaches of the Apalachicola River (Figure 1). Samples were wrapped in aluminum foil, frozen, and shipped to the Columbia National Fishery Research Laboratory for residue analysis. Three composite samples of each species other than little green heron were used for residue analysis except when too few fish (e.g., channel catfish) were collected; individual specimens of the little green heron were analyzed (Table 2). In compositing samples of adult largemouth bass and channel catfish, we selected the largest fish for Sample 1, medium sized fish for Sample 2, and the smallest fish for Sample 3. Eggs, juveniles, and adult males and females (ovaries removed) of largemouth bass and channel catfish were analyzed separately. Replicate composite samples consisting of 30 g tissue (wet-weight) were used for residue analysis of the Asiatic clam and mayfly. Only the fleshy portions of the clams were used.

Analytical Methods

Residues of organochlorine insecticides, PCBs, and metals were measured on a wet-weight basis from whole body composites. Whole organisms were blended into a homogeneous mixture, and samples of the resulting material were used for analysis (11). The extraction of specimens and chemical purification procedures used were those described by Ribick et al. (14). These procedures included extraction of the specimens on a column with dichloromethane; cleanup of the resulting lipid concentrate by gel permeation chromatography; and separation of the clear extracts first on Florisil and then on silica gel before determinations were made by gas chromatography. Details of the gas chromatographic analysis used were described by Schmitt et al. (11). Specimens were analyzed for *p,p'*-DDE, *p,p'*-DDD, *p,p'*-DDT, total DDT, Aroclors 1254 and 1260, total PCB, toxaphene, dieldrin, endrin, α-BHC, heptachlor epoxide, oxychlordane, *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, and *trans*-nonachlor. Metal residues (cadmium, lead, arsenic, mercury, and selenium)

Table 1. Species collected, species code, date collected, and method of collection of biota for residue analysis

Taxon	Common name	Name code[a]	Collection date (1978)	Collection method
Micropterus salmoides	largemouth bass	LMB, F, M, E	Feb. 23–Apr. 6	electrofishing
		LMB, J	Feb. 23–Apr. 27	electrofishing
Ictalurus punctatus	channel catfish	CCF, F, M, E	Mar. 1–Oct. 31	electrofishing, gill net, slat trap
				purchase from commercial fisherman
		CCF, J	Mar. 3–May 18	otter trawl
Dorosoma petenense	threadfin shad	TSF	Feb. 23–Apr. 27	electrofishing
Corbicula fluminea	Asiatic clam	COR	Jul. 7–Jul. 12	hand, seine
Hexagenia sp.	mayfly	MFY	Jul. 17–Jul. 19	sweep net
Natrix spp.	water snake	NAT	Aug. 15–Sept. 28	gig, mechanical grab, dipnet, wire loop, hand
Butorides virescens	little green heron	HER	Aug. 23–Oct. 2	shotgun (steel shot)

[a]F = female; E = eggs; M = male; J = juvenile.

Table 2. Number of organisms, mean weight, and percent lipid content of composite samples used for residue analysis[a]

Species and sample[b]	Number in composite sample		Mean wt, g		Lipid, %	
	Upper	Lower	Upper	Lower	Upper	Lower
Largemouth bass, Females						
1	5	5	2268	1542	7.8	4.3
2	5	5	1134	771	5.4	2.4
3	5	5	317	363	3.0	1.1
Largemouth bass, eggs						
1	1	5	90	90	10.8	9.5
2	5	5	45	45	10.9	9.4
3	5	5	9	14	9.2	7.8
Largemouth bass, males						
1	5	5	1678	1088	9.3	3.9
2	5	5	816	589	7.2	3.4
3	5	5	272	317	2.6	1.8
Largemouth bass, juveniles						
1	6	3	108	172	1.4	0.9
2	4	4	108	117	1.6	0.9
3	3	6	263	63	2.0	0.8
Channel catfish, females						
1	5	1	1678	2857	12.5	5.6
2	1	1	1043	1179	3.2	9.5
3	1	NS	680	NS	9.7	NS
Channel catfish, eggs						
1	5	1	181	226	7.2	7.9
2	NS	1	NS	226	NS	8.7
3	1	NS	136	NS	7.9	NS
Channel catfish, males						
1	5	1	1587	635	8.6	8.8
2	1	1	498	544	6.3	5.0
3	NS	NS	NS	NS	NS	NS
Channel catfish, juvenile						
1	5	10	1	35	1.3	1.3
2	10	6	4	2	2.8	1.4
3	10	10	3	3	2.6	1.4
Threadfin shad						
1	10	10	3	4	1.2	5.4
2	10	8	2	4	1.5	4.9
3	10	8	3	3	1.2	4.3
Asiatic clam						
1	NA	NA	30	30	5.0	3.2
2	NA	NA	30	30	4.7	3.6
3	NA	NA	30	30	5.0	3.5
Mayfly						
1	NA	NA	30	30	6.2	3.1
2	NA	NA	30	30	5.4	2.6
3	NA	NA	30	30	3.8	2.9
Water snake						
1	5	5	544	725	4.7	3.5
2	5	5	362	408	5.6	3.1
3	5	5	226	272	3.1	5.2
Little green heron						
1	1	1	NA	NA	24.8	14.5
2	1	1	NA	NA	14.6	10.3
3	1	1	NA	NA	9.2	29.5
4	1		NA		21.6	
5	1		NA		29.5	
6	1		NA		24.1	
7	1		NA		22.6	

[a]NA = not available; NS = no sample.
[b]Composite samples of adult largemouth bass and channel catfish were based on size, with Sample 1 consisting of the largest fish, Sample 2 the medium-sized fish, and Sample 3 the smallest fish.

were determined by atomic absorption spectrophotometry. Details of sample preparation and analytical methodology that we used for metals were described by May and McKinney (15).

Data Analysis

To conduct statistical analyses, we used the SAS General Linear Models procedure (16) available through the University of Georgia Computer Center. Residue values (wet-weight)

were normalized by using the log (X + 1) transformation, and lipid values by using the angular transformation (17). We analyzed the residue data by analysis of variance with covariance to test for location and sex main effects, 2-way interactions, and linear effects of lipid content in largemouth bass and channel catfish. Residue data from threadfin shad, Asiatic clams, mayflies, herons, and water snakes were analyzed for main effects due to location and linear effects due to lipid. Significant differences for the independent variables were determined by using the Type IV sum of squares.

Results and Discussions

The environmental significance of contaminant residues in biota is often difficult to interpret. For lack of more precise information, residue data from the Apalachicola River were compared with standards established for protection of human health and criteria recommended for protection of aquatic life. The U.S. Food and Drug Administration (FDA) has established, for the protection of human health, "action levels" for certain contaminant residues in edible portions (fillets) of fish. These levels, however, may not reflect the real or potential environmental impacts, since residue levels in fillets are generally lower than those in whole-body residues (11), which predators are exposed to in the environment. The National Academy of Science and National Academy of Engineering (NAS-NAE) (18) recommended that for the protection of aquatic life, whole-body residues (wet-weight) should not exceed 1.0 μg/g of total DDT (including DDE and DDD), 0.5 μg/g of total PCBs, or 0.1 μg/g of dieldrin, endrin, heptachlor (including heptachlor epoxide), chlordane, and toxaphene, either singly or together.

A total of 77 biological samples from the Apalachicola River were analyzed for residues. DDT was found in 100% of the samples, PCB and toxaphene in 96%, *cis*-nonochlor and *trans*-nonachlor in 90%, *cis*-chlordane in 87%, dieldrin in 86%, endrin in 39%, *trans*-chlordane in 38%, heptachlor epoxide in 35%, oxychlordane in 28%, and α-BHC in 2% of the samples (Tables 3 and 4). Overall, organic residue concentrations (wet-weight), particularly for DDT, PCB, toxaphene, and chlordane-related compounds, were higher in samples from the upper than from the lower reaches (Figure 2). Consistently, residue levels of these compounds in largemouth bass and herons were significantly ($P < 0.5$, Table 5) higher from the upper river than from the lower river. Male and juvenile channel catfish tended to follow this same pattern, although the differences between stations were not significant. However, concentrations were more often higher in female catfish from the lower river than in those from upstream.

Compositing samples by size instead of over size groups contributed to the wide range of residue values for largemouth bass and channel catfish. Much of this variation may be attributable to differences in lipid content among the composite samples (19) (Table 2). Lipid content generally increased with size (age), and interestingly, lipid content of the biota from the upper river was higher than from the lower river, except for threadfin shad and eggs from channel catfish. Residue concentrations of the dominant organic contaminants from the Apalachicola River on a lipid-weight basis (μg/g wet-weight concentration/percent lipid/100), which eliminates the differential effects of lipids (19), are shown for comparative purposes in Table 6. Lipid-weight concentrations of total DDT, total PCB, and toxaphene conform to the general trend shown by wet-weight concentrations of higher levels in the biota from the upper river than from the lower. However, concentrations (lipid-weight) in juvenile largemouth bass, juvenile channel catfish, and threadfin shad were comparatively higher than was shown by wet-weight concentrations, and concentrations in eggs from largemouth bass and channel catfish and herons were comparatively lower.

Table 3. Arithmetic mean and (in parentheses) minimum and maximum of the contaminant residue concentrations (μg/g, wet-weight) for largemouth bass (Micropterus salmoides) and channel catfish (Ictalurus punctatus) from upper and lower reaches of the Apalachicola River, Florida, 1978[a]

Contaminant and Section of River	Largemouth Bass				Channel Catfish			
	Females	Eggs	Males	Juveniles	Females	Eggs	Males	Juveniles
p,p'-DDE								
Upper	0.87 (0.45-1.17)	1.72 (0.47-3.84)	1.48 (0.41-2.66)	0.32 (0.25-0.45)	0.48 (0.11-1.04)	0.29 (0.16-0.43)	0.93 (0.23-1.64)	0.19 (0.03-0.05)
Lower	0.08 (0.04-0.17)	0.41 (0.00-1.01)	0.11 (0.05-0.20)	0.03 (0.00-0.04)	0.93 (0.25-1.62)	0.24 (0.17-0.32)	0.21 (0.21-0.22)	0.04 (0.03-0.05
p,p'-DDD								
Upper	0.19 (0.09-0.34)	0.41 (0.09-0.90)	0.16 (0.09-0.24)	0.07 (0.05-0.10)	0.11 (0.04-0.20)	0.05 (0.03-0.08)	0.20 (0.10-0.33)	0.05 (0.03-0.07)
Lower	0.03 (0.01-0.07)	0.09 (0.06-0.12)	0.04 (0.02-0.07)	0.01 (0.01-0.02)	0.23 (0.07-0.40)	0.06 (0.05-0.07)	0.08 (0.08-0.09)	0.01 (ND-0.01)
Total DDT								
Upper	1.07 (0.54-1.51)	1.86 (1.28-2.24)	1.64 (0.50-2.82)	0.39 (0.31-0.55)	0.60 (0.15-1.14)	0.35 (0.24-0.46)	1.13 (0.33-1.94)	0.24 (0.09-0.33)
Lower	0.12 (0.05-0.24)	0.50 (0.27-0.72)	0.14 (0.07-0.27)	0.04 (0.03-0.05)	1.17 (0.32-2.02)	0.30 (0.22-0.39)	0.30 (0.30-0.30)	0.05 (0.04-0.06)
Aroclor 1254								
Upper	0.40 (0.10-0.80)	0.90 (0.70-1.00)	0.66 (0.20-1.40)	0.13 (0.10-0.20)	0.30 (0.10-0.60)	0.15 (0.10-0.20)	0.30 (0.20-0.40)	0.13 (0.10-0.20)
Lower	0.06 (ND-0.10)	0.16 (0.10-0.20)	0.10 (0.10-0.10)	0.03 (ND-0.10)	0.30 (0.20-0.40)	0.15 (0.10-0.20)	0.10 (0.10-0.10)	0.06 (ND-0.10)
Aroclor 1260								
Upper	0.56 (0.30-0.90)	0.83 (0.60-1.00)	0.70 (0.20-1.00)	0.23 (0.20-0.30)	0.23 (0.10-0.40)	0.15 (0.10-0.20)	0.45 (0.20-0.70)	0.16 (0.10-0.30)
Lower	0.06 (ND-0.10)	0.23 (0.20-0.30)	0.10 (0/10-0.10)	0.03 (ND-0.10)	0.55 (0.30-0.80)	0.20 (0.20-0.20)	0.20 (0.20-0.20)	0.10 (0.10-0.10)
Total PCB								
Upper	1.03 (0.50-1.60)	1.70 (1.30-2.00)	1.40 (0.50-2.60)	0.16 (0.30-0.50)	0.50 (0.20-1.00)	0.30 (0.20-0.40)	0.70 (0.40-1.00)	0.30 (0.20-0.40)
Lower	0.10 (ND-0.20)	0.36 (0.20-0.50)	0.13 (0.10-0.20)	0.03 (ND-0.10)	0.80 (0.50-1.10)	0.30 (0.20-0.40)	0.25 (0.20-0.30)	0.10 (0.10-0.10)
Toxaphene								
Upper	0.60 (0.30-1.00)	0.53 (ND-0.80)	0.66 (0.20-1.20)	0.16 (0.10-0.20)	0.76 (0.30-1.00)	0.45 (0.40-0.50)	0.55 (0.40-0.70)	0.23 (0.10-0.30)
Lower	0.13 (0.10-0.20)	0.23 (0.20-0.30)	0.13 (0.10-0.20)	0.16 (ND-0.40)	0.80 (0.20-1.40)	0.35 (0.30-0.40)	0.50 (0.40-0.60)	0.13 (0.10-0.20)
Dieldrin								
Upper	0.02 (0.01-0.03)	0.03 (0.03-0.04)	0.02 (0.01-0.03)	0.01 (0.01-0.01)	0.01 (ND-0.03)	0.01 (0.01-0.01)	0.01 (0.01-0.02)	(ND-0.01)
Lower	0.01 (ND-0.01)	0.01 (0.01-0.02)	0.01 (ND-0.01)	0.01 (ND-0.01)	0.02 (0.01-0.03)	0.01 (0.01-0.01)	0.01 (0.01-0.01)	ND
Endrin								
Upper	0.01 (ND-0.01)	0.02 (0.01-0.04)	ND	ND	0.01 (ND-0.01)	0.01 (ND-0.01)	0.01 (0.01-0.01)	ND
Lower	ND	0.01 (ND-0.02)	ND	ND	0.01 (0.01-0.02)	0.01 (ND-0.01)	0.01 (0.01-0.01)	ND
α-BHC								
Upper	ND	ND	ND	ND	ND	ND	ND	ND
Lower	ND	0.01 (ND-0.01)	ND	ND	ND	ND	ND	ND
Heptachlor Epoxide								
Upper	ND	0.01 (ND-0.01)	0.01 (ND-0.01)	ND	0.01 (ND-0.02)	0.01 (0.01-0.01)	0.01 (0.01-0.01)	ND
Lower	ND	ND	ND	ND	0.01 (ND-0.02)	ND	ND	ND
Oxychlordane								
Upper	0.01 (ND-0.02)	0.01 (0.01-0.02)	0.01 (ND-0.02)	ND	ND	ND	ND	ND
Lower	ND	0.01 (0.01-0.01)	0.01 (ND-0.01)	ND	0.01 (ND-0.01)	ND	ND	ND
cis-Chordane								
Upper	0.06 (0.04-0.09)	0.08 (0.08-0.09)	0.07 (0.03-0.11)	0.02 (0.02-0.02)	0.06 (0.02-0.10)	0.03 (0.03-0.04)	0.07 (0.04-0.11)	0.05 (0.03-0.10)
Lower	0.01 (0.01-0.03)	0.03 (0.02-0.04)	0.01 (0.01-0.01)	0.01 (ND-0.01)	0.10 (0.02-0.18)	0.02 (0.02-0.02)	0.03 (0.03-0.03)	0.01 (0.01-0.01)
trans-Chlordane								
Upper	ND	0.01 (ND-0.01)	ND	ND	0.02 (0.01-0.04)	0.02 (0.02-0.02)	0.03 (0.02-0.04)	0.01 (0.01-0.02)
Lower	ND	ND	ND	ND	0.03 (0.01-0.05)	0.01 (0.01-0.01)	0.01 (0.01-0.01)	0.01 (ND-0.04)
cis-Nonachlor								
Upper	0.07 (0.04-0.12)	0.07 (0.06-0.10)	0.07 (0.04-0.09)	0.03 (0.02-0.04)	0.01 (0.01-0.04)	0.01 (0.01-0.01)	0.04 (0.02-0.07)	0.01 (0.01-0.02)
Lower	0.01 (ND-0.03)	0.04 (0.03-0.06)	0.05 (0.01-0.13)	ND	0.07 (0.02-0.13)	0.01 (0.01-0.01)	0.02 (0.02-0.02)	ND
trans-Nonachlor								
Upper	0.08 (0.04-0.14)	0.06 (0.04-0.10)	0.06 (0.04-0.10)	0.02 (0.02-0.04)	0.02 (0.01-0.04)	0.01 (ND-0.01)	0.04 (0.02-0.07)	0.01 (ND-0.02)
Lower	0.01 (0.01-0.03)	0.04 (0.03-0.05)	0.02 (0.01-0.03)	0.01 (ND-0.01)	0.08 (0.02-0.14)	0.01 (0.01-0.02)	0.02 (0.02-0.02)	ND

[a]ND = none detected.

Total DDT in samples from the Apalachicola River was comprised of *p,p'*-DDE and *p,p'*-DDD, with DDE being the predominant component. (Tables 3 and 4). The *p,p'*-DDT component was below detection levels, and thus was not reported. The highest total DDT residue concentration (1.86 μg/g) was found in the eggs of largemouth bass from the upper river, and the lowest (0.04 μg/g) in juvenile largemouth bass and catfish from the lower river (Tables 3 and 4). The range of total DDT residue concentrations (wet-weight) in the biota of the Apalachicola River was considerably less than the FDA action level of 5 μg/g, but in several instances exceeded the NAS-NAE (18) maximum permissable level of 1.0 μg/g.

Total PCB consisted about equally of Aroclors 1254 and 1260 (Tables 3 and 4). The highest residue concentration of total PCB was 1.72 μg/g in herons from the upper station. PCBs were at or below the detection level (0.10 μg/g) in

Table 4. Arithmetic mean and (in parentheses) minimum and maximum of the contaminant residue concentrations (μg/g, wet-weight) for threadfin shad (Dorosoma petenense), Asiatic clam (Corbicula fluminea), mayfly (Hexagenia sp.), water snake (Natrix spp.) and little green heron (Butorides virescens) from upper and lower reaches of the Apalachicola River, Florida, 1978[a]

Contaminant and Section of River	Threadfin shad	Asiatic clam	Mayfly	Water snake	Little green heron
p,p'-DDE					
Upper	0.16 (0.14-0.18)	0.16 (0.15-0.17)	0.16 (0.12-0.20)	0.98 (0.48-1.38)	1.00 (0.55-1.67)
Lower	0.05 (0.04-0.06)	0.09 (0.08-0.13)	0.10 (0.10-0.11)	0.19 (0.17-0.22)	0.26 (0.11-0.34)
p,p'-DDD					
Upper	0.03 (0.03-0.04)	0.04 (0.04-0.05)	0.04 (0.04-0.04)	0.04 (ND-0.09)	0.11 (0.07-0.27)
Lower	0.06 (0.02-0.14)	0.02 (0.02-0.02)	0.02 (0.02-0.03)	0.01 (ND-0.03)	0.02 (0.01-0.03)
Total DDT					
Upper	0.20 (0.17-0.22)	0.21 (0.20-0.22)	0.19 (0.12-0.24)	1.01 (0.48-1.47)	1.10 (0.67-1.87)
Lower	0.11 (0.06-0.20)	0.11 (0.10-0.15)	0.13 (0.12-0.14)	0.21 (0.20-0.24)	0.29 (0.13-0.39)
Aroclor 1254					
Upper	0.10 (0.10-0.10)	0.20 (0.20-0.20)	0.20 (0.20-0.20)	0.46 (0.40-0.60)	0.41 (0.30-0.70)
Lower	0.03 (ND-0.10)	0.23 (0.20-0.30)	0.10 (0.10-0.10)	0.10 (0.10-0.10)	0.26 (0.10-0.40)
Aroclor 1260					
Upper	0.10 (0.10-0.10)	ND	ND	0.56 (0.40-0.70)	1.17 (0.80-1.70)
Lower	0.10 (0.10-0.10)	ND	ND	0.33 (0.20-0.40)	0.46 (0.40-0.60)
Total PCB					
Upper	0.16 (0.10-0.20)	0.20 (0.20-0.20)	0.20 (0.20-0.20)	1.00 (0.80-1.30)	1.72 (1.20-2.30)
Lower	0.10 (0.10-0.10)	0.23 (0.20-0.30)	0.10 (0.10-0.10)	0.33 (0.20-0.40)	0.73 (0.50-1.00)
Toxaphene					
Upper	0.20 (0.20-0.20)	0.43 (0.40-0.50)	0.36 (0.30-0.40)	0.36 (0.10-0.70)	1.28 (0.40-2.60)
Lower	0.13 (0.10-0.20)	0.20 (0.20-0.20)	0.23 (0.20-0.30)	0.20 (0.20-0.20)	0.10 (ND-0.20)
Dieldrin					
Upper	0.01 (ND-0.01)	0.01 (0.01-0.01)	0.01 (0.01-0.01)	0.01 (0.01-0.02)	0.03 (0.02-0.06)
Lower	0.01 (ND-0.01)	0.01 (0.01-0.01)	0.01 (0.01-0.01)	0.01 (0.01-0.02)	0.08 (0.01-0.21)
Endrin					
Upper	ND	ND	ND	ND	0.01 (0.01-0.02)
Lower	0.01 (0.01-0.01)	ND	ND	0.01 (ND-0.01)	ND
α-BHC					
Upper	ND	ND	0.01 (ND-0.01)	ND	ND
Lower	ND	ND	ND	ND	ND
Heptachlor Epoxide					
Upper	0.01 (ND-0.02)	ND	ND	0.01 (ND-0.01)	0.01 (0.01-0.02)
Lower	ND	ND	ND	0.01 (ND-0.01)	0.02 (0.01-0.04)
Oxychlordane					
Upper	0.01 (ND-0.01)	ND	ND	0.01 (0.01-0.02)	0.01 (0.01-0.02)
Lower	ND	ND	ND	ND	0.02 (0.02-0.03)
cis-Chlordane					
Upper	0.08 (0.03-0.18)	0.04 (0.04-0.04)	0.03 (0.02-0.04)	0.01 (0.01-0.02)	0.02 (ND-0.05)
Lower	0.01 (0.01-0.01)	0.02 (0.02-0.03)	0.02 (0.02-0.02)	0.01 (0.01-0.02)	ND
trans-Chlordane					
Upper	0.02 (ND-0.05)	0.02 (0.02-0.02)	0.01 (0.01-0.01)	ND	ND
Lower	ND	0.01 (0.01-0.01)	ND	ND	ND
cis-Nonachlor					
Upper	0.06 (0.02-0.13)	0.02 (0.02-0.02)	0.01 (0.01-0.01)	0.07 (0.02-0.14)	0.06 (0.05-0.10)
Lower	0.01 (ND-0.01)	0.01 (0.01-0.01)	0.01 (0.01-0.01)	0.03 (0.03-0.04)	0.02 (0.02-0.03)
trans-Nonachlor					
Upper	0.06 (0.02-0.14)	0.01 (0.01-0.02)	0.01 (0.01-0.01)	0.08 (0.02-0.17)	0.07 (0.06-0.10)
Lower	0.01 (ND-0.01)	0.01 (0.01-0.01)	0.01 (0.01-0.01)	0.04 (0.03-0.06)	0.03 (0.02-0.04)

[a]ND = none detected.

juvenile catfish, threadfin shad, and mayflies at the lower station. Among the fish samples examined, largemouth bass males (1.40 μg/g) and largemouth bass eggs (1.70 μg/g) from the upper station had the highest PCB residues. The PCB residue levels in all of the biota of the Apalachicola River examined were lower than the FDA action level of 5 μg/g and the proposed action level of 2 μg/g, but exceeded in many instances the 0.5 μg/g level recommended by NAS-NAE (18).

The highest toxaphene residue (1.28 μg/g) was in herons collected from the upper station; herons from the lower station had toxaphene concentrations at or below the detection level (Tables 3 and 4). Eggs from largemouth bass at the upper station (0.83 μg/g) and female channel catfish (0.80 μg/g) from the lower station had the highest residue levels among the fish samples. These residue concentrations correspond to those found (0.6 μg/g) to adversely alter bone development in channel catfish fry (20). The residue levels in the biota of the Apalachicola River were below the FDA action level of 7 μg/g for toxaphene. However, toxaphene residues in all the biota examined from Apalachicola River except for herons at the lower station exceeded the NAS-NAE (18) recommended permissible level of 0.1 μg/g.

Dieldrin was highest (0.08 μg/g) in herons from the lower station and endrin was highest (0.02 μg/g) in largemouth bass eggs from the upper station (Tables 3 and 4). Dieldrin and endrin residues in the biota of the Apalachicola River were much lower than the FDA action level of 0.3 μg/g. However, the combined residues of dieldrin and endrin with toxaphene and chlordane (and chlordane related compounds) for many of the samples from the Apalachicola River exceeded the recommended permissible level of 0.1 μg/g for protection of aquatic life (18).

Chlordane and related compounds (*cis*- and *trans*-chlordane, oxychlordane, heptachlor epoxide, *cis*- and *trans*-nonachlor) were commonly found in the biota, but were concentrated at different levels (Tables 3 and 4). The variation in the residue concentrations of these compounds from the Apalachicola biota was consistent with the findings of Cardwell et al. (21) that *cis*- and *trans*-nonachlor tend to be stored to a greater extent than the other components. However, in the present study, *cis*-chlordane was also stored at concentrations comparable to those of *cis*- and *trans*-nonachlor, and higher than those of the other chlordane related compounds. Total chlordane in female channel catfish was higher at the lower station than the upper (Figure 2).

The combined effects of total organic residue concentrations (shown for the present study in Figure 2) on aquatic populations are not known. The combined residue concentrations generally exceeded the permissible level of 0.1 μg/g recommended for the protection of aquatic life (18). The highest total concentration (4.68 μg/g) was found in the eggs

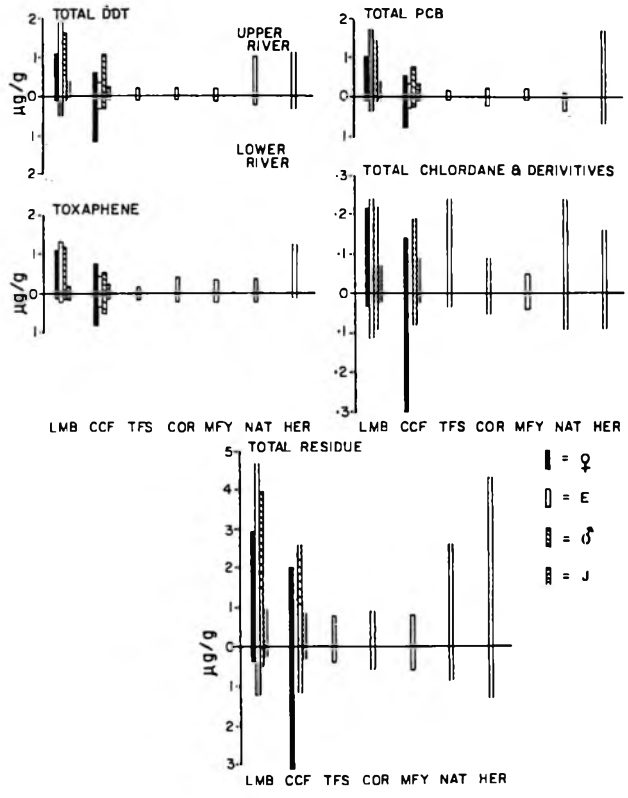

Figure 2. Organochlorine residue concentrations (μg/g, wet-weight) in biota from upper reaches (above horizontal line) and lower reaches (below horizontal line) of the Apalachicola River, Florida, 1978.

LMB = largemouth bass; CCF = channel catfish; TFS = threadfin shad; COR = Asiatic clam; NAT = water snake; HER = little green heron

Table 5. Results of analysis of variance (*F*-value significance: * = 0.01<*P*<0.05; ** = *P*<0.01) of organic and metal contaminant residues (wet-weight) and percent lipid content

Matrix[a] source	Degrees of freedom	Lipid, %	*p,p'*-DDE	*p,p'*-DDD	Total DDT	Aroclor 1254	Aroclor 1260	Total PCBs	Toxaphene	Dieldrin	Endrin	Heptachlor epoxide
LMB (*n* = 24)												
Station	1	9.74**	17.90**	3.24	23.54**	6.08*	16.61**	22.41**	5.97*	6.27*	0.26	1.68
Sex	3	25.00**	0.65	0.11	0.94	0.87	0.97	0.93	0.64	0.52	0.46	1.39
Station × sex	3	0.62	2.28	0.68	2.24	3.32*	1.52	2.41	2.95	2.86	0.59	3.24
Lipid	1		13.61**	0.55	19.64**	12.35**	15.70**	17.47**	9.27**	10.51**	0.01	3.03
Error ms	15	0.026[b]	0.386	0.197	0.319	0.235	0.149	0.324	0.223	0.0004	0.0007	0.0001
CCF (*n* = 20)												
Station	1	0.18	0.21	0.00	0.26	1.15	0.27	0.53	0.12	0.01	1.98	7.80*
Sex	3	12.17**	1.62	2.12	2.01	2.55	2.36	3.12	3.39	0.72	1.68	4.55*
Station × sex	3	0.17	1.57	1.72	1.78	0.98	2.96	3.04	0.32	0.65	1.90	1.96
Lipid	1		8.16	5.18*	9.55*	10.19**	6.93*	11.31**	20.64**	4.29	0.26	20.01**
Error ms	10	0.026[c]	0.447	0.042	0.458	0.059	0.097	0.145	0.137	0.0005	0.0002	0.0001
TFS (*n* = 6)												
Station	1	158.17**	0.96	0.00	0.11	2.78	—	0.53	0.14	11.65*	—	0.09
Lipid	1		0.02	0.02	0.01	4.51	—	0.20	0.01	13.58*	—	0.20
Error ms	3	0.0004[d]	0.0008	0.0008	0.009	0.002	—	0.004	0.005	0.0000	—	0.0002
COR (*n* = 6)												
Station	1	82.61**	4.39	4.32	6.92	3.68	—	3.68	0.98	—	—	—
Lipid	1		1.69	0.27	2.15	5.19	—	5.19	0.27	—	—	—
Error ms	3	0.0000[d]	0.0009	0.0000	0.0008	0.001	—	0.001	0.002	—	—	—
MFY (*n* = 6)												
Station	1	11.46*	7.25	4.59	4.70	—	—	—	0.13	—	—	—
Lipid	1		130.76**	0.00	29.25*	—	—	—	2.19	—	—	—
Error ms	3	0.001[d]	0.0000	0.0000	0.0005	—	—	—	0.004	—	—	—
NAT (*n* = 6)												
Station	1	0.29	9.53	0.44	8.53	28.73*	3.02	14.53*	0.39	0.00	3.30	0.39
Lipid	1		1.21	1.44	1.34	0.06	0.00	0.05	0.62	0.01	2.19	5.80
Error ms	3	0.003[d]	0.084	0.002	0.094	0.011	0.035	0.048	0.079	0.0001	0.0000	0.0000
HER (*n* = 10)												
Station	1	0.32	10.72*	4.99	11.85*	2.13	14.84**	14.02**	11.32*	3.24	23.78**	5.25
Lipid	1		0.14	2.16	0.21	0.08	0.00	0.01	1.56	5.79	1.30	7.41*
Error ms	7	0.081[e]	0.255	0.017	0.256	0.088	0.140	0.205	0.516	0.011	0.0000	0.0003

	Oxychlordane	*cis*-Chlordane	*trans*-Chlordane	*cis*-Nonachlor	*trans*-Nonachlor	Cadmium	Lead	Mercury	Arsenic	Selenium
LMB (*n* = 24)										
Station	0.03	25.39**	1.21	0.84	3.05	1.73	1.04	50.25**	0.32	6.53
Sex	2.05	3.73*	0.66	1.82	1.78	0.78	0.84	69.93**	2.02	39.66**
Station × sex*	1.25	5.88**	0.78	0.86	0.43	2.40	1.58	8.56**	1.16	0.56
Lipid	28.62**	38.88**	0.29	6.53*	9.68**	0.03	0.00	36.09**	4.76*	4.10
Error ms	0.0002	0.001	0.000	0.009	0.005	0.0001	0.0000	0.012	0.006	0.030
CCF (*n* = 20)										
Station	1.94	0.59	0.66	0.11	0.62	3.55	0.23	0.13	0.92	6.06*
Sex	1.45	1.95	1.58	3.15	2.10	10.33**	1.53	7.69**	1.35	31.91**
Station × sex*	1.65	1.04	1.01	2.12	2.05	0.63	0.92	0.34	1.57	1.33
Lipid	1.17	4.18	5.92*	5.90*	3.54	1.17	4.73	0.22	0.44	0.48
Error ms	0.000	0.012	0.001	0.004	0.006	0.0001	0.0000	0.014	0.009	0.082
TFS (*n* = 6)										
Station	0.09	0.06	0.01	0.19	0.09	0.01	1.99	0.35	5.14	0.31
Lipid	0.20	0.20	0.08	0.45	0.28	0.03	1.00	0.21	1.87	0.49
Error ms	0.000	0.011	0.001	0.005	0.007	0.0000	0.033	0.0000	0.003	0.0009
COR (*n* = 6)										
Station	—	4.21	—	—	0.80	1.17	0.05	0.23	—	—
Lipid	—	1.40	—	—	0.27	0.07	2.04	0.33	—	—
Error ms	—	0.0000	—	—	0.0000	0.001	0.001	0.0000	—	—
MFY (*n* = 6)										
Station	—	0.79	—	—	—	5.32	50.91**	32.23*	8.30	19.62*
Lipid	—	3.10	—	—	—	0.09	45.06**	0.01	0.01	0.06
Error ms	—	0.0001	—	—	—	0.004	0.008	0.0000	0.002	0.007
NAT (*n* = 6)										
Station	11.16*	14.87*	—	0.45	0.29	5.24	10.72*	2.35	0.06	53.52**
Lipid	0.06	4.13	—	0.22	0.27	0.49	3.77	0.20	0.19	4.13
Error ms	0.0000	0.001	—	0.006	0.009	0.0000	0.043	0.016	0.0003	0.0007
HER (*n* = 10)										
Station	2.75	1.49	—	16.59**	12.39**	5.08	—	2.17	0.24	0.07
Lipid	0.06	0.00	—	1.35	0.19	0.23	—	1.82	0.28	0.40
Error ms	0.0001	0.003	—	0.001	0.001	0.010	—	0.091	0.020	0.038

[a]LMB = largemouth bass; CCF = channel catfish; TFS = threadfin shad; COR = Asiatic clam; MFY = mayfly; NAT = water snake; HER = little green heron.
[b]df = 16.
[c]df = 11.
[d]df = 4.
[e]df = 8.

Table 6. Lipid-weight residues (μg/g, whole body) of total DDT, total PCBs, toxaphene, and sum (Sum miscellaneous) of dieldrin, endrin, α-BHC, heptachlor epoxide, oxychlordane, *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor and *trans*-nonachlor

	Total DDT		Total PCBs		Toxaphene		Sum Miscellaneous	
Matrix	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Largemouth bass—female	19.81	4.61	19.07	3.85	11.11	5.00	4.63	1.54
Largemouth bass—eggs	18.06	5.62	16.50	4.04	8.06	2.58	2.81	1.57
Largemouth bass—males	26.03	4.67	22.22	4.33	10.48	4.33	3.97	3.33
Largemouth bass—juveniles	24.37	5.00	22.50	3.75	10.00	20.00	5.00	3.75
Channel catfish—females	7.14	15.60	6.76	10.67	9.05	10.67	1.90	4.40
Channel catfish—eggs	4.67	3.61	4.00	3.61	6.00	0.12	1.33	0.84
Channel catfish—males	15.27	4.35	9.46	3.62	6.76	7.25	2.84	1.45
Channel catfish—juveniles	10.91	3.08	13.64	7.69	10.45	10.00	4.54	1.54
Threadfin shad	15.38	2.29	12.30	2.08	15.38	2.71	19.23	1.04
Asiatic clam	4.08	3.23	4.08	6.76	8.77	5.88	2.04	1.76
Mayfly	3.72	4.28	3.92	3.57	7.06	8.21	1.37	1.78
Water snake	22.95	5.38	22.73	8.46	8.18	5.13	5.68	2.82
Little green heron	5.26	1.60	8.23	4.03	6.12	0.55	1.29	0.93

from largemouth bass and the next highest (4.3 μg/g) in heron samples, both from the upper station. The highest total residue level for samples from the lower station was in female channel catfish (3.10 μg/g). Juvenile largemouth bass and channel catfish, threadfin shad, Asiatic clams, and mayflies had comparatively lower total residue concentrations than the other biota (< 1.0 μg/g).

Compared with residue levels in fish from NPMP stations (10, 11, 22), organic contaminant residues in biota from the Apalachicola River were considered moderately high. Mean residue concentrations of total DDT, total PCBs, and toxaphene in largemouth bass and channel catfish from the Apalachicola River generally exceeded the 1974 national geometric mean for these compounds (total DDT = 0.52 μg/g, total PCBs = 0.95 μg/g, toxaphene = 0.17 μg/g) (11). Compared with just the southeastern and Gulf Coastal NPMP stations, residue concentrations of DDT, PCBs, and toxaphene, particularly from the upper station on the Apalachicola River, generally exceeded the median concentration for all NPMP fish in 1974 (total DDT = 0.39 μg/g; total PCBs = 0.25 μg/g; toxaphene = < 0.10 μg/g).

The elevated levels of organic contaminant residues in the biota from the upper Apalachicola River may reflect the extensive agricultural and industrial developments in the tributary drainage systems (Chattahoochee and Flint Rivers) (1, 3). Municipal and industrial wastes emanating from the major metropolitan areas of Albany, Atlanta, Bainbridge, and Columbus are discharged into the river systems in addition to nonpoint source runoff from agricultural land (23). Forty-two percent of the land in these drainages is classified as farmland, with over 20% designated as cropland (24). It is presumed that reservoirs on the Chattahoochee and Flint Rivers, and particularly Lake Seminole, serve as settling basins for most of the contaminants; however, passage of material through Lake Seminole may account for the elevated levels of these contaminants in the upper river over those in the lower reaches. The Apalachicola drainage system has no major metropolitan areas and is sparsely populated (5), but runoff from agricultural developments in the northern region of the drainage may also contribute to the elevated contaminant levels in the biota of the upper reaches of the river. Agriculture is restricted primarily to the two northern counties (Jackson and Gadsen) of the drainage, with 32% of the land in Jackson county and 15% in Gadsen county utilized as cropland. Less than 4% of the land in the four southern counties is classified as cropland (25).

According to Walsh et al. (13), residues of arsenic, cadmium, lead, mercury, or selenium exceeding 0.5 μg/g in whole body components are considered potentially harmful to aquatic biota. Residue levels exceeding 0.5 μg/g occurred for cadmium and lead in mayflies at both stations, mercury in herons at the lower station, arsenic in threadfin shad and Asiatic clams at the lower station, and selenium in eggs from largemouth bass and channel catfish from both stations and in mayflies and Asiatic clams from the lower station (Tables 7 and 8). Metal residues in the biota from the Apalachicola River were generally higher at the lower than at the upper station (Figure 3).

The highest residue levels observed for arsenic were 1.75 μg/g in Asiatic clams and 1.07 μg/g in threadfin shad from the lower station (no analysis for arsenic was conducted on Asiatic clams from the upper station) (Tables 7 and 8). Our data are consistent with that of Spehar et al. (26) in that arsenic tends

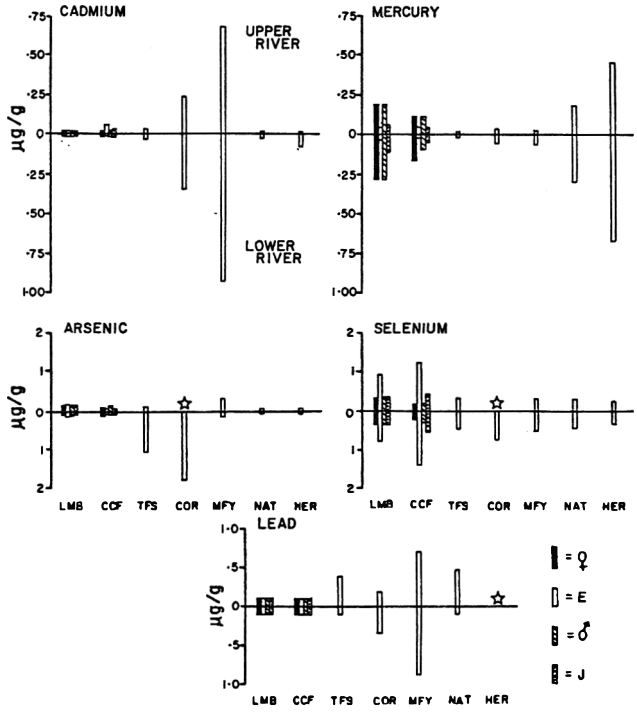

Figure 3. Metal residue concentrations (μg/g, wet-weight) in biota from upper reaches (above horizontal line) and lower reaches (below horizontal line) of the Apalachicola River, Florida, 1978.

Star = no data; LMB = largemouth bass; CCF = channel catfish; TFS = threadfin shad; COR = Asiatic clam; MFY = mayfly; NAT = water snake; HER = little green heron.

Table 7. Arithmetic mean and (in parentheses) minimum and maximum of metal residue concentrations (μg/g, wet-weight) for largemouth bass and channel catfish

Contaminant and section of river	Largemouth bass				Channel catfish			
	Females	Eggs	Males	Juveniles	Females	Eggs	Males	Juveniles
Cadmium								
Upper	0.01 (0.01–0.01)	0.01 (0.01–0.01)	0.01 (0.01–0.01)	0.01 (0.01–0.01)	0.01 (0.01–0.02)	0.07 (0.01–0.19)	0.02 (0.01–0.03)	0.03 (0.02–0.04)
Lower	0.01 (0.01–0.01)	0.02 (0.01–0.03)	0.01 (0.01–0.01)	0.01 (0.01–0.01)	0.01 (0.01–0.02)	0.01 (0.01–0.01)	0.01 (0.01–0.01)	0.03 (0.02–0.02)
Lead								
Upper	0.10 (0.10–0.10)	0.10 (0.10–0.10)	0.10 (0.10–0.10)	0.10 (0.10–0.10)	0.10 (0.10–0.11)	0.10 (0.10–0.10)	0.10 (0.10–0.10)	0.10 (0.10–0.10)
Lower	0.10 (0.10–0.10)	0.10 (0.10–0.10)	0.10 (0.10–0.10)	0.10 (0.10–0.11)	0.10 (0.10–0.10)	0.10 (0.10–0.10)	0.10 (0.10–0.10)	0.10 (0.10–0.10)
Mercury								
Upper	0.18 (0.09–0.28)	0.04 (0.01–0.11)	0.18 (0.11–0.26)	0.06 (0.05–0.07)	0.12 (0.06–0.16)	0.03 (0.02–0.04)	0.11 (0.03–0.18)	0.03 (0.03–0.04)
Lower	0.28 (0.22–0.34)	0.03 (0.02–0.04)	0.29 (0.20–0.38)	0.12 (0.10–0.13)	0.16 (0.15–0.17)	0.01 (0.01–0.02)	0.10 (0.06–0.14)	0.05 (0.04–0.06)
Arsenic								
Upper	0.11 (0.11–0.12)	0.17 (0.15–0.18)	0.14 (0.09–0.18)	0.15 (0.12–0.17)	0.08 (0.05–0.14)	0.05 (0.05–0.05)	0.12 (0.10–0.15)	0.06 (0.05–0.07)
Lower	0.12 (0.09–0.14)	0.16 (0.13–0.18)	0.14 (0.12–0.17)	0.12 (0.11–0.13)	0.10 (0.05–0.15)	0.05 (0.05–0.05)	0.05 (0.05–0.05)	0.05 (0.05–0.05)
Selenium								
Upper	0.33 (0.27–0.40)	0.94 (0.86–1.03)	0.39 (0.31–0.47)	0.36 (0.30–0.43)	0.18 (0.14–0.21)	1.25 (0.78–2.08)	0.19 (0.17–0.21)	0.42 (0.38–0.44)
Lower	0.32 (0.25–0.38)	0.70 (0.67–0.91)	0.35 (0.30–0.39)	0.36 (0.31–0.49)	0.22 (0.18–0.26)	1.39 (0.95–1.84)	0.28 (0.26–0.31)	0.53 (0.46–0.64)

Table 8. Arithmetic mean and (in parentheses) minimum and maximum of metal residue concentrations (μg/g, wet-weight) for threadfin shad, Asiatic clam, mayfly, water snake, and little green heron

Contaminant and section of river	Threadfin shad	Asiatic clam	Mayfly	Water snake	Little green heron
Cadmium					
Upper	0.02 (0.02–0.03)	0.23 (0.21–0.25)	0.68 (0.63–0.75)	0.01 (0.01–0.01)	0.01 (0.01–0.01)
Lower	0.02 (0.02–0.03)	0.34 (0.32–0.38)	0.92 (0.86–1.01)	0.02 (0.01–0.03)	0.07 (0.03–0.17)
Lead					
Upper	0.40 (0.24–0.64)	0.20 (0.19–0.20)	0.71 (0.31–1.13)	0.48 (0.22–0.87)	NA[a]
Lower	0.10 (0.10–0.10)	0.35 (0.31–0.41)	0.88 (0.76–1.08)	0.10 (0.10–0.10)	NA
Mercury					
Upper	0.01 (0.01–0.02)	0.03 (0.03–0.03)	0.02 (0.02–0.02)	0.18 (0.13–0.21)	0.46 (0.21–0.75)
Lower	0.02 (0.01–0.02)	0.05 (0.04–0.06)	0.05 (0.05–0.06)	0.29 (0.17–0.38)	0.67 (0.55–0.74)
Arsenic					
Upper	0.09 (0.08–0.10)	NA	0.33 (0.30–0.35)	0.06 (0.05–0.07)	0.07 (0.05–0.22)
Lower	1.07 (0.98–1.20)	1.75 (1.47–1.91)	0.15 (0.12–0.19)	0.05 (0.05–0.06)	0.05 (0.05–0.05)
Selenium					
Upper	0.33 (0.30–0.35)	NA	0.35 (0.32–0.37)	0.30 (0.28–0.33)	0.29 (0.15–0.45)
Lower	0.49 (0.47–0.51)	0.74 (0.69–0.79)	0.52 (0.51–0.53)	0.44 (0.42–0.48)	0.32 (0.28–0.36)

[a]NA = not analyzed.

to accumulate at lower trophic levels and does not biomagnify. Arsenic residue levels in the Apalachicola River did not exceed the FDA action level of 2 μg/g.

Mercury residues in the biota from the Apalachicola River were highest (0.67 μg/g) in herons from the lower station (Tables 7 and 8). Mercury concentrations were significantly higher in largemouth bass and mayflies from the lower river than the upper (Table 5). Concentrations in fish were less than 0.5 ppm, the FDA action level. Mercury residues less than 0.5 μg/g are generally accepted as representative of natural mercury levels in fish from unpolluted environments (27).

The highest cadmium concentrations in the biota of the Apalachicola River were in mayflies and Asiatic clams, particularly from the lower station, where Asiatic clams contained 0.34 μg/g and mayflies 0.92 μg/g (Tables 7 and 8). Fish from both the upper and lower stations had low levels (< 0.08 μg/g) of cadmium.

Mayflies had the highest lead residues (Tables 7 and 8), and the residues were significantly ($P < 0.01$) lower at the upper

(0.71 μg/g) than at the lower (0.88 μg/g) station (Table 5). Residues in fish were comparatively lower (0.10 μg/g).

Selenium was the highest metal residue in largemouth bass and channel catfish, and was highest in the eggs from these fishes. The greatest concentration (1.39 μg/g) was in channel catfish eggs from the lower station (Tables 7 and 8). Selenium residues were significantly higher in the eggs than in the other sex groups for these 2 species (Table 5). Residues in largemouth bass eggs were significantly higher at the upper station than the lower, but in channel catfish eggs, concentrations were significantly higher at the lower station than the upper.

Biota from the lower Apalachicola River were collected downstream of the confluence with the Chipola River, and this may be important in ascribing differences in residue concentrations between upstream and downstream reaches. The influence of the Chipola River on the water quality and contaminant loading of the lower Apalachicola River is not known, but could potentially be an area of concern. The most extensive agricultural developments in the Apalachicola drainage system occur in the headwater area of the Chipola River (Jackson County) (25). Abandoned and active battery salvage operations discharge into the upper Chipola River and may be implicated in the somewhat elevated metal residues in the biota of the lower Apalachicola River. Discharge of the Chipola River, however, is small in comparison with the Apalachicola. The Chipola River has an average discharge (measured at Altha) of 42 cu. m/second (1484 cfs), and the Apalachicola River at Blountstown has an average discharge of 695 cu. m/second (24 560 cfs) (28).

Conclusion

Moderately high residue concentrations of metals, organochlorine insecticides, and PCBs were measured in the biota collected from the Apalachicola River, in comparison with levels in other systems and criteria for the protection of aquatic life. Organic contaminants in the biota were more concentrated in the upper than in the lower reaches, whereas metals generally were higher in the lower reaches. Total DDT, total PCBs, and toxaphene were the contaminants with highest concentrations in the biota, but all were below 2 μg/g and did not exceed FDA action levels. However, organic residues in the biota, particularly from the upper station, exceeded NAS-NAE (18) recommended residue levels for the protection of aquatic life. Considering the persistence of the metals and organochlorine compounds, their potential for bioconcentration and bioaccumulation, and the moderate concentration of residues observed in the biota of the Apalachicola River, we conclude that this system was moderately contaminated in 1978. Additional contaminant loading from ongoing activities or future developments in the Apalachicola River watershed could pose a serious threat to the environmental quality of this already moderately contaminated system.

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DRUG RESIDUES IN ANIMAL TISSUES

Rapid Liquid Chromatographic Determination of Clopidol in Chicken Muscles

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A simple, rapid, and sensitive liquid chromatographic method is described for the quantitative determination of trace amounts of clopidol (3,5-dichloro-2,6-dimethyl-4-pyridinol) in chicken muscles. Clopidol was extracted from tissues with acetonitrile and the extract was cleaned up on an alumina column and separated on an anion exchange column, using 0.5% acetic acid in methanol as eluant. The solvent in the eluate was evaporated and the residue was dissolved in methanol containing benzamide as internal standard. Clopidol was separated on a Zorbax ODS column (4.6 × 250 mm) by using acetonitrile–0.05M phosphate buffer, pH 7.0 (15 + 85 v/v%), and detected at 270 nm with 0.005 AUFS. Recoveries of clopidol added to chicken tissues at levels of 0.02, 0.05, and 0.1 μg/g were 94.8, 94.8, and 97.0%, respectively. This method is applicable to levels as low as 0.01 ppm clopidol in chicken muscle.

Clopidol (3,5-dichloro-2,6-dimethyl-4-pyridinol), a coccidiostat in chickens, is administered in feed at 80–250 mg/kg. The medicated feed must be withdrawn 7 days before slaughter to prevent any residues from appearing in the tissues, according to Japanese regulations (1). Methods have been developed for determining clopidol in feed (2, 3) and chicken tissue (4–8) by using gas chromatography. However these methods are quite tedious and require considerable time for alumina column cleanup followed by separation on an anion exchange column before measurement by gas chromatography (2–8). Liquid chromatography (LC) has been applied to determine clopidol in feed (9) and chicken tissue (10). Because low residue levels of clopidol are expected and there is no agreement on the level of clopidol that would be a health concern, a more sensitive method was necessary. It was suggested that additional work be done with the AOAC method (5).

This paper describes a simple, rapid, and sensitive method for determination of clopidol in chicken muscle by LC at the 0.01 ppm level, with no interference from sample background.

Experimental

Reagents

All chemicals were analytical reagent grade.

(a) *Clopidol.*—Purity 99% (The Dow Chemical Co., Midland, MI 48640). *Standard solutions:* Dissolve 10.0 mg clopidol in 100 mL methanol. Dilute 1.0 mL aliquot of this stock solution to 100 mL with methanol to prepare working solution that contains 1 μg clopidol/mL.

(b) *Internal standard solution.*—Dissolve 10.0 mg benzamide (Tokyo Kasei Co., Tokyo, Japan) in 100 mL methanol. Dilute 2.5 mL of this solution to 100 mL with methanol to prepare solution that contains 2.5 μg benzamide/mL.

(c) *Alumina.*—Aluminum oxide 90, 70–230 mesh (E. Merck); use as received.

(d) *Anion exchange resin.*—Dowex 1-X8, 100–200 mesh, chloride form (Dow Chemical Co.). Convert to acetate form by procedure of Analytical Methods Committee (2).

(e) *Tissue samples.*—Commercial chicken tissues were obtained from Tokyo and Kanagawa prefectures; clopidol-free chicken tissue was supplied by Research Institute for Animal Science in Biochemistry and Toxicology, Sagamihara, Japan.

Apparatus

(a) *High-speed homogenizer.*—Ultra-Turrax tp 18/10 S2 (Janke & Kunkel GmbH & Co., Switzerland).

(b) *Meat mincer.*—2 Brev. Super Baby, Italy.

(c) *Evaporator.*—Rotary evaporator, type YR (Yazawa Co., Japan).

(d) *Centrifuge.*—Model 90-22 (Sakuma Seisakujo, Tokyo, Japan) or equivalent.

(e) *Liquid chromatographic column.—(1)* Alumina column: Place small cotton plug at bottom of 300 × 18 mm id column and add 6 g alumina. *(2)* Anion exchange column: Place small cotton plug at bottom of 300 × 10 mm id column and add 1 cm bed height Dowex 1-X8 resin. Rinse column with 20 mL 0.5% acetic acid in methanol and wash with 15 mL methanol before use.

(f) *Liquid chromatograph.*—Shimadzu LC-3A, equipped with spectrophotometric detector (Shimadzu SPD-2A) and column oven (Shimadzu CTO-2A). 4.6 × 250 mm, Zorbax ODS column (DuPont Co., Analytical Instruments Div., Westwood, NJ 07675) fitted with 2.1 × 50 mm guard column of Permaphase ODS (DuPont Co.) at 50°C column temperature. Degas acetonitrile–0.05M phosphate buffer, pH 7.0 (15 + 85) mobile phase on ultrasonic bath for 5 min and run at flow rate of 1.5 mL/min. Monitor UV absorption at 270 nm with 0.005 AUFS, and chart speed of 5 mm/min. Inject 10 μL clear solution onto LC system for analysis.

(g) *LC recorder.*—Model 056, Hitachi.

(h) *Ultrasonic generator.*—NS-300 (Nihon Seiki Co., Tokyo, Japan.)

Extraction and Cleanup

Accurately weigh 20 g minced tissue and homogenize twice for 3 min at maximum speed with 50 mL portions of acetonitrile, and then centrifuge at 3000 rpm for 10 min. Add 1.5 g anhydrous sodium sulfate and let stand 30 min. Filter through cotton, and dilute filtrate to 100 mL with acetonitrile and mix well. Place anion exchange column under alumina column. Pipet 20 mL extract onto alumina column and elute through both columns into beaker. Wash both columns twice with 10 mL portions of methanol. Remove alumina column, place 100 mL evaporating flask under anion exchange column, and elute clopidol with 20 mL 0.5% acetic acid in methanol. Evaporate to dryness on rotary evaporator at 40°C. Dissolve residue in 1.0 mL methanol containing internal standard. Calculate concentration of clopidol from calibration curve.

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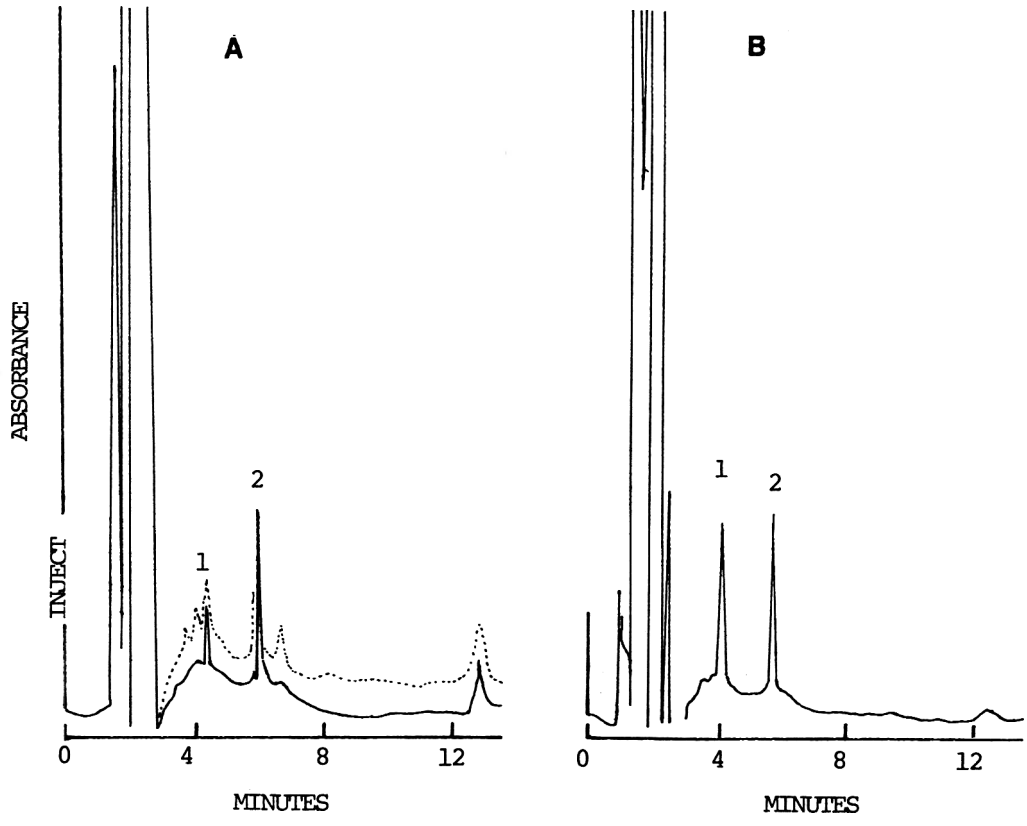

Figure 1. Typical chromatograms obtained from commercial chicken tissues: A, methanol extract (dashed line); acetonitrile extract (solid line). B, acetonitrile extract (0.05 ppm clopidol added to tissue). Peak 1, clopidol; peak 2, benzamide internal standard.

Results and Discussion

The optimum LC operating condition was derived from experiments based on parameters such as column temperature, flow rate, mobile phase composition, which contribute to a favorable column pressure. Experiments on column temperatures between 30 and 55°C confirmed 50°C as the best maximum temperature, which showed a reasonable retention time with good resolution. A flow rate of 1.5 mL/min gave a favorable retention time under the conditions used between 0.8 and 1.8 mL/min. The effect of mobile phase on separation was studied at different compositions of either methanol or acetonitrile with phosphate buffer, including ion-pair chromatography with tetra-*n*-butylammonium bromide. Optimum results for detecting clopidol were obtained using an acetonitrile–phosphate buffer system as mobile phase. Based on chosen parameters, a calibration curve was prepared over the range 0.2–10 ng clopidol. The ratio between the peak heights of clopidol and internal standard plotted against concentration of clopidol gave a straight line. Benzamide was selected as the internal standard because its retention time was sufficiently longer than that of clopidol, and it had been used in medications for chicken.

Clopidol could not be determined in chicken tissues at lower levels by the cleanup procedure by Bjerke and Herman (5). Suzuki et al. (6) could not determine clopidol at levels lower than 0.1 ppm because clopidol is detected with a faster retention time, i.e., closer to the solvent front, and therefore there is high possibility of interference by other tissue components. Several procedures have been used to establish a simple, rapid, and good cleanup procedure, using Florisil, Sep-Pak cartridge (silica, C_{18}, and Florisil provided by Waters Associates, Inc., Milford, MA 01757), alumina, anion exchange resin, as well as activated charcoal with LC detection. In all

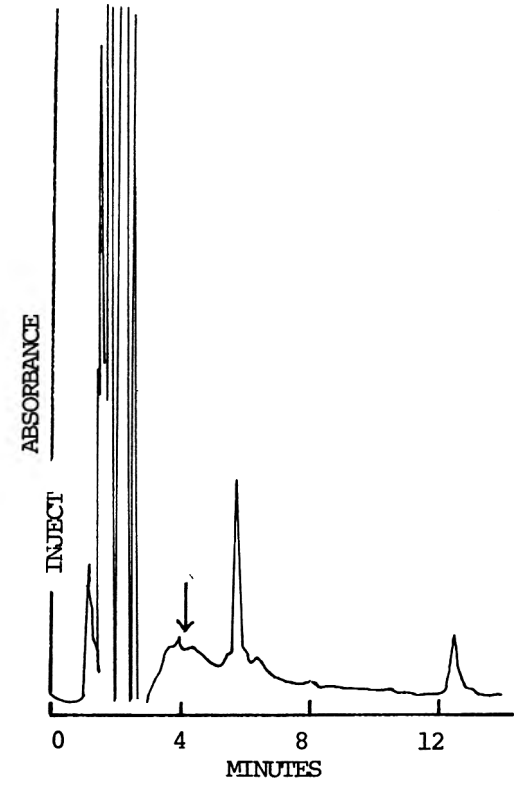

Figure 2. Typical chromatogram of acetonitrile extract obtained from clopidol-free chicken tissue. Arrow shows retention time of clopidol.

these procedures, the presence of interfering components contributed to the difficulty in obtaining good results (Nakazawa, H., Mtema, C. A., Kabasawa, Y., Inoue, Y., & Takabatake, E., unpublished data). However, conclusive results could be obtained after both alumina and anion exchange column cleanup. Based on the LC condition selected, clopidol is well separated from the solvent front and other interfering components for detection and identification. Extraction efficiency was the same for methanol and acetonitrile, but using acetonitrile gave a better chromatogram (Figure 1A, solid line) with less interference and better resolution than for methanol (Figure 1A, dashed line). Figure 1B is a chromatogram obtained from commercial chicken tissue samples with added clopidol. The chromatograms are well defined and free from any interference from tissue components.

Figure 2 shows a chromatogram of the acetonitrile extract from clopidol-free chicken tissues. Recovery studies were carried out by adding 0.02, 0.05, and 0.1 ppm clopidol to 20 g minced clopidol-free chicken tissues. These samples were analyzed by the present method. A relative retention time for clopidol derived from added clopidol was identified with good precision. As shown in Table 1, these recoveries are satisfactory for residue analysis and substantiate the validity of this method, the utility of which was demonstrated in its application to commercial chicken tissues. Determination of clopidol in commercial chicken tissues shows that this method is applicable at levels as low as 0.01 ppm. Results of analyses on 7 duplicated samples showed no clopidol detected in 3 samples; <0.01 ppm (below the detection limit) in 1 sample; 0.01 ppm in 2 samples; and 0.02 ppm in 1 sample.

Table 1. Recovery of clopidol added to chicken tissues

Clopidol added, μg/g	Av. rec., % (± SD, $n = 5$)
0.02	94.8 ± 2.3
0.05	94.8 ± 2.5
0.10	97.0 ± 2.3

Acknowledgment

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TECHNICAL COMMUNICATIONS

Comparison of Liquid Chromatography and High Performance Thin Layer Chromatography for Determination of Aflatoxin in Peanut Products

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Recent research studies concerning aflatoxin in peanut products have involved the use of new instrumental methods for greater detection sensitivity. A comparison of 2 such techniques, liquid chromatography (LC) and high performance thin layer chromatography (HPTLC), is reported here. With respect to precision, accuracy, sensitivity, recovery, and linearity of response, HPTLC appears to be equivalent to LC. These points illustrate the viability of HPTLC as an alternative technique in the determination of aflatoxin.

While thin layer chromatography (TLC) has been used for the determinative step in the analysis of aflatoxins in peanut products for many years, recently, attempts have been made to use both liquid chromatography (LC) and high performance thin layer chromatography (HPTLC) techniques. LC should naturally have an advantage over the standard TLC determinative step: It is a more objective means of determining aflatoxins in peanut products because of the elimination of the subjective human visual evaluation of the TLC plate. However, when normal phase LC is used, a number of time constraints imposed by late-eluting peaks require considerable column equilibration between successive samples. The use of HPTLC for detecting aflatoxins was reported years ago by Viteck et al. (1) but has only recently generated interest with improvements in the sensitivity of the measuring densitometer.

Experimental

We compared these 2 techniques on peanut butter samples extracted by the Pons method as reported by Pons and Franz (2). Sample cleanup also followed the Pons method, but we substituted a 2 g silica gel Sep-Pak cartridge (Waters Associates) for the specified cleanup column.

The elution solvents passing through both the column and the silica gel cartridge were the same, but volumes were only one-tenth with the cartridge. Also, the time for effective sample cleanup with the silica gel cartridge was reduced to about 15 min per sample compared with more than 30 min for the standard Pons cleanup (2). All extracts were analyzed using Pons LC system (2).

All extracts were evaluated by HPTLC using an automated micro-applicator and 250 μm silica gel G hard layer TLC plates (Merck). Plates were developed twice for 20 min in a lined tank containing chloroform–acetone (88 + 12) and water vapor. Plates were allowed to air-dry. They were then exposed to longwave UV light and scanned for fluorescence by use of a Camag monochrometer equipped TLC densitometer.

Results and Discussion

While Sep-Pak cartridges performed well about 2 years ago during initial evaluations using LC, recent studies with HPTLC gave unexpectedly low recoveries. Recovery studies were made on the cartridges alone, using the nominal conditions of the Pons method. Aliquots of a combined aflatoxin standard mixture were diluted with 2.0 mL methylene chloride and quantitatively transferred directly onto the cleanup cartridge. The cartridge was washed with 5 mL toluene–acetic acid (9 + 1) followed by 5 mL ether–hexane (3 + 1). The aflatoxins were eluted from the cartridge with ca 10 mL methylene chloride–acetone (9 + 1). This solvent was carefully evaporated, and the residue was redissolved and diluted with 0.20 mL methylene chloride. While final concentrations of aflatoxins were designed to give approximately 40 ng B_1 and G_1 and 12 ng B_2 and G_2, a loss of each of the 4 aflatoxins was evident. When the losses were traced to excessive retention by the cleanup cartridge, attempts were made to improve the recoveries but were unsuccessful even with different cartridge lots.

With a change of cartridge source, to the Analytichem International product, Bond Elute, excellent recoveries were obtained. The results reported on Table 1 are averages of 4 samples each. Subsequently, similar losses were also obtained with Bond Elute cartridges, indicating that recovery testing should be made before any new lot of silica gel cartridges is used.

Experiments were performed to establish recovery and reproducibility by the HPTLC technique. Five peanut butter samples were spiked with the mixed aflatoxin standard at a level of 10.4 ppb total aflatoxin, and samples were analyzed over a 2-day period. The results in Table 2 show recoveries of 90% or better except for aflatoxin G_2 whose recovery was about 79%.

An additional recovery study was made at various aflatoxin levels to check the linearity of the response. Peanut butter samples were spiked at levels of 5 to 52 ppb total aflatoxins and assayed as before. As can be seen in Table 3, these recoveries were consistently around 90%. No observable trend was noted within the concentration range studied.

The 2 techniques were also compared by analyzing 5 naturally contaminated peanut butters. The final extract was split and assayed by both LC and HPTLC systems. The results in Table 4 show good agreement between methods for individual aflatoxins and total aflatoxins. Another set of 5 naturally contaminated peanut butter samples (Table 5) were also compared against the results obtained by the BF method (3). These results, while showing individual sample variability, were not significantly different across methods and were all within the normal analytical error for the aflatoxin level involved.

Table 1. LC recovery comparison of mixed aflatoxin standard eluted from cleanup cartridges

	Rec., %[a]				
Cartridge	B_1	B_2	G_1	G_2	Total aflatoxins
Bond Elute	98.0	101.3	103.4	100.6	100.8
Sep-Pak	81.8	81.7	76.5	67.4	76.9

[a]Average of 4 determinations.

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Table 2. HPTLC recovery and reproducibility of aflatoxin from spiked peanut butter samples

Sample	Day	Spike, ppb	Rec., %[a] B_1	B_2	G_1	G_2	Total
1	1	10.4	84.4	94.3	97.8	81.8	89.6
2	1	10.4	84.4	90.5	94.4	74.9	86.0
3	1	10.4	90.6	92.4	91.6	74.9	87.4
4	2	10.4	95.3	102.5	91.9	81.8	92.9
5	2	10.4	95.7	99.3	88.9	82.7	91.7
	SD		5.56	5.19	3.35	3.96	2.88
	RSD		6.17	4.98	3.61	5.00	3.21
	Av.		90	96	93	79	90

[a]Using Bond Elute silica gel cleanup cartridge.

Figure 1 shows typical chromatograms by both LC and HPTLC. The time scale for the LC elution of aflatoxins is ca 15 min. For a sample having late-eluting peaks, these peaks must be removed before the next injection. The densitometer scan time for a single sample by HPTLC is 1 min. By scanning only the area of interest, using 366 nm longwave UV light, interferences from the HPTLC chromotograms are minimized. While the resolution between peaks in Figure 1 is not as good for HPTLC as for LC, this is predominantly a function of the plate development time. If time is not a constraint, resolution comparable to that obtained by LC should be possible.

Table 3. Check of linearity of HPTLC assay procedure for aflatoxins, using spiked samples

Sample	Added, ppb	Detected, ppb	Rec., %[a]
1	5.20	4.89	94.13
2	10.40	9.31	89.50
3	20.80	18.29	87.95
4	52.00	45.85	88.18
Av.	—	—	89.94

[a]Using Bond Elute silica gel cleanup cartridges.

Table 4. Comparison of LC and HPTLC analyses of aflatoxin in peanut butter

Sample	Method	Aflatoxin, ppb B_1	B_2	G_1	G_2	Total
1	LC	0.1	0.1	0.2	0.1	0.5
	HPTLC	0.2	0.1	0.2	0.1	0.6
2	LC	0.2	0.1	0.1	0.2	0.6
	HPTLC	0.2	0.1	0.1	0.0	0.4
3	LC	0.8	0.7	0.0	0.0	1.5
	HPTLC	0.7	0.6	0.0	0.0	1.3
4	LC	11.8	1.8	0.6	0.4	14.6
	HPTLC	7.6	3.8	0.2	1.1	12.7
5	LC	9.1	1.8	0.8	0.4	12.1
	HPTLC	7.1	3.1	2.6	1.1	13.9

Table 5. Comparison of HPTLC and LC method results with results by BF method (naturally contaminated peanut butter)

Sample	Total Aflatoxin, ppb TLC	LC	HPTLC
1	neg.	1.1	1.3
2	neg.	1.4	1.2
3	5	5.9	5.6
4	5	5.7	5.7
5	17.5	12.8	12.2

In summary, the HPTLC method surpasses LC with respect to reduction of total analysis time by permitting simultaneous multiple sample separations. With the same extraction and cleanup procedure for both methods, HPTLC can be as much as 3 times faster than the LC system with a group of 10 samples but requires essentially the same time to assay a single sample. With respect to precision, accuracy, and sensitivity, HPTLC appears to be equivalent to LC. The disposable nature of the stationary phase (TLC plates) with the HPTLC method eliminates the problem of residual contaminants that affect the life and performance of microparticulate silica gel LC columns. Also, the amount of solvent used is less (as much as 90% reduction in solvent used for the determinative step of a 10-sample group) for HPTLC than for LC.

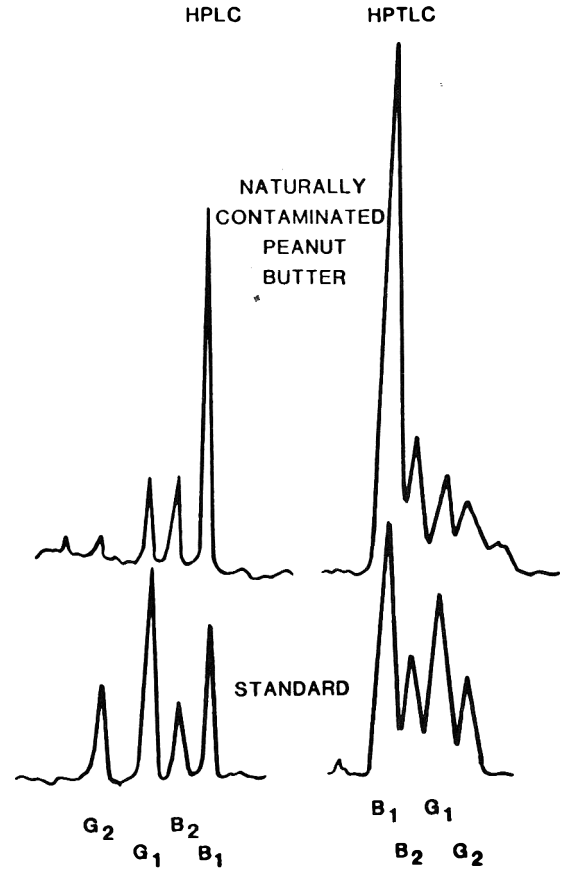

Figure 1. Comparison of resolution obtained with standard aflatoxin solution and aflatoxins extracted from naturally contaminated peanut butter for LC or HPTLC.

These points make the use of HPTLC a viable alternative technique in the determination of aflatoxins.

Acknowledgment

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Liquid Chromatographic Determination of Acetaminophen in Multicomponent Analgesic Tablets

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A simple, rapid LC method is presented for the separation and determination of acetaminophen in analgesic preparations containing up to 6 additional active components. The method uses a C_{18} reverse phase column, methanol–0.75% acetic acid (1 + 3) mobile phase, and photometric detection in the ultraviolet region. Acetaminophen was effectively separated from chlorpheniramine maleate, phenylephrine hydrochloride, caffeine, salicylamide, aspirin, and phenacetin, as well as from salicylic acid, a degradation product of aspirin. Typical chromatograms of the separation of acetaminophen from the above compounds in synthetic mixture and in commercial multicomponent analgesic preparations are presented, along with reproducibility and recovery data.

Acetaminophen is an analgesic and antipyretic agent which is commercially available in tablet, capsule, and suppository forms. Because it shows good stability in solution, this drug is also formulated as an elixir, syrup, and suspension. Acetaminophen is frequently available in combination with antihistamines, stimulants, buffers, and other analgesics (1, 2).

A number of liquid chromatographic (LC) methods have been described for the detection and determination of acetaminophen in biological fluids (3, 4) and in pharmaceutical dosage forms (5–7). Biological samples have been analyzed by adsorption chromatography on a silica gel column (3) or by reverse phase chromatography (4). Methods proposed for pharmaceutical products have relied on ion-exchange (8, 9) and on bonded reverse phase (5–7, 10–13) columns, the latter sometimes in the ion-pairing mode (10, 11, 14). LC has been applied to the simultaneous quantitation of acetaminophen in combination with other active ingredients such as aspirin, caffeine, codeine phosphate, phenacetin, and salicylamide (5), caffeine, aspirin, and salicylamide (9), aspirin, benzoic acid, and salicylamide (9), chlorzoxazone (7, 11), caffeine, aspirin, and salicylic acid (6), and salicylamide and sodium butabarbital (13).

Compendial methods for the determination of acetaminophen in pharmaceutical dosage forms (15–17) are often cumbersome and time consuming. The method for elixir and tablets (15) requires a lengthy preliminary column chromatographic separation that involves elution with water-saturated ether, evaporation of the eluate, and spectrophotometric determination in a solution of the residue. In our experience, traces of chloroform in the assay sample sometimes interfere with the determinative step. Officially, the combinations propoxyphene hydrochloride–acetaminophen and propoxyphene napsylate–acetaminophen are assayed by gas chromatographic methods (16, 17), but each drug requires its own stationary phase and its own internal standard.

This paper describes a rapid and reproducible LC method for the determination of acetaminophen in dosage forms, alone or in combination with the following drugs: aspirin, caffeine, chlorpheniramine maleate, phenacetin, phenylephrine hydrochloride, salicylamide, and salicyclic acid, which is formed upon hydrolytic degradation of aspirin. The proposed method uses a C_{18} reverse phase column, methanol–0.75% acetic acid (1 + 3) as the mobile phase, and detection in the UV region. The method is applicable to a variety of commercial tablets that contain from 2 to 6 other active ingredients. To our knowledge, LC has not been previously used to analyze a mixture of acetaminophen with all these drugs.

Experimental

Apparatus and Reagents

(a) *Liquid chromatograph.*—Tracor, consisting of Model 950 pump, Model 970A variable wavelength detector, Model TS-10 recorder (Tracor Instruments Inc., Austin, TX 78721), and Rheodyne Model 7125 injector equipped with 20 μL loop (Rheodyne Inc., Berkeley, CA 94710). Chromatographic conditions: flow rate, 2.0 mL/min at ca 2000 psi; detector wavelength, 254 or 280 nm, depending on composition of dosage form; detector sensitivity, 0.08 or 1.28 AUFS, depending on composition of dosage form; column temperature, ambient.

(b) *Column.*—C_{18} Chromegabond, 30 cm × 4.6 mm id, 10 μm particle size (E. S. Industries, Marlton, NJ 08053), or equivalent.

(c) *Mobile phase.*—Methanol–0.75% acetic acid (1 + 3, v/v), filtered through appropriate membrane filter of ca 0.5 μm pore size, and degassed. pH ca 3.0.

Sample Preparation

Weigh and finely powder not less than 20 tablets. Accurately weigh portion of powder, equivalent to ca 60 mg acetaminophen, and transfer to 100 mL volumetric flask. Dilute to volume with mobile phase, and mix. Filter solution through suitable microfilter, discarding first 10 mL filtrate. For samples containing aspirin, analyze solution within 1 h.

Standard Preparation

Accurately weigh ca 60 mg acetaminophen USP Reference Standard and transfer to 100 mL volumetric flask. Dissolve and dilute to volume with mobile phase, and mix.

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System Suitability Test

Make 5 replicate injections of standard preparation and record peak responses. Coefficient of variation is not more than 2.0% and tailing factor is not more than 2.0.

Procedure

Inject 20 μL each of standard preparation and sample preparation, in duplicate, into liquid chromatograph set at sensitivity of 1.28 AUFS. Measure peak responses and obtain corresponding average values. Calculate quantity of acetaminophen in sample by following formula:

$$\text{mg/tablet} = P_u/P_s \times C \times F \times \text{av. tablet wt, mg}$$

where P_u and P_s = average peak responses of sample preparation and standard preparation, respectively; C = concentration, μg/mL, of standard preparation; and F = numerical factor that converts entire mathematical expression to mg and accounts for any dilution made, i.e., dilution/(1000 × weight sample taken, mg).

Results and Discussion

Figures 1 and 2 are typical chromatograms of a synthetic mixture of acetaminophen with 6 other drugs that are often found in varying combinations in multicomponent analgesic tablets. Amounts of each drug in the mixture were based on published $A_{1\,cm}^{1\%}$ values (18) so they were detectable at the same sensitivity setting. In addition, salicylic acid was included to demonstrate the ability of the proposed method to verify the degradation of aspirin to this product. These separations were separately monitored at 280 and 254 nm, primarily to illustrate the detectability of acetaminophen at more than one wavelength. The selection of the detection wavelength is dictated by the drug to be assayed. If only acetaminophen (A_{max} about 247 nm) is to be assayed, the detector is set at 254 nm and a standard solution of approximately 35 μg/mL is used, with a sensitivity of 0.08 AUFS. If other drugs such as caffeine (A_{max} about 273 nm) or phenylephrine (A_{max} about 275 nm) are to be assayed, a different wavelength, such as 280 nm, should be used to compensate for the much smaller quantities of these drugs relative to acetaminophen. In this case, the concentration of acetaminophen in the sample should be approximately 600 μg/mL, and the sensitivity setting 1.28 AUFS.

To evaluate the accuracy of the proposed method, an amount of powdered composite prepared from commercial multicomponent tablets, and equivalent to one-half the average weight of a tablet, was mixed with an equivalent weight of acetaminophen, and the mixture was analyzed for its acetaminophen content. The results of this study are summarized in Table 1. The overall average recovery was 101.9% for 4 tablet samples.

The precision of the LC method was investigated by performing 10 consecutive injections of each of 3 different dilutions of an acetaminophen standard preparation. The results are shown in Table 2. The method was reproducible at the concentration levels studied, and gave acceptable relative standard deviations.

When the LC method was applied to 4 different brands of acetaminophen-containing multicomponent tablets, the difference between duplicate assay values was less than 1.1% in all cases (Table 3). None of the additional active ingredients interfered with the assay of acetaminophen because they were undetectable at the sensitivity settings used.

Because of the possible hydrolysis of aspirin to salicylic acid in the mobile phase, it is recommended that the sample

Table 1. Recovery of acetaminophen from composite samples of commercial tablets

Product[a]	Amt declared, mg/tablet	Amt added, mg	Amt recd, mg	Amt recd, %
A	128	64.3	65.5	101.9
B	250	125.3	126.9	101.3
C	325	167.7	169.8	101.3
D	325	169.2	174.4	103.1

[a]Tablets from (A) Reid-Provident Laboratories, Atlanta, GA, containing acetaminophen, salicylamide, caffeine, ascorbic acid, pyrilamine maleate, methapyrilene hydrochloride, and phenylephrine hydrochloride; (B) Bristol-Myers Co., New York, NY, containing acetaminophen, aspirin, and caffeine; (C) Bristol-Myers Co., containing acetaminophen, phenylpropanolamine hydrochloride, chlorpheniramine maleate, and dextromethorphan hydrobromide; (D) Whitehall Laboratories Inc., New York, NY, containing acetaminophen, phenylephrine hydrochloride, chlorpheniramine maleate, and caffeine.

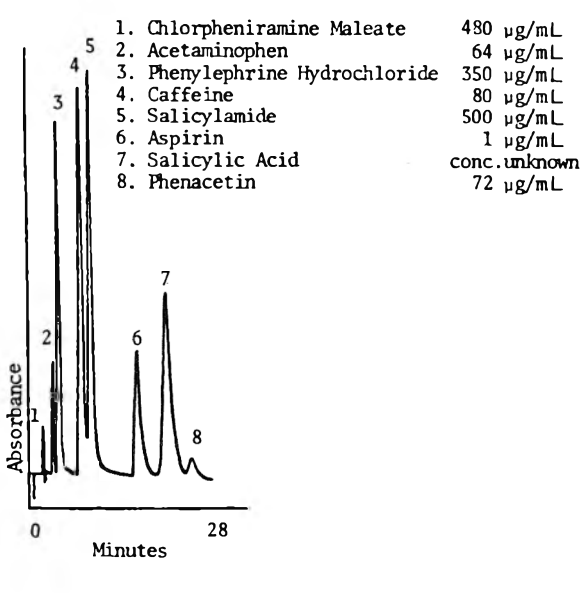

Figure 1. Liquid chromatogram of synthetic mixture of drugs found in multicomponent analgesic tablets. Sensitivity setting 0.32 AUFS.

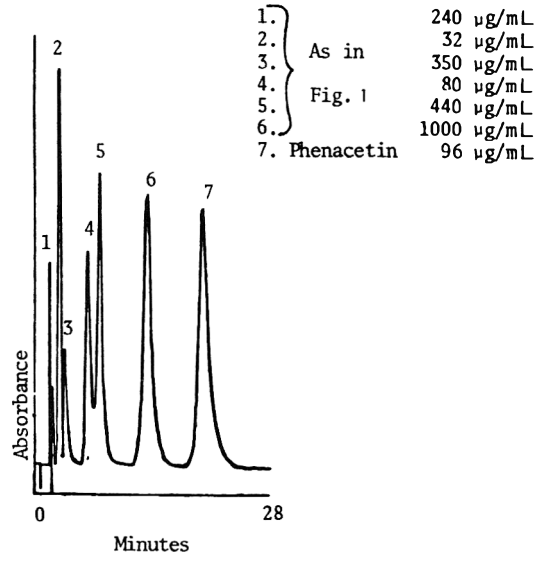

Figure 2. Liquid chromatogram of synthetic mixture of drugs found in multicomponent analgesic tablets. Sensitivity setting 0.08 AUFS.

Table 2. Reproducibility of LC method at 3 concentrations of acetaminophen

Acetaminophen, μg/mL	Detector settings		Peak height (n = 10)		
	nm	AUFS	mm	SD	CV, %
34.59	254	0.08	165.0	0.63	0.38
325.00	280	0.32	133.9	1.35	1.01
639.00[a]	280	1.28	173.5	0.51	0.29

[a]Tailing factor at this concentration was 1.34.

Table 3. Results of assay of acetaminophen in commercial multicomponent analgesic tablets

Product[a]	Amt declared, mg/tablet	Amt found	
		mg/tablet (av.)	% declared (av.)
A	128	134.3, 135.0 (134.7)	104.9, 105.5 (105.2)
B	250	253.9, 251.5 (252.7)	101.6, 100.6 (101.1)
C	325	337.9, 339.2 (338.6)	104.0, 104.4 (104.2)
D	325	341.9, 338.5 (340.2)	105.2, 104.2 (104.7)

[a]See Table 1 for commercial source and composition.

be analyzed within 1 h. The presence of any preformed salicylic acid in acetaminophen–aspirin-containing tablets was ascertained by monitoring the chromatographic separation at 280 nm.

In summary, the LC method presented can be used as a rapid and reliable means of identification of acetaminophen in the presence of up to 6 other drugs that are commonly found as ingredients of multicomponent analgesic and cold tablets, and of salicylic acid formed from aspirin. Furthermore, this method was accurate and precise for the determination of acetaminophen in these products, and of potential utility in the analysis of other active ingredients.

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ISO Working Group Studies on Filtration Problems in Crude Fiber Determinations

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Members: N. T. Crosby; A. M. Dordio; B. F. Harland; P. Hollman; F. Kadijk; P. Lutonska; D. F. Martillott; J. M. v. d. Meer; V. D. Mudgal; I. Olah; I. Pichl; S. E. Rayner

A working group of individuals from International Organization for Standardization member nations studied the use of ceramic fiber to replace asbestos as a filter aid in crude fiber determinations in 8 samples: rolled oats, 48% solvent-extracted soybean meal, alfalfa meal, degermed corn meal, an all-bran cereal, hard red winter wheat, brussels sprouts, and soy fiber. Results obtained using ceramic fiber were accurate and precise, except for one laboratory which reported very high values. Results on 4 of these samples were compared with results obtained on the same 4 samples in an earlier collaborative study. There was good correlation between the studies. In addition, 2 laboratories reported data obtained using sea sand as filter aid, and 2 laboratories analyzed samples using no filter aid.

A working group composed of individuals from International Organization for Standardization (ISO) member nations was set up to study filtration problems for crude fiber and cell wall determination. Results for the crude fiber study are reported here. Ceramic fiber (1), which was adopted official first action by AOAC to replace asbestos as a filter aid, was used in analyzing 8 samples with crude fiber content from 0.5 to 30.0%. The data agree well, except for results from one laboratory which reported extremely high values. Those data were excluded from statistical analyses.

Eight samples—rolled oats, soybean meal 48% solvent-extracted, alfalfa meal, corn meal (degermed), Kellogg's All-Bran, hard red winter wheat, brussels sprouts, and soy fiber (Enloo 2000)—were sent to 13 working members for analysis. (The soybean meal, alfalfa meal, winter wheat, and soy fiber samples were also used in the collaborative study described in ref. 1.) All 13 members (including the author) reported data obtained using ceramic fiber as a filter aid. In addition, 2 laboratories reported data obtained using the Tecator unit with no filter aid, and 2 laboratories reported data obtained using sea sand as filter aid.

Crude Fiber Determination

AOAC method **7.061–7.065** (2) was used, with either Tecator or ABC Model 150 (3) filtration unit used for vacuum control, and ceramic fiber for filter aid as described in ref. 1.

Results and Discussion

Table 1 gives the data for all samples by each of the 13 laboratories. The statistical treatment of the data was made to determine the precision among the various laboratories when ceramic fiber was used as a filter aid. Laboratory 10 reported extremely high fiber values (4); those data were excluded from further consideration. Table 2 gives the mean and standard deviation for each sample for all laboratories, a mean for the study, a pooled standard deviation, and the relative standard deviation. For some samples, the standard deviation is high because some laboratories reporting difficulties in filtering. Table 3 shows the repeatability of duplicate analyses within a laboratory and the average range for the

Table 1. Determination of crude fiber (%), using ceramic fiber as filter aid[a]

	Sample							
Lab.[b]	1	2	3	4	5	6	7	8
1	1.31	3.68	27.69	0.68	8.60	11.98	10.46	12.18
	1.34	3.68	28.18	0.64	8.82	12.19	10.14	12.90
2	1.21	3.78	26.74	0.69	7.78	10.80	10.69	12.52
	1.26	3.50	26.90	0.70	7.73	11.10	9.53	12.08
3		4.10	27.70	0.60	7.60	10.90		13.60
4	1.03	4.19	29.07	0.69	8.24	10.98	11.29	13.53
	1.04	4.21	30.34	0.77	8.26	10.96	11.45	13.59
6	0.99	3.74	25.67	0.58	6.95	10.09	9.40	11.99
7	1.18	4.26	28.08	0.82	7.84	10.52	10.27	12.35
	1.02	4.27	27.30	0.76	7.70	10.12	10.20	12.55
8	1.30	3.80	26.90	0.59	8.60	11.10	9.90	12.80
	1.30	3.80	27.50	0.63	8.50	11.20	9.90	12.60
9	2.00	4.50	29.60	0.60	9.50	13.30	9.60	12.10
		4.20	30.90	0.60	9.60	12.70	9.80	12.00
10[c]	7.50	8.90	30.20	5.10	15.20	19.80	16.50	22.30
	7.30	9.00	29.60	5.30	14.20	19.70	16.00	22.00
11	1.53	3.80	27.12	0.75	8.38	10.96	10.02	12.31
	1.45	3.93	27.51	0.74	8.20	10.79	9.87	12.05
12	0.94	4.19	26.20	0.53	5.68	8.85	8.89	9.95
	0.77	3.71	26.51	0.43	5.82	9.10	8.11	9.73
13	1.38	4.03	26.60	0.85	8.38	10.70	9.96	12.59
	1.54	4.03	26.49	1.48	8.35	10.73	9.97	12.17
14	1.27	3.99	25.68	0.78	8.01	10.25	9.64	12.77
	1.02	3.67	25.72	0.68	7.56	9.56	9.56	12.84

[a]All values on dry matter basis.
[b]Lab. 3 reported no data on samples 1 and 7, and single results on the remaining samples. Lab. 6 reported results of single determinations on all samples. Lab. 9 reported 1 value on sample 1; 2 values on all remaining samples.
[c]Data eliminated from statistical analysis.

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Table 2. Statistical evaluation of data on determination of crude fiber, using ceramic fiber as filter aid

Sample	Mean,[a] %	Std dev.
1	1.24	0.27
2	3.97	0.26
3	27.47	1.43
4	0.67	0.10
5	8.00	0.95
6	10.86	1.05
7	9.94	0.72
8	12.33	0.95
Mean	9.31	
Pooled std dev.		0.72
Rel. std dev., %		7.69

[a]Each value is average of data reported in Table 1, excluding Lab. 10 (from 20 to 22 determinations per sample).

Table 3. Precision of intralaboratory analyses

Lab.	Av. range[a]
1	0.25
2	0.31
4	0.20
7	0.23
8	0.13
9	0.32
11	0.17
12	0.31
13	0.10
14	0.24
Av.	0.23

[a]Each value is average of range between duplicate determinations on 8 samples.

study. Table 4 shows the reproducibility, or the maximum spread, among laboratories for each sample and shows the average spread for the study. A statistical summary shows that S_r (random error) or the precision standard deviation, 0.27, and S_b (bias) standard deviation (4) of the systematic error, 0.67, are satisfactory values for the study. Table 5, a comparison of data of 4 samples that were used in 2 different studies, indicates good correlation between studies. Table 6 shows results for use of the Tecator instrument without a filter aid; Table 7 shows results obtained using sea sand as a filter aid.

Table 4. Precision of interlaboratory analyses (%)

Sample	Low	High	Max.[a] spread
1	0.77	2.00	1.23
2	3.50	4.50	1.00
3	25.67	30.90	5.23
4	0.43	0.85	0.42
5	5.68	9.60	3.92
6	8.85	13.30	4.45
7	8.11	11.45	3.34
8	9.73	13.60	3.87
Av.			2.93

[a]Each value is spread between extremes of data reported in Table 1, excluding Lab. 10.

Table 5. Comparison of data on 4 samples used in 1981 AOAC collaborative study[a] and in the ISO study

	ISO		AOAC[b]	
Sample	Mean, %	Std dev.	Mean, %	Std dev.
2	3.97	0.26	4.03	0.29
3	27.47	1.43	27.70	1.41
6	10.86	1.05	10.90	0.48
8	12.33	0.95	12.39	0.87

[a]Ref. 1.
[b]Eleven labs did 2 independent analyses on each sample.

Table 6. Results for crude fiber determinations by 2 laboratories using Tecator filtration unit without filter aid

Sample	Mean, %	Std dev.
1	1.90	0.41
2	4.28	0.43
3	28.20	0.53
4	0.77	0.13
5	8.35	0.30
6	11.61	0.59
7	10.48	0.48
8	12.87	0.60
Av.	9.81	

Table 7. Results for crude fiber determinations by 2 laboratories, using sea sand (EEC official method) as filter aid

Sample	Mean, %	Std dev.
1	0.99	0.09
2	3.49	0.36
3	26.35	1.19
4	0.64	0.14
5	7.06	0.32
6	10.29	1.08
7	9.75	0.29
8	11.50	0.56
Av.	8.76	

Recommendation

On the basis of data for this study and the comparative data on the 4 samples used in the previous collaborative study, the accuracy and precision of the method with ceramic fiber are equivalent to those using asbestos or sea sand as a filter aid. Asbestos should be eliminated completely from the laboratory because of its carcinogenic nature. Sea sand does not seem to be an effective filter aid because small sand particles (pebbles) are used to separate large particles, and in a sand mat there is no lattice work as with the ceramic fiber or even asbestos fiber. Ceramic fiber would best serve the purpose as a universal filter aid at this time.

One laboratory reported a matting problem with the ceramic fiber. This problem could probably be overcome by using more care in preparing the ceramic fiber.

Two laboratories reported slow or difficult filtering, likely caused by improper vacuum control. The ability to have precise control of the vacuum and the facility for keeping sample and filtrate hot while filtering are just as essential, if not more essential, than a filter aid for accurate and precise analyses.

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I. Olah, Hungarian Office for Standardization, Budapest, Hungary

I. Pichl, Central Checking and Testing, Institute of Agriculture, Prague, Czechoslovakia

S. E. Rayner, U.S. Dept of Agriculture, Fruit and Vegetable Division, Washington, DC

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GENERAL REFEREE REPORTS: COMMITTEE A

Report on Feeds

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One collaborative study on the use of copper catalyst in protein analysis was completed this year. Two additional collaborative studies sustained some work this year.

Amino Acids in Mixed Feeds.—Associate Referee Wayne Stockland has initiated a preliminary collaborative study for amino acids in feeds by using amino acid analyzers. Depending on the outcome, he plans next to study liquid chromatographic (LC) methods for specific amino acids and a broad spectrum LC method.

Fiber, Crude, in Milk Replacers.—Associate Referee Jim G. Pierce is continuing investigation of acceptable methods.

Fat, Crude, in Pet Foods.—Pet food companies continue to ask for interpretation of the directions for the method for fat, but other than interpretation of instructions, no problems have been encountered this year.

Fiber, Crude.—Associate Referee David O. Holst continues to report on the topic. One comment this year was that new ceramic fiber needs to be dried before use.

Iodine in Feeds.—Stuart Meridian initiated a study, but it was not completed due to complications not associated with the study. A new study will be started.

Infrared Reflectance Techniques in Mixed Feeds.—There is still interest in the use of infrared analysis methods in feeds, and an Associate Referee is needed.

Minerals.—No action this year, but this is an area in which an Associate Referee is needed.

Non-Nutritive Residues in Feeds.—Associate Referee Peter J. Van Soest continues to be active in field, but no collaborative studies are reported this year.

Protein, Crude.—An excellent collaborative study was completed this year by Associate Referee Peter Kane. The copper catalyst gave comparable results to mercury if certain critical conditions were observed.

Sampling.—Associate Referee Val Midkiff retired this year. A report, summerizing his observations, would be helpful for a new Associate Referee.

Water (Karl Fischer Method).—The new Associate Referee, Raffaele Bernetti, hopes to initiate a collaborative study soon.

Enzymes in Feeds.—It has been requested that AOAC appoint an Associate Referee for enzymes in feeds. The field is so diverse, that first it is necessary to determine what areas an Associate Referee would cover and toward what purpose.

Recommendations

(1) Delete the topic Fat, Crude, in Pet Foods.

(2) Adopt as official first action the copper catalyst method for protein in feeds.

(3) Determine the need for and the scope of the topic Enzymes in Feed.

(4) Appoint Associate Referees for: Minerals, Infrared Reflectance Methods, and Sampling.

(5) Continue study on all other topics.

Presented at the 97th Annual International Meeting of the AOAC, Oct. 3–6, 1983, at Washington, DC.

The recommendations of the General Referee were approved by Committee A and adopted by the Association. See the report of the committee (and "Changes in Methods"), this issue.

Report on Fertilizers and Agricultural Liming Materials

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No reports were received from Associate Referees on Biuret in Urea and Mixed Fertilizers, Boron, Free and Total Water, Nitrogen, Potash, Slow-Release Mixed Fertilizers, Sodium, Soil and Plant Amendment Ingredients, and Sulfur.

Iron.—Associate Referee James R. Silkey has reviewed the first action method (**2.D16–2.D18**) adopted in 1982 for iron chelates as it applies to iron *N,N*-bis-(2-hydroxy-5-sulfobenzyl) glycine (DPS). Collaborative results (*J. Assoc. Off. Anal. Chem.* **66,** 952–955 (1983)) indicated greater variability for this method with FeDPS than with other chelates included in the study. The current investigation has resulted in a modification believed to improve precision for FeDPS. This modification will be incorporated in a collaborative study in 1984.

Molybdenum.—There has been no activity or need expressed over the past decade; it is recommended this subject be discontinued.

Phosphorus.—Associate Referee Frank J. Johnson has been extremely active leading a working group in defense of the AOAC method for available phosphorus before an entity of the International Standardization Organization, Working Group 2, Subcommittee 4 of Technical Committee 134. No report has been submitted requiring action by the General Referee.

Sampling and Preparation.—Douglas Caine, Associate Referee. An investigation of some complexity has been designed for study of sampling variables inherent in the AOAC sampling method of bagged blended fertilizers. This study is a joint endeavor with the fertilizer industry and fertilizer regulatory officials. It is currently underway and is expected to be completed in 1984.

Water-Soluble Methylene Ureas.—The LC method adopted first action in 1982 has been subjected to a second collaborative study involving 6 laboratories and 5 matched sample pairs. Results reported by Associate Referee Allen D. Davidson are comparable with those of the first study and he recommends adoption of the method as final action. The General Referee concurs.

Zinc.—A new Associate Referee has been appointed to this topic and will pursue a collaborative study with atomic absorption spectrophotometry for 1984.

Recommendations

(1) Continue official first action status of the spectrophotometric method for boron, **2.C01–2.C04**; continue study.

(2) Continue official first action status for chelated iron by the atomic absorption spectrophotometric method, **2.D16–2.D18**; continue study.

(3) Discontinue the topic Molybdenum.

(4) Continue official first action status of determination of K_2O by the flame photometric method, **2.D06–2.D15**; continue study.

(5) Continue official first action status of the water elution method for slow-release mixed fertilizers, **2.073–2.074**; continue study.

(6) Continue official first action status of the flame photometric method, **2.147–2.150**, and the atomic absorption spectrophotometric method, **2.019–2.022** for sodium; continue study.

(7) Continue official first action status for aluminum by the atomic absorption spectrophotometry method, **2.D23–2.D25**; continue study.

(8) Continue official first action status of the gravimetric method for total and elemental sulfur, **2.A01**; continue study.

(9) Adopt as official final action the LC method for water-soluble methylene ureas, **2.D01–2.D05**; continue study.

(10) Continue study on all other topics.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition, and "Changes in Methods" *J. Assoc. Off. Anal. Chem.* **63,** 374–423 (1980); **64,** 501–540 (1981); **65,** 450–521 (1982); **66,** 512–564 (1983).

This report of the General Referee was presented at the 97th Annual International Meeting of the AOAC, Oct. 3–6, 1983, at Washington, DC.

The recommendations of the General Referee were approved by Committee A and were adopted by the Association. See the report of the committee (and "Changes in Methods"), this issue.

Report on Pesticide Formulations: Carbamate and Substituted Urea Insecticides

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This year has been very productive for all concerned. Continuing a trend started several years earlier, all methods being considered for action are based on an internal standard liquid chromatographic (LC) procedure. The following is the present status of selected topics assigned to the Associate Referees:

Aldicarb; and Carbaryl.—William H. McDermott is working on a series of methods, both normal and reverse phase LC, directed to the analysis of these materials and combinations with other pesticides.

Aminocarb.—Stephen C. Slahck has initiated a collaborative study using an internal standard and reverse phase LC; it should be completed before the end of this year.

Bendiocarb.—Peter L. Carter is completing a collaborative study of an internal standard LC method. Initial collaborative results indicate that the method is applicable to materials in the 20–95% range with acceptable variance. Interim first action approval is anticipated.

Carbofuran; and Carbosulfan.—Edward J. Kikta, Jr, has completed a collaborative study of an internal standard, reverse phase LC method. Interim first action approval of the method is anticipated. A collaborative study for carbosulfan is contemplated for the new year.

Methiocarb.—Stephen C. Slahck has completed a collaborative study on an internal standard LC method and has recommended that the method be adopted official first action.

Methomyl.—James E. Conaway, Jr, has completed a collaborative study of a reverse phase, internal standard LC method and recommends the method be adopted official first action.

Oxamyl.—Glenn A. Sherwood, Jr, has completed a collaborative study of a reverse phase, internal standard LC method and recommends the method be adopted official first action.

Pirimicarb.—John E. Bagness, newly appointed Associate Referee, has subjected the interim AOAC–CIPAC first action method to a limited collaborative trial. The Substituted Pyrimidine Panel recommended that the method be accepted for analysis of pirimicarb technical materials, wettable powders, and water dispersible granules containing not less than 50% active ingredient.

Propoxur.—Stephen C. Slahck has completed a collaborative study and recommends that the LC method, using an internal standard, be considered for official first action status.

Recommendations

(1) Adopt as official first action the GC method for pirimicarb, **6.C04–6.C07**.

(2) Adopt as official first action LC methods for methiocarb, methomyl, oxamyl, and propoxur.

(3) Continued study on all other topics.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition, and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **63**, 374–423 (1980); **64**, 501–540 (1981); **65**, 450–521 (1982); **66**, 512–564 (1983).

This report of the General Referee was presented at the 97th Annual International Meeting of the AOAC, Oct. 3–6, 1983, at Washington, DC.

The recommendations of the General Referee were approved by Committee A and were adopted by the Association, except recommendations for methomyl and oxamyl. See the report of the committee (and "Changes in Methods"), this issue.

Report on Pesticide Formulations: Fungicides and Disinfectants

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Two new Associate Referees have been appointed in this section. Steve Slahck has been appointed to the recently created topic of Bayleton. Also, Wyatt Teubert has been appointed to the topic of Benomyl.

The following is a status report of selected topics within this section.

Bayleton.—New Associate Referee Steve Slahck reports that an in-house method will be collaborated in the near future.

Benomyl.—New Associate Referee Wyatt Teubert has reported the results of a collaborative study on this compound, with his recommendation to adopt the method as official first action.

Captan.—Aner Carlstrom reports no activity in this area and recommends inactivating the topic. Also, he recommends surplusing the gas chromatographic method, **6.215**, for this compound.

Carboxin and Oxycarboxin.—Milton Parkins reports that an in-house study on the method of choice will take place soon. A full-scale collaborative study will follow in 1984.

Chlorothalonil.—Brian Korsch reports that changes within the company have prevented him from conducting a collaborative study. He may be able to undertake this study early in 1984.

Dithiocarbamate Fungicides.—Warren Bontoyan reports that work is being done on an LC method for these compounds in preparation for a collaborative study in the future.

Folpet.—Aner Carlstrom reports no activity in this area and recommends inactivating the topic.

Thiram.—A collaborative study is being conducted on this compound by CIPAC.

Triphenyltin.—The General Referee reports no negative feedback on the GC method for this compound.

Recommendations

(1) Adopt as official first action the LC method for benomyl described by the Associate Referee. Precision for one data pair (10% active ingredient in a mixture with another fungicide) was poorer than for other data pairs. The Associate

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition.

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Referee states in his paper that, on investigation, the samples involved (2 and 6) were not as homogeneous as originally thought. Another data pair (also including a similar mixture of fungicides) of similar percent active ingredient was included in the study. This pair (5 and 8) provides precision information indicating reliability of the method at the lower level. It is noted that the method described is not specific for benomyl, because of spontaneous degradation that occurs in solution. Therefore, percent ingredient found is interpreted as the maximum benomyl that could have been present in the sample.

(2) Adopt as official first action the interim GC method for triphenyltin.

(3) Surplus the gas chromatographic method for captan, **6.215**.

(4) Inactivate the topics Captan and Folpet.

(5) Continue study in all other areas.

Report on Pesticide Formulations: Halogenated and Other Insecticides, Synergists, and Insect Repellants

JAMES E. LAUNER
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Under these topics, 4 collaborative studies were conducted this year; however, due to time restrictions, only one report was completed.

At the 1983 Collaborative International Pesticide Analytical Council (CIPAC) meeting in Brisbane, Australia, the AOAC–CIPAC provisional method for pyrethrins and piperonyl butoxide (**6.C22–6.C25**) was adopted as a full AOAC–CIPAC method. The AOAC first action LC method (**6.D05–6.D08**) was adopted as AOAC–CIPAC provisional method.

Two Associate Referees were appointed during the past year: James W. Miles, Benzene Hexachloride; A. A. de Reyke, Diflubenzuron. New Associate Referees are needed for Methyl Bromide and Methoxychlor.

The following is the present status of selected topics assigned to Associate Referees:

Benzene Hexachloride.—J. Miles has completed a collaborative study of a GC method for the gamma isomer and recommends adoption as official first action. The General Referee concurs.

Chlordane.—J. Forrette has initiated a collaborative study of the combination of total chloride with infrared to determine chlordane and heptachlor in mixtures.

Chlordimeform.—A. Hofberg has initiated a collaborative study of a GC method.

Cypermethrin.—P. Bland plans a collaborative study of a capillary GC method.

Dicofol.—A. Rothman is re-evaluating the data from the 1982 collaborative study of the LC method.

Diflubenzuron.—A . Van Rossum recommends adoption of the official first action CIPAC–AOAC liquid chromatographic method (**6.D09–6.D15**) as official final action. The General Referee concurs.

Endosulfan.—R. Watson recommends adoption of the official first action CIPAC–AOAC gas chromatographic method (**6.D16–6.D21**) as official final action. The General Referee concurs.

Fenvalerate.—R. Collins is cooperating with CIPAC on a GC method.

Fumigants.—D. Yeaman plans a collaborative study of a GC method for sulfuryl fluoride.

Nicotine.—S. Carrigan will study an LC method.

Permethrin.—H. Morris is cooperating with CIPAC on a GC study.

Rotenone.—R. Bushway is investigating modifications to the official first action AOAC–CIPAC liquid chromatographic method (**6.D05–6.D08**).

Toxaphene.—W. Clark will initiate a study of an infrared method.

Trichlorfon.—M. Sabbann is investigating an LC method.

Recommendations

(1) Continue official first action status of the following methods: (a) GC method for technical allethrin (**6.149–6.154**); (b) radioactive tracer method for benzene hexachloride (**6.202**); (c) hydrolyzable chloride method for dicofol (**6.283–6.288**); (d) GC method for fumigants (**6.143–6.148**); (e) infrared method for rotenone (**6.163–6.164**); (f) LC method for rotenone (**6.D05–6.D08**); (g) UV method for sulfoxide (**6.419**).

(2) Adopt as official first action the CIPAC GC method for the gamma isomer of benzene hexachloride described by the Associate Referee.

(3) Adopt as official final action the following first action methods: (a) CIPAC–AOAC liquid chromatographic method for diflubenzuron (**6.D09–6.D15**); (b) CIPAC–AOAC gas chromatographic method for endosulfan (**6.D16–6.D21**).

(4) Continue study of piperonyl butoxide and pyrethrins, including low levels and mixed formulations.

(5) Continue movement toward the goal of deletion of total halide methods as rapidly as possible.

(6) Continue study on all other topics.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition, and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **63**, 374–423 (1980); **64**, 501–540 (1981); **65**, 450–521 (1982); **66**, 512–564 (1983).

This report of the General Referee was presented at the 97th Annual International Meeting of the AOAC, Oct. 3–6, 1983, at Washington, DC.

The recommendations of the General Referee were approved by Committee A and were adopted by the Association. See the report of the committee (and "Changes in Methods"), this issue.

Report on Pesticide Formulations: Herbicides I; Other Organophosphate Insecticides; Rodenticides and Miscellaneous Pesticides

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Efforts put forth by the Associate Referees during the past year have resulted in one method revision, a report of one collaborative study, and 3 studies planned for the coming year. The following is a summation of activities this past year.

Herbicides I

Chlorophenoxy Herbicides.—Robert B. Grorud and John E. Forrette report on the collaborative study of liquid chromatography of liquid formulations containing, 2,4-dichlorophenoxyacetic acid, dicamba, and 2-(2-methyl-4-chlorophenoxy) propionic acids as their salts.

Dicamba.—See above comment.

Pentachlorophenol.—Elmer H. Hayes plans a collaborative study this year.

Plant Growth Regulators.—An Associate Referee is needed.

This report of the General Referee was presented at the 97th Annual International Meeting of the AOAC, Oct. 3–6, 1983, at Washington, DC.

The recommendations of the General Referee were approved by Committee A and were adopted by the Association. See the report of the committee (and "Changes in Methods"), this issue.

Other Organophosphate Insecticides

Crotoxyphos.—Wendy King plans a study for 1984.

Naled.—A. A. Carlstrom is planning a study for next year.

Dichlorvos, Mevinphos, Monocroptophos, Tetrachlorvinphos.—Associate Referees are needed on these topics.

Rodenticides and Miscellaneous Pesticides

Brodifacoum.—A method was adopted at the last meeting for all formulations except wax block formulations. A collaborative study is planned this year by Peter Bland to include the preparation of the wax block for analysis by the current AOAC method.

Diphacinone.—An Associate Referee is needed.

Warfarin.—Elmer H. Hayes reports on a method revision.

Recommendations

(1) Adopt as official first action the LC method for 2,4-D, dicamba, and MCPP.

(2) Continue study on all other topics.

Report on Pesticide Formulations: Herbicides II

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Associate referees are having difficulty finding time to carry out method development or collaborative study. After contacting the Associate Referees individually, it sounds like 1984 will be a more productive year. Glenn Sherwood is proposing an LC method for diuron, linuron, and siduron; William Ja is proposing an LC method also for bensulide; and Roger Stringham is preparing a collaborative study for benefin, fluchloralin, profluralin, trifluralin, and pendimethalin.

The General Referee is still seeking Associate Referees for benzoylprop-ethyl, dinoseb, *S*-ethyl dipropylthiocarbamate, monuron, and oryzalin.

This report of the General Referee was presented at the 97th Annual International Meeting of the AOAC, Oct. 3–6, 1983, at Washington, DC.

The recommendations of the General Referee were approved by Committee A and were accepted by the Association. See the report of the committee (and "Changes in Methods"), this issue.

Recommendations

Discontinue study for chloroxuron; use existing AOAC method. Continue study on all other topics.

Report on Pesticide Formulations: Herbicides III

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Four new Associate Referees have been appointed to this section during the year. Several of these have indicated that they will be conducting collaborative studies soon. One of the new Associate Referees, Peter Bland, in fact, has completed a collaborative study on fluazifop-butyl, and has submitted results to the Association. This compound has recently been added to the section by the Committee. In addition, William R. Betker has completed a collaborative study on metribuzin and has also submitted results to the Association.

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

Alachlor, Butachior, and Propachlor.—New Associate Referee David F. Tomkins reports that in-house work is complete, and a collaborative study will be undertaken this year on alachlor.

Amitrol.—A call for an Associate Referee for this compound was placed in the June 1983 issue of "The Referee."

Bentazon.—New Associate Referee Thomas M. Schmitt reports that an in-house method for this compound will be collaborated this year.

Bromoxynil Octanoate.—Laurence Helfant reports no change in methodology for this compound. However, he reports work will be undertaken on another formulation of bromoxynil in the future.

Cacodylic Acid.—A call for an Associate Referee for this compound was placed in the June 1983 issue of "The Referee."

Cyanazine.—New Associate Referee Ron Collins reports that an in-house method will be collaborated this year.

Dalapon.—Tim Stevens recommends that the LC method for this compound be adopted official first action.

Dichlobenil.—A call for an Associate Referee for this compound was placed in the June 1983 issue of "The Referee."

Fluazifop-butyl.—New Associate Referee Peter Bland has reported the results of a recently completed collaborative study, with his recommendation to adopt the method official first action.

Glyphosate.—Arnold J. Burns reports no negative feedback on the collaboratively studied method for glyphosate. He therefore recommends that the method be adopted official final action.

Metolachlor.—Arthur Hofberg reports that a collaborative study will be completed in the near future.

Metribuzin.—William Betker has reported the results of a recently completed collaborative study with his recommendation to adopt the method official first action.

Propanil.—Delmas Pennington reports that a collaborative study will be completed in the near future.

s-Triazine Herbicides.—Arthur Hofberg reports that the study on internal standards for these compounds will take place in the near future.

Recommendations:

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

(2) Adopt as official first action the GC method for fluazifop-butyl described by the Associate Referee. It should be noted that the data included in the statistical analysis of the collaborative study produced good precision. Some laboratories however, were eliminated before the statistical summary was made. Reasons included both equipment malfunctions and the inability to achieve required separation. The proposed method did specify use of a certain column and also specified the complete separation needed to be achieved before attempting the method.

(3) Adopt as official final action the official first action LC method for glyphosate.

(4) Adopt as official first action the GC method for metribuzin described by the Associate Referee.

(5) Continue study in all other areas.

This report of the General Referee was presented at the 97th Annual International Meeting of the AOAC, Oct. 3–6, 1983, at Washington, DC.

The recommendations of the General Referee were approved by Committee A and were adopted by the Association. See the report of the committee (and "Changes in Methods"), this issue.

Report on Pesticide Formulations: Organothiophosphate Pesticides

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One new Associate Referee was appointed during the year and another change is eminent due to a shift in job status. Finding time to do the work expected of an Associate Referee is a continuing problem for many of our pesticide chemists.

The following is a summation of activities and recommendations of the various Associate Referees:

Acephate.—A. Aner Carlstrom completed a GC study but found excessive variation in the reported results. He recommends that the method not be made official but that study be continued to improve its performance.

Coumaphos.—Linda Ruiz has a reverse phase LC method which she plans to submit to collaborative study later this year.

Diazinon.—Associate Referee A. H. Hofberg plans to study a modification of the official first action GC method for diazinon whenever time permits.

Dimethoate.—Richard S. Wayne has forwarded an LC method to the General Referee for study, preliminary to a full-scale collaborative effort.

Dioxathion.—W. H. Clark is developing an LC method for this pesticide.

EPN.—Associate Referee J. E. Forrette is continuing the study of both an LC and GC method.

Ethoprop.—Wallace Embry is unable to continue as Associate Referee. He hopes to be able to recommend a successor from among his associates.

Fenitrothion.—Associate Referee James W. Miles has received most of the reports from collaborators involved in a study of a GC method. He will evaluate these data and make a recommendation during 1984.

Fensulfothion.—The LC method for this pesticide is official first action. Margie Owen recommends that the method be adopted official final action.

Fenthion.—Study by Associate Referee W. G. Boyd, Jr, indicates that LC is the method of choice for this pesticide. Following ruggedness testing, he plans to submit the method to collaborative study in 1984.

Fonofos.—William Y. Ja has accepted appointment as Associate Referee for this pesticide. He is evaluating current methodology in this area.

Methidathion.—Tom Gale is working with a reverse phase LC method and feels that it will be ready to study collaboratively in 1984.

Parathion and Methyl Parathion.—Later this year Association Referee E. R. Jackson will conduct a collaborative study of the official first action GC method for parathion to include wettable powders.

Phorate.—Roman Grypa is planning a collaborative study of a GC method.

No further work has been done on Azinphosmethyl, Chlorpyrifos, Demeton, Demeton-*S*-Methyl, Encapsulated Organophosphorus Pesticides, Malathion, Oxydemeton, and Sulprofos.

Recommendations

(1) Adopt as official final action the official first action method for fensulfothion.

(2) Discontinue the topic chlorpyrifos; the LC method is official final action and no further work is anticipated.

(3) Continue study on a GC method for acephate and on other indicated topics.

This report of the General Referee was presented at the 97th Annual International Meeting of the AOAC, Oct. 3–6, 1983, at Washington, DC.

The recommendations of the General Referee were approved by Committee A and were adopted accepted by the Association. See the report of the committee (and "Changes in Methods"), this issue.

Report on Plants

J. BENTON JONES
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NBS may undertake the development of vegetation samples certified for fluorine so that the questions of accuracy can be addressed for the current official first action status of the potential method (**3.077–3.082**), and the semiautomated method (**3.083–3.095**) can be more fully evaluated.

A collaborative study on the determination of mineral elements in plant tissue by plasma emission spectroscopy has been completed and interim official first action is being recommended. This procedure will be an addition to the current AOAC procedures using atomic absorption (**3.006–3.009**) and spark emission spectroscopy (**49.001–49.007**).

A collaborative study for the determination of sulfur in plant tissue is being initiated, using the automated turbidimetric method for total sulfur. A call for collaborators has been issued.

It has been recommended that a collaborative study be initiated to investigate the azomethine-H colorimetric procedure for boron determination in plant tissue. This same procedure has been recently approved for first action in the determination of boron in fertilizers.

Rose A. Sweeney has been appointed Co-Associate Referee for Sulfur, replacing Larry Wall.

Oscar E. Olsen has requested that he be relieved of his Associate Refereeship, and he has recommended a possible appointee. However, the change is still pending.

Recommendation

Continue study on all topics.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition.

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The recommendation of the General Referee was approved by Committee A and was accepted by the Association. See the report of the committee (and "Changes in Methods"), this issue.

Report on Reference Materials and Standard Solutions

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Certified Reference Materials (CRMs) are homogeneous materials used in determining the accuracy of methods, calibrating instruments, and validating and referring data to a common base. CRMs are issued by the certifying organization with certificates or other documentation in which are listed values of the certified concentrations or properties.

AOAC-related CRM activities of several organizations are noted below:

Community Bureau of Reference (BCR), Commission of the European Communities, 200 rue de la Loi, B-1049 Brussels, Belgium, issues a "Catalog of BCR Reference Materials." For each reference material, the certified value is based on results of measurements by a number of laboratories applying different current state-of-the art, accurate methods. CRMs have recently been issued for use in reliably determining trace concentrations of heavy metals in environmental samples. These include *Lagarosiphon major* (aquatic plant) CRM 060, *Platihypnidum riparoides* (aquatic plant) CRM 061, and *Olea europaea* (olive leaves). The certified elements include cadmium, copper, mercury, manganese, lead, and zinc.

The International Atomic Energy Agency, Laboratory Seibersdorf, PO Box 100, A-1400 Vienna, Austria, issues 4 CRMs applicable to AOAC activities. Muscle (H-4) is certified for 14 elements; Soil (Soil-5) is certified for 33 trace elements; Lake Sediment (SL-1) is certified for 28 trace elements; and Simulated Deposition on Air Filter (Air-3/1) is certified for 13 trace elements.

CRMs issued by the *National Bureau of Standards (NBS)* are known as Standard Reference Materials (SRMs). Recent developments in SRMs applicable to AOAC activities are listed below.

Biological, Botanical, Foods

(1) Citrus Leaves, SRM 1572, has been analyzed for vanadium and thorium, using high accuracy stable isotope dilution mass spectrometric methods. The concentrations tentatively certified as V 0.245 ± 0.018 μg/g, and Th 0.015 ± 0.005 μg/g, will appear in a revised Certificate of Analysis. In addition, information on the iodine-129 content of this material will be added.

(2) Non-Fat Milk Powder, SRM 1549, is being analyzed for issue in early 1984. The Certificate will provide concentrations of nutritionally and toxicologically significant elements. These will include iodine, selenium, sodium, and zinc.

Clinical

(1) Human Serum, SRM 909, has been analyzed for sodium using an ion-exchange separation, gravimetric method. The concentration, confirmed by atomic absorption spectrometry, has been certified and is included in the revised Certificate of Analysis dated July 13, 1983. The Certificate now lists certified concentrations for 10 constituents (calcium, chloride, cholesterol, glucose, lithium, magnesium, potassium, sodium, urea, and uric acid). The Certificate also includes a method-specific value for total protein and uncertified catalytic concentrations of 7 enzymes. NBS Special Publication 260-83, describing the methods used for the enzyme determinations and details of the interlaboratory study, is available from the General Referee on request.

(2) Tripalmitin, SRM 1595, has been issued as a chemical of known purity. The certified concentration of tripalmitin is 99.5 ± 0.2 weight percent. Liquid chromatography (LC) and

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nuclear magnetic resonance methods were used for the certification. A glyceride, which could not be identified further, is the major impurity. This SRM was developed for use primarily in the calibration and standardization of procedures for the chemical analysis of serum for triglycerides.

(3) Freeze-Dried Urine, SRM 2670, is expected to be issued in early 1984. Approximately 13 elements including arsenic, cadmium, and lead will be certified depending on the agreement of the results by independent analytical methods.

Environmental

(1) Halocarbons (in methanol) for Water Analyses, SRM 1639, has been issued. The Certificate of Analysis lists the certified concentrations of 7 priority pollutant halocarbons present at ng/μL levels in methanol. The compounds are chloroform, chlorodibromomethane, bromodichloromethane, bromoform, carbon tetrachloride, trichloroethylene, and tetrachloroethylene.

SRM 1639 was developed primarily for adding known accurate amounts of the certified halocarbons to water and other samples. It is also intended for use in calibrating chromatographic instrumentation and determining response factors.

Concentrations of the 7 volatile compounds were determined by gas chromatography (GC). The certified concentrations are based on the GC analytical results and on the concentrations calculated from the mass of the halocarbon (corrected for compound purity) added to the methanol. In addition to GC, LC was used to confirm the homogeneity of the entire lot.

The certificate also provides a suggested procedure for preparing water calibration standards in the range normally found in drinking water supplies.

(2) Petroleum Crude Oil, SRM 1582, has been analyzed for organic constituents of environmental interest and is being issued in 1984. Expected to be certified as a result of concordant data by 2 independent analytical methods are phenanthrene, fluoranthene, pyrene, benz(a)anthracene, perylene, benzo(a)pyrene, and dibenzothiophene. Use of this new SRM and Organics in Shale Oil (SRM 1580), issued in 1980, will provide researchers with 2 calibration points for the determination of compounds certified in both materials. For example, the concentrations (in μg/mL) of 3 of these compounds in SRM 1582 and SRM 1580 are fluoranthene 2.5 and 54, respectively; pyrene 7.1 and 104, respectively; and benzo(a)pyrene 1.2 and 21, respectively. Based on a single analytical method and therefore listed in the certificate as information, values will only be the concentrations of benzo(e)pyrene, benzo(ghi)perylene, carbazole, indeno(1,2,3-cd)pyrene, phenol, and *o*-cresol. Except for phenol and *o*-cresol, which were determined by LC, the compounds were determined by gas chromatograph-mass spectrometry.

(3) Chrysotile Asbestoes Fibers, SRM 1876, has been issued for use in evaluating techniques used to identify and count these fibers in filter samples by transmission electron microscopy (TEM). This SRM should also be useful in evaluating the preparation of samples for the measurement of asbestos in air as described by the U.S. Environmental Protection Agency in a Provisional Methodology Manual, EPA Report 600/2-77-178, 1978 Environmental Protection Technology Series (available from NTIS, Springfield, VA 22161).

The SRM consists of four 3 mm × 3 mm sections of a 0.4 μm pore size polycarbonate filter containing chrysotile fibers mixed with an urban dust (Urban Air Particulate, SRM 1648). TEM copper 200 mesh grids are also included for use in sample preparation. The fiber loading on the SRM filter is 30 ± 9 fibers/0.01 sq. mm of filter area.

(4) Chlorinated Pesticides in Isooctane, SRM 1583, has been prepared and analyzed, and was expected to be issued late in 1983. The concentrations of 6 pesticides present at the 1 μg/mL level in the isooctane will be certified. They are δ-BHC, λ-BHC (lindane), *p,p'*-DDE, *p,p'*-DDT, aldrin, and heptachlor epoxide. The concentrations were calculated from the known masses of the compounds, corrected for compound purity, dissolved in a known volume of isooctane. GC was used to confirm the calculated concentrations.

(5) Phenols in Methanol, SRM 1584, is an ampouled solution of 11 priority pollutant phenols ranging in concentration from 15 to 65 μg/mL. The SRM should be useful for calibrating gas and liquid chromatographic instrumentation and for adding known amounts of the certified compounds to water and other samples. The SRM is expected to be issued in early 1984.

(6) Chlorinated Biphenyls in Isooctane, SRM 1585, has been ampouled and is being analyzed. Eight compounds at μg/mL levels, are present in the SRM—7 polychlorinated biphenyl (PCB) congeners and a monochlorobiphenyl compound. This SRM can assist investigators in confirming the presence of incidentally generated PCBs or commercially produced PCB mixtures (Aroclors) in environmental samples. It can also be used to add known accurate amounts of these congeners to a sample.

(7) Isotopically labeled and unlabeled priority pollutants in methanol, SRM 1586, has been prepared especially to assist chemists analyzing water samples for the certified organic compounds. The SRM consists of 2 ampouled solutions. One solution contains 10 compounds isotopically labeled with either deuterium or carbon-13, while the second contains the same compounds without labels. The compounds are distributed among the EPA compound classes: acid extractable, base/neutrals, and purgeables/volatiles.

(8) Respirable Quartz, SRM 1878, consists of quartz powder in the respirable size range. It is intended for use in determining the level of alpha quartz in an industrial atmosphere by X-ray diffraction.

The Associate Referee on Stability of Organophosphorus Pesticide Standards, G. M. Doose, Food and Drug Administration, Los Angeles, CA, has stopped working on this project because of the pressure of other activities and does not intend to resume this activity.

Recommendations

(1) Continue to investigate and report sources of available reference materials, especially certified reference material, applicable for use in the development, testing, and validation of AOAC methods.

(2) Urge Associate and General Referees of all committees to inform the General Referee of this topic of their plans to conduct collaborative studies. In these studies, the use of test samples, representative of larger lots of homogeneous material, may enable the materials to be issued by NBS as SRMs, depending on the stability of analytes and matrixes.

(3) Discontinue study on stability of organophosphorus pesticide standards unless another associate referee for this activity can be found.

Report on Tobacco

RAY F. SEVERSON
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Nicotine-Gas Chromatography.—The method for the gas chromatographic determination of nicotine on Cambridge filter pads (**3.158–3.161**) has been successfully used by several laboratories for a number of years without any adverse comments. Associate Referee John R. Wagner feels that this method can now be recommended for official final action with confidence.

Differentiation of Cigar and Cigarette Tobacco.—No recommendation has been made by Associate Referee John A. Steele on this method.

Tar and Nicotine in Cigarette smoke.—(Associate Referee Harold C. Pillsbury). No further collaborative studies have been conducted on this method.

Recommendations

Adopt as official final action the determination of nicotine by gas chromatography. Continue studies on other topics.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition.

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The recommendations of the General Referee were approved by Committee A and were adopted by the Association. See the report of the committee (and "Changes in Methods"), this issue.

GENERAL REFEREE REPORTS: COMMITTEE B

Report on Drugs, Acidic

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Acetaminophen in Drug Mixtures.—David Krieger is conducting a collaborative study on the proposed LC method. Additional collaborators are needed. If you are interested in participating in the collaborative study, please contact the Associate Referee.

Allopurinol.—Donald Shostak was recently appointed Associate Referee for this topic. He completed the development of an LC method and conducted a collaborative study. The method uses reverse phase chromatography with detection at 254 nm. The collaborative study was conducted on 2 commercial samples (100 and 300 mg) and a synthetic tablet mixture formulated at the 100 mg level. Seven collaborators analyzed blind duplicates of the 3 samples for a total of 42 determinations. A complete description of the methodology and the collaborative study results were presented at the 1983 Annual International Meeting.

Amitriptyline.—Samuel Walker has completed his study. The methodology and collaborative study results presented at the 1982 Annual International Meeting were accepted interim official first action.

Aspirin, Phenacetin, and Caffeine with Other Drugs.—Douglas V. Don intends to conduct a collaborative study on the LC procedure. Collaborators are needed. If you are interested in participating in the collaborative study, please contact the Associate Referee.

Methyldopa.—Susan Ting has completed her study.

Primidone.—Stanley E. Roberts has completed his collaborative study. A statistical analysis of the data is in process.

Sulfamethoxazole.—John W. Robinson has completed his study.

Sulfisoxazole.—Robert W. Roos has completed his study. The method was accepted interim official first action.

Sulfonamides (Thin Layer Chromatography).—Charlotte A. Brunner recommends that this study be discontinued and a new study on sulfonamides by LC be initiated. Analysts interested in serving as Associate Referee for the new topic should contact the General Referee.

Other Associate Referees report little or no progress because of other commitments.

Recommendations

(1) Adopt as official first action the LC method described by the Associate Referee for the determination of methyldopa and methyldopa-thiazide combinations in dosage forms.

(2) Continue study on all other topics.

This report of the General Referee was presented at the 97th Annual International Meeting of the AOAC, Oct. 3–6, 1983, at Washington, DC.

Recommendation 2 of the General Referee was approved by Committee B and was accepted by the Association. Committee B did not approve recommendation 1. See the report of the Committee, this issue.

Report on Drugs, Alkaloids and Related Bases

EDWARD SMITH
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Aminacrine.—Associate Referee Elaine Bunch published her proposed method for the spectrophotometric determination of aminacrine HCl in creams, jellies, and suppositories (1) and her evaluation of a fluorometric determination-TLC identification of aminacrine HCl in drug preparations (2). The initiation of any collaborative study is being delayed pending the determination of whether pharmaceuticals containing aminacrine will still be manufactured or withdrawn from the market.

Atropine in Morphine and Atropine Tablets and Injections.—Associate Referee Ira J. Holcomb reported that he is continuing his investigation of chromatographic procedures for the separation and determination of the atropine and morphine content of these pharmaceuticals.

Belladonna Alkaloids.—An Associate Referee is being sought. Methodology is needed for determining hyoscyamine and atropine individually when they are present together in the pharmaceutical. The work would also involve the determination of the other belladonna alkaloids that might be present, as well as ensuring the suitability of the procedure to detect decomposition products.

Chlorpromazine.—Associate Referee Donald Smith reports that no work was done on this project.

Colchicine in Tablets.—Associate Referee Richard D. Thompson reported the initiation of the collaborative study of his proposed liquid chromatographic procedure for the determination of colchicine. The LC system resolves colchicine from 13 associated compounds, including the related alkaloids and phototransformation products of colchicine. The collaborative study will include samples of the bulk drug and dosage forms that contain colchicine alone. A subsequent study will be made on dosage forms containing colchicine in combination with probenecid. One decomposition product, colchiceine, does not chromatograph under the proposed LC conditions. Either a TLC limits test or an alternative chromatographic procedure will have to be developed for that determination.

Curare Alkaloids.—Associate Referee John R. Hohmann reported that the collaborative study of the proposed revised LC procedure was initiated. A final report will be made when all the results are evaluated.

This report of the General Referee was presented at the 97th Annual International Meeting of the AOAC, Oct. 3–6, 1983, at Washington, DC.

The recommendations of the General Referee were approved by Committee B and were adopted by the Association. See the report of the committee and "Changes in Methods," this issue.

Dicyclomine Capsules.—Associate Referee Charles Brownell reported that no further work was done on his proposed GC method for dicyclomine in capsules and other pharmaceuticals.

Epinephrine–Lidocaine Combinations.—Associate Referee Donald Smith reported that he did no work on this project. At the 1983 Annual International Meeting, R. D. Kirchhoefer and G. M. Sullivan reported the results of the analysis of epinephrine injections and lidocaine–epinephrine dental cartridges (3). Their LC procedure uses electrochemical and UV detectors to determine the active ingredients, impurities, degradation products, and the d enantiomer.

Epinephrine and Related Compounds by LC-Electrochemical Detectors.—Associate Referee John Newton reported that he made no progress on this project this year.

Ergot Alkaloids.—Associate Referee Thomas C. Knott reported that he is investigating the cause of the low recoveries obtained with the samples used in the last collaborative study. Initial results indicate that decomposition of some samples is a cause of some low results on samples stored in certain sample containers. Once the source of the low assays is confirmed and prevented, a collaborative study is planned.

Homatropine Methyl Bromide in Tablets.—Associate Referee Duane Hughes reported that he is continuing his work on separating homatropine methyl bromide from the other active ingredients in the pharmaceuticals prior to a chromatographic determinative step.

Neostigmine.—Associate Referee Rita E. Kling Jhangiani reported that no further work was done on this project.

Phenethylamine Drugs, Semiautomated Individual Unit Analysis.—Associate Referee Percy A. McCullen reported that no further work was done on his proposed semiautomated colorimetric procedure.

Pheniramine with Pyrilamine, Phenylpropanolamine, and Phenylephrine.—Associate Referee Henry S. Scroggins reported that his proposed ion-pair LC method for pheniramine in combination with phenylalkanolamines and other amines is undergoing an intralaboratory validation. A collaborative study is planned following completion of the validation.

Phenothiazines in Drugs.—Associate Referee Edward G. Lovering reported that the current collaborative study of his proposed LC procedure was aborted due to problems with reaction of the internal standard with some sample matrixes yielding double peaks. Another internal standard is being investigated and, following the completion of an intralaboratory validation, the collaborative study will be re-initiated.

Physostigmine and Its Salts.—Associate Referee Norlin W. Tymes reported that he has initiated a collaborative study of his reverse phase LC procedure (4) for the analysis of physostigmine salicylate injection. A final report will be made when all the results are received and evaluated.

Pilocarpine.—Associate Referee Irving W. Wainer reported the results of the collaborative study of the proposed method for the simultaneous determination of pilocarpine, isopilocarpine, and pilocarpic acid, utilizing an alkylphenyl column and a UV detector. Based on the results of the collaborative study, the Associate Referee recommended that this method be adopted official first action for the determination of pilocarpine in the presence of the decomposition products isopilocarpine and pilocarpic acid.

Rauwolfia Alkaloids (Reserpine and Rescinnamine).—Associate Referee Susan Barkan reported that no work was done on this topic.

Rauwolfia serpentina.—Associate Referee Ugo Cieri reported that he is continuing the investigation of the development of a quantitative LC procedure for determining the reserpine-rescinnamine content of *Rauwolfia serpentina* tablets.

TLC Identification of Phenothiazine-type Drugs.—Associate Referee Kurt Steinbrecher reported that the collaborative study of his proposed TLC procedures for 20 phenothiazines was completed. A final report is being written. The procedures are based on those that he published in 1983 (5).

Recommendations

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

(2) Continue study on all topics.

(3) Any topic that has been inactive for any extended period (more than 2 years) should be declared open.

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(1) Bunch, E. A. (1983) *J. Assoc. Off. Anal. Chem.* **66,** 140–144
(2) Bunch, E. A. (1983) *J. Assoc. Off. Anal. Chem.* **66,** 145–150
(3) Kirchhoefer, R. D., & Sullivan, G. M. (1983) 97th AOAC Annual International Meeting, Washington, DC, Oct. 3–6, Abstract No. 152
(4) Tymes, N. W. (1982) *J. Assoc. Off. Anal. Chem.* **65,** 132–137
(5) Steinbrecher, K. (1983) *J. Chromatogr.* **260,** 463–470

Report on Drugs, General

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Sampling procedures have been prepared at the request of Sidney Williams, editor of *Official Methods of Analysis*. These procedures will replace the present sections under the head "Sampling" and will appear in Chapter 36 in the 14th Edition. The new sections include sampling of bulk lots and containers of tablets and pills; capsules; ampuls; solutions and suspensions in vials; and ointments.

Progress on current topics under this refereeship is as follows:

Ampicillin and Amoxicillin.—A proposed liquid chromatographic method for pure ampicillin is under review.

Bisacodyl.—An Associate Referee has been appointed to this new topic. A paper describing a liquid chromatographic method for analysis of tablets and suppositories was presented at the 1983 Annual International Meeting. A collaborative study is planned for 1984.

Disulfiram.—A colorimetric method was adopted official final action at the 1982 meeting.

Ethylene Oxide.—A paper has been prepared describing a sensitive gas chromatographic method for residues of the sterilant gas and its breakdown products. Plans for a collaborative study have been cancelled because the volatility of the analyte makes the determination of accurate recoveries virtually impossible.

Fluoride.—A collaborative study has been completed and Associate Referee John Marzilli, Boston District of the Food and Drug Administration, has submitted a method for fluoride in sodium fluoride preparations, using an ion-specific electrode procedure. Eleven collaborators from federal, state, and industry laboratories examined 8 samples of solutions and tablets. Recovery of fluoride from authentic solutions of known composition ranged from 97.0 to 102.6%. The average recovery of 22 determinations by the 11 collaborators was 99.6%, with a relative standard deviation of ±1.9%. The analysis of 6 commercial preparations showed satisfactory precision. The procedure is straightforward and rapid. Mr. Marzilli has recommended that the method be adopted official first action. The General Referee concurs.

Halogenated Hydroxyquinoline Drugs.—An Associate Referee has been appointed to this new topic.

Insulin; Human, Porcine, and Bovine LC Assay.—An Associate Referee has been appointed to this new topic. The General Referee recommends that this title be shortened to Insulin by LC.

Medicinal Gases.—Methods have been developed for mixtures of carbon dioxide and oxygen, and for nitrous oxide. Collaborators are needed who have gas chromatographs equipped with hot-wire thermal conductivity detectors and electronic integrators.

Menadiol Sodium Diphosphate.—The Associate Referee encountered problems with the method he was planning to collaboratively study, and is investigating a new approach.

Mercurial Diuretics.—The Associate Referee is planning no further work on this topic. The General Referee recommends that this topic be discontinued.

Mercury-Containing Drugs.—A method for assaying mercury-containing drugs (**36.D01–36.D07**) was adopted official first action at the 1982 Annual International Meeting.

Metals in Bulk Drug Powders.—A collaborative study is planned for 1984.

Microchemical Tests.—An Associate Referee has been appointed to this new topic. He has developed new microcrystalline tests for phenothiazine antiemetics, and presented a paper at the 1983 Annual International Meeting. A collaborative study is now in progress.

Protein Nitrogen Units in Allergenic Extracts.—A method (**39.C01–39.C03**) was adopted official first action at the 1981 meeting.

Thyroid and Thyroxine Related Compounds.—No report was received on this topic.

Thyroid by Differential Pulse Polarography.—Associate Referee Walter Holak, FDA New York Regional Laboratory, recommends that the official first action polarographic method for the determination of iodine in thyroid, **39.C04–39.C07**, be adopted official final action. Reports on usage of this method have all been favorable. The General Referee concurs.

Recommendations

(1) Adopt new sections on sampling procedures to replace the present sections under this heading in Chapter 36.

(2) Adopt as official final action the differential pulse polarographic method for iodine in thyroid tablets, **39.C04–39.C07**.

(3) Adopt as official first action the specific ion electrode method for fluoride.

(4) Change the title of the insulin topic to "Insulin by LC."

(5) Discontinue the topics *Ethylene Oxide, Mercurial Diuretics,* and *Disulfiram.*

(6) Continue study on all other topics.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition, and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **63,** 374–423 (1980); **64,** 501–540 (1981); **65,** 450–521 (1982); **66,** 512–564 (1983).

The recommendations of the General Referee were approved by Committee B and were adopted by the Association, except in rec. 5 the Committee recommended continued study on disulfiram. See the report of the committee and "Changes in Methods," this issue.

This report of the General Referee was presented at the 97th Annual International Meeting of the AOAC, Oct. 3–6, 1983, at Washington, DC.

Report on Drugs, Illicit

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The Associate Referee for Chemical Microscopy, Richard Ruybal, has done no work in this area for at least 5 years. The topic should be declared open.

A new topic, Diazepam, was initiated during the past year. The Associate Referee for this topic is Michael Tsougros.

The Associate Referee for Heroin, Harold Hanel, assures this General Referee that a collaborative study will be instituted in the next year.

The Associate Referee for Optical Crystallographic Properties of Drugs, Robert Ferrera, states that because of a change in employment he is unable to continue this project.

No reports were received on Benzodiazepines, Cocaine, Methaqualone Hydrochloride, or Phencyclidine Hydrochloride.

Recommendations

(1) Discontinue the topic Optical Crystallographic Properties of Drugs.

(2) Declare as open the topic Chemical Microscopy.

(3) Continue study on all other topics.

This report of the General Referee was presented at the 97th Annual International Meeting of the AOAC, Oct. 3–6, 1983, at Washington, DC.

The recommendations of the General Referee were approved by Committee B and were accepted by the Association. See the report of the committee, this issue.

Report on Drugs, Neutral

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Automated Corticosteroid Methods.—The method for prednisolone and prednisone tablets (**39.052–39.058**) has been in official final action status for several years and performs well. The Associate Referee recommends that the topic be discontinued. The General Referee concurs.

Automated Methods for Progestins in Tablets.—The Associate Referee has developed a method, but the in-house results varied too widely for the method to be acceptable. He hopes to have opportunity to do followup work on improving the method. Continued study is recommended.

Digitoxin, Automated Individual Tablet Analysis.—The Associate Referee has developed a method and sent it out for collaborative study, but he was able to enlist only 4 participants. He is not sure that there are enough qualified laboratories to assure that AOAC collaborative study standards are met. If there is hope of obtaining adequate participation, continued study is recommended.

Estrogens (Fluorometric Method).—The Associate Referee has developed an LC method and a manuscript is in press. The method involves preparing dansyl derivatives of conjugated estrogens and monitoring the column effluent by fluorometry. A collaborative study is planned for the coming year.

The Associate Referee feels that a change of the topic title to *Conjugated Estrogens, LC Methods* would better describe the project and is in order. The General Referee concurs.

Ethinyl Estradiol, Automated Individual Tablet Analysis.—The Associate Referee worked on this topic, but was unable to obtain satisfactory results. Since then, he has been assigned to entirely different work and can no longer work on this project. There does not seem to be a great need for the method; discontinuation of the topic is recommended.

Hydrocortisone.—The Associate Referee presented her report of the collaborative study at the 1982 Annual International Meeting. The method received interim approval. Adoption as official first action and continued study are recommended.

Steroid Acetates.—The Associate Referee developed and published (*J. Assoc. Off. Anal. Chem.* (1980) **63,** 1184–1188) a method that included the analysis of dosage forms. However, he has not had the opportunity to conduct a collaborative study and does not expect to be able to do so. It is recommended that another Associate Referee be appointed and the work continue.

Steroid Phosphates.—The Associate Referee has been reassigned to an entirely different type of work. He was not able to make any reportable progress, and has resigned his refereeship.

The present official method for dexamethasone phosphate (**39.047–39.051**) involves enzymatic degradation of the molecule. It needs to be replaced, and LC procedures have been published. The Referee recommends that a new Associate Referee be appointed and that study continue.

Recommendations

(1) *Automated Corticosteroid Methods.*—Discontinue topic.

(2) *Automated Methods for Progestins in Tablets.*—Continue study.

(3) *Digitoxin, Automated Individual Tablet Analysis.*—Continue study.

(4) *Estrogens (Fluorometric Method).*—Change title to *Conjugated Estrogens, LC Methods.* Continue study.

(5) *Ethinyl Estradiol, Automated Individual Tablet Analysis.*—Discontinue topic.

(6) *Hydrocortisone.*—Adopt the interim first action method as official first action. Continue study.

(7) *Steroid Acetates.*—Appoint a new Associate Referee and continue study.

(8) *Steroid Phosphates.*—Appoint a new Associate Referee and continue study.

This report of the General Referee was presented at the 97th Annual International Meeting of the AOAC, Oct. 3–6, 1983, at Washington, DC.

The recommendations of the General Referee were approved by Committee B and were adopted by the Association. See the report of the committee and "Changes in Methods," this issue.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition, and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **63,** 374–423 (1980); **64,** 501–540 (1981); **65,** 450–521 (1982); **66,** 512–564 (1983).

GENERAL REFEREE REPORTS: COMMITTEE C

Report on Coffee and Tea

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The Associate Referee on Ash in Instant Tea, Francis J. Farrell, has continued his work on a combined study of ash and moisture in instant tea. He is now obtaining collaborators. At the meeting in June 1983 of ISO/TC 34/SC 8 Tea in Balatonfured, Hungary, the United States offered to organize the ring test to establish on an international basis the repeatability and reproducibility of these methods. The Associate Referee is awaiting the names of the international participants.

ISO also adopted a draft resolution calling for interested countries to participate in a study of the repeatability and reproducibility of the method for water extract in tea. Dr. Farrell will arrange for the study in the United States. Other interested countries include France, Kenya, Malawi, Netherlands, Sri Lanka, United Kingdom, and the E.T.C.

The Associate Referee for Coffee and Tea, W.P. Clinton, whose method for mass loss determination on drying for instant coffee was recommended for final action adoption last year, reports that the method has been accepted by ISO/TC 34/SC 15.

The Associate Referee on Solvent Residues in Decaffeinated Coffee, Denis Page, is setting up a collaborative study for both coffee and tea.

We still lack an Associate Referee on Crude Fiber in Tea.

The Associate Referee on Water Extract in Tea, E. DeLaTeja, has submitted his method for publication.

John Newton, Associate Referee on Caffein in Coffee and Tea and Theophyllin in Tea, reports that he has developed a method for theophyllin and is ready to test it collaboratively.

Recommendation

It is recommended that work on all topics be continued and an Associate Referee for Crude Fiber in Tea be appointed.

This report of the General Referee was presented at the 97th Annual International Meeting of the AOAC, Oct 3–6, 1983, at Washington, DC.

The recommendations of the General Referee were approved by Committee C and were accepted by the Association. See the report of the committee (and "Changes in Methods"), this issue.

Report on Dairy Products

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The Referee has received 2 reports from Associate Referees recommending official action at this year's meeting. Dick Kleyn reports on the collaborative study of an enzymatic UV method for measuring lactose in milk. The Associate Referee recommends the method be adopted official first action as an alternative method for determining lactose in whole milk, low-fat milk, and skim milk. The Referee concurs with this recommendation.

Associate Referee D. A. Biggs reports on the collaborative study of the infrared analysis of milk with the Infra-analyzer 400D. The study demonstrates that the Infra-analyzer 400D can produce analytical results for fat, whole solids, and protein that meet AOAC specifications for IR analysis of milk, provided that suitable precautions are taken to monitor instrument performance and maintain calibration for the types of milk to be analyzed. The Associate Referee recommends that AOAC include in the official method for infrared analysis of milk the use of the Infra-analyzer 400D with the addition of appropriate sections as indicated in his report. The analysis of milk from individual cows was not included in the collaborative study and therefore is not included in the recommendation. The Referee concurs with the recommendation of the Associate Referee.

Progress continues to be made in the joint IDF/AOAC groups working on methods of analysis for milk and milk products. Collaborative studies have been completed on several topics and manuscripts are being prepared. It is anticipated that several recommendations will be received by the Referee during the forthcoming year.

At last year's meeting, the official final action for determination of chloride in cheese, **16.242–16.243,** was repealed official first action and a potentiometric method was adopted official first action. The Referee recommends the association adopt as official final action the official first action potentiometric method for determination of chloride in cheese **(16.D01–16.D04)** and further, that the present official final action method, **16.242–16.243,** be deleted official final action.

A question has arisen about the status of the Milco Tester Minor. The Associate Referee, W. F. Shipe, in his report at the 1979 annual meeting stated that the Milco Tester Minor conformed to the AOAC-recommended performance specifications for measuring milk fats by turbidimetric means in unhomogenized milks. The Referee concurred with the recommendation. Therefore, although it was not specifically stated in the report, the association did adopt the Milco Tester Minor for measuring milk fat in unhomogenized milks.

The Associate Referee, G. K. Murthy, has recommended that 3 methods currently having official first action status be

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This report of the General Referee was presented at the 97th Annual International Meeting of the AOAC, Oct. 3–6, 1983, at Washington, DC.

The recommendations of the General Referee were approved by Committee C and were adopted by the Association, except the new IR method for milk was not adopted. See the report of the committee (and "Changes in Methods"), this issue.

moved to official final action status: residual phosphatase method V, **16.125–16.126;** reactivated and residual phosphatase, differential tests, **16.129–16.130;** residual phosphatase in casein, **16.B01–16.B03.** The Referee concurs with this recommendation.

Recommendations

(1) Adopt as official first action the collaboratively studied enzymatic UV method for measuring lactose in milk.

(2) Adopt as official first action the collaboratively studied Infra-analyzer 400D method for the determination of fat, total solids, and protein in milk.

(3) Adopt as official final action the official first action potentiometric method for determination of chloride in cheese; delete the present official final action method, **16.242–16.243.**

(4) Adopt as official final action the official first action methods residual phosphatase method V, reactivated and residual phosphatase differential tests, residual phosphatase in casein.

(5) Continue study on all other topics.

Report on Decomposition and Filth in Foods (Chemical Methods)

WALTER F. STARUSZKIEWICZ, JR
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Ethanol in Seafoods.—Associate Referee Harold R. Throm reported that the analytical procedure for the headspace GC determination of ethanol as prepared by T. Hollingworth of FDA performed satisfactorily in an in-house study. A collaborative study is planned. The Referee concurs with the recommendation of the Associate Referee that the study be continued.

Gas and Liquid Chromatography.—Applications of the GC procedure for cadaverine and putrescine were made on mahimahi (*Coryphaena hippurus*) in a controlled spoilage study. Samples of mahimahi were permitted to spoil in seawater for various lengths of time at 10, 20, and 30°C and then analyzed for a range of compounds. A modified extraction procedure using 76% methanol in AOAC sec. **18.066,** paragraph 1, was used to extract amines. Cadaverine and putrescine were determined according to *J. Assoc. Off. Anal. Chem* **64**(3), 584–591 (1981). Acceptable quality samples contained less than 2 μg/g of the diamines. Early spoilage produced 18 and 8 μg/g, definite spoilage 106 and 21 μg/g, and advanced spoilage 160 and 55 μg/g of cadaverine and putrescine, respectively. Recovery of the diamines added to acceptable quality fish averaged 87%. Other extraction procedures are being evaluated. The Referee recommends that the study be continued.

Shellfish.—Because of a change in duties, Associate Referee T.L. Chambers has resigned his refereeship.

GC Determination of Volatile Amines, DMA and TMA.—Associate Referee Ronald C. Lundstrom reported on a delay in a planned collaborative study of the GC procedure. Because of interactions between formaldehyde and the internal standard *n*-propylamine, a new internal standard, diethylamine, was selected. It was also found that batch-to-batch differences in the column packing, Chromosorb 103, resulted in poor resolution of the peaks. This problem has yet to be resolved. The Referee concurs with the recommendation of the Associate Referee that the study be continued.

Although there were no reports on the topics Ammonia in Dogfish, Coprostanol, Crabmeat, Diacetyl in Citrus Products, TLC of Amines in Fishery Products, and Tomatoes, the Referee recommends that they be continued.

Recommendations

Continue study on all topics.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition.

This report of the General Referee was presented at the 97th Annual International Meeting of the AOAC, Oct 3–6, 1983, at Washington, DC.

The recommendation of the General Referee was approved by Committee C and was accepted by the Association. See the report of the committee (and "Changes in Methods"), this issue.

Report on Fish and Other Marine Products

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Crabmeat Identification—Associate Referee Judith Krzynowek reported that the topic has expanded to include the identification of crabmeat as an adulterant in other seafood products and the identification of fish in crabmeat products. Minced cod fish added at 10, 20, and 40% to canned blue crab was detectable at the 10% level of adulteration. As a further complication, some samples contained mayonnaise or similar condiments.

The Associate Referee's work has centered around the identification of species in canned fishery products, involving samples that have been heat-denatured as a result of sterilization and samples canned in a variety of packing liquids, which compounds the problem.

The Associate Referee is trying to develop an improved method and to determine its applicability to crabmeat as well as other cooked seafoods. The General Referee recommends continued study.

Determination of Fish Content in Coated Products (Breaded or in Batter).—Associate Referee Frederick J. King has been cooperating with H. Houwing, Chairman of the West European Fisheries Technologists Association's Working Group for Analytical Methods. The Working Group has been developing and comparing protocols for 3 physical methods and 1 chemical method for determining fish content of coated products. One of the physical methods is a modification of AOAC method **18.002.** During 1983, 7 laboratories took part in a trial of the 3 physical methods on breaded plaice fillets; a paper will be submitted for publication in the Journal. The General Referee recommends that study of this topic be continued.

Drained Weight of Block Frozen, Raw, Peeled Shrimp.—This topic has received very little financial and manpower support. The Associate Referee, Frederick J. King, believes that this topic should be placed in dormant status. If interest regenerates, the topic can be reactivated.

Drip Fluid in Fish Fillets and Fish Fillet Blocks.—Like the preceding topic, financial support and resources for this work are not available, and Associate Referee King proposes that the topic be declared dormant, with the option of reactivating it if interest revives.

Fish Species Identification (Thin Layer Isoelectric Focusing).—The Associate Referee, Ronald C. Lundstrom, plans a collaborative study. The General Referee recommends that work on the topic continue.

Nitrites in Smoked Fish.—Charles Cardile, the Associate Referee, has developed the method to the point at which it is ready for intralaboratory study. However, he has been reassigned to other duties and will not be able to conduct the study himself. The General Referee recommends that a new Associate Referee be appointed with a view to completing the collaborative study of the proposed method.

Organometallics in Fish.—A method for determination of methylmercury in fish by LC and atomic absorption detection has been developed by Walter Holak, the Associate Referee, and has been published (*Analyst* (1982) **107**, 1457–1461). Mr. Holak plans an intra- and interlaboratory collaborative study during the coming year. The General Referee recommends continuation.

Other Topics.—It is proposed that a new topic, Determination of Minced Fish in Fish Fillet Blocks, be initiated and that J. Perry Lane, Northeast Fisheries Center, Gloucester, MA, be appointed Associate Referee.

The General Referee recommends that the potentiometric method for sodium chloride, **18.036,** now in official first action status, be made official final action.

A number of minor editorial changes in various methods have been suggested in preparation for the 14th edition of *Official Methods of Analysis.*

Recommendations

(1) Declare as dormant the topics Drained Weight of Block Frozen, Raw, Peeled Shrimp and Drip Fluid in Fish Fillets and Fish Fillet Blocks, with the possibility that they can be reactivated at a later date.

(2) Adopt the official first action potentiometric method for sodium chloride in fish, **18.036**, official final action.

(3) Continue work on all other topics.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition.

This report of the General Referee was presented at the 97th Annual International Meeting of the AOAC, Oct 3–6, 1983, at Washington, DC.

The recommendations of the General Referee were approved by Committee C and were adopted by the Association. See the report of the committee (and "Changes in Methods"), this issue.

Report on Food Additives

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Anticaking Agents.—The General Referee was unable to obtain an Associate Referee for this topic during the past year.

Antioxidants.—No adverse comments were received during the past year concerning the Page method for determining 7 antioxidants in fats and oils. The General Referee recommends that the procedure now be considered for official final action status.

Brominated Oils.—Associate Referee J.F. Lawrence reported on the status of his brominated vegetable oils (BVO) project.

An improved analytical method developed in the Associate Referee's laboratory has been published (*J. Assoc. Off. Anal. Chem.* (1983) **66,** 1385). A feeding study has been completed to examine the tissue and fluid distribution and metabolism of BVO in the rat. The above-mentioned analytical method was employed for the determinations.

Two in-depth toxicology studies were published during the year. They examined the bromine distribution in tissues and some gross toxicological effects of animals that had been fed selected brominated fatty acids.

No work was carried out on LC of BVO during the year. It is hoped that work in this area will commence in the near future so that a comparison can be made with the present GC method.

Dichlorodifluoromethane in Frozen Foods.—Little progress has been made by the Associate Referee during the past year because of higher priority commitments.

Dilauryl thiodipropionate.—The General Referee was unable to obtain an Associate Referee for this topic during the past year.

Dressings.—Associate Referee Warner reports that because of other higher priority commitments he has not been able to continue his studies. No collaborative study of the method was initiated during this past year. The Associate Referee reports that official methods are needed for determining surfactants in salad dressings.

EDTA in Food Products.—The Associate Referee reports no progress because of other higher priority commitments.

Ethoxyquin in Foods.—The LC method for determining ethoxyquin in milk has not yet been collaboratively studied because of other commitments. It is expected that work on this project will continue in the coming year.

Gums.—The General Referee has been unsuccessful in finding an Associate Referee for this topic during the past year.

Indirect Additives from Food Packaging.—The Associate Referee reports the following progress:

Vinyl Chloride (VC): Work has continued in ASTM Committee D-20 on Plastics on the development of a rapid, quality control method to determine residual vinyl chloride in PVC at the 5 ppb level. A revised draft of the method has passed subcommittee ballot and is now undergoing main committee ballot. It is hoped that the method will be approved and published by this time next year.

Dimethyl terephthalate (DMT): Work has progressed in the development of a method to determine residual DMT in PET (polyethylene terephthalate) beverage bottles. The method involves dissolving the PET in hexafluoroisopropanol, injecting the resulting polymer solution onto a size exclusion LC column, collecting the fraction containing the DMT, and analyzing the fraction by LC. Recoveries are reasonable at a quantitation limit of 0.5 ppm DMT (polymer basis). The method is now being applied to the determination of residual DMT in various commercial PET soft drink, wine, and liquor bottles. A manuscript will be prepared describing the method and results of the analyses.

The stability of DMT in various food-simulating solvents was investigated by FDA's Indirect Additives Laboratory in preparation for a possible determination of the migration of DMT out of PET bottles. DMT was very stable in 3% acetic acid. In aqueous ethanol systems, however, DMT rapidly broke down to a number of products including methyl ethyl terephthalate, diethyl terephthalate, methyl and ethyl half esters of terephthalic acid, and terephthalic acid. The higher the aqueous content, the faster the breakdown occurred. An implication of this study is that if DMT migrates into an aqueous ethanol product, it will rapidly disappear. If the product is acidic, however, it will likely remain as DMT.

Styrene: The manuscript, "Determination of Styrene Migration from Food-Contact Polymers into Margarine Using Azeotropic Distillation and Headspace Gas Chromatography," by Sandra L. Varner, Charles V. Breder, and Thomas Fazio has been published (*J. Assoc. Off. Anal. Chem.* (1983) **66,** 1067–1073).

Toluene Diamines (TDA): A paper by Roger C. Snyder, William C. Brumley, Charles V. Breder, and Thomas Fazio titled "Gas Chromatographic and Gas Chromatographic-Mass Spectrometric Confirmation of 2,4- and 2,6-Toluenediamine Determined by Liquid Chromatography in Aqueous Extracts" appeared in *J. Assoc. Off. Anal. Chem.* (1982) **65,** 1388–1394.

Volatiles: ASTM Committee F-2 on Flexible Barrier Materials is doing a statistical workup of recent round robin data so that ASTM Method F-151 for volatile solvents in laminated films can be balloted for reapproval.

There is a need for an interest in developing a more reliable quantitative method for residual solvents. Several companies are investigating various approaches and will report their results at future F-2 meetings.

A project is underway in the Indirect Additives Laboratory at FDA to determine volatile compounds in food packaging. Three new pieces of equipment are being utilized: a dual capillary GC column chromatograph, a trapping/concentrator capillary column GC inlet system by Chrompack, and a sparge/trapping/concentrator capillary column GC inlet system by Envirochem. Coupled with a computerized relative retention volume data base for commonly used volatiles, these systems provide a powerful means to look for volatiles. A survey is currently underway for volatiles in locally purchased food packaging.

Benzene: A manuscript by Sandra L. Varner and Charles V. Breder on "Determination of Residual Levels and Migration of Benzene from Polystyrene Containing Polymers" has been accepted for publication in the *Journal.* The paper describes the development of a method to determine benzene

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in styrene-based polymer food containers and in water. Results of polymer analyses showed a residual benzene range from none detected (200 ppb limit of quantitation) to 3.5 ppm in the containers. No benzene was observed in water under simulated hot-filling and pasteurization conditions using the styrene-based containers.

New migration cell: The new migration cell, described in the last yearly report, continues to be used for studies of migration of styrene from polystyrene into various solvents. Briefly, the cell comprises a 24 mL vial topped with a Mininert cap. Polymer disks to be tested are threaded onto a wire support in the vial and solvent is added to the vial. The sealed vial is shaken in a thermostated waterbath, and aliquots of the solvent are periodically removed with a syringe through the Mininert septum and analyzed by appropriate methodology. A manuscript describing this work has been written by Roger C. Snyder and Charles V. Breder for submission to the *Journal*. The work is being expanded to investigate the migration of migrants other than styrene from polystyrene.

Hydrogen peroxide: ASTM Committee F-2 formed a new subcommittee called "Aseptic Packaging" with 4 functioning sub-groups. The Test Methods sub-group includes a Task Group responsible for developing methods to determine (1) residual H_2O_2 in the package at the 0.1 ppm level, (2) airborne H_2O_2, and (3) H_2O_2 in solution at $\geq$ 1% levels. FDA currently regulates the amount of permitted residual H_2O_2 in the package. The method used for compliance, however, is considered by some to be too slow for quality control purposes. Thus this Task Group will look for improved methods.

Maturi extraction cell: ASTM Method F-34 describes the use of the Maturi cell for 1-sided extractions of food films. Some FDA regulations reference this method. Committee F-2 voted to drop this method because the cell is no longer commercially available and no one is interested in doing the work to extend the life of the method. FDA will have to reference *Official Methods of Analysis* for this method in the future.

Other ASTM work: Several methods for indirect food additives are in various stages of work: (1) Additives in polyolefins. An LC method to determine additives in polyolefins is under development. (2) BHT in polyolefins. A method to determine BHT in polyolefins has been submitted for ASTM ballot. (3) Acrylonitrile. Two methods to determine residual acrylonitrile in polymers are up for renewal. A task group is looking into whether any changes are needed in the methods. If so, additional round-robin testing may be necessary. (4) Multi-layered films. A method to identify multi-layered films has been developed and sent out for balloting.

Official sample analyses: Two major sampling and analyses programs were carried out for potential indirect food additives. The first concerned the possible use of an unapproved catalyst component for polypropylene food contact items and the second involved the analysis of edible oils for the presence of an unapproved oil extraction solvent.

Nitrates and Nitrites.—The Associate Referee has been evaluating several techniques but has not submitted a progress report.

Nitrosamines.—Associate Referee N.P. Sen reported excellent progress in this research area. The results of these efforts are summarized below.

(1) "Determination of *N*-Nitrosodimethylamine in Nonfat Dry Milk: Collaborative Study" by N.P. Sen, S. Seaman, and R. Stapley was given interim official first action status by Committee C in April 1983 and is recommended for first action adoption.

(2) "Survey of Rubber Baby Bottle Nipples for Volatile *N*-Nitrosamines" by D.C. Havery and T. Fazio. A local market survey of 27 baby bottle rubber nipples for volatile *N*-nitrosamines was conducted. *N*-Nitrosodimethylamine, *N*-nitrosodiethylamine, *N*-nitrosodibutylamine, and *N*-nitrosopiperidine were found at levels up to 387 ppb. All 4 nitrosamines migrated from the rubber into water, milk, and infant formula when sterilized together in a manner typical of that used in the home. The source of the nitrosamines is the chemical accelerators and stabilizers added to raw rubber during the vulcanization process.

(3) "CID/MIKES in the Analysis of Volatile *N*-Nitrosamines in Rubber Nipples" by D. Andrezejewski, D. Havery, P. Dreifuss, K. White, J. Sphon, and T. Fazio. Volatile *N*-nitrosamines have been reported present in rubber baby bottle nipples. The usefulness off CID/MIKES analysis for the detection and confirmation of nitrosamines in nipple extracts was investigated. Sample extracts giving a positive response on a thermal energy analyzer (TEA) were cleaned up and analyzed by capillary GC/MS. CID/MIKES spectra were recorded for the molecular ion of the suspected nitrosoamines, nitrosodimethylamine, nitrosodiethylamine, and nitrosopiperidine. The specificity provided by the MIKES analysis reduces the need for extensive sample cleanup and maintains the sensitivity of the confirmatory method when compared with the conventional EI full mass scan GC/MS method.

(4) "Collaborative Study of the Dry Column-Thermal Energy Analyzer Method for *N*-Nitrosopyrrolidine in Fried Bacon" by W. Fiddler, J. Pensabene, A. Miller, A. J. Malanoski, and J. Phillips. A dry column method for isolating *N*-nitrosopyrrolidine (NPYR) from fried, cured-pumped bacon and detection by GC-thermal energy analyzer (TEA) was studied collaboratively. The outlier statistical analyses were carried out by AOAC recommended procedures and by a mean-ratio performance standard technique. Results obtained from 6 collaborators showed an average coefficient of variation for within-laboratory repeatability of 5.08% and reproducibility of 14.92% over the range of NPYR studied and 11.08% for the average recovery of 89.25% *N*-nitrosoazetidine (NAZET) internal standard. The method is recommended for adoption as interim official first action as an alternative to the mineral oil distillation-TEA procedure.

Polycyclic Aromatic Hydrocarbons in Foods.—Work continued on the LC method for determination of N-PAH in smoked foods. Imported smoke foods, including oysters, clams, red snappers, and two types of sardines, were analyzed. Based on LC retention time data, two of the products, oysters and clams, appeared to be contaminated with 5,7-dimethylbenzo(a)-acridine (DMBaAc) and dibenzo(a,h)-acridine (DBahAc). However, UV-visible scans of the collected peak showed different spectra from that of the standard N-PAH. In addition, these peaks were submitted for mass spectroscopy study. The results obtained indicated the presence of phthalate and adipate esters and some free sterols rather than N-PAH compounds.

The imported foods chosen for study were based on investigations conducted by Grimmer in 1980 to establish gas chromatographic profiles for N-PAH in high protein foods, including smoked meat, ham, fish, poultry, etc. In his data, several N-PAH were detected including B(c)Ac, DB(a,h,)Ac, DB(a,j)Ac, and DB(ch)Ac. Based on our studies with similar foods, no N-PAH were observed; therefore, questions must be raised regarding the identity of the peaks Grimmer reported as N-PAH.

Propylene Chlorohydrin.—The Associate Referee for this topic resigned.

No reports were received from Associate Referees for

Chloride Titrator, Chlorobutanol in milk, Dimethylpolysiloxane, and Nitrates (Selective Ion Electrode).

Recommendations

(1) Adopt as official final action the method for antioxidants in foods reported by B.D. Page; continue study.

(2) Adopt as official final action the method for *N*-nitrosodimethylamine in nonfat dry milk, collaboratively studied by the Associate Referee as Method I; continue study.

(3) Appoint Associate Referees on Anticaking Agents; Dichlorodifluoromethane in Frozen Foods; Dilauryl thiodiproprionate; Gums; Propylene Chlorohydrin; and Sodium Lauryl Sulfate.

(4) Continue study on all other topics.

Report on Meat, Poultry, and Meat and Poultry Products

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Activity on all topics compared with last year has been reduced. In addition, 2 topics (Sulfonamide in Meat and Poultry, and Organohalides in Poultry Fat) have been transferred to other General Referees. The reduction in activity can be attributed partly to the general economic condition which has resulted in more emphasis on program-related analytical work from special studies and a consequent reduction in method improvement and development work. This emphasis is having an effect on our ability to conduct collaborative studies in the meat and poultry areas. The reduced number of agency laboratories, coupled with the high cost of analytical time and instrumentation and the time required to achieve acceptable analytical results, is making it difficult to obtain a sufficient number of laboratories for the classic AOAC collaborative study. The Food Safety and Inspection Service (FSIS) is using and will continue to use the Three Analyst Validation Study coupled with analyst performance standards (1) when 6 laboratories cannot participate in the standard collaborative study.

The mechanical separation of muscle tissue from bone, resulting from Agency rules and proposals, is placing an additional emphasis in the areas of bone particle measurement and protein quality. Bone particle measurement is extremely challenging because of the heterogeneity of the sample, and an attempt to achieve a more homogeneous sample could cause a reduction in the size of the larger particles. Our laboratories are evaluating procedures to determine the sample size necessary to eliminate the need for homogenization and techniques for measuring particle size. This may be an area for a new Associate Referee topic. For regulatory control purposes, the Expert Work Group convened by FSIS has recommended that the determination of collagen be used instead of amino acid analysis as the bioassay procedure. They further suggested the use of 3-methylhistidine (2) be investigated as a measure of muscle protein and indirectly as a measure of protein quality. If the work in progress suggests regulatory uses, new associate refereeships will be recommended. Further support for this work on 3-methylhistidine comes from the FSIS proposal on the Protein Fat-Free procedure for measuring product compliance in cured pork products.

The investigational work in using 3-methylhistidine, if coupled with additional unique amino acids or marker compounds not yet identified, may make it possible to determine meat protein directly. Under this General Refereeship, it would be more consistent to work with the meat and poultry matrix identification rather than attempt to identify the individual nonmeat proteins. This problem of protein analysis is being picked up by Agriculture Research Service (ARS) and new topics may arise from this effort. Armstrong (3) has determined soy protein isolate by using electrophoresis with an internal standard. However, with all of the nonmeat proteins that are available, quantitation techniques for each would require more resources than are available. The associate refereeship for nonmeat proteins should be changed to identification and we should redirect our efforts to the quantitation of meat protein, remaining open to the possibility of new associate refereeships based on pending work in ARS. Work in Europe should also be reviewed for potential associate refereeships in this area.

Automated Methods.—Associate Referee Jon Schermerhorn has been reassigned and was unable to complete the evaluation of the collaborative study data for total protein in meat, using the Technicon AA-2 AutoAnalyzer. It is recommended that the data evaluation be completed.

Bone Content.—Associate Referee Paul Corrao recommends that the general terms "young chickens" and "chickens" replace the terms "broilers and fryers" and "fowl" in **24.D01.** He recommends that method **24.D01–24.D02** remain as official first action and that study be continued to extend the method to pork. The Referee concurs.

Chemical Antibiotic Methods.—Associate Referee Francis B. Suhre has recently been appointed and has not had an opportunity to study the topic.

Fat in Meat Products.—Associate Referee Jon E. McNeal reports that Webb Food Laboratory has recently undertaken a collaborative study on fat, using the CEM instrumental procedure. The work was not completed early enough for evaluation at this AOAC meeting. It is recommended the results should be submitted for review as interim first action status.

Fat and Moisture Analysis, Rapid Methods.—Associate Referee Julio Pettinati has been reassigned and has been delayed in completing the data evaluation and the collaborative study report. It is recommended that the Associate Referee complete the report.

Histologic Identification Methods.—Associate Referee Albert Carey recommends that the procedure for identification of cardiac musculature, soy flour, and partially defatted tissue in ground beef reported at last year's meeting be con-

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sidered for adoption as official first action. The Referee concurs.

LC Methods for Meat and Poultry Products.—Associate Referee Douglas Gillard has recently been appointed. Continued study is recommended.

Identification of Meats, Serological Tests.—A vacancy exists. The Referee recommends continued study when an Associate Referee is appointed.

Nitrates and Nitrites.—Associate Referee Francis B. Suhre, because of other assignments, was not able to report progress in the ion chromatographic procedure for the determination of nitrite and nitrate. Continued study is strongly recommended.

Nitrosamines in Bacon.—Associate Referee Earl L. Greenfield reports that artifactual formation has not been observed in the analysis of fried pumped bacon. He recommends that **24.C01–24.C07** be made official final action. The Referee concurs.

The dry column procedure for NPYR in bacon, submitted last year by Fiddler et al., has been rewritten to satisfy statistical considerations and it is recommended the procedure be considered for interim first action status.

Nonmeat Proteins in Meat.—Julio Pettinati, Chairperson for the 3-member Associate Referee Committee, has been reassigned and is unable to continue as an Associate Referee. Associate Referee Marion Greaser has reported on the determination of soy (4) and because of other assignments is also unable to continue as an Associate Referee. No activity has been reported by Associate Referee Khee C. Rhee. With the large number of nonmeat proteins and high interest, it might be more effective to discontinue the topic and investigate the determination of meat protein. Therefore, it is recommended that the topic be discontinued and a new one addressing meat protein analysis be established. Considerable time and effort on this topic has been done in Europe, particularly The Netherlands. A strong consideration from an associate referee from Europe on quantitating meat protein in a meat product, or methods for determining nonmeat proteins in a meat product, is warranted and should be pursued.

Protein in Meat.—Francis B. Suhre, Associate Referee, suggests that with the adoption of **24.B01–24.B03** as official final action and no further studies planned, the topic be discontinued. The Referee concurs.

Specific Ion Electrode Applications.—Associate Referee Randy Simpson is investigating the possibility of a collaborative study using the NOVA Biomedical ion analyzer for the determination of sodium, potassium, and chloride. He is concerned that he will not have sufficient participation for an acceptable collaborative study. It may only be possible to identify 3 laboratories who have the instrument. The Referee recommends that a collaborative study be planned with the assistance of the Committee on Statistics.

Steroid Analysis.—Associate Referee Michael Thomas has only recently been appointed. Continued study is recommended.

Sugars and Sugar Alcohols.—The topic is vacant; however, interest has been indicated by 2 individuals. The vacancy will be filled and continued study is recommended.

Temperature, Minimum Processing.—Associate Referee James Eye is evaluating isoelectric focusing based on the work of Lee et al.(5). Continued study is recommended.

The changing and seemingly increased Agency responsibilities and other activities within AOAC make it necessary for me to resign from this General Refereeship. I would like to take this opportunity to express my sincere gratitude for all the efforts of the Associate Referees in this topic area which has resulted in a substantive amount of activity these past 3 years.

Recommendations

(1) Continue as official first action method **24.D01–24.D02** for determination of calcium in beef and poultry, and replace the terms broilers and fryers with young chickens and chickens.

(2) Adopt as official final action the official first action method **24.C01–24.C07** for determination of nitrosamines in cooked bacon.

(3) Complete evaluation of the collaborative studies for Technicon AA-2 Auto Analyzer and microwave moisture determinations.

(4) Plan a collaborative study on the determination of sodium, potassium, and chloride, using the NOVA Biomedical ion analyzer.

(5) Discontinue the topics Protein in Meat, and Nonmeat Proteins in Meat and establish a new topic on meat protein analysis.

(6) Appoint an Associate Referee for Sugars and Sugar Alcohol.

(7) Approve dry column procedure for NPYR by Fiddler et al. in bacon as an interim first action method.

(8) Continue study on all other topics.

(9) Appoint a new General Referee to the topic of Meat, Poultry, and Meat and Poultry Products.

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Report on Mycotoxins

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Leonard Stoloff's annual perspective as General Referee on Mycotoxins will be missed, and as I take over this appointment my comments this year will be more modest. Nevertheless, his reports, published in the March issue of the *Journal of the AOAC* since 1971, repay rereading—they contain nuggets of advice for the Associate Referees and are a chronicle of the development of mycotoxin methodology by AOAC over the years. Leonard Stoloff was also instrumental in the publication of a valuable recent book on mycotoxin analysis: *Environmental Carcinogens—Selected Methods of Analysis, Volume 5, Some Mycotoxins* (1982) H. Egan (Ed.-in-Chief), L. Stoloff, M. Castegnaro, P. Scott, I. K. O'Neill, H. Bartsch (Eds), & W. Davis (Tech.Ed.), International Agency for Research on Cancer, Lyon, France. I urge Associate Referees to read and study the Report of the Committee on Interlaboratory Studies which includes a Final First Draft of an Outline of Interlaboratory Study Procedure to Validate Performance of a Method of Analysis (1), in addition to the well known *Statistical Manual of the AOAC* (2) and *Handbook for AOAC Members* (3), before planning new collaborative studies.

The base of mycotoxin methods built up under the previous General Refereeship I hope will not remain static but will continue to show improvements. Four new collaborative studies are nearly completed and several more are planned for 1984. It is encouraging to note new sources of reference standards—Wako Chemicals USA, Inc., 12200 Ford Rd, Suite 212, Dallas, TX 75234; and Canpro Laboratories, 77 Champagne Dr, Downsview, Ontario, Canada M3J 2C6. Included are some trichothecenes not available before commercially.

We welcome 3 new Associate Referees: Douglas Park (Food and Drug Administration (FDA), Washington) is now Associate Referee for Aflatoxin Methods, George Ware (FDA, New Orleans) has just taken over responsibility for ergot alkaloids, and Kenneth Ehrlich (U.S. Department of Agriculture (USDA), New Orleans) is Associate Referee for the new topic of Secalonic Acids. Progress reports of the Associate Referees are as follows:

Aflatoxin M.—Associate Referee Robert D. Stubblefield (USDA, Peoria, IL) reports that the alternative extraction procedure (4) for determination of aflatoxin M_1 in milk (**26.A10–26.A15**) has been submitted to 20–25 laboratories for evaluation. The procedure uses a commercially available extraction column containing an inert hydrophilic matrix. Sufficient reports have not yet been obtained to permit a decision on whether to recommend it for adoption; 5–6 laboratories so far favor the method and 2 other laboratories have no emulsion problems so I see no need for the change.

The collaborative study for the LC determination of aflatoxin M_1 in milk (5) is in progress. Problems have occurred which did not allow completion of the study at this time. The study will be continued.

The Associate Referee recommends that the first action methods for determination and confirmation of aflatoxin M_1 in dairy products (**26.A10–26.A15, 26.090–26.094**) and aflatoxin B_1 and M_1 in liver (**26.C01–26.C08**) be retained in that status, that evaluation of the alternative extraction procedure be continued, and that collaborative study of the Foos and Warren LC method be continued. The Referee concurs.

Aflatoxin Methods.—Newly appointed Associate Referee Douglas L. Park has organized a collaborative study of a negative-ion chemical ionization mass spectrometric (MS) procedure for confirmation of identity of aflatoxin B_1 (6, 7). The study is a joint effort by FDA Laboratories of the Bureau of Foods in Washington, the New York Import District, and the New York Regional Laboratory and is being conducted in the United States, Canada, England, and Germany. Twelve partially purified, dry film extracts from naturally and artificially contaminated roasted peanuts, cottonseed, and ginger root containing varying quantities of aflatoxin B_1 were distributed to participating laboratories. Extracts required additional cleanup prior to MS analysis, using either an acidic alumina column and preparative TLC or a 2-dimensional TLC procedure. Alternative MS procedures were also evaluated. The Associate Referee will make recommendations on the confirmatory procedure after completion of the study.

Alternaria Toxins.—Associate Referee Douglas King (USDA, Albany, CA) has reviewed the topic of the *Alternaria* toxins and their importance in foods. The potential for *Alternaria*-formed toxins in foods has recently been examined in grains and breads (8). Alternariol, alternariol methyl ether, and altenuene were produced on wheat, rye, whole wheat bread, and malt-extract agar by *Alternaria* isolates but tenuazonic acid was not; there were substrate and strain differences in production of the toxins. He recommends that the meager information on natural occurrence and stability of these toxins in foods and feed be expanded and that continued study on methods of analysis is needed. The Referee concurs.

Citrinin.—Associate Referee David M. Wilson (University of Georgia, Tifton, GA) reports that analysis for citrinin continues to be a problem because of poor recoveries during extraction. Three reports on methodology have been published or are in press (9–11). The papers by Gimeno and Martins (9) and Johann and Dose (10) are multimycotoxin methods and the report by Trantham and Wilson (11) concerns a fluorimetric screening method for citrinin.

The simultaneous occurrence of aflatoxin and citrinin in corn flours in Japan was reported in 1982 (12). Some of the recent papers on the biological effects of citrinin include metabolism of (14C)-citrinin by pregnant rats (13), toxic effects on chicken embryos (14), hematological changes in mice (15), and the tumorigenicity of citrinin in male rats (16).

The Associate Referee recommends further study on methods for the determination of citrinin. The Referee concurs.

Ergot Alkaloids.—The recent application of LC-MS (17, 18) and MS/MS (19) to ergot alkaloid analysis is noted. The new Associate Referee is George Ware (FDA, Washington).

Grains.—Associate Referee Odette L. Shotwell (USDA, Peoria, IL) reports that a study was made of deoxynivalenol (DON) incidence and levels in 1982 hard red winter (HRW) wheat grain in areas of Nebraska and Kansas known to have scabby wheat. One hundred and sixty-one samples represen-

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tative of the wheat harvested in the areas were collected from elevators. They were analyzed for DON by a modification of the method used by the Canadian government (20). Of the 161 samples analyzed, 42% contained ≤1 μg/g; 67% contained ≤2 μg/g; and 89% contained ≤4 μg/g. The average DON level in Grade U.S. No. 1 HRW wheat was 0.77 μg/g. Correlations were made between DON incidences and levels in HRW wheat and factors used in grading wheat. The highest correlations were between DON levels and the grading factors: percent total damaged kernels, percent total damage, and percent total scab damage. Forty-four soft red winter wheat samples were graded by the Federal Grain Inspection Service and analyzed for DON; only one had more than 1 μg/g DON (1.2 μg/g).

An analytical method has been developed by Thomas Zennie, University of Cincinnati, and the Northern Regional Research Center, to determine aflatoxin in 0.1 to 1.0 g samples of airborne dusts from corn. Toxin levels in extracts are determined by high performance thin layer chromatography (HPTLC).

Four methods of determining aflatoxin levels in a series of naturally contaminated corn samples were investigated: the CB method (**26.026–26.031**), cottonseed method (**26.A01–26.A09**), Thean's method (21), and a mixed feeds method (22). Levels of aflatoxin in extracts obtained by the four methods were measured by TLC, HPTLC, and LC. Results of the determination of aflatoxin in a set of samples run in triplicate varied in order of increased magnitude—Thean < cottonseed < mixed feeds < CB. Aflatoxin was not recovered from the lot of Sep-Pak silica gel columns used in the Thean method, although the volume of the eluting solvent was increased. Variations between lots of Sep-Pak cartridges have been reported. Results from the 4 analytical methods and 3 methods of measuring toxins are being evaluated statistically.

The Associate Referee recommends completion of the evaluation of analytical and quantitation methods for aflatoxins in corn, and if a method or combination of methods appears to be valid after the statistical evaluation, a collaborative study should be conducted. The Referee concurs with these recommendations except to note that a collaborative study of the determination of aflatoxin in corn by the CB method using TLC has already been carried out (23).

Ochratoxin.—Last year it was recommended by Associate Referee Stanley Nesheim (FDA, Washington) that the AOAC and IUPAC cooperate in conducting a collaborative study of the modified green coffee method applied to swine tissue (24). P. Krogh was to supply naturally contaminated tissue for the studies. These tissues, it was discovered, had become useless upon storing in freezers. New tissues could have been obtained from Danish slaughter houses; however, Dr. Krogh has found that tissues cannot be shipped to the United States unless first irradiated with 6000 Rads to eliminate the possibility of spreading foot and mouth disease. FDA have therefore taken steps to produce ochratoxin A-contaminated swine tissues by feeding ochratoxin to 3 mini pigs for one week. These tissues will be used for a study of the modified green coffee method, an AOAC TLC method (25). Since no published LC method has supporting validation data it has been decided to subject the best method (25) to a preliminary study in 3 or 4 laboratories before conducting the planned full collaborative study on LC of ochratoxin A in barley and corn.

The Associate Referee recommends that an AOAC/IUPAC collaborative study of the modified green coffee method applied to swine kidneys be conducted, and that a precollaborative study of the LC method of Thorpe be carried out in 3 or 4 laboratories, followed up by an AOAC/IUPAC study of the method as is, or with improvements if needed. The Referee concurs.

Penicillic Acid.—Associate Referee Charles W. Thorpe (FDA, Washington) has conducted a precollaborative study of a method for the determiniation of penicillic acid in grains (26), which was successful in that 3 of 4 participants recovered > 70% of the penicillic acid added. The fourth participant however had low recoveries that seem to be due to the silica gel used. For this reason he is modifying the method to add a step where the analyst checks recovery of standard penicillic acid from the silica gel column before proceeding with the analysis. He is also checking the long-term (up to 3 months) recovery of penicillic acid added to ground grains in preparation for a full collaborative study which will be held in early 1984.

Dr. Thorpe also notes some recent research activity on penicillic acid in other laboratories. Papers describing the in vitro metabolism of penicillic acid with mouse-liver homogenate fractions and the excretion of conjugated metabolites of penicillic acid in male mice have been published (27, 28). It was found that penicillic acid reacted with glutathione both enzymatically and non-enzymatically in the mouse-liver homogenate study and that each reaction was of equal importance. Penicillic acid metabolites in mouse urine were, for the most part, not extracted by polar organic solvents. At least 3 metabolites of penicillic acid in the bile and 3 in the urine were resolved by reverse phase LC and found to be glutathione or cysteine derivatives. An analytical method for determination of aflatoxins, citrinin, ochratoxin A, patulin, penicillic acid, and sterigmatocystin in moldy foods was reported (10). Samples were extracted with CH_3CN–4% aq. KCl (9 + 1) and cyclohexane. Sodium bicarbonate solution was added to the acetonitrile phase and this was extracted with methylene chloride. The basic CH_3CN phase was acidified and extracted with CH_2Cl_2. The CH_2Cl_2 extracts were combined and evaporated to dryness. Determination of penicillic acid was carried out by 2-dimensional TLC analysis with diphenylboric acid-2-ethanolamine spray used to form a fluorescent derivative (excitation 365 nm, emission 445 nm). Recoveries of penicillic acid added to rice at the 200 and 400 ng/g levels were 59% and 78%, respectively, and recoveries of penicillic acid added to wheat bread at the 200 ng/g level were 78%.

The Associate Referee recommends that collaborative study of a published method for determination of penicillic acid in grains (26), as modified to include a silica gel check step, be carried out in 1984. The Referee concurs.

Secalonic Acids.—This is a new topic initiated as a result of detection of secalonic acid D in corn dust obtained from grain storage elevators in concentrations ranging up to 4.5 μg/g (29). The Associate Referee Kenneth Ehrlich (USDA, New Orleans, LA) reports that no collaborative studies have been set up for the reported LC method for secalonic acid D (29, 30). The method (30) has continued to be used for analysis of secalonic acid D in grain dust (mixed grains, wheat, corn) but further work is needed to separate possible interfering compounds before the procedure would be suitable for collaborative testing. An ELISA method for secalonic acid is being developed at the Southern Regional Research Center and preliminary work on this has been reported (31). Secalonic acid D was found to be a toxin in rat lung (32). This fact plus the evidence that secalonic acid D is present in corn dust increased the need for new sensitive assay procedures. At present no assay can be unequivocally recommended. Further development of an LC method or an equivalent assay procedure is recommended. The Referee concurs.

Sterigmatocystin.—A collaborative study on the determination of sterigmatocystin in cheese, originally planned by Associate Referee Octave J. Francis, Jr (FDA, New Orleans), for 1983, is now scheduled for 1984 pending review and evaluation of the method (33), which has now been modified by the authors. In addition to earlier reports of natural contamination of cheeses with sterigmatocystin (34), investigators have reported the detection of this mycotoxin in breakfast cereal (35), corn and barley (36) and, again, in cheese (37). The first known incidence of suspected sterigmatocystin poisoning of poultry through feed ingestion has been encountered (38). Two multitoxin analytical methods applicable to sterigmatocystin were reported (10, 39). The Associate Referee recommends collaborative study of the method for sterigmatocystin in cheese and that surveys for occurrence of sterigmatocystin in susceptible commodities be continued. The Referee concurs.

Tree Nuts.—Associate Referee Vincent P. DiProssimo (FDA, New York Import District, New York) reports that confirmation of identity of aflatoxin B_1 by negative-ion chemical ionization MS has been extended from Brazil nuts, filberts, pistachio nuts, and melon seeds, to sunflower seeds and grain by-product animal feeds. Work continues on validating the method for other tree nuts. A method employing acid alumina column chromatography and preparatory TLC for preparing tree nut and other sample extracts for MS confirmation has been developed. The MS method first described by Brumley et al. (7) and employing a 2-dimensional TLC cleanup is currently under collaborative study, including the alternative alumina column-preparatory TLC cleanup (see under Aflatoxin Methods).

Work is continuing on developing an economical, accurate, general purpose method for aflatoxins in tree nuts utilizing alternative TLC and LC determinative steps. The Associate Referee recommends continued study towards developing a general quantitative tree nut method, and continued study of the confirmation of aflatoxins in tree nuts by MS. The Referee concurs.

Trichothecenes.—During the past year, considerable emphasis has been placed on development of methods for the analysis of trichothecenes, reports Associate Referee Robert M. Eppley (FDA, Washington). The widespread detection of DON in grains has resulted in further refinements of several methods for the analysis of this trichothecene. Two methods (20, 40) were recommended for collaborative study last year. A study of a TLC procedure based on a previously reported method (40) has been initiated. This study is a TLC method (41) which uses visual and/or densitometric determination of the DON. The first phase of the study is with wheat only. The study should be completed by September 1983 and the results evaluated by October 1983. Details of a collaborative study of the original GC method for DON (20), or a modification thereof, have not been formulated at this date, but are under consideration.

A second GC method (G. Ware, FDA, New Orleans, LA, personal communication to the Associate Referee) has been proposed for collaborative study. This study would include both wheat and corn and will be initiated by Mr. Ware. Details of the study are being worked out and the study will be initiated in late 1983. Another interlaboratory study has been initiated by Y. Ueno, Tokyo University of Science, under the auspices of the Commission on Food Chemistry, IUPAC. The study includes 4 naturally contaminated wheat samples which were to be analyzed by a method used in-house or that of Kamimura et al. (42). Results were due June 30, 1983; no report has been issued at this time.

The development and refinement of methods for the other trichothecenes has not been as intense as that for DON, since none of the other trichothecenes have been reported to occur widely in grains. No collaborative studies for these other trichothecenes are planned for the immediate future; however, development and refinement of methods will be continued.

The Associate Referee recommends collaborative study of a GC method for DON in grains and that studies be continued on development of analytical methods for trichothecenes including confirmation methods, improved screening methods and preparation of reference standards. The Referee concurs.

Zearalenone.—Associate Referee Glenn A. Bennett (USDA, Peoria, IL) reports that a collaborative study on the determination of zearalenone and α-zearalenol in corn by LC is in progress and that preliminary results should be available by the 1983 AOAC Meeting. This method has been used to determine α-zearalenol and zearalenone in a variety of agricultural commodities, including sorghum, and low levels (20 ng/g) of both toxins can be detected with a fluorescence detector (43).

Two additional multitoxin procedures that include zearalenone were reported in the last year. A rapid multitoxin screening procedure for zearalenone, DON, T-2 toxin, diacetoxyscirpenol, ochratoxin A, and aflatoxin uses disposable columns and small volumes of solvents, and permits the analysis of a large number of samples simultaneously (44). This method reports a detection limit of 50 ng/g of zearalenone in corn, wheat, and oats. Capillary GC has been used for the simultaneous determination of 6 fusariotoxins (DON, diacetoxyscirpenol, HT-2 toxin, T-2 toxin, fusarenone-X, and zearalenone) in corn, wheat, and swine feed (45). A specific and sensitive method for zearalenone and zearalenol uses antibodies from pigs for a radioimmunoassay procedure that is sensitive to 5 ng/mL in human serum (46).

Although zearalenone does coexist with other *Fusarium* toxins, none was detected in southeast and south central Nebraska wheat which was damaged by *Fusarium graminearum* (47).

The Associate Referee recommends that the LC collaborative study on the determination of zearalenone and α-zearalenone be completed and that studies be continued to develop rapid methods for zearalenone which use minimum amounts of solvents. The Referee concurs.

Recommendations

(1) Complete collaborative studies on the Foos-Warren method for aflatoxin M_1 in milk, the negative-ion chemical ionization MS procedure for confirmation of identity of aflatoxin B_1, the TLC method for DON in wheat, and the Bagnaris LC method for zearalenone and α-zearalenol in corn.

(2) Initiate collaborative studies on a method for aflatoxins in corn, a modification of the green coffee TLC method for ochratoxin A applied to swine kidneys, the Thorpe-Johnson GC method for penicillic acid in grains, the van Egmond et al. method for sterigmatocystin in cheese, and a GC method for DON in wheat and corn.

(3) Continue study on all other topics.

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Report on Oils and Fats

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Antioxidants.—B. D. Page is investigating procedures to confirm the presence of antioxidants detected by the LC method (1).

Chromatographic Methods.—W. G. Doeden, Jr, has resigned as Associate Referee. Because of the rather general nature of this topic, the Referee recommends that it be discontinued in favor of more specific topics as the demand arises.

The International Union of Pure and Applied Chemistry (IUPAC) Commission on Oils, Fats and Derivatives has completed collaborative study of a GC method for determination of triglyceride composition of fats and oils, using 2–4 mm id × 50–60 cm long packed columns with 3% methyl silicone liquid phase (2). Palm oil, coconut oil, and cocoa butter triglycerides were examined by 8 collaborators in an initial collaborative study. Lard, butterfat, soybean oil, hydrogenated soybean oil, and peanut oil triglycerides were analyzed by 15 collaborators in a second collaborative study. Several collaborators reported results obtained by using a capillary column. For the major triglycerides it appeared that the type of column (either packed or capillary) did not significantly influence the results. The method was adopted by the Commission. A recommendation for adoption by the AOAC will be made after publication of the collaborative results.

Cyclopropene Fatty Acids.—G. S. Fisher has reported that there is currently little or no interest in this topic and recommends that the topic be discontinued.

Emulsifiers.—H. Bruschweiler has completed a joint AOAC–IUPAC collaborative study of a GC procedure for emulsifiers involving separation of the emulsifiers from an oil sample by column chromatography, hydrolysis of the emulsifier fraction, silylation, and analysis by either packed or capillary column GC. Among the emulsifier components detected by GC are fatty alcohols; glycols and polyglycols; glycerol and polyglycerols; carbohydrates and mono- and disaccharides; and carboxylic and dicarboxylic acids. A recommendation for adoption of the method will be made after publication of the collaborative results.

This report of the General Referee was presented at the 97th Annual International Meeting of the AOAC, Oct. 3–6, 1983, at Washington, DC.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition.

The recommendations of the General Referee were approved by Committee C and were adopted by the Association. See the report of the committee (and "Changes in Methods"), this issue.

The Associate Referee has also described procedures for determination of anionic emulsifiers and surfactants (lauryl sulfate, dioctylsulfosuccinate, etc.) and nonionic emulsifiers (ethoxylated and propoxylated compounds such as polyoxyethylene fatty acid esters) as well as a procedure for specific determination of emulsifier components by capillary column GC without prior hydrolysis (3). The Associate Referee recommends that these procedures be subjected to collaborative study.

Lower Fatty Acids.—G. Bigalli is planning a collaborative study of revised methodology for quantitation of lower fatty acids by GC.

Marine Oils.—R. G. Ackman is continuing the study of methodology for analysis and identification of fish oils.

Olive Oil Adulteration.—Several methods used in Italy and supplied by the Associate Referee are under review. The IUPAC Commission on Oils, Fats and Derivatives has completed study of a method for determination of erythrodiol in olive oil, useful for detection of solvent-extracted olive oil in pressed olive oil. The method involves fractionation of extracted unsaponifiable matter by thin layer chromatography followed by silylation of the sterol and triterpenoid glycol fractions and analysis by GC. The results of the IUPAC collaborative study are under review.

Oxidized Fats.—A. E. Waltking is continuing study of procedures for oxidation products (polymers) in vegetable oils. Stephan C. Anderson, Cargill, Inc., Wayzata, MN 55391, is planning a joint AOCS/AOAC/IUPAC collaborative study of a method for determination of polymers in abused vegetable oils by gel permeation chromatography employing μ-Styragel columns to separate fatty acid methyl esters from polymer (dimer) fatty acid methyl esters. The results of collaborative study of the method for determination of polar compounds in frying fats (4) have been published in *Pure Appl. Chem.* (1983) **55**, 1381–1385.

Pork Fat in Other Fats.—There was no activity on this topic during the past year.

Spectrophotometric Methods.—A. J. Sheppard has completed study of the enzymatic method for *cis,cis*-methylene interrupted polyunsaturated fatty acids in vegetable oils. The Associate Referee recommends that the topic be discontinued and the Referee concurs.

Sterols and Tocopherols.—H. J. Slover has completed a collaborative study of the GC method for determination of tocopherols and sterols in vegetable oils. The method involves addition of an internal standard (5,7-dimethyltocol) to about 0.1 g of sample, saponification (aqueous KOH) in the presence of pyrogallol, extraction of the unsaponifiables, preparation of trimethylsilyl ethers, and chromatography of the derivatized unsaponifiables on either a packed (Apiezon L, 15 ft × 2 mm id) or capillary (50 m × 0.25 mm id Dexsil 400) column at 245–250°C. The collaborative results indicate that the method should be simplified and that a single GC column, preferably fused silica methyl silicone bonded phase, be specified for the GC analysis.

Water Content.—R Bernetti has completed work on validation of the Karl Fischer method for determination of water in oils and fats recommended by the International Standards Organization (ISO/TC 34/SC 11, N99). A final draft of the international collaborative study report has been prepared and the Associate Referee recommends that the method, granted interim first action status, be adopted official first action.

Other Topics.—The General Referee recommends that the following official first action methods be adopted official final action: Preparation of Methyl Esters—Boron Trifluoride Method, **28.052–28.056;** Methyl Esters of Fatty Acids—AOAC–IUPAC Gas Chromatographic Method, **28.057–28.065;** Docosenoic Acid—Gas Chromatographic Method, **28.066–28.069;** Polymers and Oxidation Products of Heated Vegetable Oils—Gas Chromatographic Method for Non-Elution Materials, **28.070;** *cis,cis*-Methylene Interrupted Polyunsaturated Fatty Acids, **28.071–28.074;** Polar Components in Frying Fats, **28.C01–28.C08.**

Commission on Oils, Fats and Derivatives, Applied Chemistry Division, IUPAC.—The General Referee completed a term this year as Chairman of the IUPAC Commission on Oils, Fats and Derivatives. The Commission met on August 19–21, 1983, at the Technical University, Lyngby, Denmark, during the 32nd IUPAC General Assembly. The Commission discussed 23 projects and topics, including methods for polyunsaturated fatty acids, antioxidants, emulsifiers, plastic monomers in oils, polycyclic aromatic hydrocarbons, industrial lecithin products, erythrodiol in olive oil, total sterols, mineral oil residues, solvent residues in oils and oilseed cakes, thiobarbituric acid value, triglyceride composition by GC, tocopherols and sterols, moisture by the Karl Fischer method, butterfat content (butyric acid), and metal content by atomic absorption spectroscopy.

The Commission adopted methods for determination of acetone-insoluble fraction of commercial lecithin, determination of solvent residues in oilseed cakes, and determination of triglyceride composition of fats and oils by GC. Additional collaborative data were reviewed for 2 methods adopted last year, determination of erythrodiol in pressed and solvent-extracted olive oil, and determination of total sterols by enzymatic oxidation with cholesterol oxidase. A precollaborative study is planned of a method for determination of thiobarbituric acid (TBA) as well as for GC determination of linolenic and other n-3 polyunsaturated fatty acids.

Several new projects which were discussed include: (1) determination of solvents in oils; (2) determination of butyric acid in butterfat (in cooperation with the International Dairy Federation); (3) determination of tocopherols by LC; and (4) determination of iron, copper, and nickel by atomic absorption spectroscopy. The AOAC method for determination of antioxidants by LC (1) will be considered by the Commission for adoption next year.

Methods adopted earlier which have been published in *Pure and Applied Chemistry* include erucic acid (5), linoleic acid (6), and solid content in fats by low resolution NMR (7), along with several methods for glycerol (8). In addition, plans have been made to publish a supplement to the 6th edition of the Commission's *Standard Methods* as well as a reprinting of the 6th edition itself.

With respect to procedures for organizing, conducting, and evaluating collaborative studies, the Commission has prepared tentative guidelines for conducting collaborative studies and has established a timetable for carrying out and reporting the results of collaborative studies. In addition, the Commission arranged for a presentation by William Horwitz on validation of performance of methods at a joint meeting of this Commission and the Commission on Food Chemistry during the IUPAC General Assembly. The new officers of the Commission are: Chairman, M. Naudet (France); Vice-Chairman, A. Hautfenne (Belgium); Secretary, D. Pocklington (Great Britain).

Recommendations

(1) Discontinue the topics Cyclopropene Fatty Acids, Chromatographic Methods, and Spectrophotometric Methods.

(2) Adopt as official first action the interim first action Karl Fischer method for determination of water content in vegetable oil products.

(3) Adopt as official final action the official first action methods for preparation of methyl esters, **28.052–28.056;** gas chromatographic analysis of fatty acid methyl esters, **28.057–28.065;** gas chromatographic determination of docosenoic acid, **28.066–28.069;** gas chromatographic determination of non-elution materials, **28.070;** *cis,cis*-methylene interrupted polyunsaturated fatty acids, **28.071–28.074;** and polar components in frying fats, **28.C01–28.C08.**

(4) Continue study on all other topics.

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Report on Plant Toxins

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The pyrrolizidine and solanum alkaloids continue to attract interest with regard to their potential for the contamination of food and feed crops and the resulting requirement for official analytical methods in certain commodities. Recommendations for Associate Referees for these topics are being considered, and these positions will be filled as soon as possible.

Another plant toxin area of concern is the glucosinolates and their hydrolysis products, which are present in many cruciferous plants of economic importance. The observed toxic effects of some of the compounds include growth depression, hypothyroidism, and liver and kidney toxicity. Official analytical methods for the glucosinolates and their products should be available, particularly to determine the content of these natural toxicants in new plant varieties.

Recommendations

(1) Appoint Associate Referee for Pyrrolizidine Alkaloids.

(2) Appoint Associate Referee for Solanum Alkaloids.

(3) Establish Associate Refereeship for Glucosinolates.

(4) Continue evaluation of other plant toxin problems; assess needs for official analytical methodology.

This report of the General Referee was presented at the 97th Annual International Meeting of the AOAC, Oct. 3–6, 1983, at Washington, DC.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition.

The recommendations of the General Referee were approved by Committee C and were adopted by the Association. See the report of the committee (and "Changes in Methods"), this issue.

Report on Processed Vegetable Products

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Entrapped Air in Flexible Retort Pouches: There has been no interest shown in this topic which was proposed in the previous years. Discontinuation of this topic is recommended.

Fibrous Material in Frozen Green Beans: The method of fibrous material in frozen green beans has official first action status. The Associate Referee has received no comments adverse or otherwise. The referee does not anticipate further laboratory work.

pH Determination: pH determination in acidified foods has official final action status since last year. The collaborative study was reported in *J. Assoc. Off. Anal. Chem.* **64**(2), 332–336 (1981).

A quality assurance study of the method is currently being done. In the study, 6 samples were analyzed by each of 21 laboratories. Data will be analyzed and subjected to statistical analysis. The referee recommends that work continue on this topic.

Sodium Chloride: Sodium chloride methods I (**32.023**) and II (**32.024**) were declared surplus in the 3rd Supplement to 13th edition of *Official Methods of Analysis (J. Assoc. Off. Anal. Chem.* **65**(2), 482 (1982)). There has been no change regarding the official final action status on method III, potentiometric method (**32.025**).

Salt determination is necessary for the adjustment of salt consumption and is considered a primary step in preventing hypertension. The Associate Referee is working on a paper reviewing all of the official methods on this topic and recommends that the study be continued.

Total Solids by Microwave Moisture Analyzer: This is the new topic initiated this year. Henry B. Chin was appointed as Associate Referee to study this topic.

A preliminary work, involving 10 laboratories and 3 types of tomato products, is currently being conducted, and the statistical study of the data collected is underway. Further collaborative study on this topic is anticipated.

Water Activity: The method of water activity determination has official first action status. Three quality assurance studies were conducted during the past several years, using the hair hygrometer, and several electronic instruments. A combined report will be prepared.

The referee has not submitted any laboratory work plans for 1983–84. Interested persons are invited to submit comments and participate in the continued study on this topic.

Other Topic: There is a continuing interest in the problem of direct refractometer method (for soluble solids of tomato products without filtering or centrifugation). Interested persons are invited to participate in the study of this topic.

Recommendations

(1) Discontinue the topic Entrapped Air in Flexible Retort Pouches.

(2) Adopt as official final action status the official first action method for water activity (**32.004–32.009**).

(3) Initiate the new topic Direct Refractometer Method for Soluble Solids of Tomato Products, and appoint Associate Referee on this topic.

(4) Continue study on all topics.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition.

This report of the General Referee was presented at the 97th Annual International Meeting of the AOAC, Oct. 3–6, 1983, at Washington, DC.

The recommendations of the General Referee were approved by Committee C and were adopted by the Association. See the report of the Committee (and "Changes in Methods"), this issue.

Report on Seafood Toxins

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Ciguatoxin—Biochemical Methods.—Associate Referee Yoshitsugi Hokama (School of Medicine, University of Hawaii at Manoa, Honolulu, HI) reports completion of his study of the analysis of fish tissues for ciguatoxin by the newly developed enzyme-immunoassay (EIA) procedure (1, 2). This study comprised 3 areas: (1) the examination of clinically defined and documented toxic and nontoxic consumed fish samples; (2) the assessment of freshly caught fishes from 2 sites in the leeward part of the island of Oahu where ciguatoxin is found; and (3) competitive inhibition with suspension of purified ciguatoxin and closely related polyether ionophoretic toxins.

The comparison of clinically defined and documented toxic with nontoxic consumed fishes by EIA clearly demonstrated a statistically significant difference ($p<0.001$) between the 2 populations. The result is similar to that reported for the radioimmunoassay (RIA) and EIA procedures (1–5). The assessment of the EIA with samples of each species of fish caught in the Hawaiian waters presented positive and borderline frequency values comparable to those reported earlier (5) by the RIA, and more recently (2) by the EIA.

An interesting assessment of the EIA is the demonstration that structurally known polyether compounds such as brevetoxin, okadaic acid, and monensin can inhibit the sheep anti-CTX toxic fish tissue binding. The IC 50% inhibition concentrations suggest that maitotoxin, okadaic acid, and brevetoxin are closely related to ciguatoxin, which is believed to be a polyether compound (6).

The inotropic and chronotropic properties associated with these polyether compounds (7–9) are compatible with the properties demonstrated for ciguatoxin by several investigators (10). Thus, the high level of frequency of borderline to positive results in the EIA at the present may be attributed to these or other closely related polyethers found in fish tissues.

The critical factors in the EIA procedure are as follows: (1) the size and shape of the tissue samples should be uniform, and replicate samples from each area should be tested; (2) the fixation step of the fish tissue with methyl or ethyl alcohol containing H_2O_2 is essential (isopropanol, butanol, and acetone are not satisfactory); and (3) both fresh (including liver) and cooked fish tissues may be examined by this procedure, provided the tissues are firm and not flaky. Further studies are needed for tissue sampling sites. In this regard, preliminary studies show that liver samples can be tested by the EIA procedure, unlike the RIA method (3). As shown earlier by bioassay procedures, livers tend to show greater toxicity (11–13).

For lack of an acceptable routine procedure for the direct assessment of ciguatoxin in fish tissues, the mouse bioassay (14) remains the method of choice for most laboratories doing ciguatera research. In addition, the RIA (4, 14) has proven of value in screening *S. dumerili* (amberjack, kahala), but it is precluded for routine screening because of its cost factor and technical procedure. Nevertheless, it has been of value in the survey and assessment of new fishing grounds (5).

Based on the data presented in this study, it is suggested that the EIA procedure may be an acceptable choice for the routine direct assessment of ciguatoxin in fish tissues. The procedure is sensitive (it can detect approximately 0.01–0.005 ng), practical, and specific for ciguatoxin and ciguatoxin-like polyethers. The procedure warrants further examination and evaluation. Thus, we are presently using the procedure for routine survey of developing fishing grounds and for analyzing fishes from clinically documented ciguatera outbreaks. To improve and increase the specificity of the EIA procedure, monoclonal antibodies are being prepared to ciguatoxin and each of the polyethers cited. The Referee concurs.

In a prior report (15), the General Referee noted that under an FDA contract the Associate Referee delivered 5 of the EIA kits for evaluation in the Bureau of Foods. Our incomplete findings (G. Yang and M. Cerny, FDA, Washington, DC) indicate that we are obtaining similar results on analyzed fish (liver and flesh) samples received from the Associate Referee; further evaluation is continuing.

Shellfish Poisons.—Associate Referee William L. Childress (FDA, Boston, MA) reports investigating the use of electrochemical detection, with the desire to combine it with liquid chromatography (LCEC), to determine saxitoxin (STX), neosaxitoxin (neoSTX), and gonyautoxins 1–4 (GTX 1–4). Preliminary attempts to determine the toxins directly by differential pulse polarography have given disappointing results. In the reductive mode, no useful response could be obtained when the toxins were scanned from -0.2 to -1.25V vs SCE in 1M HOAc. Other workers have been able to obtain a response at alkaline pH (R. Gajan, FDA, Washington, DC, personal communication, 1983). Therefore, a solution of STX in 1M HOAc was made alkaline by the addition of NH_4OH and allowed to stand for 2 h. When the solution was subjected to differential pulse polarography, a dual response was obtained at about -0.9V and -1.3V vs SCE, with the latter peak being the sharper of the two. Similar experiments were run with the other toxins. A response for neoSTX was obtained at about -1.4V vs SCE, but no response was obtained with the gonyautoxins.

The electrochemical detection of saxitoxin in the oxidative LCEC mode was also briefly investigated. A solution of STX in 0.1M NH_4OAc, pH 4.5, was passed directly through an electrochemical detector (glassy carbon vs Ag/AgCl). No response was seen at potentials of up to $+1.1$V vs Ag/AgCl. Further investigation into the LCEC approach has been discontinued for the moment.

Other efforts in the Associate Referee's laboratory have been directed to determining the analytical behavior of the toxins subjected to peroxide oxidation. This effort could lead to the measurement of total PSP toxicity. Solutions of STX standard were adjusted to various pH values and oxidized with H_2O_2 (about 100°C for 30 min) such that the final concentration of peroxide was 1%. The reaction mixture was analyzed by LC using a reverse phase column (C_8, 5 μm), and a mobile phase of bufferred aqueous methanol with UV detection at 340 nm. All solutions showed multiple peaks.

Two conclusions were drawn from these experiments. (1) The oxidation reaction, at least for STX, is highly dependent upon pH, with the optimum value, at least in terms of sensitivity, coming somewhere between pH 4.0 and 5.0. (2) The

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reaction, at least in the medium of dilute HCl, is a complex one in which multiple products are formed. As a further complication, the relative amounts of the various reaction products formed change fairly rapidly with time. For these reasons, the oxidation of STX in dilute HCl is not a good analytical reaction, at least for the purposes of quantitation.

Further experiments were then undertaken in an attempt to find a suitable reaction medium which would yield a stable product or products. One of the earliest attempts at a chemical assay used 0.5M trichloroacetic acid (TCA) as the extraction medium, rather than 0.1N HCl, which is used in the mouse bioassay (16, 17). When a solution of STX (10 μg/mL in 0.5M TCA) was subjected to H_2O_2 oxidation (1% H_2O_2, 30 min at about 100°), a single sharp peak was observed upon LC analysis (C_8, 5 μm, 5% MeOH/0.05M KH_2PO_4, pH 3.0, UV at 340 nm). The peak had a k' value of 2.55. The solution was injected at 30 min intervals over a period of 4 h. The peak height remained reasonably constant, with a coefficient of variation of 2.11% for 9 injections. Re-injection of the solution after 24 h showed no change in the chromatogram. The optimum condition will be determined and applied to the other toxins; the use of other reaction media needs to be explored.

In addition to the above activity, the Associate Referee commends the noteworthy work of Sullivan and Iwaoka, who have developed an LC method for the determination of the above PSP toxins (18). This method shows considerable promise for determining the toxin profile of a particular shellfish sample, as it represents a significant saving of time and effort when compared to the TLC (19) and the classical column chromatographic methods previously used (20). It also has potential for monitoring shellfish samples for total toxicity. However, some prevailing problems are still present, namely, the low sensitivity of neoSTX, GTX_1, and GTX_4 to the oxidation-fluorimetric determinative step and the instability of the LC column. These problems are being resolved through the use of PRP-1 columns and ion-pairing (J. J. Sullivan, FDA, private communication, 1983).

The General Referee reports that work is continuing on development of a fly bioassay (A. Siger, B. C. Abbott, M. Ross, and T. Wong, University of Southern California, Los Angeles, CA) and an EIA approach (R. E. Carlson and P. E. Guire, Bio-metric Systems, Inc., Eden Prairie, MN) for the detection of PSP. With respect to these methods, the response of the housefly, *Musca domestica*, to injected STX can be quantally scored. The probit-log dose response relation is linear with a slope of 4.2 (± 0.55) and an LD_{50} of 360 pg (+ 21, − 19[S.D.]) for an injection volume of 1.5 μL. A manuscript describing the fly bioassay, its application to the analysis of shellfish samples, and comparison to the mouse bioassay has been submitted by Siger et al. for publication. Investigation into the enzyme immunoassay approach for the detection of PSP is progressing with the preparation of additional immunogens of PSP for the production of immune serum, as well as the preparation of enzyme-labeled toxins. These assay components are being assembled into solid-phase test kits for the simple colorimetric, non-instrumented measurement of toxin content in shellfish at the site of the catch. Preliminary RIA measurements demonstrate a sensitivity to 4 ng of STX, equivalent to 7 μg/100 g of shellfish meats.

The General Referee further reports the investigation at FDA's Bureau of Foods (D. Park, D. Salinger, and E. King) into a procedure reported (21) for the separation and fluorimetric detection of PSP by LC through post-column derivatization of the toxin with σ-phthalaldehyde. Using this procedure, however, multiple peaks with STX standard are being observed; the significance of this observation is under study.

Tetrodotoxin.—Associate Referee Yuzuru Shimizu (College of Pharmacy, University of Rhode Island, Kingston, RI) reports that he has made no headway towards the development of a direct chemical assay for tetrodotoxin (TTX). However, he expects to evaluate a continuous TTX analyzer constructed (22) for the routine analysis of TTX in Japanese pufferfish as a replacement to the mouse bioassay currently used in Japan (23). The method is a refinement of the alkaline oxidation procedure to detect TTX as a highly fluorescent 2-aminoquinazoline derivative which was the subject of an earlier study (24). The performance of the analyzer appears to give a good linear toxicity-fluorescence response in a wide range 0.1–20 mouse units/g with 3% variance. This procedure may have value in our continuing search for the origin of TTX. The Referee concurs.

Recommendations

Continue study on all topics.

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GENERAL REFEREE REPORTS: COMMITTEE D

Report on Alcoholic Beverages

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Arthur Caputi Jr, of the E. & J. Gallo Winery, Associate Referee for Ethanol in Wine by Gas Chromatography, recommends the method be adopted official final action with the proposed specifications of only one column (6 ft × 2 mm id glass column packed with 0.2% Carbowax 1500 on 80–100 mesh Carbopak C, 15 mL/min nitrogen carrier gas, injector and detector temperature 150°C, and oven temperature 105°C) and changing the internal standard from 0.1% v/v *n*-butanol to 0.2% v/v 2-propanol. The basis for these changes is to simplify the written procedure and reduce analysis time (to less than 2 min) while retaining the original precision. The General Referee concurred with the recommendation of the Associate Referee for adoption as official final action.

The method for ethanol in beer by gas chromatography was granted interim first action status by Committee D, and the General Referee now recommends the method be adopted official first action. A.J. Cutaia, of the Anheuser-Busch Brewery, the AOAC–ASBC (American Society of Brewing Chemists) Liaison Officer and the Associate Referee for Brewing Materials orally reports that there are no additional ASBC methods proposed for AOAC adoption this year but collaborative method studies are continuing.

Based on several years of successful and continued use, the General Referee recommends that the methods for β-asarone in wine and coumarin in wine both be adopted official final action.

The associate refereeship for diethylpyrocarbonate in beverages should be discontinued; a method has been adopted official final action and the U.S. FDA has prohibited diethylpyrocarbonate as a preservative in food and beverages. No further work is required.

Guenther Henniger has been appointed Associate Referee for Sugars in Wines. Leonard Mascaro has been appointed Associate Referee for Citric Acid in Wines. Both Associate Referees report collaborative studies are planned and results based on an enzymatic analysis should be available for next year.

Robert Dowrie of the Almaden Vineyards, the Associate Referee for Malic Acid in Wine, reports a collaborative study is planned for the coming year.

Recommendations

(1) Adopt as official final action the method for β-asarone in wine and the method for coumarin in wine.

(2) Adopt as official final action, with the noted modifications, the method for ethanol in wine by GC.

(3) Adopt as official first action the method for ethanol in beer by GC.

(4) Discontinue the associate refereeship on Diethylpyrocarbonate in Beverages.

(5) Carry out collaborative studies on malic acid in wine, citric acid in wine, and sugars in wine.

(6) Continue study on all other topics.

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Report on Cereal Foods

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Phytates—Associate Referee Barbara Harland is continuing study of phytate methods and planning a collaborative study.

Iron—Associate Referee James Martin is studying a modification of method **14.012** that could be an alternative rapid method for routine analysis of samples not known to present analytical problems. A collaborative study is planned.

Starch—Associate Referee Bert D'Appolonia has resigned. There are no major problems with the starch methods, and no new methods are under study. Therefore, this topic should be discontinued.

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Recommendations

(1) Continue study of methods for phytate and iron.
(2) Discontinue study of starch methods.

Report on Fruits and Fruit Products

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Fruit Acids.—During the past year, Associate Referee E.D. Coppola has been working with liquid chromatography (LC) using 2 reverse phase C_{18} columns to separate the different acids in fruits. He has been able to separate tartaric, quinic, malic, ascorbic, and citric acids in cranberry, grape, and other juices. However, additional work is required to check the ruggedness of these 2 columns and to see how long they will last with the separation power they exhibit at the moment. Accordingly, the Referee recommends continued study.

Iso-Ascorbic Acid 'Erythorbic Acid-Antioxidant) in Fruit Purees.—This refereeship is vacant. The Referee recommends that it be terminated.

Orange Juice Content.—Associate Referee Carl Vandercook reports that the level of interest in methodology to detect adulteration in citrus juice remains high. This was demonstrated by the attendance of over 100 people at a recent 2-day workshop (New Orleans, June 16 and 17, 1983) sponsored by the Association of Food and Drug Officials. A number of new and promising techniques were discussed. The isotope ratio studies have been extended to include ^{18}oxygen. Another new technique is the application of the "phosphorous analyzer," developed by Bessman of the University of Southern California, to citrus juices. Around 25 phosphate esters were separated and identified by this procedure. In view of the continuing interest and research activity, it is recommended that the study on orange juice content be continued.

Fruit Juices, Identification and Characterization.—This refereeship is vacant. The Referee has just recently recommended that Ronald W. Wrolstad of Oregon State University be appointed as Associate Referee.

Detection of Adulteration of Apple Juice.—The Referee has just recently recommended that John Zyren of National Food Processors Association be appointed Associate Referee.

Sodium Benzoate in Orange Juice.—James Fisher of the Florida Department of Citrus has recently been appointed as Associate Referee.

Adulteration of Orange Juice by Pulpwash and Dilution.—The Referee recommends that work continue on this subject.

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Recommendations

(1) Terminate the topic Iso-Ascorbic Acid (Erythorbic Acid-Antioxidant) in Fruit Purees.
(2) Continue study on all other topics.

Report on Nonalcoholic Beverages

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J.M. Newton has investigated a procedure for simultaneously determining caffeine, theobromine, and theophylline in cola and chocolate beverages.

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Recommendations

(1) Continue study of a method that can detect the major methyl xanthenes in various nonalcoholic beverages.
(2) Appoint an Associate Referee for the quantitative assay of glycyrrhizic acid salts in licorice-derived products.
(3) Appoint an Associate Referee for the determination of quinine in various nonalcoholic beverages.

Report on Preservatives and Artificial Sweeteners

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Benzoates, Saccharin, and Caffeine (Liquid Chromatography).—Associate Referee B. Woodward and others (1,2) previously reported a successful collaborative study which was subsequently adopted official first action for soda beverages (**12.050–12.053**) in 1979. Due to other commitments, no work was performed during the past year. The work will be continued by Norma Webb, who has been recommended as an Associate Referee under the topic, Aspartame, Benzoates, Saccharin and Caffeine by Liquid Chromatography.

Formaldehyde in Olives.—Associate Referee R. J. Reina has conducted a limited, within-laboratory study for the determination of formaldehyde in olives at levels of 5 and 1 ppm. The method consisted of developing a derivative of the formaldehyde with 2,4-DNPH. The derivative was then extracted and cleaned up through an acidic alumina column and then determined by liquid chromatography using a C_{18} column with a mobile phase of CH_3CN-H_2O (55 + 45) and a UV detector at 254 nm. The limited study was performed by 3 analysts on olives spiked at 2 different levels (5 and 1 ppm). The recoveries at 5 ppm ranged from 69.1 to 83.6% and at 1 ppm ranged from 67.6 to 95.1%. Additional work is planned to improve the recoveries and reproducibility. It is recommended that study be continued.

Meats, Ground, Screening Method for Chemicals and Added Blood.—Associate Referee J.J. Maxstadt (3) previously reported on a method which was adopted official first action for chemical preservatives in ground meats (**20.A01–20.A05**). As a result of an inquiry regarding the possible interferences of phthalate esters when they were found in beef jerky, a limited study was performed to resolve the problem. The study concluded that phthalate ester interference can be eliminated by a combination of AOAC methods **20.A01** and **12.050**, where LC conditions are optimized for separation of benzoate and sorbate. It is recommended that study be continued on this topic.

Organic Preservatives (Thin Layer Chromatography).—Associate Referee C. P. Levi previously conducted a limited within-laboratory study with 6 types of food spiked within 9 common preservatives. Additional work was planned; however, no report was received during the past year. It is recommended that the study continue.

Saccharin in Foods (Differential Pulse Polarography).—Associated Referee W. Holak previously reported on a successful collaborative study which was adopted as official final action (**20.A06**) in 1982. The method should be editorially revised to correct an error in the calculations in **20.A10** to read as follows;

$$\% \text{ (w/w)} = \mu g \text{ (from std addn curve)} \times (D + E/CD) \times (B/2) \times 10^{-4}$$

It is recommended that the study be discontinued.

Aspartame, Benzoates, Saccharin, and Caffeine (Liquid Chromatography).—N. G. Webb and D. D. Beckman have developed a method for the determination of aspartame in beverages and beverage mixes by liquid chromatography. The separation was achieved on a C_{18} column using a mobile phase of acetic acid, water, and isopropyl alcohol at pH 3.0 and a UV detector at 254 nm. The beverages were filtered through 0.45 μm filters and injected directly into the chromatograph. Quantitation of the aspartame was made in the presence of other additives in beverages such as saccharin, caffeine, sodium benzoate, artificial colors, and artificial flavors. The products included in this paper included spiked soda beverages, fruit-flavored mixes, instant tea, reconstituted drink mixes, and a powdered tabletop sweetener. The products were spiked at the expected levels ranging from 0.040 to 0.066 g/100 mL. Except for the 2 instant tea samples, the recoveries ranged from 93 to 102%. The recoveries for the 2 tea samples were 122 and 130%. It is recommended that N.G. Webb be appointed Associate Referee for this topic and that study be continued.

Recommendations

(1) Appoint Associate Referees for the topics Benzoates and Hydrobenzoates in Foods; Preservatives (Quantitative Methods).

(2) Adopt as official final action the method for sodium saccharin, sodium benzoate, and caffeine by LC (**12.050–12.053**). Discontinue study.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition, and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **63**, 374–423 (1980); **64**, 501–540 (1981); **65**, 450–521 (1982); **66**, 512–564 (1983).

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(3) Continue official first action status of method for chemical preservatives in ground meats (**20.A01–20.A05**); continue study of the method for added blood.

(4) Discontinue topic for Saccharin (Differential Pulse Polarography).

(5) Appoint N.G. Webb as Associate Referee for the topic Aspartame, Benzoates, Saccharin, and Caffeine (Liquid Chromatography).

(6) Continue study on all other topics.

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Report on Spices and Other Condiments

RAYMOND WAY

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No activity was reported on any topic.

Ash and Pungent Principles in Mustard.—I have recommended that this be considered inactive at this time. I have been unable to find as Associate Referee for Ash in Mustard. The American Spice Trade Association has adopted the AOAC method **30.026(a)** as an alternative method for volatile oil in mustard seeds.

Monosodium Glutamate, and Vinegar.—I have recommended that these topics be considered inactive for lack of Associate Referees.

I recommend a new Associate Referee topic be established under the title Ethylene Oxide Residue in Spices. Preliminary investigations by several spice companies have indicated that the analysis of ethylene oxide in whole and ground spices needs to be studied on a collaborative basis. Such studies have been initiated and are being conducted in accordance with AOAC principles.

Study should contine on all other topics.

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Report on Sugars and Sugar Products

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Chromatographic Methods.—Associate Referee Michael Gray reports no new developments at present, but plans to initiate collaborative studies for chromatographic methods in several areas.

Color, Turbidity, and Reflectance—Visual Appearance.—Frank Carpenter, the Associate Referee, reports no new developments on the attempts to revise the AOAC method for color in sugars to conform to ICUMSA.

Corn Syrup and Corn Sugar.—Associate Referee Raffaele Bernetti recommends adoption as official first action of the interim official first action electrometric titration method for titratable acidity in corn syrups to replace the present phenolphthalein end point method at pH 8.3. Since the end point is taken at pH 6, some question was raised as to whether or not different entities were being measured. Dr. Bernetti believes the electrometric method redefines neutrality as pH 6.0 where the titration curves he developed show a sharp inflection. Very little additional alkali is needed to reach pH 8.3.

Dr. Bernetti reports that the results for a Corn Refiners Association collaborative study on a colorimetric procedure for the determination of sulfur dioxide in corn syrups is under review. This proposed alternative to the time-consuming Monier-Williams method is a colorimetric method using *p*-rosaniline and formaldehyde reagents to detect 50–100 ppm and 0–5 ppm SO_2 in various corn syrups.

The Associate Referee reports that a collaborative study is planned to determine cadmium and zinc in food starches and starch conversion products. Five collaborators have been assigned to examine 8 samples.

The Augustana Research Foundation has issued its reports to the Corn Refiners Association. The information for dry substance tables for corn syrups and blends has been submitted for publication in the *Journal of Chemical and Engineering Data*. Dr. Bernetti plans to recommend changes in tables **31.09** and **31.10** based on these data.

Dr. Bernetti believes that the methods for saccharides by liquid chromatography in **31.228–31.236** should be revised to reflect availability of commercial chromatographic columns and deionizing packings. Some are no longer available. He recommends that apparatus be described in a generic manner and that column packing procedures be abbreviated or eliminated.

Enzymatic Methods.—Associate Referee Marc Mason has presented a contributed paper on enzyme electrodes for LC

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detection. Collaborative studies relating to determinations by enzymatic methods are planned for next year.

Honey.—Jonathan White, the Associate Referee for Honey, submitted the results of a collaborative study on the visual classification of honeys using the Lovibond 2000 Honey Color Comparator. Fourteen collaborators classified 6 honeys and obtained favorable results. Of 47 judgments, 46 agreed with the consensus. Dr. White recommends that the classification of honey using the Lovibond 2000 Comparator be adopted as official first action.

Lactose Purity Testing.—Last year, Associate Referee Janice Saucerman presented a limited collaborative study on the quantitative LC determination of lactose purity. The study was not adopted at that time although excellent precision data were presented. The new results which she presented are data from her second study. This study shows excellent precision, repeatability, and reproducibility, with few outliers, along with demonstrated specificity, applicability, and ruggedness. The Associate Referee again recommends adoption as official first action.

Maple Sap and Syrups.—Maria Franca Morselli recommends adoption official first action of the interim official first action mass spectrometric carbon isotope ratio method for the determination of corn and cane based sugars in maple syrup. The Associate Referee presented a contributed paper on the GC determination of nonvolatile organic acids in maple syrups. A manuscript reporting sodium and chloride as sources of off-flavor in maple syrups is being revised for publication in the *Journal* of the AOAC. Dr. Morselli reports that her staff at the University of Vermont will continue with the work toward indicated revisions of the maple syrup section of Chapter 31 in an effort to provide current and improved methods of analysis.

Stable Carbon Isotope Ratio Analysis.—Associate Referee Landis Doner reports plans to examine ^{18}O values in water samples and recommends continued study.

Standardization of Sugar Methods of Analysis.—Margaret Clarke had no scheduled studies to report. She plans to institute several collaborative studies in the coming year: (1) A study to test the Roberts Copper method for dextran analyses in raw and refined sugars. (2) A study for color determination using ICUMSA Method 4, the official method for white sugars, using 2 buffers instead of acid and base adjustment to pH 7. (3) A study of an LC method for molasses and cane juices using ion exchange columns.

In addition, Dr. Clarke would like to hear from anyone interested in rapid methods for sulfite analyses which would be suitable for sugars.

She reports that at the June 1982 ICUMSA meeting in Dublin, an important topic was Subject 5, Polarimetry, for which the new 100°S point was adopted. The Chairman of ICUMSA, Dr. Reinefeld, is presently attempting to get acceptance by letter vote. The U.S. National Committee voted to accept the new 100°S point.

Sugars in Cereals.—Associate Referee Lucian Lygmunt recommends a nonsubstantive change in **14.C01–14.C04,** the LC procedure for sugars in cereals, to refer to 2 additional steps to eliminate potential interference by sodium chloride as described by DeVries, et al. (*J. Assoc. Off. Anal. Chem.* (1983) **66,** 197–198), but with the additional qualification that the remedy provides only a temporary cure (e.g., 10 injections).

The Associate Referee reports that further examination will be made of the use of mobile phases, inadequate resolution, and alternative column choices to improve the analytical results.

Sugars in Licorice Products.—Associate Referee Raymond Tuorto has presented a report describing a collaborative study of the determination of sugars in licorice products by LC. He recommends adoption as official first action.

Weighing, Taring, and Sampling.—The Associate Referee, Melvin Lerner, reports no new developments in the subject area.

Recommendations

(1) Adopt as official first action the interim official first action electrometric titration method for titratable acidity in corn syrups to replace the present phenolphthalein end point method, **31.217.**

(2) Adopt as official first action the classification of honey color using the Lovibond 2000 Comparator.

(3) Adopt as official first action, the quantitative LC method for the determination of lactose purity.

(4) Adopt as official first action the interim official first action mass spectrometric carbon isotope ratio method for the determination of corn and cane sugars in maple syrup.

(5) Make a non-substantive change in **14.C01–14.C04,** the LC method for the determination of sugars in cereals, to refer to 2 additional steps for the elimination of potential interference by sodium chloride as described by DeVries et al. in *J. Assoc. Off. Anal. Chem.* **66**(1), 197–198 (1983) but with the additional qualifying statement that the remedy provides only a temporary cure (e.g., 10 injections).

(6) Adopt as official first action the LC method for the determination of sugars in licorice products.

(7) Continue study on all topics.

Report on Vitamins and Other Nutrients

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Three stimulating vitamin analysis sessions were held at the 97th Annual Meeting of AOAC.

"Instrumental Methods for the Analysis of Vitamins" (Water-Soluble Vitamins by LC).

Poster session on vitamin and other nutrient assays.

Round table discussion for intended LC collaborative study for vitamin D in foods and infant formula.

James P. Clark, Henkel Corp., Associate Referee for Vitamin E, has resigned and Francis Amore of that organization has replaced him.

Patric Bryan of Mead Johnson and Co. has resigned as Associate Referee for Folate Activity. Lynn Hoepfinger of that organization has replaced him.

Donald P. Parrish, Manhattan State University, has resigned as Associate Referee for Vitamin A and James N. Thompson of the Canadian Food Directorate has replaced him.

James T. Tanner, FDA, and Steven A. Barnett, Mead Johnson and Co., have been appointed Co-Associate Referees for Nutrient Assay of Infant Formula.

The topics Vitamin C in Milk-Based Foods, Niacinamide (Polarography), Choline in Foods, and Energy Value of Foods (Biological) have been discontinued and the people that held these assignments are thanked for their efforts on behalf of the Association.

Recommendations

(1) Adopt as official final action the official first action method for fat in food by chloroform-methanol extraction.

(2) Adopt as official first action the collaboratively studied method for dietary fiber.

(3) Adopt as official first action the interim official first action semiautomated method for vitamin C in food products.

(4) Continued study on all other topics.

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GENERAL REFEREE REPORTS: COMMITTEE E

Report on Industrial Process Waste

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The hazardous waste management problem is one of increasingly strong concern both to the public and to those government organizations charged with protecting public health and the environment. To ensure that regulatory actions are based on sound data, it is critical that the methodology used to gather such data be capable of measuring the property of interest. To promote uniformity and reliability in analytical and other test methods needed to implement the hazardous waste management control programs mandated under the Resource Conservation and Recovery Act (RCRA), the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) (Superfund), the Canadian Transportation of Hazardous Goods Act and various state and provincial laws, the Industrial Process Waste refereeship was established this year in AOAC.

The scope of coverage of this refereeship is very broad, encompassing chemical, physical, and biological test methods. The methods developed and collaborated under this refereeship will be used to analyze rather concentrated levels, 1 ppm to 50%, of toxic organic and inorganic chemicals in industrial process wastes and other matrixes encountered during hazardous waste management. Methods to be studied will generally be applicable to the analysis of many components in more than one matrix. In addition, methods to measure hazards associated with such wastes (e.g., explosivity, corrosivity, flammability, biological activity) will be collaborated.

The General Refereeship is divided into 3 Associate Refereeships:

Inorganic Analytes—Florence Richardson
Organic Analytical Methods—James Poppiti
Physical Chemical Properties—Florence Richardson

In addition to finding an Associate Referee to replace Florence Richardson on the inorganic analytical methods, Associate Referees are needed so that the following additional topics can be established in 1984: Sampling; Bioassays.

During the past year, several single-laboratory or interlaboratory collaborative studies were initiated.

Inorganic Analytes Methods.—During the past year, 5 EPA methods for determining the presence and concentration of hexavalent chromium in the presence of trivalent chromium have been the subject of a single laboratory precision and accuracy study. The driving force for this evaluation has been EPA's contemplated change in the Extraction Procedure Toxicity (EP) (EPA Methods 1310) Characteristic from regulatory thresholds based on total chromium concentration in the extract to one based on concentration of the hexavalent species only. The 5 methods being evaluated are (1) coprecipitation/atomic absorbtion spectroscopy; (2) chelation/extraction/atomic absorbtion spectroscopy; (3) colorimetry; (4) ion chromatography/conductivity; (5) differential pulse polarography.

Spiked and unspiked EP extracts of actual wastes were analyzed for hexavalent chromium by using all 5 methods. Results of the analyses suggest that colorimetry is the most generally applicable overall. Differential pulse polarography is best where accuracy is the primary consideration, and coprecipitation rated highest where precision was primary. Chelation was ineffective, correctly analyzing only 2 of the 3 wastes, and ion chromatography was totally inadequate.

Because problems were found with all methods, before any recommendation for action can be made, further method evaluation studies are needed. EPA, under the direction of Warner Beckert (EMSL-LV), is undertaking such studies.

Organic Analytes.—The combination of EPA Solid Waste Methods 3550 (sonication extraction with CH_2C1_2) and 8270 (capillary column GC/MS) is used to determine the concentration of semivolatile organic compounds in a variety of solid waste matrixes. This method is applicable to nearly all types of samples regardless of water content, including aqueous sludges, waste solvents, oily wastes, filter cakes, and sediments. Method 8720 is designed to quantitate most neutral, acidic, and basic organic compounds that are soluble in methylene chloride and capable of being eluted without derivitization as sharp peaks from a fused silica capillary GC column with a slightly polar silicone phase (DB 5). Such compounds include polynuclear aromatic hydrocarbons, chlorinated hydrocarbons, phthalate esters, organophosphate esters, nitrosamines, haloethers, aldehydes, ethers, ketones, anilines, pyridines, quinolines, aromatic nitro compounds, and phenols. The detection limit for determining an individual compound is approximately 1 μg/g when the sample contains less than 1 mg/g of solvent extractable material.

During the past year, the combination has been subjected to an 8-laboratory collaborative test using 9 sample types. A total of 137 semivolatile compounds were spiked into the waste samples for this evaluation, with spiking concentrations ranging from 4 to 11400 μg/g.

While the results of the evaluation were generally favorable, the methods gave poor results for benzoic acids and nitrophenols. Best results were found for aromatic hydrocarbons and halocarbons. The within-laboratory component of the variability averaged about 30%, with the between-laboratory variability about twice that. Seventy percent of the high level spikes were reported by the laboratories, while only 50–60% of the low spikes were found.

A final report on the results of this collaborative study is expected by the end of this year. A recommendation would include only those compounds for which the methods were found to give acceptable precision and accuracy. Further work is being undertaken by EPA under the direction of Ann Alford-Stevens to determine the reasons why the methods did not work for certain compounds.

Physical Chemical Properties—Cyanide/Sulfide Evolution Method.—RCRA §261.23 identifies as hazardous wastes those cyanide- or sulfide-bearing wastes which, when exposed to pH conditions between 2 and 12.5, generate toxic gases or fumes in a quantity sufficient to present a danger to human health. The test method being evaluated in this effort is designed

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to be a semiquantitative means of identifying those waste materials which meet the above definition. The method uses detection tubes to measure the amount of hydrogen cyanide or sulfide generated under a defined set of conditions.

The method has been subject of a collaborative study this past year. The initial results indicate that the method may be suitable for identifying hazardous sulfide-bearing wastes; however, there appears to be a problem when the method is used for cyanide wastes. Some unidentified interference appears to temporarily stain the detector tube during analysis.

While the initial evaluation shows promise, the interlaboratory RSD was quite high. Further study, especially with respect to stirring rate, rate and method of acidification, and reaction time, appears to be necessary before the method is ready for recommendation. Such work is being conducted, at this time, under the direction of Robert Handy (Research Triangle Institute).

Explosivity.—RCRA §261.23 identifies as hazardous wastes those waste materials which are capable of detonation. The U.S. Card Gap Test and the U.S. Internal Ignition Test are under evaluation as a means of determining if a given waste material can undergo detonation under reasonable mismanagement conditions. The methods are currently the subject of a single-laboratory evaluation at the Bureau of Mines laboratory in Pittsburgh, PA.

Samples of sludge from several explosives processing waste treatment facilities have been collected and analyzed in triplicate by each test. In addition, a series of standard explosives has been obtained for use in calibrating the tests.

The sensitivity of the test procedures is being evaluated. A report summarizing the single laboratory precision of the tests is anticipated during the first quarter of 1984.

Recommendations

(1) Continue to evaluate the available methodology for analysis of hexavalent chromium in the presence of trivalent chromium.

(2) Finalize and report the performance of the sonication/capillary column GC/MS method and initiate a first action on the method.

(3) Finalize and report on the performance of the test method for hydrogen sulfide evolution and initate a first action on the method.

(4) Continue work on the method for hydrogen cyanide evolution.

(5) Finalize and report on the performance of the test method for explosivity.

(6) During the coming year, initiate single laboratory evaluations of the following EPA solid waste methods: Method 6010, inductively coupled plasma spectroscopy; Method 8280, chlorinated dibenzo-*p*-dioxin and chlorinated dibenzofurans in Wastes; Method 8150, chlorinated herbicides by gas chromatography.

(7) During the coming year, initiate interlaboratory collaborative evaluations of EPA solid waste testing Method 6010 (inductively coupled plasma spectroscopy) and of the EPA method, daphnia magna chronic bioassay.

Report on Metals and Other Elements

KENNETH W. BOYER
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Atomic Absorption Spectrophotometry (AAS).—Milan Ihnat, Associate Referee, is continuing evaluation of parameters affecting the reliability of flame AAS measurements in AOAC and other AAS methods before consolidating parts of these methods into a general AAS analytical scheme. During the past year the Associate Referee published an evaluation of the importance of acid-insoluble residues in plant analyses (*Comm. Soil Sci. Plant Anal.* (1982) **13**, 969–979). The Associate Referee has begun preparation of several well characterized agricultural materials, which will represent the wide range of matrixes and elements found in plant and animal samples. These matrixes, along with carefully prepared standard solutions, will be used both to evaluate consolidated AAS analytical schemes in the Associate Referee's laboratory and as samples for collaborative study of the most promising consolidated candiate methods resulting from this study. The Associate Referee hopes to initiate a collaborative study of a general AAS scheme in 1984.

Cadmium and Lead in Earthenware.—Benjamin Krinitz, Associate Referee, reports that he is studying leaching of decorated teacup rims for leachable Pb. The hot leach method for determining extractable Cd and Pb in ceramic cookware and enameled cookware by AAS, **25.D11–25.D17**, collaboratively studied by John Gould, Food and Drug Administration, Division of Chemical Technology, was adopted interim official first action in December 1982 after the manuscript describing the study was modified to comply with the requirements for clarification stipulated by Committee E. The manuscript was subsequently published (*J. Assoc. Off. Anal. Chem.* (1983) **66**, 610–619). The method is currently being balloted as a draft proposed method by the subcommittee for ceramic cookware of the International Standards Organization (ISO) (ISO TC 166/SC1). If it is approved at this level it will be balloted by the full ISO Technical Committee for adoption as a draft international standard.

Carbon Rod Atomization.—Robert Dabeka, Associate Referee, reports that a manuscript describing the collaborative study of a rapid screening method for Pb in canned milk and infant formula has been submitted for publication. As discussed in last year's General Referee report, this study, which was not carried out under AOAC auspices, was unsuccessful because some collaborators used instrumentation with inadequate background correction capability. The Associate Referee wants to repeat the collaborative study in 1984 with the technique he has developed for collaborators to check the adequacy of background correction being incorporated into the study protocol. The Associate Referee requests that

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the topic be renamed "Graphite Furnace Atomic Absorption Spectrophotometry" (GF-AAS) to better reflect the subject area of interest. The General Referee agrees with this request. The Associate Referee is currently evaluating GF-AAS for determination of subnanogram/gram levels of Pb and other elements in foods.

Emission Spectrochemical Methods.—Fred Fricke, Associate Referee, reports no progress during the past year toward conducting a collaborative study of an inductively coupled plasma (ICP) method for multielement analysis of foods. However, he does feel that ICP methodology is far enough advanced and that enough laboratories now have ICP capability to conduct such a collaborative study. During the coming year the Associate Referee will be reviewing candidate methods and seeking collaborators for the study.

Fluorine.—Robert Dabeka, Associate Referee, is re-evaluating the ion specific electrode method for determination of fluoride in foods. This method was previously collaboratively studied (*J. Assoc. Off. Anal. Chem.* (1981) **64,** 1021–1026), but was not adopted by the AOAC because of the necessity to freeze-dry foods, the limited concentration range, and the limited number of foods tested in the collaborative study. The method is being applied to fresh foods with the objectives of eliminating the freeze-drying step and increasing the applicable concentration range of the method. If results are promising and time permits, a collaborative study of the modified method may be conducted in 1984.

Hydride Generating Techniques.—Stephen Capar, Associate Referee, reports that after reviewing a number of hydride generating systems described in the literature, his laboratory has constructed a simple hydride generator from commonly available laboratory ware. The performance parameters of this system are currently being evaluated in conjunction with flameless AAS detection for the determination of Sn in foods. A preliminary evaluation of the system suggests that it should be useful for determination of Sn in foods ranging from low ppb to high ppm levels. It is anticipated that this work will lead to a collaborative study of a method for Sn that is applicable to a much wider range of Sn levels than can be determined by current flame AAS methods.

Mercury.—Walter Holak, Associate Referee, reports that his method for methyl mercury in fish, which uses liquid chromatography (LC) for separation coupled with flameless AAS detection, has been published (*Analyst* (1982) **107,** 1457–1461). This method overcomes some of the difficulties in sample preparation and gas chromatographic (GC) columns associated with currently used methods for methyl mercury. The Associate Referee would like to collaboratively study this method during the next year if enough laboratories with LC and AAS capabilities can be found.

Methyl Mercury in Fish and Shellfish.—Susan Hight, Associate Referee, reports that the GC method for methyl mercury in fish and shellfish, **25.D05–25.D10**, which was adopted official first action last year, has been extended to include commercially prepared freezer case seafoods, such as breaded or batter-dipped fish and shellfish products. A few laboratories have objected to the use of benzene in the method as the extraction solvent for methyl mercury. The Associate Referee is currently evaluating the use of toluene as a substitute for benzene in the method. She also intends to evaluate a new Snyder column that would not require modification for benzene evaporation. This method should be continued as official first action pending outcome of these evaluations.

Multielement Analysis of Infant Formula by ICP.—Ronald Suddendorf, Associate Referee, has completed the data evaluation of the collaboratively studied method for ICP determination of Ca, Cu, Fe, K, Mg, Mn, Na, P, and Zn in infant formulas. In this study 6 laboratories each analyzed 6 infant formulas (2 each ready-to-feed, concentrated, and powdered) as blind duplicates at the 2 levels each for 9 elements. Statistical analysis of the data indicates that the overall interlaboratory coefficients of variation (CV_x) for most of the elements in most of the infant formulas studied are consistent with CV_x values (as a function of concentration) historically associated with collaborative studies of acceptable methods for trace elements in foods. One very high CV_x (66.3%) for Fe in one powdered infant formula suggests that there may have been a homogeneity problem or perhaps laboratory contamination associated with this sample. Slightly higher than desirable CV_x values were exhibited for the following: (a) one powdered infant formula for Cu; (b) several samples, both liquid and powdered, for P; (c) 2 samples for K; and (d) 2 samples for Ca. The overall average recovery for each of the 9 elements ranged from 87% for Fe to 100% for P. This method is being recommended for adoption as official first action.

Multielement Determination After Closed System Digestion.—Walter Holak, Associate Referee, reports that the extension of method **25.A01–25.A05** to include Cu, Ni, and Cr reported last year has been published (*J. Assoc. Off. Anal. Chem.* (1983) **66,** 620–624). The Associate Referee would like to collaboratively study this method extension and is seeking participants with AAS and anodic stripping voltammetry (ASV) capabilities.

Multielement Residues by Resin Column Separation.—Richard Baetz, Associate Referee, reports no progress for the year. Since the Associate Referee has not had an opportunity to work on resin column separation of metals for several years, it now seems desirable to combine this topic with the topic "Organometallics in Foods" and to rename the combined refereeship "Separation Techniques for Trace Elements in Foods." The Associate Referee agrees with this approach. John Jones, FDA, Division of Chemical Technology, the current Associate Referee for Organometallics in Foods, has been working for several years on resin column separation techniques for trace metals in foods, and has agreed to assume responsibility for the combined refereeship. Mr. Jones anticipates being able to collaboratively study the Chelex-100 separation method for ICP determination of trace elements in foods, which has been published (*Analyst* (1982) **107,** 353–377). Mr. Jones has suggested that this study could be carried out in cooperation with the Associate Referee on Emission Spectrochemical Methods.

Organometallics in Foods.—John Jones, Associate Referee, reports no progress for the year. However, he has been actively studying resin column separation procedures for trace elements in foods (see the discussion above).

Polarography.—Raymond Gajan, the Associate Referee who has served admirably in this role, is no longer actively working in the area of trace elements in foods. However, the official first action method, **25.C01–25.C07**, for Pb and Cd in foods is currently being extensively used to gather data on these elements in canned foods. This method should be continued in official first action status pending evaluation of these data.

Tin.—Edgar Elkins, Associate Referee, cooperated with Robert Dabeka, Health and Welfare, Canada, who has completed a collaborative study of a nitrous oxide-acetylene flame AAS method (*J. Assoc. Off. Anal. Chem.* (1981) **64,** 1297–1300) for Sn in canned foods. Evaluation of the data from the collaborative study is in progress. A preliminary look at the data by the General Referee indicates that the method is satisfactory for Sn in foods at levels down to about 25 ppm.

According to the Associate Referee, the method collaboratively studied by Dabeka requires fewer laboratory manipulations and is more rapid than the current official first action method, **25.136–25.138**. After a complete statistical analysis of the data, the method will be recommended for interim official first action if appropriate.

Voltammetric Methods.—Eric Zink, Associate Referee, reports that the 2 manuscripts relating the method development and collaborative study, respectively, of official first action method **25.080–25.082** for ASV determination of Pb in evaporated milk and fruit juice have been published (*J. Assoc. Off. Anal. Chem.* (1983) **66,** 1409; 1414). This method should be adopted official final action.

Comment.—During this past year the General Referee edited and updated Chapter 25 for inclusion in the 14th edition of *Official Method of Analysis*. A format for grouping methods under the broad headings of "Multielement Methods" and "Single Element Methods" was adopted in order to logically accommodate the increasing number of multielement methods of analysis being developed.

Recommendations

(1) Continue assessment of AAS methods and develop a comprehensive AAS analytical scheme for collaborative study.

(2) Adopt as official first action the interim first action of hot leach method, **25.D11–25.D17**, for leachable Pb and Cd from cookware.

(3) Rename the "Carbon Rod Atomization" refereeship to be "Graphite Furnace-Atomic Absorption Spectrophotometry."

(4) Collaboratively study an ICP method for multielement analysis of foods.

(5) Collaboratively study the LC-AAS method for methyl mercury in fish.

(6) Continue official first action status of method **25.D05–25.D10** for GC determination of methyl mercury in fish and shellfish.

(7) Adopt as official first action the ICP method for multielement analysis of infant formula.

(8) Collaboratively study the extension of the multielement determination after closed system digestion method, **25.A01–25.A05**, to include Cu, Ni, and Cr. Continue official first action status for this method.

(9) Combine the "Multielement Residues by Resin Column Separation" refereeship with the "Organometallics in Foods" refereeship. Name the combined refereeship "Separation Techniques for Trace Elements in Foods." Collaboratively study the Chelex-100 method for ICP determination of trace elements in foods.

(10) Continue official first action status of the dry ash ASV method, **25.C01–25.C07**, for Pb and Cd in foods.

(11) Complete statistical evaluation of AAS method for Sn in canned foods. Continue official first action status for method **25.136–25.138**.

(12) Adopt as official final action method **25.080–25.082** for ASV determination of Pb in evaporated milk and fruit juice.

(13) Continue work on all other topics.

Report on Multiresidue Methods (Interlaboratory Studies)

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Four Associate Referees have reported on the status of the work on their topics. The topics Pesticides in Meat and Meat Products and Gas Chromatography (Alkaline Petroleum) have been discontinued. Transfer of the topic Pesticides in Whole Blood is recommended.

Comprehensive Multiresidue Methodology.—Leon Sawyer was appointed Associate Referee on this topic during the past year and has completed a collaborative study on the method of Luke et al. (*J. Assoc. Off. Anal. Chem.* (1981) **64,** 1187–1195) with modifications. In the study, 10 laboratories analyzed 3 commodities for 6 different pesticides, with each commodity represented by 3 separate samples. The pesticides included were α-BHC, dieldrin, chlorpyrifos, omethoate, acephate, and monocrotophos. The commodities were lettuce, tomatoes, and strawberries. Two different GC detection systems were included: the Hall 700A electrolytic conductivity detector for the organochlorine compounds (including chlorpyrifos) and the flame photometric detector (phosphorus-selective mode) for the organophorus pesticides (including chlorpyrifos). Because chlorpyrifos was quantitated on both systems, there was a total of 63 determinations per laboratory. Included in the 63 determinations were 16 pairs of blind duplicates (same level and crop, different sample code) which required a total of 19 duplicate determinations per laboratory when the chlorpyrifos results for the 2 separate GC determinations are counted. Tentative review of the data indicates a high potential for supporting a recommendation of official first action adoption. This General Referee wishes to acknowledge the outstanding accomplishment of a very significant collaborative study. Recommendation for adoption will be reviewed and processed quickly upon receipt of final statistical review of data, complete report of the collaborator study, and recommendation from the Associate Referee.

Gas Chromatography (Alkaline Pre-column).—This topic was discontinued during 1983. The past Associate Referee was not able to finalize a publication covering the overall information on the effectiveness of the alkaline precolumn (*J. Assoc. Off. Anal. Chem.* (1969) **52,** 548–564) for providing quantitative results in actual food analyses. A collaborative study involving solutions of pesticides (dichloro-bis(phenyl) ethane derivatives) had been completed much earlier and subsequent intralaboratory studies had been conducted to show effectiveness with actual food analyses.

Organophosphorus Pesticide Residues.—Associate Referee R. Laski is working toward extension of **29.039–29.043** to include additional polar organophosphorus pesticides and metabolites of current interest. During 1984, he hopes to

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investigate recoveries of approximately 14 chemicals and to compile and summarize available data on recoveries by this method.

Pollutant Phenols in Fish.—Associate Referee Larry Smith has developed a tentative procedure involving a dual column chromatographic operation to recover purified phenolic and other acidic residues by, derivatization, and an electron capture GC determinative step. A wide range of phenolic and other acidic residues are recovered by this approach. The Associate Referee plans to conduct an interlaboratory evaluation of the procedure, with a future collaborative study if the interlaboratory evaluation is successful. I concur with this course of continued study, but recommend that the method be published prior to collaborative study.

Whole Blood.—Associate Referee H. M. Stahr has been unable to complete a collaborative study of his method for chlorinated pesticides in whole blood (*J. Assoc. Off. Anal. Chem.* (1980), **63**, 965–969). Six samples of freeze-dried blood with lindane and mirex added by feeding a bovine animal, were distributed to laboratories but not completed. The Associate Referee has earlier demonstrated stability of the pesticides in the freeze-dried blood. Because of the close relationship between this residue method study and veterinary toxicology, I recommend this topic be transferred to the Committee G General Referee on Veterinary Analytical Toxicology.

Recommendations

(1) Complete the evaluation and report of the collaborative study of the method report in *J. Assoc. Off. Anal. Chem.* (1981) **64**, 1187–1195; recommend adoption as appropriate during the coming year.

(2) Continue study of **29.039–29.034** to extend the coverage of this method to additional organophosphorus pesticides and additional crops; continue study to compile and summarize available data on the recovery of organophosphorus pesticides by this method.

(3) Continue study on topic of Pollutant Phenols in Fish; publish the method prior to implementation of collaborative study.

(4) Transfer the topic pesticides in whole blood to the General Refereeship on Veterinary Analytical Toxicology, Committee G.

Report on Organohalogen Pesticides

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During the past year, a total of 25 Associate Referee topics have been dealt with by this General Referee. Written Associate Referee reports were received from 4 of the 20 referees currently assigned to topics, despite the deadline announced to them in January by the Association. The remaining referees were contacted by telephone. The current status of each topic follows.

Chlordane.—(Wilbur Saxton, FDA, Seattle, WA) The Associate Referee has been unable to devote time to this project this year.

Chlorinated Dioxins.—(David Firestone, FDA, Washington, DC) The Associate Referee has reported on progress in development of methods for part per trillion and part per quadrillion levels of 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (2,3,7,8-TCDD) in fish, municipal and industrial waste water, soil, sediment, and human tissue. Other chlorinated dioxins and furans are also measured by some of the methods. The several studies are being conducted by U.S. federal agencies with jurisdiction in such areas (FDA, EPA, and VA). The Referee acts as a consultant, formally and informally, for much of this work and thus is able to maintain an overview of the progress.

The low levels involved in these analyses necessitate the use of methods with multiple cleanup steps, determination by sophisticated instrumentation, and often the use of internal standards to compensate for expected losses through the lengthy procedures. Each laboratory follows similar schemes, but each chooses differently from among the many ways to clean up the samples and detect the residues. For these reasons, it may be very difficult to organize a traditional collaborative study for any particular method for 2,3,7,8-TCDD.

The U.S. EPA maintains a quality assurance program among the contract laboratories which work on analyses such as these. If the quality assurance program were set up to meet the statistical criteria the AOAC recommends, the long-term results of these studies may provide sufficient data to support official status for the methods without a traditional collaborative study.

Chlorinated Hydrocarbons in Poultry.—(James Ault, ABC Laboratories, Columbia, MO) This Refereeship was transferred from Committee C to Committee E in January 1983. The Associate Referee submitted during the year the results of a collaborative study of 16 organochlorine pesticides in poultry fat. The analytical method involves separation of pesticides from fat by gel permeation chromatography (GPC) and determination by electron capture gas chromatography (GC). Committee E gave interim approval to this method in July 1983, for 15 of the 16 chemicals tested. Official status for the method was withheld for toxaphene, because the collaborative study included only samples fortified with standard toxaphene. This was not considered to be a realistic test of the method's ability to measure incurred residues of toxaphene.

Directions for the method have been prepared for inclusion in the 14th edition of *Official Methods of Analysis*, in anticipation of its receiving official first action status in 1983.

The Referee's future plans include a validation study to extend the method's official status to use with beef and swine fat. He is also considering a collaborative study of the same method for analysis for polychlorinated biphenyls in the 3 meat fats.

Chlorobenzilate, Chloropropylate, and Bromopropylate.—(Roy S. Brosdal, FDA, Chicago, IL) The Associate Referee

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition, and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **63**, 374–423 (1980); **64**, 501–540 (1981); **65**, 450–521 (1982); **66**, 512–564 (1983).

This report of the General Referee was presented at the 97th Annual International Meeting of the AOAC, Oct. 3–6, 1983, at Washington, DC.

The recommendations of the General Referee were approved by Committee E and were adopted by the Association, except recommendation 5. See the report of the committee (and "Changes in Methods"), this issue.

has been unable for some time to pursue this work because of a change in work situation. He has therefore resigned. He has not had the opportunity to publish the results of the research he has completed, but is willing to pass these results on to a newly appointed Referee.

Currently, chlorobenzilate is registered for use only on citrus, with no tolerances yet established for potential residues in meat and milk. Such residues may result from use of citrus by-products in animal feed. Chloropropylate is an inactive compound, with no registration, tolerances, or (reportedly) production. Bromopropylate has never been registered in the United States, but tolerances are pending for residues in citrus, citrus pulp, meat, and milk.

Chlorophenoxy Alkyl Acids.—(Allan E. Smith, Agriculture Canada, Regina, Saskatchewan) The Associate Referee completed a collaborative study of a method for 2,4-D residues in dried green wheat, presented at the 1983 meeting. The method includes residue extraction with sodium hydroxide solution to assure removal of conjugated residues, methylation with borontrifluoride-methanol, cleanup on a Florisil column, and determination by electron capture GC. Six laboratories completed the study and results from 5 of these laboratories were statistically evaluated, the results of the sixth having been determined to be outliers. Average recoveries of 2,4-D were 83.3% at 0.5 ppm and 88.2% at 1.0 ppm. Interlaboratory coefficients of variation at the 2 fortification levels were 18.9% and 16.1%, respectively.

The Associate Referee, having completed the collaborative study, has now resigned the position. Chlorophenoxy acids are still used extensively in many different ester formulations. Additional method research on residues is still of interest.

Dicofol.—(vacant) This topic has been vacant for over 10 years. Despite repeated recommendations to fill this position, no one has ever expressed an interest in becoming the Associate Referee. At the request of R. C. Bahner of AOAC, I designated this topic as "inactive" in April 1983.

Ethylene Oxide and its Chlorohydrin.—(A. R. Stemp, Kraft Co., Glenview, IL) The Associate Referee has been unable to devote time to this project in the past year and does not foresee having time available within the next year.

Fenvalerate.—(Terry Spittler, NYS Agriculture Experiment Station, Geneva, NY) The referee was appointed to this topic during the year. He has had extensive experience in using the manufacturer's methodology for fenvalerate on food products. He has also conducted interlaboratory studies on fenvalerate residues in which the various participating laboratories used different methods. He has recently tried the manufacturer's method for the fenvalerate photodegradation product, called SD54597 by the manufacturer. Data on residues of this photoproduct are now required by EPA to support petitions for tolerances for this chemical on leafy products.

Fumigants.—(James Daft, FDA, Kansas City, MO) The referee was appointed to this topic during the year. He has already had considerable experience with the current official method for fumigants, **29.056–29.057**, and has published a paper describing some improvements to that methodology (*J. Assoc. Off. Anal. Chem.* (1983) **66,** 228–233). He has prepared a research proposal, for approval by the management of his laboratory, outlining an approach which would meet the goals set in the past for this refereeship. Unfortunately, the limited research time available in his laboratory will prevent any significant work on this topic before late 1984.

Gel Permeation Chromatography.—This topic was created in 1982, but was still unfilled at the time that the topic on Chlorinated Hydrocarbons in Poultry was transferred to this Committee and General Refereeship. Since the Associate Referee on Chlorinated Hydrocarbons in Poultry has collaborated a method using GPC, it is no longer necessary to have this topic on GPC under this General Referee.

Inorganic Bromides in Grain.—(King T. Zee, Environmental Protection Agency, Beltsville, MD) The Associate Referee was unable to work on this project during the past year.

Kepone.—(Francis D. Griffith, Jr, Division of Consolidated Laboratory Services, Richmond, VA) The Associate Referee's laboratory is developing a new method for Kepone and other residues in fish. The method specifies traditional blender extraction, but residues are cleaned up and separated on an aliquot of the sample by means of a cartridge-type column cleanup device. At this point, the method is undergoing testing to compare its results on incurred residues with those obtained by traditional residue methods. The Referee anticipates continued development and use of that method in his laboratory, because its micro-scale aspects result in considerable savings of time and materials over other methods.

Low Moisture–High Fat Samples (Extraction Procedure).—(Leon Sawyer, FDA, Minneapolis, MN) The Associate Referee was unable to collaborate the oilseed extraction procedure during the past year. He now plans to collaborate the method during 1984.

Multiresidue Methodology, Miniaturization.—(D. Ronald Erney, FDA, Detroit) The Associate Referee has scheduled an interlaboratory test of a miniaturized version of method **29.001–29.018** as applied to nonfatty foods. Several state and FDA laboratories, which participate in the Central States Association quality assurance program, will test the method beginning in November 1983. Assuming a successful method trial, a collaborative study will be undertaken in 1984.

Pentachlorophenol.—(George Yip, FDA, Division of Chemical Technology, Washington, DC) The former Associate Referee, Arnold Borsetti, resigned during the year and a new Referee was appointed. A method for PCP in gelatin was written by Borsetti and Thurston and submitted for publication in the AOAC *Journal*. A collaborative study of that method is currently underway in 12 laboratories. A report of this study will be prepared when all the results have been received. The Associate Referee will continue to refine the method for PCP as applied to fish, eggs, and milk, with a goal of eventually collaborating the method on these commodities.

Pentachlorophenol in Animal and Poultry Tissue.—(Douglas Gillard, U.S. Department of Agriculture, Beltsville, MD). This Refereeship was transferred from Committee C to Committee E in Janaury 1983. The Associate Referee ran a collaborative study of a method for PCP in liver during 1981 and presented the results at the 1982 annual meeting. He will complete the written report of this study during the coming year and submit it for interim first action approval.

Permethrin.—(Vacant) Request for an Associate Referee was published in *The Referee* in June 1983, but no response has yet been received.

Photochemical Derivatization for Confirmation of Residue Identity.—(Paul Ward, FDA, Atlanta, GA).—The Associate Referee completed the work under this topic and presented it at the 1982 meeting. He has now resigned the refereeship. The results of his work should be made available as a guide to those using similar methodology to confirm pesticide residues. Since the AOAC *Journal* has declined to publish the paper, the material may be included in a future revision of FDA's *Pesticide Analytical Manual*, Vol. I.

Polychlorinated Biphenyls.—(Leon D. Sawyer, FDA, Min-

neapolis, MN) No further work on this topic has been necessary during the past year.

Polychlorinated Biphenyls (PCBs) in Blood.—(Larry L. Needham, Centers for Disease Control, Atlanta, GA) This topic was created as a result of a 1982 recommendation and the Associate Referee appointed. The referee has developed and used a method for the analysis for PCBs in blood serum. A previous interlaboratory study of this method produced interlaboratory coefficients of variation of 92.7, 67.6, and 25.8% at levels of approximately 10, 25, and 75 ppb, respectively. The referee noticed in that study that those laboratories with experience in analysis of this type produced more accurate results than the others.

The Associate Referee plans to collaborate this method during the coming year. Collaborators will be selected from among laboratories with experience in analysis for low levels of PCBs.

Resmethrin.—(Calvin Corbey, Environmental Protection Agency, Beltsville, MD) The Associate Referee has not had the opportunity to work on this project in the past year.

Root-Absorbed Residues (Extraction Procedures).—(Vacant) This topic has been vacant for several years. As of April 1983, it was declared inactive until such time as interest in it revives. The problem of adequate extraction of root-absorbed residues still needs to be explored.

Sodium Monofluoracetate.—This topic was transferred during the past year to Committee G, General Referee on Veterinary Analytical Toxicology.

Tetradifon, Endosulfan, and Tetrasul.—(Lawrence R. Mitchell, FDA, Atlanta, GA) The Associate Referee has been unable to devote time to this project in the past year.

Toxaphene.—(Larry Lane, Mississippi State Chemical Laboratories, Mississippi State, MS) The Associate Referee's laboratory is pursuing research to choose appropriate toxaphene components for use in quantitating residues. Samples of fish of 6 species have been prepared by solvent extraction, Florisil column cleanup as in **29.015,** and LC fractionation of the 6% Florisil eluate. Each LC fraction has then been examined by capillary column electron capture GC for occurrence of major peaks common to all species. Five of these major peaks are now being studied with mass spectrometry. These peaks from both standard toxaphene and fish extracts will be compared to show whether they are caused by the same chemical in both cases. If so, these components will be used as part of a quantitation scheme. Other sample types, including human adipose tissue, will be examined for these same components.

Recommendations

(1) Continue work on the application of capillary column gas chromatography to the analysis of residual chlordane. It is also recommended that the Associate Referee initiate a collaborative study of a method combining multiresidue extraction, **29.011–29.012,** acetonitrile partitioning cleanup, **29.014,** Florisil column chromatographic cleanup and residue separation, **29.031–29.033,** and electron capture liquid chromatography, **29.018,** for determining residues of *cis*-chlordane, *trans*-chlordane, octachlor epoxide (oxychlordane), and heptachlor epoxide in butter, eggs, fish, and poultry fat.

(2) Continue to monitor progress on development of methods for 2,3,7,8-TCDD, and encourage the use of AOAC criteria in EPA quality assurance programs so that the data could be used to support official status at some time in the future.

(3) Adopt as official first action the GPC method for chlorinated hydrocarbons in poultry, presented by Ault at the 1982 AOAC meeting, for α-BHC, *cis*-chlordane, *trans*-chlordane, *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDT, dieldrin, endrin, hexachlorobenzene, heptachlor epoxide, lindane, methoxychlor, mirex, octachlor epoxide, and *p,p'*-TDE in poultry fat. It is also recommended that the Associate Referee validate the applicability of this method for use on beef and swine fat by means of an interlaboratory study. Such a study would not be as extensive as a collaborative study since the 15 pesticides to be included will be those already tested by the collaborative study on poultry fat. It is further recommended that the title of this topic be changed to GPC Methods for Organochlorine Pesticides in Meats to reflect the specific type of methodology involved and to broaden the substrate type from poultry to meat.

(4) Change the topic Chlorobenzilate, Chloropropylate, and Bromopropylate to Chlorobenzilate and Bromopropylate to reflect the compounds which are of interest, and appoint a new Associate Referee to this topic. The focus of future work should be on methods for low level residues in meat and milk.

(5) Adopt as official first action the method for 2,4-D in dried green wheat, as presented by Allen E. Smith, and appoint a new referee to the topic Chlorophenoxy Alkyl Acids.

(6) Delete the topic *Dicofol.*

(7) Revive the study of the GC method of Scudamore and Heuser (*Pestic. Sci* (1971) **2,** 80–91) for determining ethylene oxide, ethylene chlorohydrin, and ethylene bromohydrin in foods.

(8) Continue to examine methods for fenvalerate and its photodegradate SD54597 with a goal of selecting and testing method(s) suitable for collaborative study.

(9) Continue to prepare for research to improve the current official method for fumigants. Long-range goals should include extension of the method to 1,2-dichloroethane and tetrachloroethylene and to additional foods (citrus fruits, milled products, and baked goods).

(10) Delete topic Gel Permeation Chromatography from this General Refereeship.

(11) Restart studies on methods for inorganic bromides in grains.

(12) Continue to study the new microscale method for residues of kepone in fish.

(13) Collaboratively study the published method for extraction from oilseeds and some related low moisture–high fat samples (Sawyer, L. D. (1982) *J. Assoc. Off. Anal. Chem.* **65,** 1122–1128).

(14) Proceed with the planned interlaboratory and collaborative studies of a miniaturized version of the AOAC official multiresidue method for nonfatty foods.

(15) Complete the evaluation of collaborative study results on pentachlorophenol, and, if warranted, submit the method for interim first action approval; continue work on refinement of the methodology for PCP in fish, eggs, and milk.

(16) The Associate Referee for Pentachlorophenol in Animal and Poultry Tissue should prepare a written report of his collaborative study and submit it for interim first action approval.

(17) A new Associate Referee should be appointed to evaluate and collaboratively study methods for residues of permethrin in foods.

(18) Delete the topic Photochemical Derivatization for Confirmation of Residue Identity.

(19) Maintain the topic Polychlorinated Biphenyls so that a focal point will exist for future questions about methodology for PCBs.

(20) Collaboratively study the method developed by the Associate Referee for polychlorinated biphenyls in blood.

(21) Continue to study methods for residues of resmethrin.

(22) Inactivate the topic Root-Absorbed Residues (Extraction Procedure) until a referee is found to study it.

(23) Complete the intralaboratory trials on **29.029–29.034,** the method for tetradifon, endosulfan, and tetrasul, to validate the use of this method on all the foods for which **29.001–29.018** is official.

(24) Continue the work on developing a practical means for quantitating toxaphene residues.

(25) It is also recommended that the following change be made in the 14th edition of *Official Methods of Analysis,* **29.017,** to read:

29.017 **Saponification**

(Applicable only to those chems stable to hot alkali treatment. Use as supplemental cleanup if 15% eluate or MgO–Celite eluate is not substantially free from oily materials.)

Conc. pet. ether–ether (85 + 15) fraction under current of air to 2 mL, add 1 mL 2% alc. KOH, attach micro-Snyder column, and carefully reduce to ≤1 mL on steam bath. Reflux sample 15 min, remove, and cool. Add 2 mL alcohol–H_2O (1 + 1) and 5 mL hexane, and shake 1 min. Centrif. to sep. layers. Transfer as much hexane layer as possible to second tube, using disposable Pasteur pipet, and repeat extn with 5 mL hexane. Conc. combined hexane to appropriate vol. for GC analysis.

This paragraph is recommended as a replacement for the macro-scale saponification step, **29.017,** in the 13th edition, and also replaces a previously approved micro-scale version of **29.017** (*J. Assoc. Off. Anal. Chem.* (1980) **63,** 395). The recommended paragraph is taken from the method described by Erney (*J. Assoc. Off. Anal. Chem.* (1983) **66,** 969–973). This report of a collaborative study led to the approval of the method **29.D01–29.D06** (*J. Assoc. Off. Anal. Chem.* (1983) **66,** 536). The application of this saponification step in Erney's collaborative study was the same as that for which **29.017** is recommended, i.e., supplemental cleanup of a 15% Florisil eluate from analysis of a fatty product. This applicability makes it superior to the previously approved micro-scale alkaline hydrolysis which was never tested on the 15% eluates of fatty foods.

If this change is approved, then **29.D06** should also be changed as follows to cross-reference to **29.017:**

29.D06 **Additional Cleanup**

Often addnl cleanup is required for second fraction (85 + 15) to prevent deterioration of GC column. Use **29.017.**

It is recommended that the official first action method for chlorinated pesticides and polychlorinated biphenyls in fish, **29.D01–29.D06,** be continued as official first action for another year.

(26) Finally, it is recommended that the Associate Referee topics assigned to this General Refereeship be divided between 2 General Referees. The large number of active topics in this refereeship make it almost impossible to do justice to each. Associate Referees deserve the attention of an interested General Referee who can help set research goals, assist in planning interlaboratory studies, and review manuscripts as requested, all in a timely manner.

As the Association strives to improve its operation, repeated requests for information are being made of the General Referees. We are now also the designated editors for our chapters in the *Official Methods of Analysis.* For all of these reasons, I recommend that another General Referee be appointed and approximately half the topics be assigned there. Dividing the topics into multiresidue methods and single-compound methods is one approach to this, but some other criteria for division may be preferred by the Committee.

Report on Organonitrogen Pesticides

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Associate Referee reports were received on 4 topics. The Associate Referees for 2 topics, Carbofuran and Organotin Fungicides, have tendered their resignations.

Anilazine.—An Associate Referee is required to conduct a collaborative study of the liquid chromatographic method of Lawrence and Panopio (*J. Assoc. Off. Anal. Chem.* (1980) **63,** 1300) for anilazine on potato and tomato crops.

Benzimidazole-Type Fungicides.—Associate Referee Mikio Chiba reports that collaborators are required to test the liquid chromatographic method for benomyl and methyl-2-benzimidazole carbamate described previously (*J. Assoc. Off. Anal. Chem.* (1980) **63,** 1291). The method involves extraction of the freeze-dried crop material in the presence of propyl isocyanate at 1°C and is capable of determining both the parent compound and its major degradation product.

Captan and Related Fungicides.—Associate Referee Dalia Gilvydis reports having obtained recoveries exceeding 83% for captan, captafol, and folpet from tomato, apple, lettuce, or strawberry samples fortified with each fungicide and carried through the PAM I, 232.4 and 212.2, procedure. The compounds were eluted from the Florisil cleanup column in the combined 15 and 50% ethyl ether fractions and were quantitated by gas chromatography with electron capture detection on a 5% SP-2401 column. Collaborators are required to study this method as applied to the analysis of captan, captafol, and folpet in 2 commodities, lettuce and strawberries.

Carbamate Herbicides.—An Associate Referee is required to select an analytical method and subject it to collaborative study.

Carbamate Insecticides, Gas Chromatographic Methods.—The Associate Referee Roderick Young did not submit a report on his topic.

Carbamate Insecticides, Liquid Chromatographic Methods.—Associate Referee Richard Krause reports that a collaborative study of his liquid chromatographic method (*J. Assoc. Off. Anal. Chem.* (1980) **63,** 1114) is in progress with 8 laboratories participating. The method is being applied to

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The recommendations of the General Referee were approved by Committee E and were accepted by the Association. See the report of the committee (and "Changes in Methods"), this issue.

grapes and potatoes, each fortified with 7 carbamate insecticides at 2 levels.

Carbofuran.—Associate Referee Sujit Witkontin has resigned.

Chlorothalonil.—An Associate Referee is required to apply established multiresidue procedures to the determination of chlorothalonil and to conduct a collaborative study of the resulting method.

Daminozide.—An Associate Referee is required to develop and test a chromatographic method specific for succinic acid 2,2′-dimethylhydrazide.

Dinitro Compounds.—An Associate Referee is required to study methods for dinitro aryl herbicides.

Diquat and Paraquat.—Associate Referee Brian Worobey reports that his borohydride reduction method has been modified to include the use of 2,2′-bipyridine as an internal standard to correct for adsorptive effects observed with "aged" diquat and paraquat residues. No interference was observed with nonspiked potato samples carried through the reduction, extraction, and gas chromatographic steps. Details of the procedure were presented at the 8th AOAC Spring Workshop (April 1983). An interlaboratory study will be conducted, pending ruggedness testing.

Dithiocarbamates, General Residue Method.—An Associate Referee is required to test a method capable of determining dimethyl and ethylenebis(dithiocarbamates) as separate entities in crop material.

Maleic Hydrazide.—An Associate Referee is required to study a liquid chromatographic or gas chromatographic method for maleic hydrazide residues in crops.

Organotin Fungicides.—The Associate Referee, Richard Cannizzaro, reports that he will be unable to carry out the proposed collaborative study for fenbutatin and triphenyltin and has resigned the position.

Sodium o-Phenylphenate.—An Associate Referee is required to develop a specific method for *o*-phenyl phenol in foods.

Substituted Ureas.—An Associate Referee is required to select and subject to collaborative study a method for urea herbicides.

Thiocarbamate Herbicides.—This topic is vacant and requires an Associate Referee.

s-Triazines.—An Associate Referee is required to conduct a collaborative study of a method for atrazine and cyanazine in crop material.

Trifluralin.—An Associate Referee is required to apply a multiresidue method to the analysis of trifluralin and to test the method collaboratively.

Recommendations

(1) Appoint Associate Referees to work as indicated on the topics Anilazine; Carbofuran; Chlorothalonil; Daminozide; Dinitro Compounds; Dithiocarbamates, General Residue Method; Maleic Hydrazide; Sodium *o*-Phenylphenate; Substituted Ureas; Thiocarbamate Herbicides; *s*-Triazines; and Trifluralin.

(2) Initiate collaborative studies as indicated on the topics Benzimidazole-Type Fungicides; Captan and Related Fungicides.

(3) Provide documentation of the on-column silylation gas chromatographic method for carbamate insecticide residues to General Referee for comment and approval prior to initiation of a collaborative study.

(4) Complete the collaborative study presently in progress on carbamate insecticides, liquid chromatographic method, and prepare a report and recommendation regarding adoption.

(5) Study validity of an internal standard for quantitation of diquat and paraquat. Prepare report for review by General Referee. Initiate a collaborative study employing either a calibration curve or internal standard.

(6) Transfer the topic Organotin Fungicides to General Referee for Metals and Other Elements to be included under the topic of Organometalics in Foods.

Report on Organophosphorus Pesticides

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The General Referee has continued to monitor the literature for publications on the determination of organophosphorus pesticide residues. Alawi (1) reported the determination of acephate in water and buffer solutions by liquid chromatography (LC) with UV detection. Jenkins et al. (2) determined azinphos-methyl residues in soil by gas chromotography (GC) with flame photometric detection (FPD). Residues were extracted with acetone and cleaned up on a silica gel (Adsorbosil CAB) microcolumn eluted with 4% ethyl acetate in benzene. LC and anticholinesterase assay were compared for measuring azinphos-methyl oxon in biological systems (3). Both methods selectively measured the compounds to the same degree of accuracy and precision.

Chlorpyrifos was determined in citrus fruit by GC with nitrogen-phosphorus detector (NPD) following acetone extraction, partitioning into hexane, removal of the hexane, and solution of the residue in acetone (4). Total 3,5,6-trichloro-2-pyridinol was determined following alkaline methanol extraction, derivatization with diazomethane, and cleanup on Florisil. Some residue stability studies were also reported (4). Residues of chlorpyrifos and its oxon were stable in laboratory-treated fruits stored at 8°C and chopped rind from these fruits stored in a freezer for 7 days and 5 weeks, respectively. Chlorpyrifos residues were stable in chopped rind from field-treated oranges kept under frozen storage for 95 days. Residues were stable in acetone extracts prepared from orange rind and stored for 5 weeks at 8°C.

Lehmann et al. (5) described methods for the determination of DEF in water and fish tissue. Water was buffered to pH 4 with acetate buffer and extracted with dichloromethane, and residues were determined by GC with electron capture (EC) detector. Fish tissue was extracted with dichloromethane and DEF was separated from lipid material by gel permeation chromatography. Additional cleanup was done on a silica gel column with elution of DEF with 5% acetone in hexane and determination by GC with either EC or thermionic specific detectors. Dimethoate residues were determined in citrus leaves by GC with NPD (6). Following extraction with acetone, the extract was acidified and extracted with light petroleum. Dimethoate residues were extracted from the aqueous phase with chloroform and cleaned up on Kieselgel with elution of dimethoate with dichloromethane–acetone (80 + 20) and omethoate with dichloromethane–acetone (40 + 60).

Szeto and Brown (7) reported methods for the determination of residues of disulfoton, phorate, oxydemeton-methyl, and their metabolites in asparagus and soil by GC-NPD. Asparagus and soil were extracted with ethyl acetate, except oxydemeton-methyl residues were extracted from soil with 20% methanol in ethyl acetate. Residues were cleaned up on a column of Nuchar C charcoal and Whatman CF-11 cellulose (2 + 5). Disulfoton residues were eluted in 2 fractions: 40% ethyl acetate in hexane eluted disulfoton oxon, disulfoton sulfone, and disulfoton oxon sulfone; 20% methanol in ethyl acetate eluted disulfoton sulfoxide and disulfoton oxon sulfoxide. Phorate and its metabolites (oxon, sulfone, oxon sulfone, sulfoxide, and oxon sulfoxide) were eluted with 20% methanol in ethyl acetate as one fraction. Oxydemeton-methyl sulfone was eluted with 60% ethyl acetate in hexane and oxydemeton-methyl was eluted with 40% methanol in ethyl acetate. Before GC analysis, the fractions containing the 2 sulfoxides of disulfoton or oxydemeton-methyl were oxidized to their corresponding sulfones with potassium permanganate.

Krzymien (8) measured atmospheric fenitrothion residues by GC-NPD, following collection on Tenax GC. Residues were thermally released from the adsorber and transferred by a stream of carrier gas directly into a GC equipped with a special adaptor for ease of adsorber coupling. Mallet and Volpé (9) determined fenitrothion and aminofenitrothion residues in air and water, collected on Amberlite XAD-2 and XAD-4 resins, respectively. Residues were eluted with ethyl acetate and determined by GC-FPD.

A method (10) was reported for the determination of glyphosate in soil and water by LC with fluorescence detection following derivatization with 9-fluorenylmethyl chloroformate. Soil was extracted with 0.1N NaOH and glyphosate was isolated from the soil extract and water samples on Bio-Rad AG 1-X8 anion exchange resin and eluted from the resin with 0.2N NaCl prior to derivatization.

Moye et al. (11) reported that acetone–acetonitrile (1 + 1) provided the best overall recovery of phenthoate and its oxon from soil when compared with acetone, methanol, ethyl acetate, ethyl acetate–methanol (1 + 1), ethyl acetate–2-propanol (1 + 1), ethyl acetate–benzene (1 + 1). Overnight Soxhlet extraction showed no improvement in recovery over the shaking procedure. For fresh orange peel, acetone–acetonitrile (1 + 1) was superior to ethyl acetate, methylene chloride, chloroform, and acetonitrile.

Terbufos and its metabolites (terbufos sulfoxide, terbufos sulfone, terbufoxon, terbufoxon sulfoxide, and terbufoxon sulfone) were extracted from soil with hexane–acetone (2 + 1) and identified by GC with an alkali flame ionization detector and by GC-mass spectrometry (MS) (12). Terbufos sulfoxide and terbufoxon sulfoxide had to be oxidized to the more stable sulfones before GC analysis. Terbufos sulfoxide was oxidized with potassium permanganate and terbufoxon was oxidized with meta-chloroperbenzoic acid. Tetrachlorvinphos residues were determined in apples by either GC-ECD or GD-FPD (13). Following acetonitrile extraction, residues were partitioned into chloroform and cleaned up by either column chromatography or freezing at −20°C.

Luke and Doose (14) modified the Luke multiresidue procedure for low moisture, nonfatty products. Compounds were extracted with water–acetone (35 + 65). Polar residues such as acephate, methamidophos, and dimethoate were cleaned up on a carbon column (carbon, Celite, and magnesium oxide (1 + 4 + 2)) and eluted with acetone–dichloromethane (2 + 1). Holland and McGhie (15) reported a multiresidue method for the determination of pesticides (included 13 organophosphorus compounds) in fruit. Residues were extracted with methanol and partitioned into toluene. Further cleanup was done on a carbon–cellulose–Florisil column with residue elution with toluene. Sawyer (16) reported an extraction procedure for oilseeds and related high fat/low moisture prod-

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This report of the General Referee was presented at the 97th Annual International Meeting of the AOAC, Oct. 3–6, 1983, at Washington, DC.

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ucts. Samples were serially extracted with petroleum ether, ethyl ether–petroleum ether (1 + 1), and ethanol. Residue cleanup was done by AOAC multiresidue procedures. Diazinon and malathion were the organophosphorus compounds used in this study. Krause and August (17) determined the applicability of the carbamate insecticide multiresidue method to additional types of pesticides in fruits and vegetables. The organophosphorus compounds azinphos-ethyl, azinphos-methyl, azinphos-methyl oxygen analog, phosalone, and phosalone oxygen analog were determined by LC with fluorescence detector. An additional 9 organophosphorus compounds were determined by GC-FPD. Hopper (18) described an automated gel permeation system using Bio-Beads SX-3 for the separation of organophosphorus pesticides from fats. Elution profiles of 30 organophosphorus pesticides were given, using a methylene chloride–*n*-hexane (50 + 50) solvent system. Differences between batches of SX-3 beads existed and the solvent composition ratio must be adjusted to get good fat separation.

Ferreira and Tainha (19) described a method for some organophosphorus pesticide residues in olives. Residues were serially extracted with acetonitrile and hexane. Diazinon, parathion, and methidathion were partioned into hexane, and dimethoate and phosphamidon were extracted from the aqueous acetonitrile phase with ethyl acetate. The hexane extract was cleaned up on 2% deactivated Florisil with residue elution with ethyl ether–hexane (2 + 3). A second Florisil (1% deactivated) column was used for additional cleanup. Dimethoate and phosphamidon were cleaned up on a 1% deactivated Florisil column with acetone elution. Residues were determined with GC-NP and GC thermionic detectors. A method for the determination of 11 organophosphorus pesticides in onion and related crops has been published (20). Residues were extracted with acetonitrile and cleaned up on an Amberlite XAD-8 resin column. Residues were eluted with methanol and, following partitioning into dichloromethane–benzene (1 + 4), further cleaned up on a carbon column followed by determination by GC-FPD. This method removed organosulfides from extracts of aromatic vegetables such as onions, leek, garlic, etc., that strongly interfere in the analysis of organophosphorus pesticide residues by GC-FPD.

Petzinger et al. (21) identified prothiophos in preserved cabbage by GC-MS. Residues were extracted and cleaned up by the AOAC multiresidue procedure for nonfatty foods. Stan and Kellner (22) reported the negative ion mass spectra of 52 organophosphorus pesticides. Bonner (23) used parathion contaminated lettuce as an example of the use of quadrupole MS/MS in residue analysis. Cairns et al. (24) reviewed the application of mass spectrometry in the regulatory analysis of pesticides and industrial chemicals in foods and feed commodities. Examples were cited of its application in the identification of unknown analytical responses as well as the identification of the organophosphorus compounds trichloronate,demeton-*s*-sulfone, and tris-2-chloroethyl phosphate.

Cabras et al. (25) determined dimethoate, phosalone, and tetrachlorvinphos in grapes by reverse phase LC with variable wavelength UV detector. Krause (26) reported the determination of azinphos-methyl, coumaphos, phosalone, thionazin, and their oxygen analogs and azinphos-ethyl by LC with a C_8 reverse phase column and fluorescence detector. LC with a photoconductivity detector was evaluated for some organophosphorus (azinphos-methyl, phosmet, coumaphos, dialifor, leptophos, chlorfenvinphos, dichlorvos) pesticides (27).

Tenax GC was used to extract diazinon, methyl parathion, and malathion from water (28). Residues were eluted with ethyl ether or were thermoeluted directly on the GC column.

Temephos was determined in environmental water samples by densitometry on pre-adsorbent reverse phase thin layer plates (29). Water was acidified, extracted with chloroform, and spotted on TLC plates without cleanup. Residues were detected with magnesium chloride-*N*,2,6-trichlorobenzoquinoneimine reagent and quantitated with a Kontes Model 800 fiber optics scanner. Sherma and Charvat (30) described a procedure for optimizing mobile phase selection for reverse phase TLC. Phorate and its 5 metabolites were the compounds used in the study. Wood and Kanagasabapathy (31) evaluated TLC procedures for the estimation of 7 organophosphorus pesticides in fruit and vegetables. Semiquantitative estimation by visual comparison with standards was possible. The use of reverse phase TLC was sometimes essential to avoid co-extraction interference. The extraction and cleanup procedures utilized were also suitable for use by GC analysis. Sherma (32) reviewed recent advances in the technique and application of TLC for the separation, detection, and quantitative determination of pesticides.

Egli (33) reported on the storage stability of pesticide residues. The organophosphorus compounds studied were azamethiphos, isazofos, methacrifos, piperophos, and profenofos. Methods for their determination were also given. Residue stabilities could be derived from hydrolysis data. Residue stability studies can be performed with fortified samples.

Daft (34) compiled retention tables for 15 industrial phosphates (aryl/alkyl phosphates) on 4 general purpose GC column packings. These data will facilitate the identification of residues during the analysis of foods.

A brief summary of each Associate Referee topic follows:

Confirmation Procedures.—(Bill Lee, Environment Canada, Burlington). The Associate Referee completed the evaluation of the pentafluorobenzyl bromide derivatization procedure (Coburn and Chau, *J. Assoc. Off. Anal. Chem.* (1974) **57,** 1272–1278; and Coburn & Chau, *Environ. Lett. (1975)* **10,** 225–236) for organophosphorus pesticides.

Briefly the method is as follows: Extracts from water samples prepared by the method of Ripley et al. (*J. Assoc. Off. Anal. Chem.* (1974) **57,** 1033–1042) were hydrolyzed with 10% methanolic KOH at 60°C for 2 h. Following acidification, the hydrolysis products (phenols) were extracted into benzene. Prior to derivatization of the phenols with pentafluorobenzyl bromide at 60°C for 30 min, the solvent was diluted with acetone to give a 20% benzene in acetone solution. The derivatized phenols were cleaned up on a 5% deactivated silica gel column. Elution with 25% toluene in hexane eluted the pentafluorobenzyl ethers of thiophenol (dyfonate), 4-chlorothiophenol (carbophenothion), 2-chloro-4-tert-butylphenol (crufomate), and 2,4,5-trichlorophenol (ronnel). The pentafluorobenzyl ethers of 4-cyanophenol (cyanophos), 4-nitrophenol (methyl parathion and parathion), and 3-methyl-4-nitrophenol (fenitrothion) were eluted with 75% toluene in hexane. These fractions were analyzed by GC with electron capture detection. Recoveries of the phenol derivatives were 75–90% except for 2-chloro-4-tert-butylphenol and 4-cyanophenol derivatives which were 60–70%. The lower confirmation level of this method is 0.1 ppb for the 9 organophosphorus pesticides in a 1 L water sample. A protocol for a collaborative study on this version of the method is being prepared.

High Fat Samples.—(R. Scharfe, Agriculture Canada, Ottawa). The Associate Referee has reported no progress on the topic this year. He plans to evaluate available gel systems and their applicability to organophosphorus pesticide residues in high fat samples.

Phosphine.—(T. Dumas, Agriculture Canada, London). The

Associate Referee reported the evaluation of a portable gas chromatograph (Photovac 10A10) for the analysis of fumigant gases (including phosphine) in the field (*J. Agric. Food Chem.* (1982) **30**, 986–988). The instrument was operated at ambient temperature, used a photoionization detector, and conditions for analyzing phosphine at concentrations below 0.1 ppm were established.

Sweep Codistillation.—(B. Luke, Australian Government Analytical Laboratories, Melbourne). During the past year, Randall Watts resigned and Barry Luke was appointed as the Associate Referee. Scientific Glass Engineering of Australia introduced a commercial sweep codistillation unit, Unitrex, at the 1983 Pittsburgh Conference in Atlantic City. The Associate Referee reports that a unit is fully operational in routine analysis of fats for organochlorine and organophosphorus pesticide residues in his laboratory and that other laboratories in Australia have acquired units.

Thin Layer Chromatography.—(M. Getz, USDA, Beltsville). The Associate Referee reports that the status of the topic is unchanged from last year. He has been unable to organize an interlaboratory study because laboratories with quantitative thin layer chromatographic equipment do not have the time or are not interested in participating in this study. Quantitative thin layer chromatography is used in other analytical areas but there appears to be little interest in the area of pesticide residues (preference is to use GC methods). The Associate Referee recommends that the topic be put in inactive status.

Other Topics.—The topics Azinphos-methyl, Disulfoton, Extraction Procedures, General Method for Organochlorine and Organophosphorus Pesticides, Methamidophos, Monocrotophos, Phorate, and Soils are vacant.

Recommendations

(1) Appoint an Associate Referee to evaluate and collaboratively study analytical methods for determining residues of azinphos-methyl in foods.

(2) Prepare protocol for approval and collaboratively study the modified Coburn and Chau (*J. Assoc. Off. Anal. Chem.* (1974) **57**, 1272–1278; and *Environ. Lett.* (1975) **10**, 226–236) pentafluorobenzyl bromide derivatization procedure for organophosphorus pesticides recovered from water by a method such as that of Ripley et al. (*J. Assoc. Off. Anal. Chem.* (1974) **57**, 1033–1042).

(3) Appoint an Associate Referee to evaluate and collaboratively study analytical methods for determining disulfoton and its metabolites in foods.

(4) Appoint an Associate Referee to study the efficiency of extraction procedures for residues of organophosphorus pesticides in crops, i.e., to extend the extraction efficiency studies of Watts (*J. Assoc. Off. Anal. Chem.* (1971) **54**, 953–958) to additional pesticides and crops.

(5) Appoint an Associate Referee to evaluate the applicability of the AOAC multiresidue method, **29.001–29.018**, for determining additional organophosphorus pesticide residues in fatty and nonfatty foods and study the use of the nitrogen phosphorus (N/P) detector as an alternative detector for the gas chromatographic determinative step of this method.

(6) Continue study to evaluate gel permeation chromatography as a cleanup technique for the determination of organophosphorus pesticides and their metabolites in fatty foods and evaluate the N/P detector as an alternative to the alkali flame and flame photometric detectors for determining organophosphorus pesticide residues by gas chromatography.

(7) Appoint an Associate Referee to evaluate and collaboratively study analytical methods for determining residues of methamidophos and its residues in foods.

(8) Appoint an Associate Referee to evaluate and collaboratively study analytical methods for determining residues of monocrotophos in foods.

(9) Appoint an Associate Referee to evaluate and collaboratively study analytical methods for determining phorate and its metabolites in foods.

(10) Continue study of methods for determining residual phosphine in fumigated products, including the GC method reported by the Associate Referee (J. Assoc. Off. Anal. Chem. (1978) **61**, 5–7) and the GC method reported by Nowicki (J. Assoc. Off. Anal. Chem. (1978) **61**, 829–836) for determining the total residue of intact phosphine and phosphine derived from residual aluminum phosphide in wheat.

(11) Appoint an Associate Referee to develop multiresidue extraction and cleanup methods for determining residues of organophosphorus pesticides and their metabolites in soils.

(12) Continue study to extend the official final action sweep codistillation method for determining organophosphorus pesticide residues in crops, **29.044–29.049**, to the analysis of fatty foods; carry out an interlaboratory study on the Unitrex apparatus and if successful and sufficient laboratories have acquired the Unitrex initiate a collaborative study for organophosphorus pesticides in fatty foods.

(13) Discontinue the topic Thin Layer Chromatography with reactivation in the future if sufficient interest develops in quantitative thin layer chromatography determination of organophosphorus pesticide residues.

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Report on Radioactivity

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Carbon-14.—This topic has been discontinued for the present.

Cesium-137.—The method for the determination of cesium-137 in milk by gamma-ray spectroscopy, **48.025–48.029,** has been extended to include the determination of cesium-137 in other foods. The collaborative study shows the method should be considered for official first action. A new Associate Referee will be considered. A study and evaluation of methods for determining cesium-137 in milk, other foods, and biological materials at lower levels should be considered for study.

Iodine-131.—The method for the determination of iodine-131 in milk has also been evaluated and collaboratively tested for iodine-131 in foods. This method is an extension of AOAC method **48.025–48.029.** The study shows the method should be considered for official first action.

The ruggedness testing for the more sensitive method as outlined by the General Referee (*J. Assoc. Off. Anal. Chem.* (1979) **62,** 387–389) and recommended by the Nuclear Regulatory Commission for determining iodine-131 in milk is in progress. If warranted by the results of the study, a collaborative study protocol will be prepared for review by the General Referee.

Neutron Activation Analysis.—Results from 5 collaborators have been received. The sixth collaborator will be sending in the results. When all the results are in, the Associate Referee will perform a statistical analysis of the data for determining sodium in neutron-irradiated biological materials. Results will be presented at the next annual AOAC meeting. Also, the Associate Referee plans to perform a study on multi-elements (metals), using neutron activation and, if feasible, design a collaborative study for review and comment by the General Referee.

Plutonium.—An Associate Referee is being considered for this topic to study the method of the Dept of Energy for determining plutonium in foods, biological materials, and water (HASL = 300-Ed 25, EML Procedures Manual (1982) pp. E-Pu-01-01) as outlined previously by the former General Referee (*J. Assoc. Off. Anal. Chem.* (1977) **60,** 378–379) for possible application to foods and environmental samples.

Radium-228.—Ruggedness tests and an interlaboratory trial should be conducted of the method reported by Baratta and Lumsden (*J. Assoc. Off. Anal. Chem.* (1982) **65,** 1424–1428) for determination in foods and water. If successful in interlaboratory trial, a collaborative study protocol should be prepared for review and comment by the General Referee and the Statistical Consultant to Committee E.

Strontium-89 and 90.—An Associate Referee will be appointed this year to iniate a collaborative study 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.—This topic was reviewed and it was decided that it is still needed. An Associate Referee is needed to study the literature for a possible method for ruggedness testing and collaborative study.

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The recommendations of the General Referee were approved by Committee E and were accepted by the Association. See the report of the committee (and "Changes in Methods"), this issue.

GENERAL REFEREE REPORTS: COMMITTEE F

Report on Analytical Mycology of Foods and Drugs

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To increase the yield and to impart unique textural characteristics to the product, the tomato processor attempts to use a unique combination of pulpers, mills, and homogenizers. Unfortunately, particle size reduction (comminution) results in breaking mold hyphae and rot fragments that are counted in AOAC methods **44.096** and **44.099.** Counts may increase or decrease depending on the degree of comminution.

Two approaches to this problem are currently being investigated by the General Referee: *(1)* Baseline particle size reduction using the X-Press tissue homogenizer, and *(2)* particle size determination by liquid sedimentation.

By using the X-Press, it would be theoretically possible to achieve a baseline comminution level. The X-Press works on the principle of forcing a frozen 25 mL sample through a small 2 mm orifice at a pressure of 6000–28 000 psi. Howard counts of stabilizer-water suspensions of mold hyphae of 13.3, 42.2, and 65.0% were reduced to 0.7, 1.7, and 1.5%, respectively. Catsup samples processed through a pulper, a mill, and a homogenizer had respective mold counts of 7.6, 10.4, and 20.4% which were reduced to a zero mold count. Use of the X-Press with the current official methods is clearly out of the question.

The use of liquid sedimentation for determining relative particle size is yielding promising results. A simple settling test will distinguish among samples of catsup that were pulped through an 0.033 in. screen, Urschel milled, or homogenized at 1800 psi. Suspending 2 g deaerated catsup in 100 mL water in a glass-stopper graduated cylinder will result after 75 min in sediment volumes of 33, 42, and 90 mL, respectively. By suspending 50 g catsup in a 100 mL dispensing buret, 150 mL portions of sample may be drawn off at 5 min intervals, or a quantitative particle size distribution can be generated by using the insoluble solids method, **32.011.** As an alternative procedure, mold counts may be made on each 150 mL sample portion. One advantage of sedimentation analysis over baseline comminution is that the mold in the sample remains unchanged and does not undergo further comminution in the analytical process.

The tomato rot fragment method, **44.099,** is a sizing operation that involves wet sieving delicate tomato tissue and rot fragments. Rough handling of the product during the sieving and tissue transfer steps could lead to a loss of countable rot fragments. Inadequate washing may result in the opposite: retention of small rot fragments with a corresponding increase in counts. As a result of ruggedness testing, the Associate Referee on Tomato Rot Fragment Count, Gerald E. Russell (FDA, Detroit District), has recommended procedural changes that are intended to standardize sample handling in the method (see Procedural Changes).

The calibrated 10X huygenian eyepiece prescribed in the Howard mold count method, **44.096,** is no longer available from microscope manufacturers. It has been replaced by the widefield eyepiece, which has a field of view greater than 1.382 mm. The counting diameter, 1.382 mm, has been achieved by inscribing a circle on an inserted reticle eyepiece disk. For counting purposes, the analyst then considers only the mold within the inscribed circle. Advantages of using the widefield eyepiece are less analyst eyestrain because of a flatter field, and superior optical resolution. Associate Referee Rosamond D. Scott (Superior Lab Services, Portland, IN) has compared the 2 types of eyepieces and found them equivalent. Other aspects of the Howard method are also being examined by the Associate Referee.

Procedural Changes

Change **44.099,** Rot Fragments in Comminuted Tomato Products, to read:

"Weigh sample directly into 400 mL beaker using an electric top loading balance with readability of 0.1 g, precision (std dev.) of ± 0.05 g, and 1200 g capacity (Sartorius No. 3706, or equiv.). In the case of puree or paste, add H_2O to make mixt. or tomato sol. solids content that gives refractive index of 1.3448–1.3454 at 20° (1.3442–1.3448 at 25°). Use 5.0 g catsup, sauce, or dild puree or paste; and 10.0 g juice.

"Add ca 100 mL H_2O to sample in beaker and stir thoroughly using glass rod until sample material appears well dispersed. Add 12 drops crystal violet soln, **44.033(p),** stir, and let stand 5 min. Add 200 mL H_2O and pour directly from beaker onto a 3 in. diam. No. 60 sieve (Dual Manufacturing Co., 2033 W Charleston, Chicago, IL 60647). Spread samples evenly over sieve. Rinse beaker with 200 mL H_2O, and pour H_2O evenly over tomato tissue on sieve. Tilt sieve to ca 30° angle and carefully wash tissue to lower edge using stream of H_2O from polyethylene wash bottle (500 mL Nalgene No. 2402-0500 wash bottle with delivery tube molded on side, or equiv.). Let tissue drain. If necessary, repeat washing and draining steps until tomato tissue is concentrated at lower edge of sieve. Transfer tissue, portion-wise, with a micro-spoon (Scientific Products No. S1571, or equiv.), or small scoop-style spatula to bottom of a graduated tube ca 12 × 3 cm. Tissue remaining on sieve should be washed to lower edge as before. Hold sieve at ca 80–90° angle so that some H_2O and tissue is retained at edge of sieve. Immediately take up H_2O and tissue with eyedropper having tip cut off at 2 mm id, and transfer to graduated tube. Repeat process of washing tissue to lower edge of sieve with wash bottle and transferring with dropper until all tissue has been transferred. Bring vol. of H_2O and tissue to 10 mL with H_2O. Add stabilizer soln, **44.003(gg),** to bring vol. to 20 mL and mix well using the micro-spoon or spatula. Pipet 4 sep. 0.5 mL portions using pipet, **44.002(s),** stirring sample preparation with pipet before drawing up each portion and pipetting from ca center of preparation. Spread 0.5 mL portion evenly over each 4 counting slides, **44.002(t),** letting material flow slowly, and spread uniformly in center of slide to cover area ca 6 × 2 cm. Touch lower end of pipet to slide several times to ensure complete removal of material. Blow out last drop if necessary. Cover preparation with glass plate and examine each slide for rot fragments at 40–45×, using transmitted diffused light. Rot

This report of the General Referee was presented at the 97th Annual International Meeting of the AOAC, Oct. 3–6, 1983, at Washington, DC.

Recommendation 2 of the General Referee was approved by Committee F and was accepted by the Association. The committee did not approve rec. 1. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition.

fragment is defined as particle of tomato cellular material with one or more mold filaments attached. Some may appear as almost solid masses of mold. See Figs. **44:06–44:08.**

"Count number of rot fragments on each of the 4 slides. Add results from the 4 slides to obtain number of rot fragments/g juice. Add result from 4 slides and multiply by 2 for 5.0 g samples (catsup, sauce, or dild puree or paste). Report number of rot fragments/g product."

Recommendations

(1) Revise **44.099** as described.

(2) Continue study on all topics.

Report on Disinfectants

RETO ENGLER

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Use-Dilution Test.—During the past year, several working sessions were held by Associate Referee Gayle Mulberry. The working group consisted of experts from state and federal governments, from industry, and from independent testing laboratories. The use-dilution test was carefully analyzed for procedural shortcomings as well as for problems related to the interpretation of test results. Major advances and insights were achieved in both areas. The procedural issues proposed for resolution or inclusion in the test were *(1)* standardization of drying time to 40 min rather than the present range of 20–60 min; *(2)* inclusion of the published method for culling defective carriers; *(3)* a standardized method of removal of *Ps. aeruginosa* pellicle by suction; *(4)* inclusion of a standardized method to determine neutralization of residual disinfectant; *(5)* a method to determine the number of viable organisms on a carrier; *(6)* standardization of hard water and "organic soil" load as additional challenges; *(7)* clarification on growth medium that is appropriate for organisms.

No conclusion could be reached concerning the interpretation of test results. However, we realized that we have to design a testing regimen that allows a quantitative statement on probability of a product to disinfect a hard surface carrier *and* on the probability that it will not. This problem is largely related to test design and statistical methods to establish limits of confidence, rather than to the procedural aspects of the test. Test designs to establish quantitative inferences on acceptance or rejection of products are available. Their applicability to the use-dilution test needs to be investigated.

Tuberculocidal Test.—Associate Referee J. Ascenzi has reported a major breakthrough concerning this test, which for a long time has been plagued by similar inconsistencies as reported for the use-dilution test, i.e., a particular product was not consistently capable of killing all organisms in 10 min. The Associate Referee has developed a quantitative assay that determines the time necessary to inactivate a standardized number of organisms. In summary, he determined that for some products inactivation of organisms in 10 min at 20°C is not complete, albeit several orders of magnitude. This new approach has not been tested in a collaborative study, but it is expected that such a study will be done as soon as possible. The quantitative approach to tuberculocidal testing does not use hard surface carriers. This deviation from prior testing must be evaluated. We hope that the program in tuberculocidal test methods will positively influence the program on the use-dilution test.

Virucidal Test.—An interim report was prepared by Associate Referee D. Fredell. Substantive progress is being made to improve this test: *(1)* A test in liquid medium is being considered (drying of viruses presents a problem). *(2)* An inactivation curve determination is being considered rather than a determination of the end point only. *(3)* Consideration is being given to testing virus groups rather than virus by virus; this approach can lead to a determination that a product is virucidal in general or specific for certain closely related viruses.

Sporicidal Test.—No Associate Referee report was received.

Fungicidal Test.—There is no Associate Referee for this topic. This test needs to be improved, maybe along the lines of progress made with the use-dilution test. Recommendations for an Associate Referee are solicited.

Recommendation

Continue study on all topics.

The report of the General Referee was received too late for consideration by Committee F at their meeting.

This report of the General Referee was presented at the 97th Annual International Meeting of the AOAC, Oct. 3–6, 1983, at Washington, DC.

Report on Drug and Device Related Microbiology

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Biological Indicator Testing Standardization.—Associate Referees Robert R. Berube and Gordon S. Oxborrow are working together on this topic to provide broader input to the complicated testing protocols. Progress in 1983 includes *(1)* agreement on priority of effort. *(2)* Protocols have been written for referee methods for steam and ethylene oxide biological indicators which include (a) population assay on paper strips, and (b) population assay in suspension. *(3)* Methodology has been written for determining survivor-kill characteristics on steam and ethylene oxide biological indicators, including a test plan for D-value determination by plate count methods and by quantal response assay.

Future studies are planned as follows: *(1)* in-house comparison of results by both Associate Referees on the above items; *(2)* expansion to full collaborative study of test procedures; *(3)* additional evaluation of problem areas such as (a) the effect of media lots and manufacturer, (b) heat activation techniques for various biological indicators, (c) the effect of blender speed on spore recovery, and (d) the effect of various diluents on the recovery of spores; and *(4)* development of methodology for population assay and resistance testing for non-paper strip carriers (inoculated product, etc.).

Additional Associate Refereeships will be requested for the following topics in 1984: package integrity microbiology; chemical sterilization indictors; industrial clean room microbiology; and ethylene oxide residual toxicity.

Limulus Amebocyte Lysate Tests for Endotoxin.—This topic was reassigned to this refereeship with Christine W. Twohy as Associate Referee. Progress in 1983 included publication of one paper and acceptance of another: "Comparison of Limulus Amebocyte Lysate from Different Manufacturers," C. W. Twohy, A.P. Duran, T. E. Munson, and M. L. Nierman, *Parenteral Science and Technology* (1983) **37**, 93–96. "Endotoxin Contamination of Parenteral Drugs and Radiopharmaceuticals Detected by Limulus Amebocyte Lysate Method," C. W. Twohy, A. P. Duran, and T. E. Munson, accepted for publication by the *Journal of Pharmaceutical Sciences*.

Current research includes 2 topics: *(1)* extraction of gram negative bacterial endotoxin from medical devices. A procedure has been established for extracting endotoxin from IV catheters, and was presented in a paper at the 1983 AOAC Symposium on Hospital Disinfectants and Sterilization Tests. Using the semiquantitative gel-tube LAL analysis (±50%), the average recovery was 54%. A collaborative study is scheduled to begin in 1984 on this methodology.

(2) Use of Multiskan microplate reader for the quantitative detection of gram negative bacterial endotoxin. Quantitative testing using the semiqualitative gel-tube method and quantitative spectrophotometric method (turbidometric and chromogens) is being conducted on drugs and medical devices. Comparisons of turbidometric and chromogenic LAL are nearly completed for endotoxin extraction from medical devices. Initial studies show a 25% increase in the amount of endotoxin detected by the chromogenic method.

Future studies will include *(1)* collaborative study on the extraction of gram negative bacterial endotoxin from medical devices; *(2)* comparison of chromogenic lysate from different manufacturers; *(3)* comparison of lysate activity on raw and purified endotoxins derived from various microorganisms that are common contaminants of medical products.

Recommendation

Continue study on all topics.

This report of the General Referee was presented at the 97th Annual International Meeting of the AOAC, Oct. 3–6, 1983, at Washington, DC.

The recommendation of the General Referee was approved by Committee F and was accepted by the Association. See the report of the committee, this issue.

Report on Extraneous Materials in Foods and Drugs

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Successful collaborative studies were carried out on methods for the extraction of light filth from unground marjoram and whole leaf peppermint.

Collaborative studies will be initiated on *(1)* a modification of the method for light filth in chocolate liquor, *(2)* the addition of 5 botanical products found applicable to the new method for whole leaf peppermint, and *(3)* modifications of the TLC visualization spray reagents for methods **44.A04–44.A07** (urine stains) and **44.183–44.185** (uric acid).

Associate Referees were appointed to the following new topics: Filth in Spirulina, and Performance Evaluation of Methods for Filth.

Methods development research will continue on all other topics.

Recommendations

(1) Adopt as official first action the method for the extraction of light filth from unground marjoram, and delete marjoram in method **44.142,** 13th ed.

(2) Adopt as official first action the method for the extraction of light filth from whole leaf peppermint.

(3) Based on the results of a methods use questionnaire sent to all FDA field laboratories, the following methods should be designated surplus: **44.013** (tea), **44.028** (pecans), **44.030** (coconut), **44.050(b)** (soy flour), **44.124(a)** (ground cinnamon), **44.125** (ground pepper), **44.132** (ground capsicums), **44.169** (bromothymol blue test for urine), and **44.178–44.182** (uric acid in flour).

(4) Continue study on all other topics.

This report of the General Referee was presented at the 97th Annual International Meeting of the AOAC, Oct. 3–6, 1983, at Washington, DC.

Recommendation 4 of the General Referee was approved by Committee F and was accepted by the Association. The committee did not approve recs. 1–3. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition, and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **63,** 374–423 (1980); **64,** 501–540 (1981); **65,** 450–521 (1982); **66,** 512–564 (1983).

Report on Food Microbiology

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Collaborative Studies

Six collaborative studies were performed; all are recommended for adoption official first action. They are as follows:

(1) Comparative collaborative study of the helium leak test and the vacuum leak test, using canned foods. Seven collaborators examined 400 dented cans for leaks, using the helium leak test and the commonly used vacuum test. The helium test identified 267 leaking cans, whereas the vacuum test identified 181.

(2) Comparison of *Salmonella* recovery from nonfat dry milk rehydrated under rapid and reduced conditions for preenrichment: Collaborative study. Twelve collaborators compared the AOAC method for *Salmonella* in nonfat dry milk with a modified method that involves a reduced rate of rehydration of the dry milk. Two shipments were made of samples that contained the desired levels of *Salmonella*. The modified method detected 47% more positive samples than did the AOAC method. The official method, **46.054–46.067,** should be revised to incorporate the method modification for dry milk.

(3) Comparison of a semi-automated method (Fossomatic-90) with the official optical somatic cell counting method III for somatic cells in milk. The Fossomatic-90 is a bench top, lower cost, slower machine that operates on the same basic principles as does the Fossomatic-215, an instrument that is the basis for an official method for counting somatic cells. Two Fossomatic-90 instruments were run in parallel with the 215 model and the direct microscopic somatic cell method. The Fossomatic-90 model was found to have acceptable precision, lack of bias, and agreement of results with direct microscopic somatic cell counts.

(4) Detection of *Escherichia coli* enterotoxins by using mouse adrenal cell and suckling mouse assays: Collaborative study. Eight analysts assayed 10 *E. coli* strains in duplicate for their ability to produce heat labile (LT) and heat stabile (ST) enterotoxins. At the 95% confidence level, the overall percentage of correct results for ST was 96.3 ± 2.9 and 95.0 ± 3.4 for LT.

(5) Enumeration of total coliforms, fecal coliforms, and *Escherichia coli* in foods by hydrophobic grid membrane filter (HGMF): Collaborative study. Eighteen laboratories assessed the HGMF method against the AOAC method for the enumeration of total coliforms, fecal coliforms, and *E. coli*. The membrane procedure gave slightly better recovery of the target organisms, and random error was lower than with the reference method in over 30% of the samples in a paired series.

(6) Genetic methods for the detection of microbial pathogens: Collaborative study to identify enterotoxigenic *Escherichia coli* by DNA colony hybridization. Thirteen laboratories received 25 cultures of *E. coli* to be tested for the heat labile toxin gene. Of the 325 cultures tested, 315 (96.9%) were identified correctly. Of the ten that were misclassified, there were 4 false negatives and 6 false positives.

This report of the General Referee was presented at the 97th Annual International Meeting of the AOAC, Oct. 3–6, 1983, at Washington, DC.

The recommendations of the General Referee were approved by Committee F and were adopted by the Association, except rec. 8, which the committee did not approve. See the report of the committee and "Changes in Methods," this issue.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition, and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **63,** 374–423 (1980); **64,** 501–540 (1981); **65,** 450–521 (1982); **66,** 512–564 (1983).

Associate Referee Reports

Bacillus cereus Enterotoxin.—Serological tests are being developed and the results are being correlated with the results of biological tests for the diarrheagenic enterotoxin of *B. cereus*. Further research is indicated before collaborative study.

Bacillus cereus, Enumeration and Confirmation.—The Associate Referee recommends adoption official final action of the method for differentiation of members of *B. cereus* group (**46.D01–46.D03**).

Canned Foods.—The Associate Referee has discussed official canned food methods with others in the field and no suggestions have been made for improvement of the current method.

Clostridium perfringens, Isolation and Enumeration.—Comments on the official methods continue to be favorable. Accordingly, no further studies are contemplated at this time.

Escherichia coli and Other Coliforms.—The Anderson/Baird-Parker procedure for *E. coli* has been examined using naturally contaminated foods inoculated with *E. coli*. The major problems encountered are *(1)* low productivity, *(2)* false positive reactions, and *(3)* finding naturally contaminated samples with statistically valid numbers of *E. coli*.

Parasitology.—The Associate Referee has evaluated a new method for recovery of parasites from sludge and food crops. The method proved unsatisfactory and a search for another method continues.

Salmonella.—The Associate Referee has been contacted by representatives from five firms or organizations about different proposed collaborative studies. Studies continue on the applicability of the soak method for dry milk to other dry foods.

Staphylococcal Enterotoxin.—A collaborative study is being planned to evaluate a radial diffusion method for screening staphylococci for their ability to produce entertoxin.

Staphylococcus.—An improved broth medium for *S. aureus* is under development and a collaborative study is planned.

Sugars.—The Associate Referee has received several requests for a comparative study on the merits of substituting PE-2 medium for liver broth in the AOAC method for thermophiles in sugar. About 10 pounds of naturally contaminated sugar is needed. However, to date, no suitable lot has been found.

Yeasts, Molds, and Actinomycetes.—Studies were conducted on the usefulness of NaCl to inhibit mold spreading on agar. A collaborative study on the use of NaCl is planned.

Yersinia enterocolitica.—A collaborative study is planned on a method to detect *Y. enterocolitica* in food.

Recommendations

(1) *Escherichia coli and other coliforms.*—Adopt as official first action biological methods for detecting heat-stabile and heat-labile enterotoxins.

(2) *Genetic methods for detection of bacterial pathogens.*—Adopt as official first action a method for detecting *E. coli* producing heat labile enterotoxin. Continue study.

(3) *Helium leaks in canned foods.*—Adopt as official first action a method for detecting leaks in cans. Discontinue topic.

(4) *Hydrophobic grid membrane filter methods.*—Adopt as official first action a membrane method for detecting total coliforms, fecal coliforms, and *E. coli*. Continue study.

(5) *Identification of microorganisms by biochemical kits.*—A collaborative study is planned. Continue study.

(6) *Salmonella.*—Revise official first action the official final action method for the detection and identification of *Salmonella*, **46.054–46.067,** to modify the preparation of sample for nonfat dry milk. Continue study.

(7) *Salmonella, fluorescent antibody technique.*—Discontinue topic.

(8) *Somatic cell, Fossomatic counting method.*—Adopt as official first action a method for somatic cells, using the Fossomatic-90. Continue study.

(9) *Spore-formers and non-spore-formers in low acid canned foods.*—A collaborative study is under way. Continue study.

(10) *Staphylococcal toxin.*—Continue study.

(11) *Staphylococcus.*—Adopt as official final action the official first action surface plating method for isolation and enumeration of *Staphylococcus aureus* in food, **46.075–47.076.**

(12) *Bacillus cereus; Enumeration and Confirmation.*—Adopt as official final action the official first action method for differentiation of members of *B. cereus* group, **46.D01–46.D03.**

(13) In the official final action method for *Salmonella*, **46.054–46.067,** in **46.061(a)** change the incubation period of lysine decarboxylase broth from 96 ± 2 hr to 48 ± 2 hr.

(14) Editorially revise the applicability statement for method **46.117–46.119,** Fecal Coliforms, by adding the following: "Because geographical differences may affect performance of the medium A-1 method, determination of comparability with the LST-EC tube method should be performed prior to using Medium A-1. Moreover, this medium must be made from individual ingredients. Pre-formulated Medium A-1 is unacceptable."

(15) Transfer the topic Endotoxins by Limulus Amebocyte Lysate to the General Refereeship for Drug and Device Related Microbiology.

(16) Continue study on all other topics.

GENERAL REFEREE REPORTS: COMMITTEE G

Report on Antibiotics

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This year there have been few collaborative studies. Instead, there has been modest movement in establishing procedures that were adopted official first action and recommending adoption as official final action.

β-Lactam Antibiotics in Milk: Although no further collaborative studies were performed, the Associate Referee for the affinity quantitative determination of penicillin in milk, Stanley Charm, recommended adoption of the method as official final action, based on correspondence from several laboratories reporting no difficulties and that the procedure performed as described. The General Referee concurs and recommends adoption as official final action with a lower limit of detectability of 0.01 IU/mL.

James Messer, Associate Referee for qualitative determination of β-Lactam residues in milk, recommended official final action status based on his experience of "widespread and satisfactory use of the method as well as the lack of adverse comments." The General Referee concurs and recommends official final action with the level of detectability of 0.008 IU/mL. This level was consistently detectable, 100% of the time. This recommendation is based on use of antibiotic medium 4 and incubation at 55 or 64°C.

Recommendations

(1) Adopt as official final action the affinity quantitative determination of penicillin in milk, and the qualitative determination of β-lactam residues in milk.

(2) Continue study on all other topics.

This report of the General Referee was presented at the 97th Annual International Meeting of the AOAC, Oct. 3–6, 1983, at Washington, DC.

The recommendations of the General Referee were approved by Committee G and were adopted by the Association. See the report of the committee (and "Changes in Methods"), this issue.

Report on Cosmetics

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Essential Oils and Fragrance Materials, Composition.—Associate Referee Harris H. Wisneski has completed work on the development of an LC/fluorometric method for the determination of cinnamaldehyde in fragrances. The aldehyde fraction of the fragrance is first isolated by extraction with a basic solution of 6-aminocaproic acid. An aliquot containing the aldehydes is reacted with 1,2-diaminonaphthalene sulfate under acid conditions to form the fluorophore 2-styrylnaphth (1,2-d) imidazole. The mixture is then analyzed by reverse phase LC-fluorometry to determine cinnamaldehyde. No interferences were noted. Recoveries at 0.01, 0.05, and 0.1% levels averaged 103%.

Nitrosamines.—Associate Referee Hardy J. Chou has completed a method for the rapid screening of cosmetic products for nitrosamines. The cosmetic is partitioned between water and methylene chloride to separate the polar and nonpolar nitrosamines. Each solution is then treated with a reducing agent to destroy nitrite. An aliquot of one of the solutions is placed in a sparging tube and a chemical reduction-cleavage reagent is added. The nitric oxide formed from the nitrosamine(s) is swept from the apparatus with inert gas to a nitric oxide analyzer.

Recommendations

(1) Continue official first action status of method **35.019–35.023**, and method **35.001–35.006**.

(2) Discontinue the following topic: Deodorants, Aluminum and Zirconium in.

(3) Continue study on all other topics.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition.

This report of the General Referee was presented at the 97th Annual International Meeting of the AOAC, Oct. 3–6, 1983, at Washington, DC.

The recommendations of the General Referee were approved by Committee G and were accepted by the Association. See the report of the committee (and "Changes in Methods"), this issue.

Report on Drug Residues in Animal Tissues

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No reports were received on the topics of Carbadox and Dimetridazole. The topics Diethylstilbestrol and 3,5-Dinitrobenzamide are vacant.

Screening Methods

Sulfonamides.—The TLC densitometric screening method for sulfonamides in animal tissues was adopted first action for sulfaquinoxaline in turkey tissues; sulfadimethoxine in turkey, swine, and duck tissues; and sulfamethazine in turkey and swine tissues. Food Safety and Inspection Service (FSIS), U.S. Department of Agriculture, reports no problems with the extensive use of this procedure during the last year.

The Manuel and Steller GC method (*J. Assoc. Off. Anal. Chem.* **64,** 794–799 (1981)) and the Suhre et al. GC/MS method (*J. Agric. Food Chem.* **29,** 727–729 (1981)) were adopted official first action for sulfamethazine in swine liver. Since 1981, both methods have been in use in some FDA laboratories. The GC/MS method has been in use in FSIS laboratories. A paper by S. J. Stout et al. (*J. Assoc. Off. Anal. Chem.* **67,** 142–144 (1984)) reports a GC/MS procedure to confirm sulfamethazine if present in the extracts from the GC method.

Tiamulin.—The Food and Drug Administration recently approved tiamulin fumarate (14-deoxy-14-((2-diethylamino-ethyl)mercaptoacetoxy)mutilin hydrogen fumarate) for dosing swine. The approved GC method requires more than one day to analyze 5 or 6 samples. A shorter screening procedure would be helpful in monitoring programs.

Benzimidazoles.—Substituted benzimidazoles are used as anthelmintics in horses, cattle, swine, and sheep. The analytical methods for some of these substances need improvement. It would be helpful if multi-residue procedures could be developed to identify and quantitate several of these drugs in a single extract of edible animal tissue. Under the sponsorship of the Food and Drug Administration, Leon LeVan, Hazleton-Raltech, is pursuing the development of a multi-residue method for the determination of residues of thiabendazole, albendazole, oxfendazole, mebendazole, and fenbendazole in cattle tissue.

LeVan has agreed to serve as Associate Referee for the development of multi-residue methods for benzimidazole residues in edible animal tissue.

Recommendations

(1) Discontinue the topics Carbadox, Diethylstilbestrol, Dimetridazole, 3,5-Dinitrobenzamide, Nitrofuran.

(2) Adopt the sulfonamide screening method official final action.

(3) Establish the new topics Tiamulin, and Benzimidazole Multi-Residues.

(4) Adopt the GC/MS and GC methods for sulfonamides official final action.

This report of the General Referee was presented at the 97th Annual International Meeting of the AOAC, Oct. 3–6, 1983, at Washington, DC.

The recommendations of the General Referee were approved by Committee G and were accepted by the Association. See the report of the committee (and "Changes in Methods"), this issue.

Report on Drugs in Feeds

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The proliferation of the use of in-house methods necessitates that greater communication will be needed among Associate Referees and other scientists concerned with the same analyte. A survey was conducted to determine methods currently in use. A summary was distributed to all participants and all Associate Referees. The survey should be useful in identifying areas of activity to the referees.

Carbadox.—(Mark S. Litchman). LC methodology is currently under study. The earliest date for a collaborative study is 1985.

Dimetridazole.—(Glenn M. George). A collaborative study based on the method reported in *J. Assoc. Off. Anal. Chem.* (1974) **57,** 343–344, is planned for 1984.

EDDI.—(Gary Ross). A colorimetic procedure is currently being investigated.

Furazolidone.—(Robert Smallidge). A method is ready for collaborative study.

Lasalocid.—(Edward H. Waysek). An LC method is being studied.

Larvadex.—This topic has been transferred to Committee A.

Morantel Tartrate.—(James Peters). Work on an LC method will be initiated after work on carbadox and pyrantel tartrate has been completed.

Pyrantel Tartrate.—(James A. Braswell). An LC method is being developed.

Sulfa Drug Residues.—(Robert K. Munns). A collaborative study is underway.

Sulfamethazine and Sulfathiazole.—(Dwight Lowie). A method will be circulated preliminary to a collaborative study.

No activity was reported on other topics. Referees are needed for Amprolium, Arsanilic Acid, and Ethopabate.

Recommendations

(1) Appoint Referees on the topics Amprolium, Arsanilic Acid, and Ethopabate.

(2) Continue study on all topics.

This report of the General Referee was presented at the 97th Annual International Meeting of the AOAC, Oct. 3–6, 1983, at Washington, DC.

The recommendations of the General Referee were approved by Committee G and were accepted by the Association. See the report of the committee (and "Changes in Methods"), this issue.

Report on Veterinary Analytical Toxicology

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The AOAC Refereeship in Veterinary Analytical Toxicology has been in existence for 2 years. During this period, interest has been steadily and rapidly increasing. An example was the session held at the 1983 AOAC Spring Workshop in Indianapolis, IN. Featured at this session were presentations on nitrates, sampling, monensin, quality control, samples, copper, and general discussion. The speakers presented basic data and information in a manner that invited audience participation through discussion and frank comments. Of particular interest were the differing views on nitrate analysis as well as a lively exchange on the preferred approach for screening diagnostic samples for monensin. The most striking information of the session was presented in a paper on quality control. A survey of diagnostic laboratories conducted by the speaker revealed that quality control procedures were used in only 66% of the routine toxicology analyses.

Demonstrations by scientists performing diagnostic toxicology analyses were another feature of the spring workshop. Included were demonstrations of the nitrate electrode, a mycotoxin cleanup method, and TLC detection of monensin.

Two other meetings held in the past year have also offered excellent opportunities for technical presentations, information exchange, and discussion. These include the 1982 Annual Meeting of the American Association of Veterinary Diagnosticians (AAVLD) held in Nashville, TN, in November 1982 (1) and the 1983 Midwest Regional AOAC meeting held in Ames, IA, in June 1983 (2). On both occasions, the AAVLD Committee on Veterinary Toxicology met and heard interim reports on activities of Associate Referees. In addition, discussions were held and recommendations were made on specific questions concerning methods, samples, and diagnostic levels of specific toxicants.

The frequent meeting and information exchange of concerned scientists has been the most outstanding feature in all that has been accomplished to date in veterinary analytical toxicology.

New techniques and applications that provide practical and useful diagnostic information continue to appear:

Hewlett et al. (3) has recently described an LC technique for the determination of glycolic acid in serum and urine from dogs poisoned by ethylene glycol. Excretion kinetics and diagnostic levels are also presented. Reynolds (4) has reported on techniques for 2 classes of poisons: the LC determination of creosote in rumen contents and the GC and infrared determination of carbon tetrachloride and carbon disulfide in fumigated grains. Stowe (5) has reported on an LC method for vitamin A analysis in animal tissues. Included in his report are data from clinical cases and diagnostic ranges of vitamin A.

Schock and Braselton (6) have presented a method to partially answer the classic diagnostic request, "Check for poisons." Using GC/MS and gel permeation chromatography, the authors have developed a powerful technique which may be used to screen for and positively identify a broad range of organic compounds pertinent to diagnostic toxicology. This method is a useful technique for checking the "negative" sample as well as the positive one.

Techniques in thin layer chromatographic detection, perhaps the single most powerful tool to be used in any laboratory, were featured in a recent workshop (7).

The individual Associate Referee areas have been very active. The topics listed here certainly do not represent every area of interest or activity. More volunteers are being sought in an effort to broaden the base of support. A summary of each topic follows:

Arsenic in Animal Tissues.—Tracy Hunter (Division of Consolidated Labs, Richmond, VA) reports that a collaborative study of a modification of the existing method (**41.009–41.012**) for As residues has been conducted. Twelve participating laboratories analyzed 8 liver samples for arsenic. Levels ranged from less than 1 ppm As to 30 ppm As. Results from the study were extremely varied with respect to both precision and accuracy. The variation between laboratories ranged from 122% at 4 ppm to 27% at 24 ppm. Recovery ranged from 90 to 175% on the samples spiked with As in the form of arsanilic acid to less than 80% for samples containing As in a naturally incurred form.

The Associate Referee recommends that the study be repeated with the following changes: (1) Add the cooling step back to the procedure prior to arsine evolution; (2) add a step to include a total reagent and glassware blank; (3) add a lower standard of 0.5 μg As to better define the lower limit of detection; (4) add instructions to report low level samples as less than 1 ppm As and not as an exact number.

Cholinesterase.—Associate Referee Paula Martin (Iowa State University, Ames, IA) reports she has completed a survey of several diagnostic laboratories conducting cholinesterase determinations. The aim of the survey was to determine normal values, standard samples, frequency of analysis, and species variation. As expected, results were quite varied and point to a broad range of opinion as to the proper sample for analysis and what normal values are. Considerable variation exists in units used to report results.

Methods used in most situations were variations of either the modified Michel (8) or the Ellman (9) methods, both of which provide reliable data.

The Associate Referee is currently preparing large batches of freeze-dried blood and freeze-dried brain for an interlaboratory study and comparison.

Copper in Animal Tissues.—Associate Referee David L. Osheim (National Veterinary Services Laboratories, Ames, IA) reports on a busy year. A study comparing the official serum copper method (**25.D01**) with those used by participating laboratories was conducted. Four serum samples were sent to 11 laboratories for analysis by their method and the official method. Some laboratories did not run the samples by 2 methods because of insufficient amount of sample or they used only the official method. Comparison of the 2 sets of data is difficult because of the mixed nature.

The CV values of the samples at a concentration of 200 μg Cu/100 mL serum were comparable between methods (6–9%), while the sample at less than 30 μg Cu/100 mL serum had a wider range of CV values, 23% for the official method and 66% for all other methods.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition, and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **63**, 374–423 (1980); **64**, 501–540 (1981); **65**, 450–521 (1982); **66**, 512–564 (1983).

This report of the General Referee was presented at the 97th Annual International Meeting of the AOAC, Oct. 3–6, 1983, at Washington, DC.

The recommendations of the General Referee were approved by Committee G and were accepted by the Association. See the report of the committee (and "Changes in Methods"), this issue.

The other methods included wet ash/ICP, wet ash/AA, wet ash/furnace AA, direct dilution/furnace AA, and dry ash/AA.

Several interesting points were generated from the study. Some laboratories did not use a control sample, some laboratories reported results even though the control sample was not within the specified range, and one laboratory reported 0.0 for one answer with no sensitivity specified.

In addition to the comparison study, the Associate Referee also conducted a collaborative study of a low temperature ashing/AA method (10) for determining Cu in liver tissue. Prior to the collaborative study, 4 laboratories analyzed identical samples by the proposed method. The range of sample concentration was 6.1–306 ppm Cu with CV values ranging from 15.6% to 9.2%

Based on the interlaboratory study and further evaluation, a collaborative study was conducted. Data are currently being analyzed.

Lead in Animal Tissues.—Associate Referee Robert Everson (Purdue University, West Lafayette, IN) reports that he is currently conducting an interlaboratory study of several methods for the determination of Pb in whole blood. The methods include wet digestion/AA flame, Delves cup/AA flame, extraction/AA flame, extraction/AA furnace, wet digestion/ICP, neutron activation, and anodic stripping voltammetry. Several diagnostic and clinical laboratories are conducting analysis of 5 sheep blood samples which contain high Pb levels. After the results have been compiled and analyzed, the Associate Referee will select the best 4 methods for a collaborative study.

A collaborative study design for studying 4 different methods has been submitted and approved. The design tests each method separately, not for comparison purposes.

Molybdenum.—Associate Referee Tracy Hunter (Division of Consolidated Labs, Richmond, VA) the newly assigned referee for this topic is currently studying the literature for methods applicable to animal tissues. The existing method **(3.056–3.058)** for plants suffers from insensitivity when applied to animal tissues.

The Associate Referee recommends continued study and welcomes any comments or suggestions from the field concerning this topic.

Monensin.—Associate Referee Ronda Moore (Iowa State University, Ames, IA) reports on the development of a minicolumn cleanup screening method for monensin (G. E. Rottinghaus, private communication). By using the charcoal/alumina column developed by Romer (11) for tricothecene mycotoxins, monensin is extracted in methanol–H_2O (9 + 1), applied to the column, and eluted with carbon tetrachloride. By using TLC with vanillan or *p*-anisaldehyde spray for detection, 1 ppm detection in mixed feeds has been reproduced consistently by several laboratories.

The Associate Referee is currently studying extraction efficiency of several techniques. Recovery problems have been encountered with very concentrated samples, depending on the extraction system used (shaking vs blending).

A study to determine between-laboratory variation is planned prior to a possible collaborative study of the minicolumn screening method.

Multielement Analysis.—Associate Referee W. Emmett Braselton (Michigan State University, East Lansing, MI) continues to demonstrate the power of the ICP technique. In a study conducted by the Associate Referee and his colleagues, the effect of hemolysis on 7 element values was determined by ICP on bovine serum. Serum from several cattle was subjected to varying degrees of hemolysis and correlated with Fe and hemoglobin. In a second study, the variability of subsample aliquots was illustrated by ICP analysis of a mineral premix. Paying close attention to instrument precision, 12 samples were analyzed for 10 different elements, with Cu (136%) showing the greatest range of variability. A similar study was also conducted on subsamples of ground corn (12).

Another study demonstrated the variability of serum element values due to storage for various lengths of time in commonly used vacutainer tubes (13).

In each study, several simultaneous analysis were performed on the same sample preparation, eliminating variation due to method or sample preparation. Compared with classical methods, ICP is more rapid and accurate and less time consuming.

The Associate Referee recommends continued study.

Multiple Anticoagulant Screening.—Associate Referee John David Reynolds (Animal Disease Laboratory, Centralia, IL) reports on a design for a collaborative study of a screening method for 7 different anticoagulants in baits. The basic procedure involves extraction of the anticoagulants with acetonitrile and detection by LC and UV. The LC system includes 2 isocratic setups because of the general unavailability of gradient capabilities in participating laboratories.

Prior to the study, laboratories will be provided with standards and practice samples so that they may become familiar with the procedure. The study, which will provide information on reproducibility, repeatability, accuracy, and the rate of false negatives and false positives, has been approved.

The Associate Referee also reports on some data from anticoagulant poisoning cases. Diphacinone has been detected in livers of dogs at levels greater than 1 ppm 3 to 4 weeks after exposure and treatment. Brodifacoum has also been detected in livers of poisoned animals at less than 1 ppm. Baits containing more than one anticoagulant are encountered frequently; this supports the need for a multiple screening method.

Nitrates and Nitrities.—Associate Referees Michael Carlson and Norman Schneider (University of Nebraska, Lincoln, NE) report on further study of the nitrate electrode. A study of forage nitrate determination conducted by McMahon (14) in 1982 provided the basis for comparison of the nitrate electrode and the cadmium (Cd) reduction column **(7.045)**. In the study, 15 laboratories analyzed the same 4 forage samples by either the nitrate electrode (9 labs) or by Cd reduction (6 labs). A comparison of the average results from the 2 methods indicates that the Cd reduction performs poorly at higher concentrations of KNO_3. This problem may be corrected in the Cd procedure by diluting the sample and repeating the analysis, resulting in an increased analysis time of up to 2 h. The nitrate electrode requires only 20 min for a complete analysis.

Interlaboratory precision results from the study clearly showed the more reliable performance of the electrode. Variation for the electrode ranged from 17% to 53% for samples containing 5.77% and 0.32% KNO_3, respectively. The respective variations for the Cd reduction were 41% and 43%.

Work by Baker and Smith (15) has previously addressed the problems of interfering anions on the nitrate electrode. Use of low pH and a standard addition of KNO_3 to the extracting solution negates any interferences that may occur due to increase in salt level from the forage extraction.

The Associate Referees have concluded the nitrate electrode meets the analytical and diagnostic requirements for determining nitrates in forage and recommend that a collaborative study be conducted.

Poisonous Plants.—Associate Referee George Rottinghaus (University of Missouri, Columbia, MO) reports on the development of a C_{18} minicolumn cleanup general alkaloid screening technique (16). Included in the screen are strychnine, nicotine, and atropine. The classical strong acid hydrolysis and extraction has been eliminated in favor of a milder acidic extraction and buffered neutralization prior to cleanup with a 1 g C_{18} minicolumn and detection by TLC.

The Associate Referee recommends further study and expansion of the method to other plant alkaloids, depending on availability of standards.

Selenium in Animal Tissues.—Associate Referee James Roof (Bureau of Animal Industry, Harrisburg, PA) is currently conducting an interlaboratory study of a GC Se method (17). The method employs dry ashing, complex formation, and GC/electron capture detection. Eight laboratories have been sent 4 liver samples for selenium determination by the method.

The results of the study will be used for determining if a collaborative study is possible.

Sodium Monofluoroacetate.—Associate Referee H. Michael Stahr (Iowa State University, Ames, IA) reports on the development of a defluorination procedure for screening for sodium monofluoroacetate (1080) in stomach contents and baits. The procedure is indirect and measures only organic fluoride. Suspected positive cases should be confirmed by GC/MS.

Study of background and normal levels of organic fluoride in tissues is necessary to eliminate possible false positive results from the method. Data are also needed concerning the relative levels of fluorocitrate and fluoroacetate in tissues of known primary and secondary poisoned animals.

The Associate Referee recommends continued study on the optimum conditions for defluorination of fluoroacetate in addition to the study of applicability to tissues.

Fluoride in Animal Tissues.—David L. Osheim (National Veterinary Services Laboratories, Ames, IA) is the newly appointed Associate Referee for this topic. A literature study and survey of the field is planned to establish the need for study of this topic and to determine the diagnostic tissues and levels.

Recommendations

(1) Make additions to the method on arsenic in animal tissues as outlined by the Associate Referee and conduct a collaborative study.

(2) Continue as official first action the serum copper method (**25.D01**) and submit results of collaborative study of copper in tissue for interim approval.

(3) Conduct collaborative study of nitrates in forage by the nitrate electrode.

(4) Continue study on all other topics.

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TRANSACTIONS OF THE ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS

The ninety-seventh annual meeting of the Association of Official Analytical Chemists was held at the Shoreham Hotel, Washington, DC, on October 3, 4, 5, and 6, 1983. The following reports, along with the actions of the Association, were given at the business meeting, held Thursday, October 6, 1983, W. R. Bontoyan presiding.

Report of the Official Methods Board

ELMER GEORGE, JR, *Chairman,*
NY State Department of Agriculture & Markets, Food Laboratory, Albany, NY 12235

Other Members: A. R. Hanks, D. E. Coffin, J. C. Kissinger, J. A. Burke, E. Sarnoff, P. M. Brickey, R. J. Noel

The Official Methods Board met twice during the year to conduct business. The first meeting was held May 20, 1983, at the AOAC office, Arlington, VA; the second, at the Annual Meeting on October 2, 1983.

Official Methods Committees received 58 collaborative study reports during the year; 14 were given interim official first action approval. Further recommendations from the various Methods Committees are to be announced in subsequent reports. The final number of collaborative study reports to receive official recognition awaits the voting by the membership during the Association's Business Meeting. By way of contrast, 40 collaborative study reports were submitted in 1981 and 39 in 1982. An average of 48 per year were submitted during the decade ending 1980.

The customary letters of instruction were sent to all Associate and General Referees in January and June.

Official Methods Committees nominated the following Associate Referees for the Associate Referee of the Year Award: Peter F. Kane, Committee A; Michel Margosis, Committee B; Dick H. Kleyn, Committee C; (No nomination), Committee D; James A. Ault, Committee E; Walter E. Hill, Committee F; (No nomination), Committee G. Dr. Walter E. Hill was selected as the winner for his outstanding report. The award was presented by President Warren R. Bontoyan at the General Session, October 3, 1983. Dr. Hill is the first microbiologist to receive the award.

Members of the Official Methods Board recommend to the Board of Directors the following actions regarding use of performance parameters:

(1) Discontinue use of inter- and intra-laboratory standard deviations, in the *Book of Methods*.

(2) Use CV_o and CV_x (i.e., the coefficients of variation corresponding to S_o and S_x, respectively).

(3) If, however, standard deviations are not discontinued, the following should be used: S_o, within-laboratory standard deviation; S_L, pure among-laboratory standard deviation; S_x, overall among-laboratory standard deviation.

Report of Committee A on Recommendations for Official Methods

ALAN R. HANKS (Office of the Indiana State Chemist, Purdue University, Department of Biochemistry, West Lafayette, IN 47907), *Chairman;*
WARREN R. BONTOYAN (Environmental Protection Agency, Office of Pesticide Programs, Beltsville, MD 20705);
A. ANER CARLSTROM (Chevron Chemical Co., 940 Hensley St, Richmond, CA 94804);
LOUIS W. FERRARA (IMC Corp., 1401 S 3rd St, Terre Haute, IN 47808);
FRANK J. JOHNSON (TVA/National Fertilizer Development Center, Muscle Shoals, AL 35660);
MARY MAUD SHARPE (State Department of Agriculture and Consumer Services, Feed Laboratory, Tallahassee, FL 32301);
RICHARD H. COLLIER (Department of Biochemistry, Purdue University, West Lafayette, IN 47907), *Secretary;*
EDWIN M. GLOCKER (14697 Roxbury Rd, Glenelg, MD 21737), *Statistical Consultant*

The Committee met individually with the General Referees during the annual meeting of the Association. Methods recommendations and other topics of interest to the Committee and the General Referees were discussed.

The Committee views these sessions as valuable opportunities for interchange of ideas and will continue the practice of holding these meetings next year. The Committee is pleased to report positive reocmmendations on 11 of 12 collaborative studies presented for consideration during this year. One study was rejected because of inadequate statistical support for the method.

During this year, Dean Hill was appointed General Referee for Hazardous Substances and Ray Severson was appoitned General Referee for Tobacco. The Committee anticipates increased activity in these areas as a result of the initiatives of these new General Referees.

FEEDS

(1) *Amino Acids in Mixed Feeds:* Continue study.
(2) *Fat, Crude, in Pet Foods:* Appoint Associate Referee and continue study.
(3) *Fiber, Crude:* Continue study.
(4) *Fiber, Crude, in Milk Replacers:* Continue study.
(5) *Infrared Reflectance Techniques in Mixed Animal Feeds:* Continue study.
(6) *Iodine in Feeds:* Continue Study.
(7) *Minerals:* Continue study.
(8) *Non-Nutritive Residues:* Continue study.
*(9) *Protein, Crude:* (a) Adopt as official first action the copper catalyst method described by the Associate Referee. (b) Continue study.
(10) *Sampling:* Appoint Associate Referee and continue study.
(11) *Water (Karl Fischer Method):* Appoint Associate Referee and continue study.
(12) *Other Topic:* Study need for new topic "Enzymes in Feeds."

FERTILIZERS

(1) *Biuret in Urea and Mixed Fertilizers:* Continue study.
(2) *Boron:* (a) Continue official first action status of the spectrophotometric method (**2.C01–2.C04**). (b) Continue study.
(3) *Free and Total Water:* Continue study.
(4) *Iron:* (a) Continue official first action status of the atomic absorption method for chelated iron in iron chelate concentrates (**2.D16–2.D18**). (b) Continue study.
(5) *Molybdenum:* Discontinue topic.
(6) *Nitrogen:* Continue study.
(7) *Phosphorus:* Continue study.
(8) *Potash:* (a) Continue official first action status of the flame photometric method (**2.D06–2.D15**). (b) Continue study.
(9) *Sampling and Preparation of Sample:* Continue study.
(10) *Slow-Release Mixed Fertilizers:* (a) Continue official first action status of the water elution method (**2.073–2.074**). (b) Continue study.
(11) *Sodium:* (a) Continue official first action status of the atomic absorption method (**2.D19–2.D22**) and the flame photometric method (**2.147–2.150**). (b) Continue study.
(12) *Soil and Plant Amendment Ingredients:* (a) Continue official first action status of the atomic absorption method for aluminum in aluminum sulfate soil acidifiers (**2.D23–2.D25**). (b) Continue study.
(13) *Sulfur:* (a) Continue in official first action status of the gravimetric method for total and elemental sulfur (**2.A01**). (b) Continue study.
*(14) *Water-Soluble Methylene Ureas:* (a) Adopt as official final action the official first action liquid chromatographic method (**2.D01–2.D05**). (b) Continue study.
(15) *Zinc:* Continue study.

HAZARDOUS SUBSTANCES

(1) *Ammonia as a Product Ingredient:* Continue study.
(2) *Benzene in Consumer Products:* Continue study.
(3) *Carbolic Acid (Phenolic) Compounds:* Continue study.
(4) *Chlorinated Hydrocarbons:* Continue study.
(5) *Diethylene Glycol and Ethylene Glycol:* Continue study.
(6) *Flammable Substances in Pressurized Containers:* Continue study.
(7) *Flash Point of Solids and Semisolids:* Continue study.
(8) *Formaldehyde:* Continue study.

*An asterisk before a recommendation indicates that official action on that recommendation resulted in its appearance in "Changes in Methods."

The recommendations submitted by Committee A were adopted by the Association.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition; and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **63**, 374–423 (1980); **64**, 501–540 (1981); **65**, 450–521 (1982); **66**, 512–554 (1983).

(9) *Hazardous Components in Resin Systems:* Continue study.
(10) *Pentachlorophenol in Toy Paints:* Continue study.
(11) *Petroleum Distillates in Mixtures:* Continue study.
(12) *Selenium:* Continue study.
(13) *Toxic Metals in Paints:* Continue study.
(14) *Turpetine:* Continue study.
(15) *Viscosity of Liquids:* Continue study.
(16) *Other topic:* (a) Continue official first action status of the method for volatile denaturants in alcoholic products **(5.012–5.013)**. (b) Continue study.

PESTICIDE FORMULATIONS: CARBAMATE INSECTICIDES AND SUBSTITUTED UREA INSECTICIDES

(1) *Aldicarb:* Continue study.
(2) *Aminocarb:* Continue study.
(3) *Carbaryl:* Continue study.
(4) *Carbofuran and Carbosulfan:* Continue study.
(5) *2,2-Dimethyl-1,3-benzodioxol-4-yl Methylcarbamate (Bendiocarb):* Continue study.
*(6) *4-Methio-3,5-xylyl Methylcarbamate (Methiocarb):* (a) Adopt as official first action the liquid chromatographic method described by the Associate Referee. (b) Continue study.
*(7) *o-Isopropoxyphenyl Methylcarbamate (Propoxur):* (a) Adopt as official first action the liquid chromatographic method described by the Associate Referee. (b) Continue study.
(8) *Methomyl:* Continue study.
(9) *Oxamyl:* Continue study.
(10) *Pirimicarb:* Continue study.

PESTICIDE FORMULATIONS: FUNGICIDES AND DISINFECTANTS

(1) *Bayleton:* Continue study.
*(2) *Benomyl:* (a) Adopt as official first action the liquid chromatographic method described by the Associate Referee with the provision that the following explanatory statement be added: "This method determines the benomyl equivalent of the benomyl and methyl-2-benzimidazole carbamate present." (b) Continue study.
*(3) *Captan:* (a) Designate as surplus the gas chromatographic method **(6.215)**. (b) Discontinue topic.
(4) *Carboxin and Oxycarboxin:* Continue study.
(5) *Chlorothalonil:* Continue study.
(6) *Copper Naphthenate:* Continue study.
(7) *Dinocap (2,4-Dinitro-6-octyl Phenyl Crotonate):* Continue study.
(8) *Dithiocarbamate Fungicides:* Continue study.
(9) *Folpet:* Discontinue topic.
(10) *Pentachloronitrobenzene:* Continue study.
(11) *o-Phenylphenol:* Continue study.
(12) *Quaternary Ammonium Compounds:* Continue study.
(13) *Thiram:* Continue study.
*(14) *Triphenyltin:* (a) Adopt as official first action the interim first action gas chromatographic method. (b) Continue study.

PESTICIDE FORMULATIONS: GENERAL METHODS

(1) *Atomic Absorption Spectroscopy:* Discontinue topic.
(2) *Contaminants in Pesticide Formulations:* Discontinue topic.
(3) *Dioxins (2,3,7,8-Tetrachloro-p-dibenzo-p-dioxin in 2,4,5,-T):* Continue study.
(4) *Nitrosamines:* Discontinue topic.
(5) *Pesticide in Spray Tank Dispersions:* Merge this topic into the topic, Sampling.
(6) *Physical Properties of Pesticides:* Continue study.
*(7) *Sampling:* (a) Adopt as official first action the sampling procedures for fertilizers **(2.001–2.002)** for Fertilizer-Pesticide Mixtures. (b) Continue study.
(8) *Sampling of Pressurized Cans (Aerosols):* Merge this topic into the topic, Sampling.
(9) *Volatility of Hormone-Type Herbicides:* Discontinue topic.
(10) *Water-Soluble Copper in Water-Insoluble Copper Fungicides:* Continue official first action status of the CIPAC–AOAC atomic absorption and bathocuproine methods **(6.B01–6.B08)**.

PESTICIDE FORMULATIONS: HALOGENATED INSECTICIDES

*(1) *Benzene Hexachloride and Lindane:* (a) Adopt as official first action the gas chromatographic method for BHC, gamma isomer (lindane) described by the Associate Referee. (b) Continue official first action status of the benzene hexachloride radioactive tracer method **(6.212)**. (c) Continue study.
(2) *Chlordane:* Continue study.
(3) *Chlordimeform:* Continue study.
(4) *Dicofol (1,1-Bis(chlorophenyl)-2,2,2-trichloroethanol):* (a) Continue official first action status of the hydrolyzable chloride method **(6.283–6.288)**. (b) Continue study.
*(5) *Diflubenzuron:* Adopt as official final action the official first action CIPAC–AOAC liquid chromatographic method **(6.D09–6.D15)**.
*(6) *Endosulfan:* (a) Adopt as official final action the official first action CIPAC–AOAC gas chromatographic method **(6.D16–6.D21)**. (b) Continue study.
(7) *Fenvalerate:* Continue study.
(8) *Heptachlor:* Continue study.
(9) *Methoxychlor:* Continue study.
(10) *Perthane:* Continue study.
(11) *Toxaphene:* Continue study.
(12) *Trichlorfon (Dylox):* Continue study.

PESTICIDE FORMULATIONS: HERBICIDES I

*(1) *Chlorophenoxy Herbicides:* (a) Adopt as official first action the liquid chromatographic method for combinations of 2,4-D, dicamba, and MCPP amine salts described by the Associate Referee, provided that data from all collaborators are included in the publication of the method. (b) Continue official first action status of the following liquid chromatographic methods: 2-methyl-4-chlorophenoxyacetic acid **(6.A18–6.A22)**; 2,4,5,-trichlorophenoxyacetic acid **(6.A23–6.A26)**; 2,4-dichlorophenoxyacetic acid esters and amine salts **(6.275–6.279)**. (c) Continue study.
(2) *Dicamba:* Continue study.
(3) *Pentachlorophenol:* Continue study.
(4) *Plant Growth Regulators:* Continue study.
(5) *2,3,6-Trichlorobenzoic Acid:* Continue study.

PESTICIDE FORMULATIONS: HERBICIDES II

(1) *Alanap:* Continue study.
(2) *Barban:* Continue study.
(3) *Bensulide (S-(O,O-Diisopropyl) Phosphorodithioate Ester of N-'2-Mercaptoethyl Benezenesulfonamide):* Continue study.
(4) *Benzoylprop-ethyl:* Continue study.
(5) *Bromacil and Lenacil:* Continue study.
(6) *Chloroxuron:* Discontinue topic.
(7) *Dimethyl Tetrachloroterephthalate:* Continue study.
(8) *Dinoseb:* Continue study.
(9) *Diuron:* Continue study.
(10) *S-Ethyl Dipropylthiocarbamate:* Continue study.
(11) *Fluchloralin, Profluralin, Benefin, Trifluralin and Penoxalin:* Continue study.
(12) *Fluometuron:* Continue study.
(13) *Linuron:* Continue study.
(14) *Methazole:* Continue study.
(15) *Monuron:* Continue study.
(16) *Oryzalin (3,5-Dinitro-N,N-(dipropyl)sulfanilamide):* Continue study.
(17) *Paraquat:* Continue study.
(18) *Siduron:* Continue study.
(19) *Thiocarbamate Herbicides:* (a) Continue official first action status of the gas chromatographic method for thiocarbamate herbicides (**6.426–6.430**). (b) Continue study.

PESTICIDE FORMULATIONS: HERBICIDES III

(1) *Alachlor, Butachlor, and Propachlor:* Continue study.
(2) *Amitrol:* Continue study.
(3) *Bentazone:* Continue study.
(4) *Bromoxynil:* Continue study.
(5) *Cacodylic Acid:* Continue study.
(6) *Cyanazine (Bladex®):* Continue study.
*(7) *Dalapon:* (a) Adopt as official first action the interim first action liquid chromatographic method. (b) Continue study.
(8) *Dichlobenil:* Continue study.
(9) *Disodium Methane Arsenate:* Continue study.
*(10) *Fluazifop-butyl:* (a) Adopt as official first action the gas chromatographic method described by the Associate Referee. (b) Continue study.
*(11) *Glyphosate:* (a) Adopt as official final action the official first action liquid chromatographic method (**6.D22–6.D27**). (b) Continue study.
(12) *Metalochlor:* Continue study.
*(13) *Metribuzin (4-Amino-6-(1,1-dimethyl-ethyl)-3-(methylthio)-1,2,4,-triazin-5(4H)one):* (a) Adopt as official first action the gas chromatographic method described by the Associate Referee, but limited to glass columns and flame ionization detectors.
(14) *Monosodium Methane Arsenate:* Continue study.
(15) *Propanil (3',4'-Dichloropropionanilide):* Continue study.
(16) *s-Triazine Herbicides:* Continue study.

PESTICIDE FORMULATIONS: INORGANIC PESTICIDES

(1) *Aluminum Phosphide:* Continue study.
(2) *Sodium Chlorate:* Continue study.

PESTICIDE FORMULATIONS: OTHER INSECTICIDES, SYNERGISTS, AND INSECT REPELLANTS

(1) *Allethrin:* (a) Continue official first action status of the gas chromatographic method for technical allethrin (**6.149–6.154**). (b) Continue study.
(2) *2,3,4,5-Bis(2-butylene)tetrahydro-2-furfural (MGK Repellant 11®):* Continue study.
(3) *Cypromethrin:* Continue study.
(4) *Dipropyl Isocinchomeronate (MGK Repellant 326®):* Continue study.
(5) *Fumigants:* (a) Continue official first action status of the gas chromatographic method for fumigant mixtures (**6.143–6.148**). (b) Continue study.
(6) *Nicotine:* Continue study.
(7) *Permethrin:* Continue study.
(8) *Piperonyl Butoxide and Pyrethrins:* Continue study, including low levels and mixed formulations.
(9) *Resmethrin:* Continue study.
(10) *Rotenone and Other Rotenoids:* (a) Continue official first action status of the liquid chromatographic method (**6.D05–6.D08**) and the infrared method (**6.163–6.164**). (b) Continue study.
(11) *Other topics:* (a) Continue official first action status of the UV method for sulfoxide (**6.419**). (b) Establish a new topic for Methyl Bromide and appoint an Associate Referee. (c) Continue study.

PESTICIDE FORMULATIONS: ORGANOTHIOPHOSPHORUS PESTICIDES

(1) *Acephate:* Continue study.
(2) *Azinphosmethyl:* Continue study.
(3) *Chlorpyriphos:* Discontinue topic.
(4) *Coumaphos:* Continue study.
(5) *Demeton:* Continue study.
(6) *Demeton-S-Methyl:* Continue study.
(7) *Diazinon:* (a) Continue official first action status of the gas chromatographic method for diazinon (**6.331**). (b) Continue study.
(8) *Dimethoate:* Continue study.
(9) *Dioxathion:* Continue study.
(10) *Disulfoton:* Discontinue topic.
(11) *Encapsulated Organophosphorus Pesticides:* (a) Continue official first action status of the gas chromatographic method for encapsulated diazinon (**6.C12–6.C15**). (b) Continue study.
(12) *EPN:* Continue study.
(13) *Ethion:* Discontinue topic.
(14) *Ethoprop:* Continue study.
(15) *O-Ethyl O-(4-Methylthio) Phenyl S-Propyl Phosphorothioate:* Continue study.
*(16) *Fensulfothion:* (a) Adopt as official final action the official first action liquid chromotographic method (**6.D28–6.D32**). (b) Continue study.
(17) *Fenthion:* Continue study.
(18) *Fonophos:* Continue study.
(19) *Malathion:* Continue study.
(20) *Methidathion:* Continue study.
*(21) *Parathion and Methyl Parathion:* (a) Continue official first action status of the volumetric (**6.388–6.394**), colorimetric (**6.395–6.399**), gas chromatographic (**6.379–6.383**). and liquid chromatographic (**6.384–6.387**) methods for parathion. and the gas chromatographic (**6.405–6.408**) methods for methyl parathion. (b) Designate as surplus the volumetric

(**6.388–6.394**) and colorimetric (**6.395–6.399**) methods. (c) Continue study.

(22) *Phorate:* Continue study.

(23) *Temephos:* (a) Continue official first action status of the CIPAC liquid chromatographic method (**6.C16–6.C21**). (b) Continue study.

(24) Establish new topics and appoint Associate Referees for Oxydemeton and Sulprofos.

PESTICIDE FORMULATIONS: OTHER ORGANOPHOSPHORUS INSECTICIDES

(1) *Crotoxyphos:* Continue study.

(2) *Cruformate:* Continue study.

(3) *Dichlorvos (2,2-Dichlorovinyl Dimethyl Phosphate):* (a) Continue official first action status of the infrared methods, **6.324–6.327** and **6.328–6.330**. (b) Continue study.

(4) *Mevinphos:* Continue study.

(5) *Monocrotophos (Dimethyl Phosphate of 3-Hydroxy-N-Methyl-cis-Crotonamide):* Continue study.

(6) *Naled:* Continue study.

(7) *2-Chloro-1-(2,4,5,-trichlorophenyl)vinyl Dimethyl Phosphate (Tetrachlorvinphos):* Continue study.

PESTICIDE FORMULATIONS: RODENTICIDES AND MISCELLANEOUS PESTICIDES

(1) *Brodifacoum (Talon®):* (a) Continue official first action status of the liquid chromatographic method (**6.D01–6.D04**). (b) Continue study.

(2) *Chlorophacinone:* Continue study.

(3) *Diphacinone:* Continue study.

(4) *Alpha-Naphthylthiourea:* (a) Continue official first action status of the surplus method (**6.139**). (b) Continue study.

(5) *N-3-Pyridyl-N'-p-nitrophenyl Urea (Vacor®):* Continue study.

(6) *Strychnine:* Continue study.

(7) *Warfarin:* Continue study.

PLANTS

(1) *Ashing Methods:* Continue study.

(2) *Atomic Absorption Methods:* (a) Continue official first action status of the atomic absorption method for calcium, copper, iron, magnesium, manganese, potassium, and zinc (**3.006–3.009**). (b) Continue study.

(3) *Boron:* Continue study.

(4) *Chromium:* Continue study.

(5) *Copper and Cobalt:* Discontinue topic.

(6) *Emission Spectroscopy:* Continue study.

(7) *Fluoride:* (a) Continue official first action status of the potentiometric method (**3.077–3.082**) and the semi-automated method (**3.083–3.095**) for fluoride. (b) Continue study.

(8) *Nitrogen, Non-Protein:* Appoint an Associate Referee and continue study.

(9) *Selenium:* Continue study.

(10) *Starch:* Continue study.

(11) *Sulfur:* Appoint an Associate Referee and initiate topic.

(12) *Zinc:* Discontinue topic.

REFERENCE MATERIALS AND STANDARD SOLUTIONS

Stability of Organophosphorus Pesticide Standards: Continue study.

TOBACCO

(1) *Differentiation of Cigar and Cigarette Tobacco (Sequential Differential Solvent Extraction):* Continue study.

(2) *Humectants in Cased Cigarettes:* (a) Continue official first action status of the method for glycerol, propylene glycol, and triethylene glycol in cased cigarette cut filler and ground tobacco (**3.147–3.150**). (b) Continue study.

*(3) *Nicotine, Gas Chromatography:* (a) Adopt as official final action the official first action method for nicotine on Cambridge filter pads (**3.158–3.161**). (b) Continue study.

(4) *Tar and Nicotine in Cigarette Smoke:* Continue study.

Report of Committee B on Recommendations for Official Methods

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At the meeting of Committee B on October 4, 1983, the following subjects were discussed.

(1) The statistical considerations of data analysis and, more important, the need for guidance from the statistical consultants in setting up a collaborative study.

(2) Flexibility in the description of chromatographic procedures and the resultant leeway afforded collaborators and subsequent users of methods. Such flexibility must conform to the accepted style of *Official Methods of Analysis*.

(3) The General Referee's responsibility for appointment of Associate Referees.

(4) The realignment of Associate Refereeships to correspond to the 14th Edition, *Official Methods of Analysis*.

The following Associate Refereeships are currently vacant: Amphetamines in Mixtures, Belladona Alkaloids, Benzothiazine Derivatives, Chemical Microscopy, Chlorpromazine, Epinephrine-Lidocaine Combinations, Ethinyl Estradiol Individual Tablet Analysis, Lysergic Acid Diethylamide (LSD), Methamphetamine, Methocarbamol, Methylphenidate Phenidine Hydrochloride, Steroid Acetates, Steroid Phosphates, Sulfonamides by LC.

DRUGS, ACIDIC

(1) *Acetaminophen in Drug Mixtures:* Continue study.
(2) *Allopurinol:* An Associate Referee was appointed; continue study.
*(3) *Amitriptyline Hydrochloride in Dosage Forms (LC):* Adopt as official first action the interim first action liquid chromatograhic method for the determination of amitriptyline in tablets and injectables, **37.D01–37.D05.** Continue study.
(4) *Aspirin, Phenacetin, and Caffeine with Other Drugs:* Continue study.
(5) *Aspirin and Salicylic Acid in Aspirin Products (Semiautomated Analysis):* Continue study.
(6) *Barbiturates:* Continue study.
(7) *Benzothiazine Derivatives:* Appoint an Associate Referee: Continue study.
(8) *Benzthiazide by LC:* Continue study.
(9) *Methyldopa:* Continue study.
(10) *Primidone:* Continue study.
(11) *Probenecid:* Continue study.
(12) *Sulfamethoxazole:* Continue study.
*(13) *Sulfisoxazole:* Adopt as official first action the interim first action liquid chromatographic method for the determination of sulfisoxazole in tablets, solutions, and ointments, **37.D06–37.D11.** Continue study.
(14) *Sulfonamides (Thin Layer Chromatography):* Discontinue topic.Appoint an Associate Referee for a new topic, Sulfonamides by LC. Continue study.
(15) *Thiazide Diuretics, Semiautomated Individual Dosage Units Analysis:* Continue study.

DRUGS, ALKALOIDS AND RELATED BASES

(1) *Aminacrine:* Continue study.
(2) *Atropine in Morphine and Atropine Tablets and Injections:* Continue study.
(3) *Belladona Alkaloids:* Appoint an Associate Referee; continue study.
(4) *Chlorpromazine:* Appoint an Associate Referee; continue study.
(5) *Colchicine in Tablets:* Continue study.
(6) *Curare Alkaloids:* Continue study.
(7) *Dicyclomine Capsules:* Continue study.
(8) *Epinephrine-Lidocaine Combinations:* Appoint an Associate Referee; continue study.
(9) *Epinephrine and Related Compounds by LC-Electrochemical Detectors:* Continue study.
(10) *Ergot Alkaloids:* Continue study.
(11) *Homatropine Methyl Bromide in Tablets:* Continue study.
(12) *Neostigmine:* Continue study.
(13) *Phenethylamine Drugs, Semiautomated Individual Unit Analysis:* Continue study.
(14) *Pheniramine with Pyrilamine, Phenylpropanolamine, and Phenylephrine:* Continue study.
(15) *Phenothiazines in Drugs:* Continue study.
(16) *Physostigmine and Its Salts:* Continue in official first action status the LC method for the solution and ointment dosage forms, **38.C01–38.C06** (rev. 1983). Continue study of method for the injection dosage form.
*(17) *Pilocarpine:* Adopt as official first action the LC method for the simultaneous determination of pilocarpine, isopilocarpine, and pilocarpic acid described by the Associate Referee. Continue study.
(18) *Rauwolfia Alkaloids:* Continue study.
(19) *Rauwolfia serpentina:* Continue study.
(20) *TLC Identification of Phenothiazine-Type Drugs:* Continue study.

DRUGS, GENERAL

(1) *Ampicillin and Amoxicillin:* An Associate Referee has been appointed; continue study.
(2) *Disulfiram:* Continue study.
(3) *Ethylene Oxide:* Discontinue study.
*(4) *Fluoride:* Adopt as official first action the ion-specific electrode method for fluoride in sodium fluoride preparations. Continue study.

*An asterisk before a recommendation indicates that official action on that recommendation resulted in its appearance in "Changes in Methods."

The recommendations submitted by Committee B were adopted by the Association.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition, and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **63,** 374–423 (1980); **64,** 501–540 (1981); **65,** 450–521 (1982); **66,** 512–564 (1983).

(5) *Halogenated Hydroxyquinoline Drugs:* An Associate Referee has been appointed; continue study.
(6) *Insulin; Human, Porcine, and Bovine LC Assay:* An Associate Referee has been appointed. Change topic name to Insulin by LC. Continue study.
(7) *Medicinal Gases:* Continue study.
(8) *Menadiol Sodium Diphosphate Injection:* Continue study.
(9) *Mercurial Diuretics:* Discontinue topic.
(10) *Mercury-Containing Drugs:* Continue in official first action status the atomic absorption method for determining total mercury in mercury-containing drugs (**36.D01–36.D07**). Continue study.
(11) *Metals in Bulk Drug Powders:* Continue study.
(12) *Microchemical Tests:* An Associate Referee has been appointed; continue study.
(13) *Protein Nitrogen Units in Allergenic Extracts:* Continue in official first action status of the protein nitrogen unit precipitation method for allergenic extracts (**39.C01–39.C03**). Continue study.
(14) *Thyroid and Thyroxine Related Compounds:* Continue study.
*(15) *Thyroid by Differential Pulse Polarography:* Adopt as official final action the differential pulse polarographic method for iodine in thyroid tablets (**39.C04–39.C07**). Continue study.
*(16) *Other Topic:* Adopt a revised sampling procedure to replace the present sections under this heading in Chapter 36.

DRUGS, ILLICIT

(1) *Amphetamines in Mixtures:* Appoint an Associate Referee; continue study.
(2) *Benzodiazepines:* Continue in official first action status the LC method for the determination of oxazepam in dosage forms. Continue study.
(3) *Chemical Microscopy:* Appoint an Associate Referee; continue study.
(4) *Cocaine:* Continue study.
(5) *Diazepam:* An Associate Referee has been appointed; continue study.
(6) *Dimethyltryptamine (DMT), Diethyltryptamine (DET), and Dipropyltryptamine (DPT):* Continue study.
(7) *Heroin:* Continue in official first action status the method for determining diacetylmorphine (heroin) in tablets (**40.006**). Continue study.
(8) *Lysergic Acid Diethylamide (LSD):* Appoint an Associate Referee; continue study.
(9) *Marihuana and Synthetic Tetrahydrocannabinol (THC):* Continue study.
(10) *Methadone:* Continue study.
(11) *Methamphetamine:* Appoint an Associate Referee; continue study.
(12) *Methaqualone Hydrochloride:* Continue study.
(13) *Methylphenidate Phenidine Hydrochloride:* Appoint an Associate Referee; continue study.
(14) *Optical Crystallographic Properties:* Discontinue topic.
(15) *Phencyclidine Hydrochloride (PCP):* Continue study.

DRUGS, NEUTRAL

(1) *Automated Corticosteroid Methods:* Discontinue topic.
(2) *Automated Methods for Progestins in Tablets:* Continue study.
(3) *Digitoxin, Automated Individual Tablet Analysis:* Continue study.
(4) *Estrogens:* Combine with Estrogens (Fluorometric Method); change title to Conjugated Estrogens, LC Methods. Continue study.
(5) *Estrogens (Fluorometric Method):* Combine with Estrogens; change title to Conjugated Estrogens, LC Methods. Continue study.
(6) *Ethinyl Estradiol, Automated Individual Tablet Analysis:* Change title to Ethinyl Estradiol, Individual Tablet Analysis. Appoint an Associate Referee; continue study.
*(7) *Hydrocortisone:* Adopt as official first action the interim first action LC method for the determination of hydrocortisone in drug substance and tablets. Continue study.
*(8) *Methocarbamol:* Adopt as official first action the interim first action LC method for the determination of methocarbamol in pharmaceutical dosage forms. Appoint an Associate Referee; continue study.
(9) *Steroid Acetates:* Appoint an Associate Referee; continue study.
(10) *Steroid Phosphates:* Continue official first action status of method **39.047–39.051**. Appoint an Associate Referee; continue study.

Report of Committee C on Recommendations for Official Methods

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MICHAEL W. O'DONNELL (Division of Mathematics, Food and Drug Administration, Washington DC 20204), *Statistical Consultant*

COFFEE AND TEA

(1) *Ash in Instant Tea:* Continue study.
(2) *Caffeine:* Continue study.
(3) *Crude Fiber in Tea:* Appoint Associate Referee; continue study.
(4) *Moisture in Coffee and Tea:* Continue study.
(5) *Solvent Residues in Decaffeinated Coffee and Tea:* Continue study.
(6) *Theophylline in Tea:* Continue study.
(7) *Water Extract in Tea:* Continue study.

DAIRY PRODUCTS

(1) *Adulteration of Dairy Products with Vegetable Fat:* Continue study.
(2) *Casein and Caseinates:* Continue study.
(3) *Chocolate Milk, Fat Test:* Continue study.
(4) *Cryoscopy of Milk:* Continue study.
(5) *Fat, Automated Methods:* Continue study.
(6) *Fat in Milk (AutoAnalyzer):* Continue study.
(7) *Infrared Milk Analyzer (IRMA):* Continue study.
(8) *Lactose in Dairy Products (Chromatographic Determination):* Continue study.
*(9) *Lactose in Dairy Products (Enzymatic Determination):* Adopt as official first action, the collaboratively studied enzymatic, ultraviolet method for measuring lactose in milk; continue study.
(10) *Moisture in Cheese:* Continue study.
(11) *Moisture in Cheese (Karl Fischer Method):* Continue study.
(12) *Nitrates in Cheese:* Continue study.
(13) *Phosphatase (Rapid Method):* Continue study.
(14) *Phosphatase (Reactivated):* Continue study.
(15) *Phosphorus:* Continue study.
(16) *Protein Constituents in Processed Dairy Products:* Continue study.
(17) *Protein in Milk (Rapid Tests):* Continue study.
(18) *Protein Reducing Substance Tests:* Continue study.
(19) *Solids-Not-Fat:* Continue study.
(20) *Vapor Pressure Osmometry:* Continue study.
*(21) *Other Topics:* Adopt as official final action the official first action potentiometric method for determination of chloride in cheese (**16.D01–16.D04**); delete the present official final action method (**16.242–16.243**); adopt as official final action the official first action methods for phosphatase: Method V (**16.125–16.126**), reactivated and residual phosphatase, differential test (**16.129–16.130**), and residual phosphatase in casein (**16.B01–16.B03**).

DECOMPOSITION AND FILTH IN FOODS (CHEMICAL METHODS)

(1) *Ammonia in Dogfish:* Continue study.
(2) *Coprostanol:* Continue study.
(3) *Crabmeat:* Continue study.
(4) *Diacetyl in Citrus Products:* Continue study.
(5) *Ethanol in Seafoods:* Continue study.
(6) *Gas and Liquid Chromatography:* Continue study.
(7) *GC Determination of Volatile Amines (TMA and DMA):* Continue study.
(8) *Shellfish:* Appoint Associate Referee; continue study.
(9) *TLC Identification of Amines in Fishery Products:* Continue study.
(10) *Tomatoes:* Continue study.

FISH AND OTHER MARINE PRODUCTS

(1) *Crabmeat Identification:* Continue study.
(2) *Determination of Fish Content in Coated Products (Breaded or in Batter):* Continue study.
(3) *Drained Weight of Block Frozen, Raw, Peeled Shrimp:* Continue study.
(4) *Drip Fluid in Fish Fillets and Fish Fillet Blocks (Quantitation):* Continue study.
(5) *Fish Species Identification (Thin Layer Isoelectric Focusing):* Continue study.
(6) *Minced Fish in Fish Fillet Blocks:* Continue study.
(7) *Nitrites in Smoked Fish:* Continue study.
(8) *Organometallics in Fish:* Continue study.
*(9) *Other Topic:* Adopt as official final action the official first action potentiometric method, **18.036**, for determination of salt in fish.

FOOD ADDITIVES

(1) *Anticaking Agents:* Appoint Associate Referee; continue study.
*(2) *Antioxidants:* Adopt as official final action the official

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The recommendations submitted by Committee C were adopted by the Association.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition, and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **63**, 374–423 (1980);**64**, 501–540 (1981); **65**, 450–521 (1982); **66**, 512–564 (1983).

first action LC method for antioxidants in fats and oils, **20.D01–20.D04**; continue study.

(3) *Brominated Oils:* Continue study.

(4) *Chloride Titrator:* Continue study.

(5) *Chlorobutanol in Milk:* Appoint Associate Referee; continue study.

(6) *Dichlorodifluoromethane in Frozen Foods:* Appoint Associate Referee; continue study.

(7) *Dilauryl Thiodipropionate:* Appoint Associate Referee; continue study.

(8) *Dimethyl Polysiloxane:* Appoint Associate Referee; continue study.

(9) *Dressings:* Continue study.

(10) *EDTA in Food Products:* Continue study.

(11) *Ethoxyquin in Meat and Eggs:* Continue study.

(12) *Gums in Foods:* Appoint Associate Referee; continue study.

(13) *Indirect Additives from Food Packages:* Continue study.

(14) *Mineral Oil in Raisins:* Continue study.

(15) *Nitrates (Selective Ion Electrodes):* Continue study.

(16) *Nitrates and Nitrites:* Continue study.

*(17) *Nitrosamines:* Adopt as official first action the interim official first action method for determination of *N*-nitrosodimethylamine in nonfat dry milk, collaboratively studied by the Associate referee as Method I; continue study.

(18) *Polycyclic Aromatic Hydrocarbons:* Continue study.

(19) *Polysorbates:* Continue study.

(20) *Propylene Chlorohydrin:* Appoint Associate Referee; continue study.

(21) *Sodium Lauryl Sulfate:* Appoint Associate Referee; continue study.

MEAT, POULTRY, AND MEAT AND POULTRY PRODUCTS

(1) *Automated Methods:* Complete evaluation of data from collaborative studies on Technicon AA-2 Auto-Analyzer; continue study.

(2) *Bioassay Methods for Meat and Poultry Products:* Appoint Associate Referee; continue study.

*(3) *Bone Content:* Retain in official first action status the titrimetric method for calcium in mechanically separated meat (**24.D01–24.D02**); replace the terms "broilers" and "fryers" with the terms "young chickens" and "chickens" in **24.D02** and continue study to extend the method to pork; continue study.

(4) *Chemical Antibiotic Methods:* Continue study.

(5) *Fat in Meat Products:* Continue study.

(6) *Fat and Moisture Analysis, Rapid Methods:* Continue study.

(7) *LC Methods for Meat and Poultry Products:* Continue study.

(8) *Histological Identification Methods:* Continue study.

(9) *Identification of Meats, Serological Tests:* Appoint Associate Referee; continue study.

(10) *Nitrates and Nitrites:* Continue study.

*(11) *Nitrosamines in Bacon:* Adopt as official final action the official first action mineral oil, vacuum distillation, thermal energy analyzer method, **24.C01–24.C07**, for determination of nitrosamines in fried bacon; continue study.

(12) *Non-Meat Proteins in Meat:* Discontinue topic.

(13) *Protein in Meat:* Discontinue topic.

(14) *Specific Ion Electrode Applications:* Initiate collaborative study using the NOVA Biomedical ion analyzer to determine sodium, potassium, and chloride; continue study.

(15) *Steroid Analysis for Meat and Poultry Products:* Continue study.

(16) *Sugars and Sugar Alcohols:* Appoint Associate Referee; continue study.

(17) *Temperature, Minimum Processing:* Continue study.

*(18) *Other Topic:* Adopt as official first action the collaboratively studied dry column, thermal energy anayzer method for determination of *N*-nitrosopyrrolidine in fried bacon by authors Fiddler, Pensabene, Miller, Malanoski, and Phillips.

MYCOTOXINS

(1) *Aflatoxin M:* Complete collaborative study on the Foos-Warren method for aflatoxin M_1 in milk; retain first action status of methods for the determination and confirmation of aflatoxin M_1 in dairy products (**26.A10–26.A15**) and (**26.090–26.094**) and for aflatoxins B_1 and M_1 in liver (**26.C01–26.C08**); continue study.

(2) *Aflatoxin Methods:* Complete collaborative study for negative ion chemical ionization MS procedure for confirmation and identity of aflatoxin B_1; continue study.

(3) *Alternaria Toxins:* Continue study.

(4) *Citrinin:* Continue study.

(5) *Ergot Alkaloids:* Continue study.

(6) *Grains:* Complete evaluation of analytical and quantitation methods for aflatoxins in corn; continue study.

(7) *Ochratoxins:* Initiate collaborative study of the modified green coffee TLC method for ochratoxin A applied to swine kidneys; continue study.

(8) *Penicillic Acid:* Initiate collaborative study of the Thorpe-Johnson GC method for penicillic acid in grains; continue study.

(9) *Secalonic Acid:* Continue study.

(10) *Sterigmatocystin:* Initiate collaborative study of the van Egmond method for sterigmatocystin in cheese; continue study.

(11) *Tree Nuts:* Continue study.

(12) *Tricothecenes:* Initiate collaborative study of a GC method for DON in wheat and corn; continue study.

(13) *Zearalenone:* Complete study of the Bagnaris LC method for zearalenone and α-zearalenone in corn; continue study.

OILS AND FATS

(1) *Antioxidants:* Continue study.

(2) *Chromatographic Methods:* Discontinue topic.

(3) *Cyclopropene Fatty Acids:* Discontinue topic.

(4) *Emulsifiers:* Continue study.

* (5) *Karl Fischer Method for Water in Oils and Fats:* Adopt as official first action the interim official first action Karl Fischer method for the determination of water in oils and fats; continue study.

(6) *Lower Fatty Acids:* Continue study.

(7) *Marine Oils:* Continue study.

(8) *Olive Oil Adulteration:* Continue study.

(9) *Oxidized Fats:* Continue study.

(10) *Pork Fats in Other Fats:* Continue study.

(11) *Spectrophotometric Methods:* Discontinue topic.

(12) *Sterols and Tocopherols:* Continue study.

*(13) *Other Topic:* Adopt as official final action the following official first action methods: Preparation of Methyl Esters–Boron Trifluoride Method, **28.053–28.056**; Methyl Esters of Fatty Acids–AOAC/IUPAC GC Method, **28.057–28.065**; Docosenoic Acid GC Method,

28.066–28.069; Polymers and Oxidation Products of Heated Vegetable Oils, GC Method for Nonelution Materials, **28.070;** *cis,cis*-Methylene Interrupted Polyunsaturated Fatty Acids, **28.071–28.074;** Polar Components in Frying Fats, **28.C01–28.C08**.

PLANT TOXINS

(1) *Glucosinolates:* Initiate topic; appoint Associate Referee.
(2) *Pyrrolizidine Alkaloids:* Appoint Associate Referee; continue study.
(3) *Solanaceous Alkaloids:* Appoint Associate Referee; continue study.

PROCESSED VEGETABLE PRODUCTS

(1) *Fibrous Material in Frozen Green Beans:* Continue study.
(2) *pH Determination:* Continue study.
(3) *Sodium Chloride:* Continue study.
(4) *Total Solids by Microwave Moisture Analyzer:* Continue study.
(5) *Volume of Entrapped Air in Flexible Retort Pouches:* Discontinue topic.
*(6) *Water Activity Determination:* Adopt as official final action the official first action method for water activity (**32.004–32.009**); continue study.
*(7) *Other Topics:* Adopt as official first action the collaboratively studied method for the determination of the loss of mass on drying of quick frozen french-fried potatoes as described by the authors David O. Biltcliffe and Roger Wood; initiate the new topic *Soluble Solids of Tomato Products (Direct Refractometer Method)* and appoint an Associate Referee.

SEAFOOD TOXINS

(1) *Ciguatoxins (Biochemical Methods):* Continue study.
(2) *Paralytic Shellfish Poisoning (Immunoassay Method):* Continue study.
(3) *Shellfish Poisons:* Continue study.
(4) *Tetradotoxins:* Continue study.

Report of Committee D on Recommendations for Official Methods

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DENNIS RUGGLES (Food and Drug Administration, Division of Mathematics, Washington, DC 20204), *Statistical Consultant*

ALCOHOLIC BEVERAGES

(1) *Acetate in Wines and Fruit Juices (Enzymatic Assay):* Continue study.
(2) *Alcohol Content by Oscillating U-Tube Density Meter:* Continue study.
(3) *Alcohol Content of High Solids Distilled Spirits:* Continue study.
*(4) *β-Asarone:* Adopt as official final action the official first action method, **11.081-11.083;** discontinue study.
(5) *Bromide Ion in Wine:* Discontinue study.
(6) *Carbon Dioxide in Wine:* Continue study.
(7) *Citric Acid in Wine:* Continue study.
(8) *Color Intensity for Distilled Alcoholic Beverage Products:* Continue study.
*(9) *Coumarin in Wine:* Adopt as official final action the official first action method, **11.079–11.080;** discontinue study.
(10) *Diethylpyrocarbonate in Beverages:* Discontinue study.
*(11) *Ethanol in Wine by GC:* Amend the official first action GC method for ethanol in wine, **11.D01–11.D02,** to specify use of only one column by deleting **11.D01 (a)**(*1*) and **(a)**(*3*); change internal std soln, **11.D01(c)** from 0.1% v/v *n*-butanol to 0.2% v/v 2-propanol as described by the Associate Referee; continue study.
(12) *Flavor Compounds in Malt Beverages:* Continue study.
(13) *Glycerol in Wine:* Continue study.
(14) *Hydrogen Cyanide:* Continue study.
(15) *Malic Acid in Wine:* Continue study.
*(16) *Malt Beverages in Brewing Materials:* Adopt as official first action the interim action method for ethanol in beer as described by the Associate Referee; continue study.
(17) *Sorbic Acid in Wine:* Continue study.
(18) *Sugar in Wine:* Continue study.
(19) *Sulfur Dioxide in Wine:* Continue study.
(20) *Tartrates in Wine:* Continue study.
(21) *Vanillin and Ethyl Vanillin in Alcoholic Beverages:* Continue study.
(22) *Volatile Acidity in Wine:* Continue study.
(23) *Volatile Congeners in Alcoholic Beverages:* Continue study.
(24) *Other Topics:* Continue official first action status of the following methods: **9.094,** artificial colors; **9.101–9.103,**

*An asterisk before a recommendation indicates that official action on the recommendation resulted in its appearance in "Changes in Methods."

The recommendations submitted by Committee D were adopted by the Association.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition, and "Changes in Methods," *J. Assoc. Off. Anal. Chem.***63,** 374–423 (1980); **64,** 501–540 (1981); **65,** 450–521 (1982); **66,** 512–564 (1983).

cyanide; **9.119,** total acidity; **9.123,** total malic acid; **9.129,** thujone; **10.148,** aphids; **10.182–10.185,** yeast; **11.047,** citric and malic acids; **11.057,** cyanide; **11.058–11.062** and **11.063–11.065,** carbon dioxide, manometric and volumetric methods, respectively.

CACAO PRODUCTS

(1) *Caffeine and Theobromine:* Continue study.
(2) *Carbohydrates in Chocolate Products:* Continue study.
(3) *Moisture in Cacao Products:* Continue study.
(4) *Shell in Cacao Products, Micro Methods:* Continue study.
(5) *Other Topics:* Continue official first action status of the following methods: **13.002,** moisture; **13.045,** lecithin; **13.050,** glucose; **13.040,** unsaponifiable matter in cocoa butter.

CEREAL FOODS

(1) *Iron:* Continue study.
(2) *Phytates:* Continue study.
(3) *Starch in Raw and Cooked Cereals:* Discontinue study.
(4) *Other Topics:* Continue official first action status of the following methods: **14.049–14.053,** α-amylase; **14.103–14.115,** lactose; **14.117–14.120,** mineral oil; **14.150–14.153,** sterols.

FLAVORS

(1) *Additives in Vanilla Flavoring:* 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 Flavorings:* Continue study.
(7) *Vanillin and Ethyl Vanillin in Foods:* Continue study.
(8) *Other Topics:* Continue official first action status of the following methods: **12.031,** essential oils; **12.032–12.036,** caffeine; **19.001–19.002,** alcohol; **19.033–19.035,** vanilla resins; **19.067,** oils of lemon and orange in extracts; **19.070,** oils of lemon, orange, or lime in oil-base flavors; **19.096, 19.097,** and **19.098,** almond extract; **19.099,** benzaldehyde; **19.104,** benzoic acid; **19.113, 19.114,** and **19.115,** ginger extract; **19.117, 19.118, 19.119,** peppermint, spearmint, and wintergreen extracts; **19.125, 19.126,** and **19.127–19.128,** other extracts and toilet preparations.

FRUITS AND FRUIT PRODUCTS

(1) *Adulteration of Orange Juice by Pulp Wash and Dilution:* Work closely with General Referee and statistician in designing a study that will reduce or eliminate possible subjectivity in spectra evaluation; include method for standardizing spectrophotometers so that spectra obtained match those provided with reference material; explore ways of quantitatively evaluating spectra; continue study.
(2) *Fruit Acids:* Continue study.
(3) *Fruit Juices, Identification and Characterization:* Continue study.
(4) *Isoascorbic Acid (Erythorbic Acid-Antioxidant) in Fruit Purees:* Discontinue study.
(5) *Orange Juice Content:* Continue study.
(6) *Other Topics:* Establish the new topics: Sodium Benzoate in Orange Juice, and Determination of Adulterants in Apple Juice and appoint Associate Referees.

NONALCOHOLIC BEVERAGES

(1) *Caffeine and Methyl Xanthenes in Nonalcoholic Beverages:* Continue study.
(2) *Lasiocarpine and Pyrrolizidines in Herbal Beverages:* Continue study.
(3) *Other Topics:* Establish the new topics: Glycyrrhizic Acid Salts in Licorice-Derived Products, and Quinine in Various Nonalcoholic Beverages and appoint Associate Referees.

PRESERVATIVES AND ARTIFICIAL SWEETENERS

(1) *Benzoates and Hydroxybenzoates in Food:* Continue study.
*(2) *Benzoates, Saccharin, and Caffeine, Liquid Chromatography:* Adopt as official final action the official first action LC method for determination of benzoates, saccharin, and caffeine, **12.050–12.053;** discontinue study.
(3) *Formaldehyde:* Continue study.
(4) *Meats, Ground, Screening Methods for Chemical Preservatives:* Continue study.
(5) *Organic Preservatives (Thin Layer Chromatography):* Continue study.
(6) *Preservatives (Quantitative Methods):* Continue study.
*(7) *Saccharin and Its Salts:* Editorially change the saccharin, differential pulse polarographic official final action method, to correct formula in **20.A10** to read: %(w/w) = μg (from std addn curve) × ((*D* + *E*/*CD*) × (*B*/2) × 10^{-4}); discontinue study.
(8) *Other Topics:* (a) Establish the new topic Aspartame, Benzoates, Saccharin, and Caffeine by LC and appoint Associate Referee. (b) Continue official first action status of the following methods: **20.A01–20.A05,** preservatives in ground beef; **20.024–20.028,** benzoic acid by TLC; **20.042–20.045,** boron by atomic absorption spectrophotometry; **20.056–20.057,** soluble fluorides by fluorescence quenching of aluminum 8-hydroxyquinolate; **20.062–20.064,** formaldehyde; **20.073–20.075,** nitrites; **20.077–20.078,** qualitative tests for quaternary ammonium compounds (QAC); **20.090–20.092,** eosin yellowish method for QAC; **20.098–20.101,** sorbic acid oxidation method; **20.121–20.122,** thiourea in frozen peaches; **20.157–20.161,** identification of non-nutritive sweeteners; **20.162,** cyclohexylsulfamate qualitative test; **20.168–20.172,** cyclohexylamine in cyclamates; **20.173–20.176,** dulcin; **20.177,** P-4000; **20.187,** saccharin by sublimation; **12.050–12.053,** benzoate, caffeine, and saccharin in soda beverages, LC.

SPICES AND OTHER CONDIMENTS

(1) *Ash and Pungent Principles in Mustard:* Continue study.
(2) *Extractable Color in Capsicum Spices and Oleoresins:* Continue study.
(3) *Moisture in Dried Spices:* Continue study.
(4) *Monosodium Glutamate in Foods: Continue study.*
(5) *Pungency of Capsicums and Oleoresins:* Continue study.
(6) *Vinegar:* Continue study.
*(7) *Other Topics:* Delete the surplus official first action method for sucrose, **30.039;** amend the official first action method for extractable color, **30.002–30.004,** to delete **30.002(b),** std color soln, and other references to that section in the method.

SUGARS AND SUGAR PRODUCTS

(1) *Chromatographic Methods:* Continue study.
(2) *Color, Turbidity, and Reflectance - Visual Appearance:* Continue study.

*(3) *Corn Syrup and Corn Sugar:* Adopt as official first action the interim official first action electrometric titration method for titratable acidity in corn syrups, limiting the method to high fructose corn syrups; continue study.
(4) *Enzymatic Methods:* Continue study.
(5) *Honey:* Continue study.
*(6) *Lactose Purity Testing:* Adopt as official first action the quantitative LC method for the determination of lactose purity as described by the Associate Referee; continue study.
*(7) *Maple Sap and Syrups:* Adopt as official first action the interim official first action mass spectrometric carbon isotope ratio method for the determination of corn and cane sugars in maple syrup as described by the Associate Referee; continue study.
(8) *Standardization of Sugar Methods of Analysis:* Continue study.
*(9) *Sugars in Cereal:* Make a nonsubstantive change in **14.C01–14.C04,** the LC method for the determination of sugars in cereals, as indicated by the Associate Referee; continue study.
*(10) *Sugars in Licorice Products:* Adopt as official first action the LC method for sugar in licorice extracts as described by the Associate Referee; continue study.
(11) *Weighing, Taring, and Sampling:* Continue study.

VITAMINS AND OTHER NUTRIENTS

(1) *Amino Acids:* Continue study.
*(2) *Automated Nutrient Analysis:* Adopt as official first action the interim semiautomated fluorometric method for vitamin C in food products; continue study.
(3) *Biotin:* Continue study.
(4) *Carotenoids:* Continue study.
(5) *Choline in Feeds:* Discontinue study.
(6) *Dietary Fiber:* Conduct limited collaborative study on proposed method including rice; include starch test to ensure complete removal of starch; determine level at which method is not reproducible; continue study.
(7) *Energy Value of Foods (Biological):* Discontinue study.
*(8) *Fat in Food by Chloroform–Methanol Extraction:* Adopt as official final action the official first action method, **43.D01–43.D02;** continue study.
(9) *Folic Acid:* Continue study.
(10) *LC Assay for Total Vitamins A, D, and E Content in Foods, Feeds, and Pharmaceuticals:* Continue study.
(11) *Iodine in Foods:* Continue study.
(12) *Niacinamide (Polarography):* Discontinue study.
(13) *Nutrient Assay of Infant Formula:* Provide documentation for changes made in AOAC methods and why they were made: supply ruggedness test data where substantive changes in AOAC methods were made; complete statistical analysis of data; continue study.
(14) *Pantothenic Acid, Total Activity in Foods:* Continue study.
(15) *Protein Quality Evaluation of Foods:* Continue study.
(16) *Sodium in Foods:* Continue study.
(17) *Thiamin Column Packing Material and Enzyme:* Continue study.
(18) *Vitamin A in Foods and Feeds:* Continue study.
(19) *Vitamin C in Milk-Based Foods:* Discontinue study.
(20) *Vitamin D:* Continue study.
(21) *Vitamin E in Pharmaceuticals (Gas Chromatography):* Continue study.
(22) *Vitamin K_1 in Foods and Feeds:* Continue study.

Report of Committee E on Recommendations for Official Methods

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Committee E recommends use of the following symbols for standard deviations used as measures of precision for results obtained at each analyte level in collaborative studies of analytical methods: S_o, for the within-laboratory standard deviation; S_L, for the pure among-laboratories standard deviation; and S_x, for the overall among-laboratories standard deviation. The subscripts are identical to those used by E. H. Steiner in the *Statistical Manual of the AOAC* (pp. 78–81).

Committee E recommends the following changes in the summary of performance parameters presently required in reports on collaborative studies of methods submitted for adoption as official methods of the AOAC: (a) include CV_o and CV_x (i.e., the coefficients of variation corresponding to S_o and S_x, respectively) expressed as percents; (b) discontinue inclusion of the intralaboratory standard deviation (s_r) and the interlaboratory standard deviation (s_x), which are symbolized as S_o and S_x, respectively, in the notation system recommended above. (Coefficients of variation, also called

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relative standard deviations when expressed in percents, are more immediately meaningful indicators of method performance than the corresponding standard deviations and are better suited for comparisons of the relative variabilities of analytical methods. CV_x is the performance parameter that best expresses in a single summary statistic the variability of a method.)

INDUSTRIAL PROCESS WASTES

(1) *Bioassays:* Initiate topic and appoint Associate Referee to study biological methods for identifying wastes classified as hazardous under the Resource Conservation and Recovery Act (RCRA); conduct an interlaboratory trial of the *Daphnia magna* chronic bioassay proposed by the Office of Solid Waste (OSW), U.S. Environmental Protection Agency (EPA).

(2) *Inorganic Analytes:* Initiate topic for study of methods for determining toxic inorganic chemicals in industrial process wastes and other matrixes analyzed in hazardous waste management programs; complete the intralaboratory evaluation of 5 candidate methods for determining hexavalent chromium in the presence of trivalent chromium for use in conjunction with EPA-OSM Method 1310 (extraction procedure (EP)) for assessing the RCRA EP toxicity characteristic of wastes; continue intralaboratory study of EPA/OSW Method 6010 (inductively coupled plasma spectroscopy) for multielement analysis of wastes and waste extracts, and proceed to interlaboratory trial if warranted.

(3) *Organic Analytes:* Initiate topic for study of methods for determining toxic organic chemicals in industrial process wastes and other matrixes analyzed in hazardous waste management programs; complete the statistical evaluation of the performance of EPA/OSW Methods 3550 (sonication extraction with dichloromethane) and 8720 (capillary gas chromatography/mass spectrometry) as combined for the 8-laboratory collaborative study performed using 9 waste sample matrixes spiked with a total of 137 organic compounds; if appropriate, prepare report with recommendation for interim official first action adoption of EPA/OSW Methods 3550 and 8270 for determining those compounds for which acceptable precision and accuracy were demonstrated in the collaborative study; continue study of EPA/OSW 3570 and 8720 to determine the reasons for the large number of false negatives and the poor results for substituted benzoic acids and nitrophenols; continue study of EPA/OSW Methods 8280 (chlorinated dibenzo-*p*-dioxins and chlorinated dibenzofurans in wastes) and 8150 (chlorinated herbicides by gas chromatography) for analysis of industrial process wastes and waste extracts.

(4) *Physical/Chemical Properties:* Initiate topic to evaluate methods for measuring explosivity, corrosivity, flammability, and other characteristics of hazardous wastes; complete the evaluation of the H_2S/HCN evolution method collaboratively studied for identifying hazardous sulfide or cyanide bearing wastes; continue study of the U.S. Card Gap Test and the U.S. Internal Ignition Test for determining the RCRA explosivity characteristic of hazardous wastes.

(5) *Sampling:* Initiate topic and appoint an Associate Referee to study procedures for obtaining representative samples of wastes from sources as diverse as a single drum to a waste-holding lagoon, and to assist in the design of collaborative studies of methods for determining the composition and characteristics of hazardous wastes.

METALS AND OTHER ELEMENTS

(1) *Atomic Absorption Spectrophotometry (AAS):* Continue study to evaluate factors affecting the reliability of flame AAS measurements and to consolidate parts of present AOAC official methods using AAS for the determination of individual elements into a unified AAS analytical scheme for multielement analysis of foods and other biological substrates.

*(2) *Cadmium and Lead in Earthenware:* Adopt as official first action the interim official first action hot leach atomic absorption method, **25.D11–25.D17** (*J. Assoc. Off. Anal. Chem.* (1983), **66**, 610–619), for determining extractable cadmium and lead in ceramic and enameled cookware; continue study to evaluate methods for determining leachable lead in decorated teacup rims.

(3) *Carbon Rod Atomization Techniques:* Change title of topic to Graphite Furnace-Atomic Absorption Spectrophotometry (GF-AAS); conduct second collaborative study (after approval of the study design by the General Referee) of the GF-AAS method outlined by the Associate Referee (*J. Assoc. Off. Anal. Chem.* (1982) **65**, 1005–1009) for determining lead in canned milk and infant formulas, with the method instructions revised to ensure that the instruments used by the collaborators have adequate background correction capability for use in the study; continue study to evaluate GF-AAS for determining sub-ng/g levels of lead and other elements in foods.

(4) *Emission Spectrochemical Methods:* Continue study to evaluate candidate methods for multielement analysis of raw agricultural commodities by inductively coupled plasma (ICP) emission spectroscopy and to develop for review and comment by the General Referee a collaborative study protocol for testing the selected method.

(5) *Fluorine:* Prepare protocol for second collaborative study of the microdiffusion and fluoride-specific electrode method previously studied by the Associate Referee (*J. Assoc. Off. Anal. Chem.* (1981) **64**, 1021–1026) for determining fluoride in infant foods, incorporating the improvements suggested in the report on the first study; after approval of the study protocol by the General Referee, initate collaborative study.

(6) *Hydride Generating Techniques:* Evaluate the performance of the simple hydride generator constructed in the Associate Referee's laboratory from commonly available laboratory ware for use in conjunction with flameless AAS for determining tin in foods; continue study to evaluate hydride generation—AAS methods for determining arsenic, selenium, and tin in foods.

(7) *Mercury:* Prepare protocol for collaborative study of the Associate Referee's method combining liquid chromatography (LC) with AAS for the determination of methyl mercury in fish (*Analyst* (1982) **107**, 1457–1461) and, if protocol is approved by the General Referee, initiate collaborative study of the LC-AAS method.

(8) *Methyl Mercury in Fish and Shellfish:* Continue official first action status of the electron capture gas chromatographic method. **25.D05–25.D10** (*J. Assoc. Off. Anal. Chem.* (1983) **66**, 531–533), for determining methyl mercury in fish and shellfish; continue study of **25.D05–25.D10** to extend the applicability of the method to

commercially prepared seafood products, such as frozen breaded or batter-dipped fish, and to evaluate the use of toluene as a replacement for benzene in the method.

*(9) *Multielement Analysis of Infant Formula:* Adopt as official first action the inductively coupled plasma (ICP) emission spectroscopic method used in the collaborative study reported by the Associate Referee in 1983, for the determination of calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc in infant formulas, *provided* that the General Referee approves the manuscript on the collaborative study after it is revised to include (a) instructions and operating parameters for the ICP determinative step under *Method* in the form required for insertion in *Official Methods of Analysis,* and (b) a summary of method performance parameters (including CV_o and CV_x) expressed in terms consistent with those used by E. H. Steiner in the AOAC *Statistical Manual,* for each element studied.

(10) *Multielement Determination After Closed System Digestion:* Continue official first action status of the multielement method, **25.A01–25.A05** (*J. Assoc. Off. Anal. Chem.* (1980) **63,** 388–391), for determining arsenic, cadmium, lead, selenium, and zinc in foods; initiate collaborative study to extend the applicability of **25.A01–25.A05** as described by the Associate Referee in *J. Assoc. Off. Anal. Chem.* (1983) **66,** 620–624 to include the determination of copper by anodic stripping voltammetry and the determination of chromium and nickel by differential pulse polarography, after approval of the collaborative study protocol by the General Referee.

(11) *Multimetal Residues by Resin Column Separations:* Combine this topic with the topic Organometallics in Foods under the new topic name Separation Techniques for Trace Elements in Foods; continue study of the ICP emission spectroscopic method for determining trace elements in foods after separation on Chelex-100, as described in *Analyst* (1982) **107,** 353–377.

(12) *Organometallics in Foods:* Combine this topic with the topic Multimetal Residues by Resin Column Separations under the new topic name Separation Techniques for Trace Elements in Foods; continue study as described in rec. 11.

(13) *Polarography:* Continue official first action status of the dry ash, anodic stripping voltammetric method for determining cadmium and lead in foods other than fats and oils, **25.C01–25.C07** (*J. Assoc. Off. Anal. Chem.* (1982) **65,** 476–478); continue study to evaluate the performance of **25.C01–25.C07** in its currently extensive use for the analysis of canned foods.

(14) *Tin:* Continue official first action status of the AAS method for determining tin in canned fruits, vegetables, and juices, **25.136–25.138**; evaluate the performance of the nitrous oxide-acetylene flame atomic absorption spectrophotometric method for the determination of tin in canned foods (*J. Assoc. Off. Anal. Chem.* (1981) **64,** 1297–1300) in the collaborative study conducted by the Associate Referee for the topics Carbon Rod Atomization and Fluorine (with the cooperation of the Associate Referee for Tin) and, if appropriate, recommend the method for adoption as interim official first action.

*(15) *Voltammetric Methods:* Adopt as official final action the official first action anodic stripping voltammetric method for determining lead in evaporated milk and fruit juice, **25.080–25.082.**

MULTIRESIDUE METHODS (INTERLABORATORY STUDIES)

(1) *Comprehensive Multiresidue Methodology:* Complete the statistical analysis of results from the collaborative study of an improved version of the pesticide multiresidue method of Luke, et al. (*J. Assoc. Off. Anal. Chem.* (1981) **64,** 1187–1195), as tested for the determination of acephate, α-BHC, chlorpyrifos, dieldrin, monocrotophos, and omethoate in lettuce, strawberries, and tomatoes; if warranted by the collaborative study results, submit report recommending adoption of the improved method under the interim first action approval procedure and continue study to extend the applicability of this method to additional pesticides and additional nonfatty foods.

(2) *Gas–Liquid Chromatography (Alkaline Precolumn):* Discontinue topic.

(3) *Organophosphorus Pesticides Residues:* Continue study of the official final action carbon column cleanup method for residues of parathion, paraoxon, EPN, carbophenothion and its oxygen analog in apples and green beans. **29.039–29.043,** to extend the coverage of this method to additional organophosphorus pesticides and additional crops; continue study to compile and summarize available recovery data for organophosphorus pesticide residues in this method and to develop recovery data for additional organophosphorus pesticides and metabolites.

(4) *Pesticides in Meat and Meat Products:* Discontinue topic.

(5) *Pollutant Phenols in Fish:* Conduct an interlaboratory study of the method proposed by the Associate Referee for the analysis of fish in which residues of phenols classified as priority pollutants by EPA are cleaned up by gel permeation chromatography and cesium silicate adsorption chromatography, converted to their pentafluorobenzyl bromide derivatives, and determined by electron capture gas chromatography; if warranted by the results of the interlaboratory study, initiate collaborative study of the method after approval of the study protocol by the General Referee.

(6) *Whole Blood:* Transfer topic to the General Referee for Veterinary Analytical Toxicology, Committee G.

ORGANOHALOGEN PESTICIDES

(1) *Chlordane:* Initiate collaborative study of the method combining multiresidue extraction, **29.011–29.012.** acetonitrile partitioning cleanup, **29.014,** Florisil column chromatographic cleanup and residue separation. **29.031–29.033,** and electron capture gas chromatography,**29.018,** for determining residues of *cis*-chlordane, *trans*-chlordane, octachlor epoxide (oxychlordane), and heptachlor epoxide in butter, eggs, fish, and poultry fat; continue study of capillary column gas chromatography for determining the terminal residues of chlordane in foods.

(2) *Chlorinated Dioxins:* Continue study to develop and evalute analytical methods for determining and confirming the identity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) at parts-per-trillion levels in fish, milk, and other foods; continue effort to encourage use of AOAC criteria for collaborative studies in the design

of EPA quality assurance programs for contract laboratories performing TCDD residue analyses; continue study to evaluate methods for determining hexachloro-, heptachloro- and octachloro-substituted dibenzo-*p*-dioxins and dibenzofurans in foods.

*(3) *Chlorinated Hydrocarbons in Poultry:* (a) Adopt as official first action the interim official first action gel permeation chromatographic method collaboratively studied by the Associate Referee in 1982, for determining α-BHC, *cis*-chlordane, *trans*-chlordane, *p,p'*-DDT, *o,p'*-DDT, dieldrin, endrin, hexachlorobenzene, heptachlor epoxide, lindane, methoxychlor, mirex, octachlor epoxide, and *p,p'*-TDE in poultry fat; (b) continue study of this method to validate the extension of its applicability to beef fat and pork fat through interlaboratory testing and to develop generic performance standards for determining the suitability of a gel permeation chromatography (GPC) system, whether automated or manual, for use in the method; (c) prepare protocol for collaborative study to assess the performance of the GPC method for determining residues of polychlorinated biphenyls (PCBs) in the fat of cattle, swine, and poultry, and if the protocol is approved by the General Referee, initiate collaborative study; (d) change title of topic to Gel Permeation Chromatography (GPC) Cleanup for Organochlorine Residues. (This topic was transferred from Committee C to Committee E in January 1983.)

(4) *Chlorobenzilate, Chloropropylate, and Bromopropylate:* Change title of topic to Chlorobenzilate and Bromopropylate; appoint an Associate Referee to continue study to incorporate the determination of chlorobenzilate and bromopropylate into the multiresidue method, **29.001–29.028**, and to evaluate and collaboratively test methods for determining low levels of these compounds in milk and meat.

(5) *Chlorophenoxy Alkyl Acids:* Appoint new Associate Referee to develop, evaluate, and collaboratively study methods for determining residues of chlorophenoxyalkanoic acid herbicides in foods; continue study of the room temperature alkaline extraction procedure used in the method for determining residues of 2,4-D in dried green wheat, as collaboratively studied and presented in 1983 by the present Associate Referee, to assess the effectiveness of the extraction procedure for releasing and recovering bound or conjugated residues of 2,4-D and for converting 2,4-D esters to the 2,4-dichlorophenoxyethanoic anion.

(6) *Dicofol:* Discontinue topic.

(7) *Ethylene Oxide and Its Chlorohydrin:* Continue study to evaluate the gas chromatographic method of Scudamore and Heuser (*Pestic. Sci* (1971) **2,** 80–91) for determining ethylene oxide, ethylene chlorohydrin, and ethylene bromohydrin in foods.

(8) *Fenvalerate:* Continue study to evaluate and collaboratively test analytical methods for determining residues of fenvalerate and its photoproduct in foods.

(9) *Fumigants:* Continue study to improve the official final action gas chromatographic method for determining volatile fumigants in grain, **29.056–29.057**, and to extend the applicability of this method to additional fumigants (1,2-dichloroethane, tetrachloroethylene) and additional foods (citrus fruits, milled products, baked goods).

(10) *Gel Permeation Chromatography (GPC) Cleanup for Organochlorine Residues:* Continue study as outlined in the recommendation for the topic Chlorinated Hydrocarbons in Poultry (see rec. 3).

(11) *Inorganic Bromides in Grains:* Continue study of the gas chromatographic method of Heuser and Scudamore (*Pestic. Sci* (1970) **1,** 244–249) for determining inorganic bromides after conversion to 2-bromoethanol, as tested in interlaboratory studies on grain (*Analyst* (1976) **101,** 386–390) and lettuce (*J. Assoc. Off. Anal. Chem.* (1979) **62,** 1155–1159).

(12) *Kepone:* Continue study to develop and evaluate for collaborative study methods for determining residues of Kepone in fish and shellfish; continue study of the proposed micro-scale cleanup procedure for isolating Kepone and other organochlorine pesticides from fish extracts on disposable C_8 or C_{18} bonded phase silica gel cartridge-type columns.

(13) *Low Moisture–High Fat Samples, Extraction Procedure:* Initiate collaborative study of a method combining the Associate Referee's sample preparation/extraction procedure (*J. Assoc. Off. Anal. Chem.* (1982) **65,** 1122–1128) with acetonitrile partitioning, **29.014**, Florisil column cleanup, **29.015** or **29.029–29.034**, and electron capture gas chromatography, **29.018**, for determining organochlorine residues in oil seeds and other low moisture–high fat products.

(14) *Multiresidue Methodology, Minaturization:* Continue official first action status of the miniaturized multiresidue method, **29.D01–29.D07** (*J. Assoc. Off. Anal. Chem.* (1983) **66,** 535–536), for determining *p,p'*-DDE, *p,p'*-DDT, *p,p'*-TDE, dieldrin, heptachlor epoxide, and polychlorinated biphenyls in fish; conduct an interlaboratory trial of a miniaturized version of the multiresidue method, **29.001–29.018**, as applied to nonfatty foods; if this trial is successful, initiate collaborative study of the miniaturized method for nonfatty foods after approval of the collaborative study protocol by the General Referee.

(15) *Pentachlorophenol:* Complete the collaborative study of the former Associate Referee's gas chromatographic method for determining pentachlorophenol, underivatized, in gelatin and evaluate the results; if warranted by the results of this study, submit collaborative study report with recommendation for adoption of the method as interim official first action; continue study to improve the former Associate Referee's gas chromatographic method for determining pentachlorophenol, underivatized, in milk and blood (*J. Agric. Food Chem.* (1980) **28,** 710–714) and to apply the improved method to the analysis of fish, eggs, and milk.

(16) *Pentachlorophenol in Animal and Poultry Tissue:* Prepare a written report on the collaborative study of the method for determining pentachlorophenol in liver, as presented by the Associate Referee in 1982, and submit report for consideration of this method under the interim first action approval procedure. (This topic was transferred from Committee C to Committee E in January 1983.)

(17) *Permethrin:* Appoint an Associate Referee to evaluate and collaboratively study analytical methods for determining residues of permethrin in foods.

(18) *Photochemical Derivatization for Confirmation of Residue Identity:* Discontinue topic.

(19) *Polychlorinated Biphenyls (PCBs):* Continue study of analytical methods for determining residues of PCBs in foods and for isolating PCBs from the organochlorine

pesticide residues that are not separated from them in the multiresidue method, **29.001–29.018**.

(20) *Polychlorinated Biphenyls (PCBs) in Blood:* Submit protocol for collaborative study of the method developed by the Associate Referee for determining PCBs in blood serum to the General Referee for review and comment; if the protocol is approved, initiate collaborative study.

(21) *Resmethrin:* Continue study to evaluate analytical methods for determining residues of resmethrin in foods.

(22) *Root-Absorbed Residues, Extraction Procedures:* Appoint an Associate Referee to review available information on extraction efficiency for root-absorbed residues and to develop improved extraction procedures for root-absorbed residues, for incorporation into the multiresidue method, **29.001–29.018**.

(23) *Tetradifon, Endosulfan, and Tetrasul:* Continue study of the official final action method for determining endosulfan I, endosulfan II, endosulfan sulfate, tetradifon, and tetrasul in apples and cucumbers, **29.029–29.034**, to develop the intralaboratory data for the 11 nonfatty foods that remain to be tested to determine if the method is applicable to all Group I and Group II nonfatty foods (Table **29:02**).

(24) *Toxaphene:* Continue study to develop and evaluate quantitation procedures for the terminal residues of toxaphene, for use in conjunction with the multiresidue methodology, **29.001–29.018**; continue study of the approach combining Florisil cleanup, **29.015**, with high resolution liquid chromatography and capillary column gas chromatography to isolate toxaphene components from fish extracts and to determine by mass spectrometry whether the major components of the residues in fish are the same as those similarly isolated from standard toxaphene and may be used as part of a quantitation scheme for altered toxaphene residues.

*(25) *Other Topic:* Adopt as official first action the revisions recommended by the General Referee in 1983 to replace the micro-scale saponification procedure, **29.017** (*J. Assoc. Off. Anal. Chem.* (1980) **63,** 395), with the micro-scale saponification procedure used for additional cleanup in the miniaturized multiresidue method, **29.D01–29.D07** (*J. Assoc. Off. Anal. Chem.* (1983) **66,** 535–536) and to replace the instructions in **29.D06** with a reference to the revised **29.017**. To effect this recommendation, replace **29.017** and **29.D06** with the following revised sections **29.017** and **29.D06**, respectively:

29.017 *Saponification*

(Applicable only to those chems stable to hot alkali treatment. Use as supplemental cleanup if 15% eluate or MgO–Celite eluate is not substantially free from oily materials.)

Conc. pet. ether–ether (85 + 15) fraction under current of air to 2 mL, add 1 mL 2% alc. KOH, attach micro-Snyder column, and carefully reduce to ≤1 mL on steam bath. Reflux sample 15 min, remove, and cool. Add 2 mL alcohol–H_2O (1 + 1) and 5 mL hexane, and shake 1 min. Centrf. to sep. layers. Transfer as much hexane layer as possible to second tube, using disposable Pasteur pipet, and repeat extn with 5 mL hexane. Conc. combined hexane to appropriate vol. for GC analysis.

29.D06 *Additional Cleanup*

Often addnl cleanup is required for second fraction (85 + 15) to prevent deterioration of GC column. Use **29.017**.

ORGANONITROGEN PESTICIDES

(1) *Anilazine:* Appoint an Associate Referee to conduct a collaborative study of the liquid chromatographic method reported by Lawrence and Panopio (*J. Assoc. Off. Anal. Chem.* (1980) **63,** 1300–1303) for determining anilazine in potatoes and tomatoes.

(2) *Benzimidazole-Type Fungicides:* Initiate collaborative study of the liquid chromatographic method reported by the Associate Referee (*J. Assoc. Off. Anal. Chem.* (1980) **63,** 1291–1295) for the simultaneous determination of intact benomyl and its major degradation product methyl 2-benzimidazole carbamate in crops.

(3) *Captan and Related Fungicides:* Initiate collaborative study of the method combining the extraction/cleanup procedures described in sec. 212.2 of the *FDA Pesticide Analytical Manual* with electron capture gas chromatography for determining captan, captafol, and folpet in lettuce and strawberries.

(4) *Carbamate Herbicides:* Appoint an Associate Referee to evaluate and collaboratively study methods for determining residues of carbamate herbicides in crops.

(5) *Carbamate Insecticides, Gas–Liquid Chromatographic Methods:* Change title of topic to Carbamate Insecticides, Gas Chromatographic Methods; submit documentation on the on-column silylation gas chromatographic method for determining carbamate insecticide residues as their silylated derivatives to the General Referee for review and comment; if the method description and supporting intralaboratory data are approved by General Referee, conduct intralaboratory trial of the method.

(6) *Carbamate Insecticides, Liquid Chromatographic Methods:* Complete the collaborative study of the Associate Referee's liquid chromatographic, post-column derivatization method for determining residues of *N*-methylcarbamate insecticides in crops (*J. Assoc. Off. Anal. Chem.* (1980) **63,** 1114–1124); evaluate the collaborative study results for grapes and potatoes fortified with 7 carbamate insecticides and, if warranted by the results, submit recommendation for adoption of the method under the interim first action approval procedure.

(7) *Carbofuran:* Appoint an Associate Referee to evaluate and collaboratively study analytical methods for determining carbofuran and its carbamate and phenolic metabolites in foods.

(8) *Chlorothalonil:* Appoint an Associate Referee to evaluate and collaboratively study the extension of existing gas chromatographic multiresidue methods to the determination of chlorothalonil residues in crops.

(9) *Daminozide:* Appoint an Associate Referee to develop and collaboratively study a gas chromatographic or liquid chromatographic method for determining residues of daminozide in foods.

(10) *Dinitro Compounds:* Appoint an Associate Referee to evaluate and collaboratively study methods for determining residues of dinitroaryl herbicides in foods.

(11) *Diquat and Paraquat:* Prepare protocol for interlaboratory study of the sodium borohydride reduction, gas chromatographic method for the simultaneous determination of diquat and paraquat residues in potatoes, as presented by the Associate Referee at the 8th AOAC Spring Workshop, for consideration by the General Referee of the proposal to use an internal standard or recovery factor to correct for adsorption of the residues on potatoes.

(12) *Dithiocarbamates, General Residue Methods:* Appoint an Associate Referee to develop residue methods for determining dimethyldithiocarbamate and ethylene bisdithiocarbamate fungicides as separate entities in foods.

(13) *Maleic Hydrazide:* Appoint an Associate Referee to develop or select for collaborative study a gas chromatographic or liquid chromatographic method for determining maleic hydrazide in crops.

(14) *Organotin Fungicides:* Appoint a new Associate Referee to evaluate and collaboratively study gas chromatographic methods for determining residues of tricyclohexyltin hydroxide, triphenyltin hydroxide, and fenbutatin oxide in foods.

(15) *Sodium o-Phenylphenate:* Appoint an Associate Referee to develop or select for collaborative study a specific gas chromatographic or liquid chromatographic method for determining *o*-phenylphenol in foods.

(16) *Substituted Ureas:* Appoint an Associate Referee to evaluate and collaboratively test multiresidue methods, such as that described by Baunok and Geissbuchler (*Bull. Environ. Contam. Toxicol.* (1968) **3,** 7–17), for determining substituted urea herbicides and their metabolites in foods.

(17) *Thiolcarbamate Herbicides:* Appoint an Associate Referee to evaluate and collaboratively study methods for determining residues of thiolcarbamate herbicides in crops.

(18) *s-Triazines:* Appoint an Associate Referee to conduct a collaborative study of the gas chromatographic method outlined by the General Referee in *J. Assoc. Off. Anal. Chem.* (1980) **63,** 273 for determining residues of atrazine and cyanazine in corn and potatoes.

(19) *Trifluralin:* Appoint an Associate Referee to evaluate and collaboratively test methods (preferably from among existing pesticide multiresidue methods such as **29.001–29.018**) for determining residues of trifluralin in crops.

ORGANOPHOSPHORUS PESTICIDES

(1) *Azinphos-methyl:* Appoint an Associate Referee to evaluate and collaboratively study analytical methods for determining residues of azinphos-methyl in foods.

(2) *Confirmation Procedures:* Prepare protocol for collaborative study of the Associate Referee's improved version of the pentafluorobenzyl bromide derivatization method of Coburn and Chau (*J. Assoc. Off. Anal. Chem.* (1974) **57,** 1272–1278; and *Environ. Lett.* (1975) **10,** 225–236) for review and comment by the General Referee; if approved by the General Referee, initiate collaborative study of the improved method for confirming the identity of organophosphorus pesticides recovered from water by a method such as that of Ripley et al. (*J. Assoc. Off. Anal. Chem.* (1974) **57,** 1033–1042).

(3) *Disulfoton:* Appoint an Associate Referee to evaluate and collaboratively study analytical methods for determining disulfoton and its metabolites in foods.

(4) *Extraction Procedures:* Appoint an Associate Referee to extend the extraction efficiency studies of Watts (*J. Assoc. Off. Anal. Chem.* (1971) **54,** 953–958) to additional organophosphorus pesticide residues and additional crops.

(5) *General Method for Organochlorine and Organophosphorus Pesticides:* Appoint an Associate Referee; continue study to extend the applicability of the AOAC multiresidue method, **29.001–29.018**, to additional organophosphorus pesticide residues in fatty and nonfatty foods; continue study to validate the use of the nitrogen/phosphorus-selective detector (NPD) as an alternative to the KCl thermionic detector used in the gas chromatographic determinative step of this method.

(6) *High Fat Samples:* Continue study to evaluate gel permeation chromatography for cleanup of organophosphorus pesticides and their metabolites in fatty foods; continue study to assess the utility of the NPD as an alternative to the KCl thermionic and flame photometric detectors used in AOAC official methods for determining organophosphorus pesticide residues by gas chromatography.

(7) *Methamidophos:* Appoint an Associate Referee to evaluate and collaboratively study analytical methods for determining methamidophos and its metabolites in foods.

(8) *Monocrotophos:* Appoint an Associate Referee to evaluate and collaboratively study analytical methods for determining residues of monocrotophos in foods.

(9) *Phorate:* Appoint an Associate Referee to evaluate and collaboratively study analytical methods for determining phorate and its metabolites in foods.

(10) *Phosphine:* Continue study of gas chromatographic methods for determining residual phosphine in fumigated products, such as the method reported by the Associate Referee (*J. Assoc. Off. Anal. Chem.* (1978) **61,** 5–7) and the method reported by T. W. Nowicki (*J. Assoc. Off. Anal. Chem.* (1979) **61,** 829–836).

(11) *Soils:* Appoint an Associate Referee to develop and collaboratively study a multiresidue extraction and cleanup method for determining residues of organophosphorus pesticides and their metabolites in soils.

(12) *Sweep Codistillation:* Continue study to extend the official final action sweep codistillation method for determining organophosphorus pesticide residues in crops, **29.044–29.049**, to the analysis of fatty foods; conduct an interlaboratory study of the Unitrex sweep codistillation apparatus developed in Australia; if warranted by the results of the interlaboratory study, initiate collaborative study of sweep codistillation cleanup for organophosphorus pesticide residues in fatty foods, after approval by the General Referee of the collaborative study design.

(13) *Thin Layer Chromatography:* Discontinue topic.

RADIOACTIVITY

(1) *Carbon-14:* Discontinue topic.

(2) *Cesium-137:* Defer consideration of collaborative studies reported jointly in 1983 by the Associate Referees for Cs-137 and I-131 in support of extending the official final action method, **48.025–48.029**, for determining Ba-140, Cs-137, and I-131 in milk by gamma-ray spectroscopy, using simultaneous equations (*J. Assoc. Off. Anal. Chem.* (1982) **65,** 1039–1043) to the determination of Cs-137 and I-131 in other foods, pending submission under the interim first action approval procedure of a revised collaborative study report that includes (a) a description of the sample preparation procedure as part of the method, (b) the results of a statistical analysis of the method's performance in the format and terminology recommended for reports on AOAC collaborative studies, and (c) information to clarify the nature of the food samples used in the collaborative studies and to justify the broad range of foods to which the method would apply; after submission of the revised collaborative study report, appoint an Associate Referee to

evaluate and collaboratively test radiochemical methods for determining Cs-137 in milk, other foods, and other biological materials at lower levels than determinable with **48.025–48.029**.

(3) *Iodine-131:* Defer consideration of collaborative studies supporting the extension of the official final action method for Ba-140, Cs-137, and I-131 in milk, **48.025–48.029** (*J. Assoc. Off. Anal. Chem.* (1982) **65,** 1039–1043), to the determination of Cs-137 and I-131 in other foods, pending revision of the collaborative study report (see rec. 2); complete the ruggedness testing of the more sensitive method recommended by the Nuclear Regulatory Commission and outlined by the former General Referee (*J. Assoc. Off. Anal. Chem.* (1979) **62,** 387–389) for determining I-131 in milk and, if warranted by the results, prepare protocol for collaborative study of the method for review and comment by the General Referee and Statistical Consultant to Committee E.

(4) *Neutron Activiation Analysis:* Complete the collaborative study of the Associate Referee's method for determining sodium in neutron-irradiated biological materials and evaluate the results; determine the availability of data from the collaborative study conducted by the former Associate Referee of the method outlined in *J. Assoc. Off. Anal. Chem.* (1976) **59,** 350–351 for determining chlorine and bromine in neutron-irradiated biological materials and, if data are available, perform statistical analysis to evaluate performance of the method; continue study to assess the feasibility of conducting collaborative studies of simultaneous multimetal determinations in neutron-irradiated foods and, if feasible, design collaborative study for review and comment by the General Referee.

(5) *Plutonium:* Appoint an Associate Referee to evaluate the applicability of the Department of Energy method for determining plutonium in urine, feces, and water *(Energy Monitoring Laboratory Procedures Manual* (1982) *HASL-300-Ed 25, E-Pu-01-01)* to the determination of plutonium in foods.

(6) *Radium-228:* Conduct ruggedness test and interlaboratory trial of the method reported by Baratta and Lumsden (*J. Assoc. Off. Anal. Chem.* (1982) **65,** 1424–1428) for determining radium-228 in foods and water; if method is successful in interlaboratory trial, prepare collaborative study protocol for review and comment by the General Referee and Statistical Consultant to Committee E.

(7) *Strontium-89 and -90:* Appoint an Associate Referee to design and conduct a collaborative study of the method described by Baratta and Reavey (*J. Agric. Food Chem.* (1969) **17,** 1337–1339) for determining strontium-89 and -90 in foods.

(8) *Tritium:* Appoint an Associate Referee to evaluate and collaboratively study methods for determining tritium in foods and biological materials.

WATER

(1) *Chemical Pollutants in Water and Wastewater:* Continue to review analytical methods collaboratively studied by ASTM, EPA, and others for possible adoption by AOAC as official methods for determining chemical pollutants in water and wastewater.

(2) *Chlorinated Solvents in Water:* Continue study to evaluate and collaboratively test methods for determining chlorinated solvents in water and wastewater.

(3) *Herbicides in Water and Sediment:* Continue study to evaluate and test through collaborative study analytical methods for the determination of herbicides in water and sediment.

(4) *Major Ions and Nutrients in Water:* Appoint an Associate Referee to evaluate and collaboratively study analytical methods for determining sodium, potassium, calcium, aluminum, phosphate, sulfate, and other major ions and nutrients in water.

(5) *Organophosphorus Pesticides in Water:* Appoint an Associate Referee to evaluate and collaboratively test the method proposed by the U.S. Environmental Protection Agency or other methods for determining organophosphorus pesticides in water and wastewater.

(6) *Triazine Herbicides in Water:* Appoint an Associate Referee to evaluate and collaboratively test methods for determining triazine herbicides in water and wastewater.

*(7) *Other Topic:* Adopt as official final action the official first action atomic absorption spectrophotometric method for determining cadmium, chromium, copper, iron, lead, magnesium, manganese, silver, and zinc in water, **33.089–33.094**, and replace the first paragraph of **33.090**, "(Use Pyrex glassware . . . in method)," with the following paragraph (as recommended by the General Referee for Water with the concurrence of the General Referee for Metals and Other Elements): "(Use Pyrex, quartz, or Teflon labware exclusively; clean thoroly with detergent and H_2O; soak in HNO_3 (1 + 1) for 1 week; rinse with H_2O, dil. HNO_3, and H_2O, in that order. Use deionized distd H_2O wherever H_2O is specified in method.)"

Report of Committee F on Recommendations for Official Methods

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ANALYTICAL MYCOLOGY OF FOODS AND DRUGS

(1) *Baseline Mold Counts by Blending:* Continue study.
(2) *Chemical Method for Detecting Mold:* Continue study.
(3) *Geotrichum candidum Morphology:* Continue study.
(4) *Geotrichum Mold in Canned Fruits, Vegetables, and Fruit Juices:* Continue study.
(5) *Geotrichum Mold in Frozen Fruits and Vegetables:* Continue study.
(6) *Howard Mold Counting, Use of Widefield Eyepiece:* Continue study.
(7) *Howard to Viable Mold Counts of Frozen Fruits and Vegetables, Comparison:* Continue study.
(8) *Microscopic Appearance of Mold Hyphae, Effect of Freezing:* Continue study.
(9) *Microscopic Mold Count Method, Use of Compound Microscope:* Continue study.
(10) *Microscopic Mold Counts, Effects of Interfering Plant Material:* Continue study.
(11) *Mold in Spices:* Continue study.
(12) *Molds and Yeasts in Beverages:* Continue study.
(13) *Refractive Index of Tomato Products:* Continue study.
(14) *Standardization of Plant Tissue Concentrations for Mold Counting:* Continue study.
(15) *Tomato Rot Fragment Count:* Continue study.

DISINFECTANTS

(1) *Antimicrobial Agents Used by Laundries on Fabrics and Materials:* Continue study.
(2) *Sporicidal Tests:* Continue study.
(3) *Textile Antibacterial Preservatives:* Continue study.
(4) *Tuberculocidal Tests:* Continue study.
(5) *Use-Dilution Test, Variation and Amendments:* Continue study.
(6) *Virucide Tests:* Continue study.

EXTRANEOUS MATERIALS IN FOODS AND DRUGS

(1) *Botanical Drugs, Adulteration by Foreign Plant Materials:* Continue study.
(2) *Botanicals:* Continue study.
(3) *Brine Extraction Techniques:* Continue study.
(4) *Chocolate Products:* Continue study.
(5) *Cocoa Powder and Press Cake:* Continue study.
(6) *Fecal Sterols:* Continue study.
(7) *Food Supplement Tablets:* Continue study.
(8) *Grains, Whole, Cracking Flotation Methods:* Continue study.
(9) *Insect Excreta in Flour:* Continue study.
(10) *Soluble Insect and Other Animal Filth:* Continue study.
(11) *Meats, Processed:* Continue study.
(12) *Mite Contamination Profiles and Characterization of Damage to Foods:* Continue study.
(13) *Mites in Stored Foods:* Continue study.
(14) *Mushroom Products, Dried:* Continue study.
(15) *Mushrooms, Canned:* Continue study.
(16) *Particulates in Large-Volume Parenterals:* Continue study.
(17) *Performance Evaluation of Methods for Filth:* Continue study.
(18) *Rye Bread:* Continue study.
(19) *Shrimp:* Continue study.
(20) *Spices:* Continue study.
(21) *Spirulina:* Continue study.
(22) *Urine, Detection:* Continue study.
(23) *Vegetable Products, Dehydrated:* Continue study.
(24) *Vertebrate Excreta, Chemical Identification Tests:* Continue study.

DRUG AND DEVICE RELATED MICROBIOLOGY

(1) *Biological Indicator Testing and Standardization:* Continue study.
(2) *Limulus Amebocyte Lysate Tests for Endotoxin:* Continue study.
(3) *Sterility Testing of Medical Devices:* Continue study.

FOOD MICROBIOLOGY

(1) *Automated Methods:* Continue study.
(2) *Bacillus cereus Enterotoxin:* Continue study.
*(3) *Bacillus cereus, Isolation and Enumeration:* Adopt as official final action the official first action method for differentiation of members of *B. cereus* group, **46.D01–46.D03.**
(4) *Campylobacter Species:* Continue study.
(5) *Canned Foods:* Continue study.
(6) *Clostridium botulinum and its Toxin, Detection:* Continue study.
(7) *Clostridium perfringens, Isolation and Enumeration:* Continue study.
(8) *Enteropathogenic Escherichia coli, Fluorescent Antibody Technique:* Continue study.
*(9) *Escherichia coli and Other Coliforms:* Adopt as official first action biological methods for detecting heat stabile and heat labile enterotoxins.
*(10) *Genetic Methods for Detection of Bacterial Pathogens:* Adopt as official first action a method for detecting heat labile toxin-producing *Escherichia coli.* Continue study.
*(11) *Helium Leaks in Canned Foods:* Adopt as official first action a method for detecting leaks in cans. Discontinue topic.

*An asterisk before a recommendation indicates that official action on that recommendation resulted in its appearance in "Changes in Methods."
The recommendations submitted by Committee F were adopted by the Association.
Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition.

*(12) *Hydrophobic Grid Membrane Filter Methods:* Adopt as official first action a membrane method for detecting total coliforms, fecal coliforms, and *E. coli.* Continue study.
(13) *Identification of Microoganisms by Biochemical Kits:* A collaborative study is planned. Continue study.
(14) *Parasitology:* Continue study.
*(15) *Salmonella:* Revise official first action the official final action method for *Salmonella,* **46.054–46.067**, to incorporate a modification of the method for nonfat dry milk. Continue study.
(16) *Salmonella, Fluorescent Antibody Technique:* Discontinue topic.
(17) *Somatic Cell, Automated Optical Counting Method:* Continue study.
(18) *Somatic Cell, Fossomatic Counting Method:* Continue study.
(19) *Spore-formers and Non-spore-formers in Low Acid Canned Foods:* A collaborative study is under way. Continue study.
(20) *Staphylococcal Toxin:* Continue study.
*(21) *Staphylococcus:* Adopt as official final action the official first action surface plating method for isolation and enumeration of *S. aureus* in food, **46.075–46.076.**
(22) *Sugars:* Continue study.
(23) *Vibrio parahaemolyticus:* Continue study.
(24) *Virology and Animal Oncology:* Continue study.
(25) *Yeast, Molds, and Actinomycetes:* Continue study.
(26) *Yersinia enterocolitica:* Continue study.
*(27) *Other Topics:* (a) Editorially revise the applicability statement for method **46.117–46.119,** Fecal Coliforms, by adding the following: "Because geographical differences may affect performance of the Medium A-1 method, determination of comparability with the LST-EC tube method should be performed prior to using Medium A-1. Moreover, this medium must be made from individual ingredients. Pre-formulated Medium A-1 is unacceptable." (b) In the official final action method for *Salmonella,* **46.054–46.067,** in **46.061**(a), change the incubation period of lysine decarboxylase broth from 96 ± 2 hr to 48 ± 2 hr. (c) Transfer the Associate Refereeship for Endotoxins by Limulus Amebocyte Lysate to the General Refereeship for Drug and Device Related Microbiology.

Report of Committee G on Recommendations for Official Methods

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ANTIBIOTICS

*(1) *Affinity Quantitative Determination of Penicillin in Milk:* Adopt the official first action method, **16.C01–16.C05**, official final action.
(2) *Bacitracin in Feeds:* Continue study.
(3) *Bacitracin in Feeds and Premixes (Chemical Determination):* Continue study.
(4) *Bambermycins:* Continue study.
(5) *Chloramphenicol in Animal Tissues:* Continue study.
(6) *Chlortetracycline in Feeds:* Continue study.
(7) *Erythromycins:* Continue study.
(8) *Lasalocid Sodium (Microbiological Assay):* Continue study.
(9) *Lincomycin in Feeds:* Continue study.
(10) *Monensin:* Continue study.
(11) *Oxytetracycline:* Continue study.
(12) *Qualitative Determination of β-Lactam Residues in Milk:* Continue study.
*(13) *Quantitative Determination of β-Lactam Antibiotic Residues in Milk:* Adopt as official final action the official first action method, **16C06–16.C11.**
(14) *Screening Procedures for Antibiotics in Feeds:* Continue study.
(15) *Statistics of Microbiological Assay:* Continue study.
(16) *Tetracyclines in Tissues (Chromatographic Assay):* Continue study.
(17) *Tetracyclines in Tissues (Microbiological Assay):* Continue study.
(18) *Tylosin:* Continue study.
(19) *Virginiamycin, Tubidimetric Assay:* Continue study.

BIOCHEMICAL METHODS

(1) *Aminoglycosides in Animal Tissue:* Continue study.
(2) *17β-Estradiol and Diethylstilbestrol in Tissues (Immunochemical Methods):* Extend work on this topic to include Affinity Chromatography.
(3) *Hormones in Tissues (Immunospecific Affinity Chromatography):* Continue study.
(4) *Immunochemical Methods for Staphylococcal Enterotoxin:* Continue study.
(5) *Immunochemical Species Identification of Meat:* Continue study.

*An asterisk before a recommendation indicates that official action on that recommendation resulted in its appearance in "Changes in Methods."
The recommendations submitted by Committee G were adopted by the Association.
Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition, and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **63,** 374–423 (1980); **64,** 501–540 (1981); **65,** 450–521 (1982); **66,** 521–564 (1983).

(6) *Performance Evaluation Methods for Non-RIA Procedures Measuring Human Chorionicgonadotropin:* Continue study.
(7) *Performance Evaluation Protocols for Clinical Chemical and Immunochemical Diagnostic Products:* Continue study.
(8) *Steroid Quantitation (Enzymatic Methods):* Continue study.
(9) *Sulfa Drugs in Animal Tissues (Immunoassay):* Continue study.

COLOR ADDITIVES

(1) *Arsenic and Heavy Metals:* Continue study.
(2) *Atomic Absorption:* Continue study.
(3) *Color in Candy and Beverages:* Continue study.
(4) *Color in Cosmetics:* Continue study.
(5) *Color in Drugs:* Continue study.
(6) *Color in Other Foods:* Continue study.
(7) *FD&C Red No. 4 in Maraschino Cherries:* Continue study.
(8) *Liquid Chromatography:* Continue study.
(9) *Inorganic Salts:* Continue study.
(10) *Intermediates, Uncombined, in Certifiable Triphenylmethane Colors:* Change name of topic to Intermediates, Uncombined, in Other Certifiable Colors.
(11) *Intermediates, Uncombined, in Certifiable Water-Soluble Azo Colors:* Continue study.
(12) *Subsidiary Colors in Certifiable Color Additives:* Continue study.
(13) *X-Ray Fluorescence Spectroscopy:* Continue study.
(14) *Colors in Nonfrozen Dairy Desserts:* Establish new topic.

COSMETICS

(1) *Deodorants, Aluminum and Zirconium in:* Discontinue topic.
(2) *Essential Oils and Fragrance Materials, Components:* Continue study.
(3) *Nitrosamines:* Continue study.
(4) *Preservatives:* Continue study.

DRUG RESIDUES IN ANIMAL TISSUES

(1) *Carbadox:* Discontinue topic.
(2) *Diethylstilbestrol:* Discontinue topic.
(3) *Dimetridazole:* Discontinue topic.
(4) *3,5-Dinitrobenzamide:* Discontinue topic.
(5) *Nitrofurans:* Discontinue topic.
*(6) *Screening Methods:* Adopt as official final action the densitometric screening procedure for the determination of multi-sulfonamides by TLC in swine, turkey, and duck, **41.D01–41.D06.**
(7) *Steroids:* Continue study.
(8) *Sulfa Drugs:* Continue study.
*(9) *Sulfonamide Drugs:* Adopt as official final action methods **41.C01–41.C08** and **41.C09–41.C12.**
(10) *Tiamulin:* Establish new topic.

DRUGS IN FEEDS

(1) *Amprolium:* Appoint an Associate Referee.
(2) *Arprinocid:* Discontinue topic.
(3) *Arsanilic Acid:* Appoint an Associate Referee.
(4) *Carbadox:* Continue study.
(5) *2-Chloro-1-(2 4,5-trichlorophenyl) Vinyl Dimethyl Phosphate (Rabon®):* Discontinue topic.
(6) *Dibutyltin Dilaurate:* Discontinue topic.
(7) *1,2-Dimethyl-5-nitroimidazole (Dimetridazole):* Continue study.
(8) *Ethopabate:* Appoint an Associate Referee.
(9) *Ethylenediamine Dihydroiodide:* Continue study.
(10) *Furazolidone and Nitrofurazone:* Continue study.
(11) *Ipronidazole:* Delete topic.
(12) *Larvadex:* Transfer topic to Committee A. (This compound is considered a pesticide, not a drug.)
(13) *Melengestrol Acetate:* Continue study.
(14) *Microscopy:* Delete topic.
*(15) *Nifursol:* Adopt method **42.098–42.104** as official final action.
(16) *Phenothiazine:* Continue study.
(17) *Pyrantel Tartrate:* Continue study.
(18) *Roxarsone:* Continue study.
(19) *Sulfa Drug Residues:* Continue study.
(20) *Sulfadimethoxine-Ormetoprin Mixtures:* Discontinue study of sulfadimethoxine; continue study of ormetoprin.
(21) *Sulfamethazine and Sulfathiazole:* Continue study.
(22) *Sulfaquinoxaline:* Delete topic.
(23) *Lasalocid:* Continue study.
(24) *Morantel Tartrate:* Continue study.

FORENSIC SCIENCES

(1) *ABO Blood Typing:* Continue study.
(2) *Biological Fluids (Immunoelectrophoresis):* Continue study.
(3) *Blood:* Continue study.
(4) *Bloodstains, ABH Typing:* Continue study.
(5) *Bloodstains, Species Determination of Dried:* Continue study.
(6) *Bomb Residues:* Continue study.
(7) *Documents:* Continue study.
(8) *Fingerprints:* Continue study.
(9) *Firearms:* Continue study.
(10) *Flammable Fluids:* Continue study.
(11) *Gunshot Residues:* Continue study.
(12) *Gunshot Residues by AAS:* Continue study.
(13) *Hair Examination:* Continue study.
(14) *Infrared Spectroscopy:* Continue study.
(15) *Microscopic Methods and Glass Products:* Continue study.
(16) *Paints, Pyrolysis-Gas Chromatographic Methods:* Continue study.
(17) *Safe Insulation:* Continue study.
(18) *Serial Number Restoration (Chemical Etching Techniques):* Continue study.
(19) *Soil Analysis:* Continue study.
(20) *Voice Print Identification:* Continue study.

Since no report has been received from the General Referee in at least 3 years, it is recommended that a letter of appreciation be sent and that a new General Referee be selected.

VETERINARY ANALYTICAL TOXICOLOGY

(1) *Cholinesterase:* Continue study.
(2) *Copper in Animal Tissues:* Continue study.
(3) *Lead in Animal Tissues:* Continue study.
(4) *Multiple Anticoagulant Screening:* Continue study.
(5) *Multielement Analysis by ICP:* Continue study.
(6) *Nitrate/Nitrite:* Continue study.
(7) *Selenium in Animal Tissues:* Continue study.
(8) *Poisonous Plants:* Continue study.
(9) *Arsenic in Animal Tissues:* Continue study.
(10) *Molybdenum:* Continue study.
(11) *Rumensin:* Continue study.

TOXICOLOGICAL TESTS

It is recommended that an advisory committee be set up to make recommendations to Committee G on the "State-of-the-Art" for this topic instead of attempting any collaborative studies at this time. It is further recommended that current General Referee Samuel I. Shibko be selected to serve as Chairman of the advisory committee.

Report of the Executive Director

DAVID B. MacLEAN
AOAC, 1111 N 19th St, Suite 210, Arlington, VA 22209.

The Board of Directors met on January 12–13, 1983, in Arlington, VA.

The Board approved the following policies: *(1)* that AOAC be an open scientific society serving regulatory, research, academic, industry, and private laboratories in the broad areas of agriculture, public health, and the environment, and providing official scientific methods of analysis, method validation procedures, exchange of scientific ideas and knowledge, and other related activities, on an international basis; *(2)* that local sections set up their own dues system; *(3)* that benefits for individual members include the following: receipt of *The Referee*, mailings, annual meeting discount on a preregistration basis, discounts on special workshops and training courses when offered, membership directory, 10% discount on all AOAC publications, methodology information and referral, free job ads and "Tips of the Trade" published in *The Referee* on a space available basis; and *(4)* that Referees and Committee members be required to become members but that their dues be waived on their written request.

The Board of Directors accepted for information the following 1982 committee reports: Official Methods Board, Committees A, B, C, D, E, F, and G, Editorial Board, Long Range Planning, Interlaboratory Studies, Joint Mycotoxin, Treasurer and Finance, Intersociety on Air Sampling, Constitution, Safety, Centennial, Ways and Means, Symposia and Special Programs, International Cooperation, and Laboratory Quality Analysis. The Board instructed that Committees A–G be designated as Committees on Official methods.

The Board took the following actions:

Approved the updated bank resolutions at the Bank of Virginia.

Approved a revised point system for selecting candidates for Fellows of the AOAC.

Approved the budget request of $85,000 for the Centennial Committee.

Approved the 1984 Fund Raising Goal for the Ways and Means Committee of $300,000 with $235,000 for the Wiley Fund and $65,000 for scientific aspects of the AOAC Centennial, with acknowledgments to substantial contributors.

Approved hiring Howard Moore as an AOAC Liaison Representative for the Midwest and Northeast (Canada–U.S.).

Approved employment of a statistical consultant to extract information on performance parameters from collaborative studies of methods approved in 1979 through 1983. Approved publication of those summaries of method performance in the 14th edition of *Official Methods of Analysis*.

Approved requiring Associate Referees to provide statistical summaries of performance of methods with methods submitted for adoption beginning in Fall 1983.

Approved hiring a statistical consultant to write a caution statement regarding the statistical significance source, interpretation, and use of performance parameters.

Approved registration fees for the 1983 Annual International Meeting.

Established a subcommittee of the Board of Directors to approve capital equipment acquisitions in the $3,000–$10,000 per item range and personnel actions.

Directed the staff to publish, in *The Referee* and the *Journal of the AOAC*, the attorney's statement that AOAC does not approve equipment and recommended that future methods contain performance descriptions of equipment and materials used therein.

The Board of Directors met on April 21, 1983, in Indianapolis, IN.

Actions taken were as follows:

Approved Seattle, WA, as the site for the 1986 Spring Training Workshop and asked the Pacific Northwest Regional Section (Canada–U.S.) to organize it.

Amended the terms of reference of the Long Range Planning Committee to include in its membership the Immediate Past President and his/her predecessor.

Approved the inclusion of Alberta in the Pacific Northwest Section (Canada–U.S.).

Charged the Committee on the Constitution to clarify *present* classes of membership and voting categories before considering changes in classes of membership and voting categories.

Approved changing publication date of *Official Methods of Analysis*, 14th edition, from January 1985 to October 1984.

Approved continuance of present multi-editor system for the *Journal of the AOAC* with reevaluation annually in the spring.

Approved obtaining an editor for the new section of the *Journal*, "Chemical Contaminants Monitoring."

Approved increasing the 1984 nonmember *Journal* subscription rate inside the United States by 10% to a total of $82.50.

Approved the policy that the names of nominees for the Board of Directors and Officers and the name of the President-Elect, who will become president, and the fact that the Bylaws permit nominations from the floor be published in *The Referee* before the Annual International Meeting.

The Board of Directors met on August 23–24, 1983, in Arlington, VA, and took the following actions:

Approved the budget for the fiscal year beginning October 1, 1983.

Approved the concept that AOAC follow U.S. federal government with respect to across-the-board staff pay increases in 1983–84.

Approved a hospitalization insurance plan with 100% coverage for staff members and 75% partial coverage for their families.

Approved purchase of liability insurance coverage of up to $3 million.

Directed that all AOAC funded travel comply with U.S. federal travel guidelines and procedures.

Approved two $1000 ($500 per year) scholarships for 1983–84.

Approved the concept of the proposal for a short course for analytical laboratories on Developing and Managing Quality Assurance Programs, the first course to be held March 27–28, 1984, in Arlington, VA.

Approved payment of room and board not exceeding $300 for a visitor from Rumania to attend the 1983 Annual International Meeting.

Approved the following constitutional amendment for consideration at the 1983 Annual International Meeting:

Membership in a regional section shall consist of members, associate members, and honorary members of the Asso-

ciation residing 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 of the section. No person shall be a member of more than one regional section.

The membership approved the amendment on October 7, 1983.

The Board of Directors met on October 2, 1983 in Washington, DC, and took the following actions:

Approved the Editorial Board recommendation that Helen Reynolds be appointed editor for the *Journal* section on Chemical Contaminants Monitoring.

Directed the staff to recommend and document price and print run for *Official Methods of Analysis* at the January 1984 Board of Directors Meeting.

Approved conducting the 1988 Spring Training Workshop in San Francisco, CA, with George Tichelaar as orgainzer.

Approved the bank resolution for the Northeast (Canada–U.S.) Regional Section.

Approved retention of Bissel and Meade as accountants/auditors for 1983–84.

Approved the following dates for the 1984 Board of Directors meetings:

January 11–12, 1984—Arlington, VA
May 2–3, 1984—Philadelphia, PA
September 12–13, 1984—Arlington, VA
October 28, 1984—Washington, DC

Approved continuance of the Administrative Committee of the Board of Directors for one more year, with Richard Ronk replacing James Kottemann.

Sources of financial support up to December 1983 were:

Government

Association of Public Analysts
Environmental Protection Agency
  Office of Pesticide Programs
Food and Drug Administration
Health and Welfare Canada
  Health Protection Branch
Laboratory of the Government Chemist
Ministry of Agriculture, Fisheries and Food
National Marine Fisheries Service
U.S. Department of Agriculture
  Agricultural Research Service
  Food Safety and Inspection Service

Alabama Department of Agriculture and Industries
Alberta Agriculture
Arkansas State Plant Board
Arizona Office of the State Chemist
California Department of Food and Agriculture
Delaware Department of Agriculture
Florida Department of Agriculture and Consumer Services
Georgia Department of Agriculture
Hawaii Department of Health
Illinois Department of Agriculture
Indiana Office of the State Chemist
Indiana State Board of Health
Iowa Department of Agriculture
Iowa State Veterinary Diagnostic Lab
Kentucky Agricultural Experiment Station
  Division of Regulatory Services
Kentucky Department of Agriculture
Maryland Department of Agriculture
Michigan Department of Agriculture
Mississippi State Chemical Laboratory
Missouri Experiment Station Chemical Laboratory
Montana Department of Agriculture
Nebraska State 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 State Department of Agriculture
Ontario Ministry of Agriculture
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 Agricultural Experiment Station
Utah State Department of Agriculture
Vermont Agricultural Experiment Station
Virginia Division of Consolidated Laboratory Services
Wisconsin Department of Agriculture, Trade, and Consumer Affairs
Wyoming Department of Agriculture

Industry

3M Medical Products Division
Agrico Chemical Co.
Agway, Inc.
Alcon Laboratories Inc.
American Cyanamid Co.
Andersons, The
A/S N. Foss Electric
Bacardi Corp.
Boehringer Mannheim Biochemicals
Borden, Inc.
Bristol-Myers Co.
Campbell Inst. of Res. & Technol.
Campbell Taggart
Cargill, Inc.
Carnation
Chevron Chemical Co.
Ciba-Geigy Corp.
Coca-Cola Co., The
Comibassal International
CPC North America-CPC Intrnt'l, Inc.
Dickey-John Canada, Inc.
Dow Chemical Co.
Duphar BV
DuPont Co.
DuPont Pharmaceuticals
E. & J. Gallo Winery
Eastman Chemical Products, Inc.
Eli Lilly and Co.
  Elanco Products Company, Division
FBC Ltd
FMC Corp.
Fertilizer Institute, The
GB Fermentation Industries, Inc.
General Foods Corp.
General Mills, Inc.

Gerber Products Co.
Hazleton-Raltech
Heinz U.S.A.
Hershey Foods Corp.
Hoechst-Roussel Pharm., Inc.
Hoffmann-La Roche, Inc.
ICI Americas, Inc.
IMC Corp.
ITT Continental Baking Co.
Kellogg Co.
Kraft, Inc.
Kroger Co., The
Lancaster Laboratories, Inc.
Leatherhead Food R.A.
Lehn & Fink Products Co.
Life Savers, Inc.
Lipton, Thomas J., Inc.
McKee Baking Co.
McNeil Consumer Products Co.
Marion Laboratories, Inc.
Mead Johnson & Co.
Merck, Sharpe & Dohme Research Labs.
Monsanto Agricultural Products Co.
Moorman Manufacturing Co.
Nabisco Brands, Inc.
National Food Processors Assn.
Norwich Eaton Pharmaceuticals
O.M. Scott & Sons Co.
Orion Research Inc.
Ortho Pharmaceutical Corp.
Pennwalt Corp.
Pfizer, Inc.
Philip Morris
Pillsbury Co., The
Procter & Gambel Co., The
Quaker Oats
Ralston Purina Co.
Rhone-Poulenc Chemical Co.
Royster Co.
Salsbury Laboratories, Inc.
Schenley Distillers, Inc.
Seagram (Joseph E.) & Sons, Inc.
Shaklee Corp.
Smith Kline Corp.
Stauffer Chemical Co.
Sunkist Growers, Inc.
Swift & Co.
Tastybird Foods
Technicon Industrial Systems
Union Carbide Agric. Products Co.
Upjohn Company, The
Velsicol Chemical Corp.

Report of the Secretary/Treasurer and the Finance Committee

PRINCE G. HARRILL, *Secretary/Treasurer of AOAC, Chairman of the Finance Committee*
Food and Drug Administration, Division of Food Technology, Washington, DC 20204

Other members: T. G. Alexander, Jr, J. E. McNeal

The Secretary/Treasurer and the Finance Committee have confirmed in Fiscal Year 1983 (1) the actuality of the Association's claimed assets, in the form of cash and securities, and (2) by means of selective checks, the reliability of the Association's financial reports. In the past two years, the year-end financial statements have been completed for the Annual Meeting. This year, the earlier date of the Annual Meeting did not allow enough time to have all of the financial statements for the meeting. The Finance Committee met on September 2, 1983, and the Quarterly Financial Report for the three months ending June 30, 1983 was reviewed and judged to be satisfactory. Your Association had an investment portfolio of $1,123,132.55 and the excess of income over expenses was $39,189.00 as of June 30, 1983. The Statement of Financial Condition is summarized below.

The Finance Committee has had the task of tracking and advising on the Association's investing and safeguarding of its funds. The Committee believes that the Association's staff has done this in a conscientious and satisfactory manner. During the last year, the Committee also has had the task of monitoring the Association's conversion from a manual system to a computerized system. As of this date, the Journal Renewal System, Membership System, Advertising and Promotion Tracking, Sales, Accounts Receivable, Inventory Control, General Ledger, Fourteenth Edition Advertising and Promotion List, Membership Directory, and Talent Tracking have been computerized.

The Finance Committee recommended to the Board of Directors that the Association's present accounting firm be retained for Fiscal Year 1984.

STATEMENT OF FINANCIAL CONDITION—JUNE 30, 1983

Assets		
Current Assets:		
Cash, Bank of Virginia*		$61,838.14
Cash, Bank of Virginia, payroll		12,868.74
Cash, office fund		500.00
Cash, Mid-West fund		2,890.41
Accounts receivable, books and publications		22,165,13
Accounts receivable, contracts and grants		52,185.80
Accounts receivable, Private Sustaining Members		8,500.00
Accrued interest receivable		20,510.18
Inventory, books and publications—at cost		79,877.02
Prepaid expenses		7,166.05
Advances		7,812.34
Total Current Assets		$276,313.81
Investments:		
Securities	$89,783.01	
Certificates of deposit	1,031,915.29	
Savings	1,434.25	1,123,132.55
Fixed Assets:		
Office furniture, fixtures and equipment	$143,454.94	
Less: Accumulated depreciation	79,613.54	63,841.40
Deferred Costs:		
Methods of Analysis, 14th Edition	$71,509.45	
Spring Workshop, 1983	5,515.73	
Spring Workshop, 1984	685.25	
Quality Assurance Manual	3,347.94	
Centennial	2,116.48	
Annual Meeting, 1983	17,686.95	100,861.80
Total Assets		$1,564,149.56

*Interest bearing account

Liabilities and Fund Balance		
Current Liabilities:		
Accrued and withheld payroll taxes		$476.95
Total Current Liabilities		$476.95
Deferred Income:		
Journal subscriptions	$137,106.53	
Annual Meeting, 1983	20,000.00	
Private Sustaining Members	27,832.21	
Membership	13,593.75	
Spring Workshop, 1983	6,800.00	
Northwest Meeting	2,242.00	
Total Deferred Income		$207,574.49
Reserve for Publications		200,000.00
Restricted Reserve for the 14th Edition		341,261.58
Restricted Fund—Harvey Wiley		44,823.78
Restricted Fund—Centennial		100.00
Restricted Reserve for FAAM		361.81
Fund Balance:		
Balance, October 1, 1982	$775,812.27	
Less: Prior year Adjustment	22,500.00	
Adjusted Balance, October 1, 1982	753,312.27	
Less: Restricted Reserve for the 14th Edition	22,950.00	
	730,362.27	
Add: Excess of income over expenses for the nine months ended June 30, 1983	39,188.68	
Balance, June 30, 1983		769,550.95
Total Liabilities and Fund Balance		$1,564,149.56

Report of the Editorial Board

ROBERT C. RUND, *Chairman*
Office of the Indiana State Chemist, Purdue University, Department of Biochemistry, West Lafayette, IN 47907

Other members; C. W. Gehrke, A. R. Hanks, K. R. Hill, M. Ihnat, C. F. Jelinek, J. Pomerantz, C. H. Van Middelem, K. Helrich, O. L. Shotwell

The Editorial Board convened on two occasions during the past year. A full day session was held on April 18, 1983, at the outset of the spring Association meeting in Indianapolis and the second Board meeting was a half-day session on October 2, 1983, at the Shoreham Hotel, Washington, DC.

Sales of publications continue to show an increase in volume and financial returns are in line with budgeted estimates. As of the end of July, 1983, a total of 16 163 copies of the 13th edition of *Official Methods of Analysis* had been sold. This is 1575 more copies than the 12th edition over the same time interval; 60% of the current edition sales were made within the United States. A slight decrease in current edition sales beyond the U.S. may reflect world economic conditions.

Manufacturing costs of the *Journal* increased 9.5% in 1982 and will nearly equal this in 1983. As a consequence the Board has proposed a 10% increase in subscription price for 1984, which will set this fee at $82.50.

In an effort to conserve expenditures and to usher in the centennial year of the Association a new format for the *Journal* will appear with the first issue in 1984. This will include an increase in page size and a newly designed silver and blue cover. Further, invitations have been extended and accepted by noted individuals in six different subject areas for review papers to highlight the association of AOAC with development of methodology in these areas.

The spirit of cooperation of the Editor of *Official Methods of Analysis*, General Referees, and editorial staff is hereby recognized. This cooperation will enable publication of the 14th edition of *Official Methods of Analysis* several months early in order to feature it as a centennial issue in 1984.

The Board also approved for publication in 1984 a Quality Assurance Manual authored by F. M. Garfield and sponsored by the Quality Assurance Committee. K. Helrich, upon invitation by the Centennial Committee, has authored a history of the AOAC entitled, "The Great Collaboration: The First 100 Years of the Association of Official Analytical Chemists" to be published and distributed to all members of the Association. A new Statistical Manual authored by G. Wernimont and edited by W. Spendley is under review and will also be published in 1984.

The Board responded to a need expressed by many for a residence and exhibit of monitoring data on residues, including total diet studies. The response has developed into a new section of the *Journal* to be known as Chemical Contaminants Monitoring. A search was conducted for an editor of this section and a candidate proposed to the Board of Directors for appointment.

The current editor for book reviews in the *Journal* has requested to be relieved of this appointment. The Board recognizes the many hours of work contributed by T. Alexander to the Association in this endeavor and expresses appreciation on behalf of the Association to him for the excellent results achieved. Unfortunately, the Board has not been successful in locating a successor and has acted to discontinue this section.

The Board has evaluated the reorganized structure of the *Journal* and found it working well and to the satisfaction of editorial staff and Board members. Performance evaluations were made in April on each section editor and an expression of confidence, satisfaction, and encouragement was communicated to all editors. Another evaluation of the arrangement of editorial assignments will be made next spring.

Informal sessions between the Board Chairman, Managing Editor and Section Editors at each annual Association meeting have proven to be productive in resolving common questions of procedure in the review process and administration of the office. Other actions taken by the Board in the past year include:

(1) Adopted a manuscript review policy.

(2) Adopted an advance publication notice as a service to sustaining members.

(3) Adopted a policy to guide staff on acceptance of advertising.

(4) Updated Terms of Reference pertaining to the Board.

(5) Agreed to publish the 6th edition of FDA's *Bacteriological Analytical Manual*.

(6) Agreed to publish a section in the *Journal* entitled, New Products, to be limited to 1–2 pp. each issue. Within this section, the product would be named and a one-line description and source of further information given. This action will be reviewed in one year.

(7) Agreed to provide a committee from within the Board to assist staff in the review of proposed agreements with outside agencies to avoid compromising publication objectivity and publication policies and independence of AOAC.

(8) Agreed to consider a proposed concept for publishing summaries of collaborative analytical studies of interest published elsewhere, upon request of the author. Format and procedure for implementation are yet to be developed.

(9) Reaffirmed earlier action to accept manuscripts describing simplified methods and established procedure for designating same.

(10) Adopted a book publication policy for future proposed AOAC publications.

(11) Agreed to negotiate through the Executive Director with Health Protection Branch Canada and Agriculture Canada for a contract to publish a sampling manual.

The Board wishes to express support and appreciation to the editorial staff of the Association without whose professional services we could not long survive.

Report of the Committee on the Constitution

D. EARLE COFFIN, *Chairman*
Health and Welfare Canada, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2

Other members: R. Frank, A. Hofberg, F. J. Johnson, J. B. Kottemann, E. Martin, M. Rhodes, E. D. Schall, A. W. Tiedemann

The Committee on the Constitution met twice during the year; on April 20 at the Spring Workshop in Indianapolis and on October 5.

As a result of concerns expressed at the Annual Business Meeting in 1982, the Committee considered revision of the Bylaws regarding membership in regional sections and recommended to the Board of Directors a revision of Article X, Section 2 of the Association Bylaws to provide for expanded membership in regional sections.

The Committee recommended to the Board of Directors a plan for the accreditation of voting delegates for annual and other general meetings of the Association. The Board of Directors has requested that the Committee on the Constitution institute these procedures as soon as possible.

The Committee held initial discussions with Virginia Schlotzhauer, a professional parliamentarian, who has been retained to advise the Association on the interpretation and inherent problems in the Bylaws of the Association.

The Committee continued consideration of the implementation of and alternative approaches to the definition of membership and voting rights in the Association.

In view of the increasing duties and complexity of issues being considered by the Committee on the Constitution, this Committee will recommend that its membership be increased so that several sub-committees may be formed within the Committee to deal with such varied issues as model bylaws for regional sections; recommendations for granting of regional section charters; consideration of options for membership and voting in the Association; accreditation of voting members; and requests for bylaw changes or interpretations.

Bylaws Changes

Article X: Subsidiary Organizations

Section 2. Membership in Regional Sections

Membership in a regional section shall consist of Members, Associate 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.

Adopted

Report of the Centennial Committee

WILLIAM HORWITZ, *Chairman*
Food and Drug Administration, Bureau of Foods, Washington, DC 20204

Other members: T. G. Alexander, W. R. Bontoyan, K. W. Boyer, C. A. Brunner, P. Bulhack, E. Elkins, F. M. Garfield, J. Levine, H. Miller, H. L. Reynolds

The committee is finalizing many of the items which were listed in the report published last year, *J. Assoc. Off. Anal. Chem.* (1983) **66**, 446–447.

A number of international organizations will meet in Washington the week prior to the AOAC meeting to permit their members to attend the Centennial celebration. The Collaborative International Pesticide Analytical Committee will meet in Baltimore and Washington the week of October 22. A number of the commissions of the Analytical Division of the International Union of Pure and Applied Chemistry (IUPAC) will also meet at that time. The International Symposium on Harmonization of Collaborative Studies will be held October 25–27, 1984, at the National Academy of Sciences and will be opened by AOAC President Gehrke.

The opening session will be addressed by Nobel Laureate, Dr. Linus Pauling, and by the famous attorney specializing in regulatory affairs, Peter Barton Hutt. Dr. Cyril Ponnamperuma will be the banquet speaker on "Transfer of Science and Technology to the Developing World."

Dr. Kenneth Helrich has prepared a 100-year history on AOAC, which should be available for distribution to AOAC members and to Sustaining Members in early spring, 1984. The AOAC *Journal* will feature special articles and symposia topics during the year.

Action on all other items mentioned in the previous report is continuing.

Report of the Committee on Collaborative Interlaboratory Studies

WILLIAM HORWITZ, *Chairman*
Food and Drug Administration, Bureau of Foods, Washington, DC 20204

Other members: R. Albert, P. W. Britton, P. R. Caudill, C. J. Dahl, T. Dols, H. Egan, R. Ellis, D. W. Fink, E. M. Glocker, R. Grappin, M. Ihnat, S. A. Katz, M. Margosis, K. McCully, A. Munson, J. O'Rangers, H. S. Ragheb, S. Sherkin, E. Smith, W. Stellar, L. Stoloff, B. K. Thompson, L. Williams, J. Winbush, E. S. Windham, J. Winter

The Committee recommends: (1) That the "Outline of Interlaboratory Collaborative Study Procedure to Validate the Characteristics of a Method of Analysis," published in *J. Assoc. Off. Anal. Chem.* (1983) **66**(2), 455–466, as revised at this meeting, be approved with respect to the conduct of the study, sections 1, 3, 4, 5, 7, and 8. Those aspects dealing with the design and statistical analysis, sections 2 and 6, are still considered temporary.

(2) That the set of letters prepared by D. Fink be accepted as suitable models for use by Associate Referees, to be modified in accordance with the conditions of the specific study under consideration.

(3) That the symbols and nomenclature as used by Steiner in the *Statistical Manual of the AOAC* be accepted as the approved symbols and nomenclature of AOAC in its publications and documents. These are:

S_o = Repeatability (within-laboratory) standard deviation

S_L = Among-laboratories standard deviation

S_x = Reproducibility (including repeatability) standard deviation

where,

$$S_x^2 = S_L^2 + S_o^2$$

The corresponding coefficients of variation, based on the above standard deviations, which should be used as the performance parameters of methods of analysis, are indicated by CV with the appropriate subscript. A table is attached relating the various symbols and terms that have been used in AOAC publications for these measures of precision. The use of Youden's S_d as a reportable parameter should be discontinued. This recommendation was developed in conjunction with the Statistics Committee and is endorsed by the Statistics Committee. The same recommendation was developed independently by Subcommittee E and by the Official Methods Board.

(4) That the issues involved in the statistical design and analysis of collaborative studies be delineated, circulated to the Committee, and discussed prior to making a final decision at the next meeting.

Precision parameters recommended and not recommended for use in AOAC documents and publications

Recommended nomenclature	Recommended symbol	Symbols and terms not recommended
Repeatability (within-laboratory) standard deviation	S_o	S_w (within); S_r (random); S_e (error)
Among-laboratories standard deviation	S_L	S_b (between) (bias)
Reproducibility (including repeatability) standard deviation	S_x	S_d (data) (difference)

CV_o, CV_L, and CV_x are the corresponding recommended symbols for the coefficients of variation.

Guidelines for Interlaboratory Collaborative Study Procedure to Validate Characteristics of a Method of Analysis

Second Draft

(Incorporates symbols and terminology recommended at meeting 02 Oct 1983 and other revisions approved at meeting of 01 December 1983. New or substantially revised sections are designated with*.)

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, intra- and interlaboratory testing, evaluating, and recommending a method of analysis. Other organizations that wish to use this document may prefer to substitute their own terminology.

SUMMARY STATEMENT OF AOAC CURRENT MINIMUM RECOMMENDED GUIDELINE FOR DESIGN OF A COLLABORATIVE STUDY:

"(No. of materials × No. of laboratories) = 30, with a minimum of 5 laboratories providing usable data, with single determinations. However, it must be recognized that such a minimum study can result in a relatively wide confidence interval for the statistical estimates of the method characteristics."

Guide to Validation Procedure

(Section numbers correspond to Outline; not all section numbers are included in Guide.)

1. Preliminary Work

1.1 Determine Purpose of the Study

Purpose of the study (e.g., to determine attributes of a method, proficiency of analysts, comparison of methods, or reference values of a material), the type of method (empirical, screening, practical, reference, definitive), and the probable use of the method (enforcement, surveillance, monitoring, acceptance testing, quality control, research) determine the relative importance of the various method attributes (bias, precision, specificity, limit of determination) and 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 Choose Method

1.2.1 Sometimes obvious (only method available)

1.2.2 Critical literature review (reported attributes are often optimistic)

1.2.3 Survey laboratories to obtain candidate methods; compare 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 by 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 (Within One Laboratory)

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 formulated by committees that 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 potential variability that may be expected in practice.

1.3.2 Approaches to optimization:

(a) Conduct trials on basis of changing one variable at a time.

(b) Conduct ruggedness testing for 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 method for optimizing a method.]

(c) Use Deming simplex optimization to identify critical steps. See Dols and Armbrecht (*8.3*).

1.4 Protocol Development on Selected Methods (as Applicable)

(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) to determine applicability to commodity(ies) of interest.

1.4.3 Interference testing (specificity):

(a) Test effects of impurities, ubiquitous contaminants, flavors, additives, and other components which may be expected to be present and at expected concentrations.

(b) Test nonspecific effects of matrixes.

*(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 (not necessary when method itself defines the property or component).

1.4.5 Develop defined performance specifications for instruments and systems suitability tests 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 in routine analysis (ruggedness).

1.4.7 Estimate limits of reliable measurement (if applicable).

1.4.8 If any of the characteristics are unacceptable, revise, retest, and reiterate such items as are necessary.

1.4.9 Conduct laboratory trial of method by analyst not familiar with method.

1.4.10 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 several laboratories on identical materials.

1.5.1 Prepare method in format and style given in instructions in the *Handbook for AOAC Members* (1982), or other recognized manual, e.g., ISO Guide 18 (*8.4*).

1.5.2 Clearly describe and explain every step in the analytical method so as to discourage deviations. Use imperative directions, avoiding subjunctive and conditional expressions as options.

1.5.3 Check editorially for completeness, credibility (e.g., buffer pH consistent with specified chemicals, volumes not greater than capacity of container), continuity, and clarity.

1.5.4 Check for inclusion of performance specifications and systems suitability tests (1.4.5), and convenient stopping points.

1.5.5 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 who are experienced in the technique 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.

1.6.2 *Letter of invitation.* Address formal letter to the individual responsible for assignment of laboratory effort. State reason for selecting that laboratory, estimated number of person-hours for performance required, 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. Enclose copy of the method, and a return form or card (with postage affixed, if domestic), requiring only check mark for acceptance or refusal of invitation, signature, 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 (1.5.5) is also useful to uncover 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 form for each laboratory and provide sufficient space for as much analytical detail as may be required for proper evaluation of the results, including a check of the calculations. Indicate number of significant figures to be reported. When recorder 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 (identification with respect to item analyzed, axes, date, submittor, experimental conditions, instrument settings, etc.). Include in the report forms a signature line for the analyst and lines for a printed or typed version of the name and address for correct acknowledgment. An example of a completed form would be 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 if the source of the problem is in the calibration or in the analysis.

1.8 Familiarization or Practice Materials

If deemed necessary, supply familiarization materials, with instructions, as far ahead as practicable, before actual materials are sent. When familiarization materials have been submitted, supply forms for reporting progress to 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 indication of the attributes of a method, particularly the systematic and random deviations, to be expected when the method is used in actual practice. It will usually provide information on the best performance that will be achieved.

2.1.2 The design should attempt to identify and to measure the possible sources of significant variability that may occur in actual practice, including between days, if this is a significant factor. (Within-laboratory performance on different days may provide a clue with respect to between-laboratory performance and is required information for quality control.) The best measure of within-laboratory variability is through the use of blind replicates or through the use of Youden pairs. The design must take into account how the data will be analyzed statistically.

*2.1.3 Present material 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 of which 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 20% (1 laboratory failure out of 6, when a minimum Youden design is used).

*2.1.5 The preparation of reference standards and standard solutions can provide a significant portion of the analytical error. A decision must be made whether this error is to be considered separately or as part of the method, i.e., will the analyst procure his own standard solutions or will they be provided by the Associate Referee.

2.2 Laboratories

Laboratories must be impressed with the importance of the study. A large investment is being made in testing the method and it probably will be the only such study that will be performed. It is important to give a fair and thorough evaluation of the method.

2.2.1 *Type:* Most appropriate type of laboratory is one with a responsibility related to the analytical problem. Other 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.

2.2.2 *Number:* Minimum of 6 (to avoid unduly large confidence bands). Fewer laboratories will widen the confidence limits of the mean and of the variance components (see design considerations 2.4.1 and 2.4.2). Most desirable number of laboratories, balancing logistics and costs against information obtained, is 8–10.

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, etc.

2.3 Materials

2.3.1 *Materials must be homogeneous. This is critical.* Establish by testing a representative number of laboratory samples taken at random before shipment to provide sound evidence of this point. (A collaborator who reports an outlying value will almost always initially claim receipt of a defective laboratory sample.)

2.3.2 *Label at random* so that there is no preselection from order of presentation.

2.3.3 *Concentration range:* Choose materials to cover concentration range of interest. If concentration range of interest is a tolerance limit or a specification level, bracket it with materials of appropriate concentration. If design includes the determination of absence of analyte, include blank (not detectable) materials as part of level of interest.

*2.3.4 *Number of materials:* Minimum number of materials is 5 to use the Youden rule of (laboratories × materials) = 30. This anticipates no analyses will be discarded as outliers and Youden pairs are not used exclusively (which requires an even number of materials). Blind replicates are counted as separate materials.

2.3.5 *Nature of materials:* Materials should consist of a representative set of commodities usually analyzed with customary and extreme values for the analyte and potential interfering substances, unless interference has been ruled out by interference testing (2.3.7).

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 collaborators 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 1.4.3). 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 known 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 within a given range. However, it should be pointed out that one of the assumptions of analysis of variance is that the results are 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, independent replication can be performed by supplying at least one of the following:

2.4.1 *Blind duplicate laboratory samples, randomly coded.*

*Analysis of blind duplicates is the best way of obtaining the within-laboratory precision component.

*NOTE: Triplicate and higher replication are relatively inefficient when compared with duplicate laboratory samples because replication provides additional information only on individual within-laboratory variability, which is usually the less important component of error. It is more efficient to use resources for the analysis of more levels and/or materials rather than for analysis of replicates.

PRACTICAL PRINCIPLE: With respect to replication, the greatest net marginal gain is always obtained in going from 1 to 2 as compared to going from 2 to 3, 3 to 4, etc.

2.4.2 *Youden pairs:* A useful design is an even number of materials arranged as Youden pairs—each pair consisting of a set of 2 materials of slightly different composition obtained either naturally or by diluting (or by fortifying) one of the materials with a small amount of diluent (or of analyte). Each laboratory sample should be analyzed only once; replication defeats the purpose of the design.

2.4.3 *Independent materials* (NOTE: Unrelated independent materials may be used as a Youden pair in the calculations or for plotting, but the more they differ, particularly with respect to repeatability, the less information they provide on within-laboratory variability.)

2.5 Other Design Considerations

2.5.1 The design may be reduced in the direction of less work or less cost, but at the sacrifice of reduced confidence in the reliability of the developed information.

2.5.2 More work 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 Thirty data points will only permit estimation of the standard deviation *of an individual reading* 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 0.80–1.34; for 200, 0.89–1.14.

2.5.4 The validity of extrapolating the use of a method beyond concentrations and components tested can only be estimated on the basis of the amount of signal change 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 used. Naturally, 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 Unit-to-unit variability within a series of laboratory samples 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 absorb analytes or other components from the matrix, e.g., water.

3.1.3 The materials must be stabilized, if necessary, preferably by physical means (freezing, dehydrating), or by chemical means (preservatives, antioxidants) which do not affect the performance of the method.

3.1.4 Moisture changes must be avoided, where necessary, by the use of vapor-tight containers.

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 and, if available, they are expensive. Sometimes the standards organization is interested in making reference materials available for the analyte under study, in which case it may provide the material for the study.

3.2.3 *Synthetic materials* may be especially formulated for the study with known amounts of analytes by actual preparation. Best used for macro constituents 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.

(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. The preparation of liquid materials susceptible to segregation should be done 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 bottles. Each collaborator is instructed to use the entire portion for the analysis, transferring the contents quantitatively. (This is the preferred alternative to spiked solid materials at trace (ppm) levels, at the expense of considerably more work.)

*Use individual coded solutions to be added to portions of the materials for single analyses by each laboratory. All solutions ideally should have the same volume and appearance. This type of material is analogous to that of 3.2.4 except for the source of matrix. This case should be used only for perishable commodities that are altered by all available preservation techniques.

(c) *Concentrated unknown solutions for direct addition by collaborators to their own commodities:* Should be used only as a last resort, because it introduces an indeterminable (material × laboratory) interaction component. This procedure also permits compromising the design of the study by direct analysis of the spiking solution. When used, supply individual coded solutions to be added to portions of the materials for single analyses by each laboratory. All solutions ideally 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 the matrix. This case should be used only for perishable commodities that are altered by all available preservative 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. It is almost impossible to avoid the personal bias introduced by knowledge of the composition of the material.

*In those instances where the collaborating laboratories are permitted to prepare study materials, the materials should be prepared by an individual who will not be involved in the analysis of the study materials.

3.3 Blanks

When the absence of a component is as important as its presence, when determinations must be corrected for the presence of the component or 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 two types of blanks: commodity blanks and reagent blanks. Since laboratories will often use reagents from different sources, each laboratory should perform reagent blanks. The number of blanks to be included in a collaborative study can be calculated based on the estimated values for the standard deviations 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 special design, with special attention being given to the number of blanks, and to the necessity for interpreting false positives and false negatives.

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%) of laboratory samples than there are collaborators. Some packages may never arrive, some materials may spoil, and some may be lost. New laboratories may have to be substituted for those which are unable to complete the promised work. Some 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.* If the containers are breakable, pack well to minimize possibility of breakage. If 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, etc.), 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 it is being sent. Hazardous materials should be packed, labeled, and conveyed as required by transportation regulations. Animal and plant products sent across international borders may require special certification from health authorities.

4.2 *A return slip to confirm safe receipt, should be included* with each package. If not sent previously, include copy of method, instructions, and report forms.

4.3 *Provide instructions for proper storage* of laboratory samples between unpacking and analysis.

4.4 *When it is important to have all instruments calibrated with the same reference material,* supply reference material to all collaborators.

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 because of the necessity to substitute reagents, apparatus, instruments, etc., must be recorded at the time and subsequently reported. If collaborator has no intention of following submitted method, he or she should not participate in the study. If the collaborator wishes to check another method on the same materials, additional samples should be requested for that purpose.

**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. If a laboratory cannot follow instructions as to number of analyses to perform, it raises a question as to the 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 "trace" or "less than."

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,* by reanalysis, if permitted by the protocol. Call Associate Referee to discuss suspicious values. If Associate Referee indicates that a value may be an outlier, also review the determination promptly to the extent possible, by reanalysis, recalculation, or preparation of new standards. If time and materials are available, obtain new laboratory samples for repeat analysis.

Since collaborators may not necessarily know if a value is an outlier, a useful suggestion is to indicate that 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 necsssary.

5.7 NOTE: The sooner an apparent outlier is investigated, the greater the likelihood of finding a reason for its occurrence. The most frequent causes of definable outliers are:

5.7.1 Incorrect calculations and arithmetic errors.

5.7.2 Editing errors, such as transposition of numbers.

5.7.3 Incorrect standards due to weighing or volumetric errors (check physical constants or compare against freshly prepared standard solutions).

5.7.4 Adventitious contamination.

6. Statistical Analysis

6.1 Initial Review of Data

The Associate Referee should first plot the collaborative data material by material (or as Youden pairs), values vs laboratory, preferably in ascending or descending order of reported average concentration. Usually major discrepancies will be apparent: displaced means, spread replicates, outlying values, differences between methods, consistent laboratory rankings, etc.

6.2 Statistical Approach

Perform calculations on each material individually or as Youden pairs, if appropriate. Only if the variances are not significantly different from each other can the results across materials be pooled for analysis of variance. If the coefficients of variation of the different materials are not significantly different, it may be convenient to average them over the range tested and to thereby report just a single coefficient of variation. 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, determinations). Rejection of more than 20% 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 1 or more laboratories from a 5–6 laboratory study. For larger studies, a smaller 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 the following statistical tests:

6.3.1 *Dixon test* for removal of isolated individual (or replicate) values or laboratory averages at either end of the set.

*Ordinarily do not iterate more than twice. Note that the Dixon tables in the Statistical Manual (*8.1*) are appropriate for one-tail only. If outliers are removed from both ends by the Dixon test, the correct wording is, for the 5% significance level, "Outliers were removed from both ends by the application of the one-tail Dixon test twice (once at each end) at the 95% confidence level." This is equivalent to a test at the 90% confidence level.

6.3.2 *Cochran test* for removal of laboratories (or indirectly for removal of extreme individual values from a set of laboratory values) showing significantly greater variability than the other laboratories for a given material.

6.3.3 *Rank sum test* for determining if a laboratory is reporting values which are consistently high or low over all materials. This test is ordinarily applied only when there are at least 5 materials in the study. This test is usually applied by the Associate Referee only as a basis for requesting the indicated laboratory(ies) to investigate potential sources of the systematic deviations.

*The decision as to whether or not a value(s) should be removed as an outlier is not ultimately 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.

6.4 Bias (Systematic Deviations) of Individual Results

6.4.1 Bias = amount found − amount added (or known or assigned value)

% Recovery = (measured concentration in fortified material − measured concentration in unfortified material) × 100/(known increment in concentration)

The amount added must be a substantial fraction or more than the amount present in the unfortified material.

6.4.2 The true value is known only in cases of spiked or fortified materials, 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 must be used for the reference point.

6.4.3 NOTES:

(a) Youden equates "true" between-laboratory variability (not including the within-laboratory variability) to bias or systematic error.

(b) The presence of random error limits the ability to estimate the systematic error. To detect the systematic *error of a single laboratory when its magnitude 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., coefficient of variation (CV) or relative standard deviation (RSD)) is usually the useful measure of precision in analytical work because it is often independent of concentration or amount of analyte over a wide range of levels. Moreover, the use of CV facilitates comparison of variabilities at different concentrations. When the CV begins to increase rapidly with lower concentration or amount, it delineates the limit of usefulness of the method (limit of reliable measurement). The most important types of precision (currently utilizing the definitions and terminology of Steiner, *8.1*) are:

6.5.1 *Reproducibility*—Among-laboratories (including within-laboratories) precision, designated as S_x.

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*, page 19). The correction term must be extracted by an analysis of variance technique (Steiner, *8.1*, pages 78–81). However, this crude, overall calculation of the standard deviation of all the data may serve as a check on the arithmetic, since the two values are usually fairly close.

6.5.2 *Repeatability*—Within-laboratory random error, designated as S_o.

6.5.3 *Among-laboratories (not including within-laboratory, variability)*, Youden's systematic error, designated as S_L.

6.5.4 NOTES:

*(a) The relationship among these 3 parameters is:

$$S_x^2 = S_L^2 + S_o^2$$

The parameters, S_x^2, S_L^2, and S_o^2, must be nonnegative by definition. The *estimate* of S_L^2, however, can be negative. This frequently occurs in practice when S_o^2 is so large (poor replication) 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 estimates of S_x^2. If this occurs with a number of the materials in the collaborative study, the method is probably unsatisfactory due to poor replication. If this occurs infrequently, the bias introduced by a single occasion is overshadowed by the other positive estimates of S_L^2.

(b) When only single determinations are performed on each material (except in the case of Youden pairs), *there is no rigorous basis for calculating S_o^2, and within-laboratory variability cannot be estimated directly.

(c) The ISO definitions for repeatability (r) and reproducibility (R), *8.5*, appear to be considerably different from the above measures of precision, although no less useful. 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 is the absolute difference between single test results of identical test material may be expected to lie with a specified probability (usually 95%); in other words, assuming normal distribution, 95% of the second readings from the same laboratory (repeatability) or from a different laboratory

(reproducibility) on the same material will be expected to lie within the calculated interval (r or R) from the first reading. The relationship between the two definitions are:

*Repeatability (r) = $2\sqrt{2}\,S_o = \pm 2.83\ m\ CV_o/100$

*Reproducibility (R) = $2\sqrt{2}\,S_x = \pm 2.83\ m\ CV_x/100$

where S_o and S_x are repeatability and reproducibility standard deviations, respectively; CV_o and CV_x are the corresponding coefficients of variation; and m is the mean.

6.5.5 *Confidence limits for precision terms.* Standard deviations and coefficients of variation 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 the Youden minimum criterion of *30 values (laboratories × materials), the standard deviation can be estimated to only about 25%. The standard deviation of the CV, where the CVs are expressed as a fraction, is:

$$= CV[(1 + 2(CV)^2)/2n]^{1/2},$$

where n is the number of values entering into the calculation of the CV. Approximately 200 values are required to estimate a CV = 10% to within 10% (relative).

6.6 Incorrect, Improper, or Illusory Values (False Positive and False Negative Values)

These 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 detectable) 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 CV increases, the percent of false negatives increases from an expected 2% at a CV = 50% to 17% at a CV = 100%, merely from normal distribution statistics alone.

When false positives and/or false negatives exceed about 10%, 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. When the distribution of zeros (not necessarily false negatives) becomes greater than approximately 30%, the distribution can become bimodal and even more uninterpretable (is the material positive or negative?).

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 data, including outliers. When replication is performed, the individual values, not averages, must be tabulated, unless the method requires averages (e.g., microbiological methods). Values not used for specified reasons (decomposition, failure to follow method, contamination, lost, etc.) should not be included in the table since they may be included erroneously in subsequent recalculations. The report should include the statistical parameters with and without specified outliers excluded. Both the standard deviations (and corresponding means) and the CVs should be reported. Tables should be proofread very carefully since many errors are of typographical origin. Names of the participants and their organizations should be given, if agreement has been obtained for their acknowledgment.

7.2 The final report should be published in an accessible publication or availability from the organization sponsoring the method should be indicated in the published method. Without public documentation, the usefulness of the method 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 from the original submission are submitted, they must be accompanied by an explanation.

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 third printing (1982) contains several explanatory footnotes.

8.2 *Handbook for AOAC Members* (1982) Available 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, and other national standards organizations.

8.5 Ibid, ISO 5725-1981.

Report of the Committee on Instrumental Methods and Data Handling

KENNETH R. HILL, *Chairman*
U.S. Department of Agriculture, Agricultural Research Service, Beltsville, Maryland 20705

Other members: W. Furman, W. J. Hurst, W. J. Morris, R. J. Gajan, R. Wayne, H. M. McNair, M. Walters, T. Gale, P. Jung, M. Margosis, L. Gelber, W. Y. Ja, W. Trujillo, G. MacEachern, P. Kane, L. Bailey, A. Conetta, P. Whittier, L. Hambleton, R. Issac, C. Gehrke, R. Sweeney, S. A. Barnett, R. Beine, L. Dusold, P. Caudill, W. Hyde

During the Association Year 1982–83, the Committee published a major report on liquid chromatography in the March 1983 issue of the *Journal*. The report, prepared by the Subcommittee on Gas and Liquid Chromatography and Column Specifications, covered general considerations and recommendations for liquid pumping systems, detectors, columns and column packings, and quantitation. The Committee also published two discussion articles in *The Referee*, "Automated Methods and Performance Data" by Jack Plimmer in the March 1983 issue and "Instrument Specification in Official Methods" by Peter Kane in the May 1983 issue.

The Committee regretfully accepted, with appreciation for service rendered, the resignations of J. Plimmer as Chairman, R. Greenhalgh as Vice-chairman, and B. Leonhardt as Secretary. New appointees are: K. R. Hill, Chairman; P. Kane, Vice-chairman; and G. Hanes, Rapporteur. The composition of the Subcommittees is undergoing revision and enlargement, and a new proposed membership list is in preparation.

The Committee organized a small symposium of 5 invited speakers and a panel discussion entitled, "Instrumental Methods and Data Handling," held in conjunction with the October 1983 Annual Meeting. The purpose of the symposium was to focus attention on the requirement of AOAC to change the style and format of published method descriptions from the use of specific brand names to the use of performance parameters, and to better acquaint General Referees and Associate Referees and other AOAC members with the structure, goals, and functions of the Committee.

The Committee met from 1–4:30 pm on October 5th, with 15 members and 3 guests in attendance. It was agreed to:

(1) Request that the AOAC staff send reprints or photocopies of the Report on Liquid Chromatography in the March 1983 issue of the Journal and the Report on Gas Chromatography published in 1973 to all General Referees on record.

(2) Seek approval and funding from the AOAC staff to purchase 35 reprints each of the ASTM Committee E-19 Standards on gas and liquid chromatographic nomenclature for the purpose of distribution to all members of the Committee.

(3) Proceed with the preparation of a chapter on Instrument Performance Specifications for a future edition of the AOAC *Methods Book*.

(4) Continue the present approach of providing guidelines and specific examples for writing method performance parameters to General Referees, Associate Referees, and section editors, rather than seek more rigid controls such as requesting that methods submitted for Official First Action be screened and/or approved by the Committee for compliance with proposed style and format.

(5) Designate Warren Bontoyan as the Committee Liaison with OIML with concurrent membership on the Subcommittee on Gas and Liquid Chromatography and Column Specification, subject to his acceptance.

In discussing proposed limits on years of service and the introduction of staggered terms of service, the Committee agreed that, due to the limited availability of required expertise willing to serve on the Subcommittees and the observed voluntary attrition of members, it request permission to continue appointing occasional new members or replacements as in the past and defer on the automatic turnover scheme at present.

The Committee took note of the Report of the Centennial Committee in the March 1983 issue of the *Journal* concerning the proposal that it suggest one of four major technical topics for the Fall 1984 meeting. A topic entitled "Advances in Analytical Instrumentation" will be proposed. It is hoped that this would result in papers on current developments in such techniques as MS-MS (and other hyphenated methods), microbore column liquid chromatography, electrochemical procedures and detectors, auto-correlation chromatography, critical phase chromatography and extraction, and inductively coupled plasma technology which will likely become part of future official or regulatory methods.

The Committee received and accepted the minutes of meetings of the Subcommittees held earlier in the week.

Report of the Automated Methods Subcommittee of the Committee on Instrumental Methods and Data Handling

PETER F. KANE, *Chairman*
Purdue University, Department of Biochemistry, West Lafayette, IN 47907

Other members: R. Isaac, A. Conetta, R. Sweeney, P. Whittier, L. Hambleton, S. Barnett. Also present, B. Furman

Relative to subcommittee membership rotation, the subcommittee does not favor any particular mechanism, but voiced the concern that turnover not be so frequent so as to disturb continuity.

The subcommittee discussed an interim automated method which was a particularly good example of how not to write automated methods. The need is clear: to reach people before the method gets to this stage. This can be done by more educational contact with Associate Referees and General Referees, and/or by a more official method screening role performed by the subcommittee.

Two methods written in performance terms, by B. Isaac and R. Sweeney, were discussed. An attempt was made to distill some general principles of their performance style of method writing, but with limited success. It was decided that more examples of such a style are needed before general principles can be established. What is needed at this point are more such methods to use as examples, and more exposure to Associate Referees and General Referees.

The Subcommittee wishes to point out that its area of responsibility needs to be more closely defined. The subcommittee does not wish to become too broad on the one hand, but on the other hand what should be done about instrumentation other than CFA and chromatographic?

Report of the Committee on International Cooperation

BEN BORSJE, *Chairman*
P.H. van Rynstraat 44, 3904 HJ Veenendaal, The Netherlands

Other members: P. Martin, R. Wood, D. Smith, R. Coleman, B. Larsen, D. Park, W. Horwitz, W. Bontoyan, R. Rund, R. Weik, B. Smith

The Committee, at its recent session, was pleased to welcome several members and guests from outside North America: R. Wood of the UK Ministry of Agriculture, Fisheries, and Food; R. Coleman of the UK Laboratory of the Government Chemist; P. Martin, representing the Association of Public Analysts of the UK; and L. F. Corominias of Fertimex, S.A., Mexico.

The Committee was pleased to confirm the appointment of B. Borsje as Chairman of the Committee. The former Chairman, B. L. Smith, was confirmed as Committee Secretary.

The Committee endorsed 2 motions put forward by the recent Joint Meeting of the Committee on International Cooperation and the Long Range Planning Committee. The first motion involved AOAC taking on a truly international status, and the second involved developing the necessary framework within the Constitution to permit the establishment of national and multinational divisions of the Association. These recommendations will be formally submitted to the Board of Directors.

The Committee also agreed to change the name of the Committee from "Committee on International Cooperation" to "International Coordination Committee." This recommendation will also be submitted to the Board.

Reports on the status of joint actions with other bodies indicated a continuing high level of cooperation. These international organizations included: International Standards Organization (ISO); Association of Public Analysts; Analytical Division of the Royal Society of Chemistry; Collaborative International Pesticide Analysis Commission (CIPAC); Food and Agriculture Organization (FAO); International Dairy Federation (IDF); Nordic Committee on Food Analysis (NMKL); and American Association of Cereal Chemists (AACC).

The Committee agreed, in principle, with adopting Category A Liaison status with ISO as an alternative mechanism to the current Technical Advisory Group structure through the American National Standards Institute. This change in status will also be submitted to the Board. The Committee also agreed that the current status for ISO/TC 134 should be maintained through the end of 1984.

The Chairman also appointed a small working group to deal with the subjects of committee appointments and travel support policy. These proposals should be available for the spring meeting of the Board of Directors.

The Committee received, with interest, the report of M. Wehr concerning his recent visit to the Far East. He emphasized that many opportunities are available for increased collaboration with scientists in Japan, Taiwan, and the Philippines.

Report of Intersociety Committee (ISC) on Methods of Air Sampling and Analysis

BERNARD E. SALTZMAN, *Representative*
University of Cincinnati, Kettering Laboratory, Cincinnati, OH 45267

During the past year, the committee held one meeting on June 21, 1983 in Atlanta, GA. This was its 20th anniversary, having been first funded in March 1963. Dr. Richard Thompson (representing ACS) took office as the new elected chairman for the next 3 years. G. Kupchik was reappointed as Executive Secretary. M. Katz, who had served as Editor since 1969, tendered his resignation because of illness, and a committee was appointed to seek a replacement. The representative of the Health Physics Society, L. Schwendiman, had passed away in March, and a replacement for him will be sought. The ISC currently has representatives from 8 societies (ACS, AIChE, AOAC, APCA, APWA, ASCE, ASME, ISA).

The Secretary reported that 1800 copies of the 2nd edition of the manual remained on hand, 3900 copies were sold, and 200 were given as complimentary or lost. APHA regards this as reasonably good sales for a laboratory manual. It was decided that the next edition will be published through a commercial publisher. D. McLean did not believe that AOAC could compete with a commercial offer.

The editor submitted 12 new methods for the 3rd edition of the manual. Some of the subcommittees have almost completed their review and revision of old methods, and preparation of new methods; others have been inactive. New or expanded general sections being considered deal with laboratory automation, data handling, quality control, sensory odor perception, ion chromatography, ICP spectroscopy, GCMS, neutron activation, and electron microscopy. General sections were completed on chemiluminescent analyzers, X-ray diffraction, X-ray fluorescence analyzers, and anodic stripping voltammetry. A target deadline for submission of manuscripts to the Editor was established at April 1, 1984. The approval procedure was streamlined to expedite publication of the 3rd edition.

Report of the Joint AOAC–AOCS–AACC–IUPAC Mycotoxin Committee

PETER M. SCOTT, *Chairman*
Health and Welfare Canada, Food Research Division, Health Protection Branch, Ottawa, Ontario K1A 0L2

Other members: J. W. Dickens, D. L. Park (AOAC); T. R. Romer, J. Routh, O. L. Shotwell (AACC); L. A. Goldblatt, R. D. Stubblefield, A. E. Waltking (AOCS); A. E. Pohland (IUPAC)

The Annual Joint Mycotoxin Committee Meeting was held on October 6, 1983, in Washington, DC. AOAC activities include several collaborative studies in progress or near completion on: (1) a negative chemical ionization MS procedure for confirmation of identity of aflatoxin B_1 (D. L. Park); (2) a TLC method for deoxynivalenol in wheat (R. M. Eppley); and (3) an LC method for zearalenone and α-zearalenol in corn (G. A. Bennett). Collaborative studies on aflatoxin M_1 in milk, ochratoxin A in meat, penicillic acid in grains, sterigmatocystin in cheese, and deoxynivalenol in grains, are expected to be undertaken in 1984. Reports from the AOCS Committee on Mycotoxins, AACC Mycotoxin Committee, and IUPAC Commission on Food Chemistry Mycotoxin Working Group were then presented. The latter group also is planning several collaborative studies, including one jointly with AOAC. The Terms of Reference of the Joint Mycotoxin Committee had recently been amended and were approved. Summaries were given of three major conferences on mycotoxins that took place in 1983: The International Conference on Mycotoxins in Cairo, Egypt, March 1983, followed by a workshop where much interest was expressed concerning control of mycotoxins; the highly successful Gordon Research Conference on Trichothecenes in Plymouth, NH, June 1983; and a Mycotoxin Symposium held in 1983 during the 3rd International Mycological Congress in Tokyo, Japan, that included papers on patterns of mycotoxin production by *Fusarium* from varying geographical locations and by *Penicillium*, confirmation of carcinogenicity of ochratoxin A in mice, a study on the carcinogenicity of aflatoxin M_1 relative to B_1, and the involvement of *Candida albicans* in the etiology of human arteritis (Kawasaki disease).

Other topics brought to the attention of the Committee included a study on the mutagenic potential of aflatoxin-related decontamination by-products resulting from ammoniation of cottonseed meal (D. L. Park); a study on the aflatoxin B_1 to M_1 conversion ratio in dairy cattle carried out in Arizona; epidemiological data on primary liver cell cancer and aflatoxin exposure in the U.S. (L. Stoloff); a new EEC directive on aflatoxin in dairy feed and feed ingredients; observations by T. R. Romer on unknown mycotoxins in animal feeds; surveys by FDA (M. Trucksess) of corn, corn meal, flour, bread, and snack foods for deoxynivalenol; correlation of deoxynivalenol contamination in soft, red winter wheat and grading factors (O. L. Shotwell); and establishment of new guidelines by Health and Welfare Canada for deoxynivalenol in soft winter wheat to be used in adult nonstaple foods and infant foods. A new journal *Food Additives and Contaminants*, published in England, was announced.

The following meetings in 1984 will include papers on mycotoxins: Annual Meeting FASEB, St. Louis, MO, April 1–6, 1984; AOAC Spring Training Workshop, Philadelphia, PA, April 29–May 2, 1984; AOCS Annual Meeting, Dallas, TX, April 29–May 3, 1984; AOAC Midwest Regional Meeting, Minneapolis, MN, June 11–13, 1984; Sixth International Biodeterioration Symposium, Washington, DC, August 5–10, 1984; Amer. Phytopathol. Soc. Annual Meeting, Guelph, Ontario, August 12–16, 1984; and the AOAC Annual International Meeting, Washington, DC, October 28–November 2, 1984. For 1985, a Gordon Conference covering certain aspects of mycotoxins and the next IUPAC Symposium on Mycotoxins and Phycotoxins, to be held in Pretoria, South Africa, are in the planning stages.

Report of the Committee on Laboratory Quality Assurance

KEITH A. McCULLY, *Chairman*
Health and Welfare Canada, Field Operations Directorate, Tunney's Pasture, Ottawa, Ontario, Canada K1A 1B7

Other Members: R. Alvarez, J. Ault, A. S. Y. Chau, R. D. Fishbeck, F. M. Garfield, E. E. Martin, J. E. McNeal, S. Sherken, K. Smith

The Committee met October 4, 1983, in Washington, DC. F. M. Garfield reported to the Committee on the status of the *Quality Assurance Handbook*. The draft manuscript received very positive reviews from several members of the Committee and the AOAC Editorial Board, as well as outside reviewers. The reviewers' comments have been incorporated and the manuscript is now undergoing editorial review with publication expected early in 1984.

Last year the Committee recommended that AOAC assume a leadership role in analytical laboratory quality assurance. As one of the ways to achieve this role and as an extension to the *Quality Assurance Handbook*, a proposal has been made to the Board of Directors to develop and conduct a short course on Analytical Laboratory Quality Assurance Plan Development. A draft course outline prepared by F. M. Garfield was discussed in detail at the meeting. The following major headings were recommended for inclusion in the course: Quality Assurance Planning and Management, Statistics, Sampling, Role of Personnel Management in Quality Assurance, Preventive Maintenance, Sample Analysis, Analytical Charting, Records and Reporting, Proficiency Testing, and Audit Procedures. Items to be included under these headings were also discussed. In addition, sessions for general discussion and review as well as a course evaluation are to be included. This course should be dynamic and involve the participants, rather than strictly a lecture course. It is recommended that the first short course be directed toward management (i.e., managers, supervisors, quality assurance staff) and not to actual hands-on analysts. The course emphasis is to be on how the manager can assure that a quality product (analytical data) is being produced in the laboratory. A two-day course is recommended and suggestions were made as to the type of instructors required. Instructors should be guided very closely as to content of their presentations. Following experience with this short course for managers, one intended for the bench analyst could be developed.

The committee reviewed topics proposed for the Symposium on Quality Assurance to be held at the 98th AOAC Annual International Meeting in 1984. The committee believes that the proposed topic areas are too general and the subject matter is too similar to that in the *Quality Assurance Handbook* and that presented at the 1980 Symposium on Optimizing Chemical Laboratory Performance Through the Application of Quality Assurance Principles. It is recommended that AOAC assume a leadership role in quality assurance activities and organize the symposium to permit various national and international organizations involved in quality assurance to present their philosophies and programs. A one-day symposium finishing with a round table discussion is suggested.

Report of the Long-Range Planning Committee

H. MICHAEL WEHR, *Chairman*
Oregon Department of Agriculture, Salem, OR 97310

Other members: C. Andres, F. Baur, G. Boone, R. Case, W. Cobb, R. Ellis, F. Garfield, W. Kadis, J. Minyard, A. Munson, H. Reynolds

The Committee met three times during the past year: March 3–4, 1983; June 28–29, 1983 and October 3 and 5, 1983.

The Long Range Planning Committee provides recommendations to the Board of Directors relating to future directions of the Association. During the past year the committee has focused on three areas: Regional/International meeting structure; international activities; and scientific priorities. In addition, the committee also prepared recommendations relating to public sustaining membership benefits.

Regional/International Meeting Structure

The establishment of regional sections, potentially, can have impact on the current meeting structure of the Association. No guidelines currently exist regarding permitted meeting formats of regional sections. Regional meeting formats can and have assumed a format similar to the Spring Training Workshop.

In conjunction with representatives from the 3 established regional sections, the Long Range Planning Committee posed and discussed the following questions:

(1) What should the meeting structure of AOAC be?

(2) What is the relationship between the Spring Training Workshop and regional/international meetings and, further, between the Annual International Meeting and other meetings of the Association?

(3) Should guidelines be set for regional and/or international meetings?

The committee and regional section representatives recommended the following:

(1) At the present time the Spring Training Workshop should be retained. The primary meetings of AOAC will thus continue to be the Annual International Meeting and the Spring Training Workshop.

(2) The structure of the meetings should be flexible and responsive to needs. The structure of the Annual International Meeting with its increasing emphasis on poster sessions and symposia appears to satisfy current needs. Although the Spring Training Workshop is generally satisfactory, it would be preferable for the meeting to return more towards an actual workshop approach.

(3) Meeting structure of regional sections should not be prescribed but should rather be the prerogative of the section. However, it was felt that regional sections should be careful to avoid structuring a meeting that would impair the success of the Spring Training Workshop or the Annual International Meeting.

(4) Consideration should be given to holding the Spring Training Workshop in those areas where there is no regional sections, but where a base for a potential section exists.

(5) Regional sections should also be encouraged to host the Spring Training Workshop.

International Activities

The changing and increasing role of AOAC as an international organization has focused the need for AOAC to review its policies and programs in this most important area. Jointly, with the Association's Committee on International Cooperation, the Long Range Planning Committee reviewed several areas including:

(1) The role of AOAC in international activities.

(2) The relationship of AOAC to other methods and/or standards organizations.

(3) Appropriate ways to work with "developing" and "developed" countries.

(4) The nature of regional sections, worldwide.

Recommendations were made as follows:

(1) AOAC should become a truly international organization without excessive focus on any one part of the world. As a corollary, AOAC should enhance participation of the international community in all phases of its activities (collaborative studies, committee activities, etc.)

(2) AOAC international efforts should reflect its strengths, especially the methods development and validation area. Related activities such as training and laboratory accreditation are also very appropriate.

(3) AOAC international activities should augment, not duplicate, activities of other organizations. This is especially true in areas such as education, where numerous other organizations provide services.

(4) AOAC, specifically the Committee on International Cooperation, should identify countries with potential for AOAC involvement (either in the collaborative study area or in related areas such as training, laboratory accreditation, etc.), inventory specific involvement, and prioritize foreign involvement.

(5) AOAC should work towards obtaining an international identity with other methods and standards organizations. Specific consideration should be given to changing to an "0" status with the International Standards Organization.

(6) AOAC should utilize differing approaches with different countries, depending upon the degree of development. Emphasis can be as follows:

(a) *For developed countries.*—Participation in a high technology information exchange; Increase emphasis in collaborative study participation; Expansion of cooperative agreements with methods organizations; Involvement with laboratory accreditation.

(b) *For developing countries.*—Participation in training programs either through direct sponsorship or through contract and/or cooperative agreements with other organizations offering needed courses; Encouragement to participate in collaborative studies where expertise exists or is developed through training; Participation in laboratory accreditation as appropriate; Assistance in providing information on methodology or related needs.

(7) AOAC should establish a divisional structure within AOAC to represent national or multinational geographic areas. Examples of such divisions would be AOAC Europe and AOAC North America. Regional sections would then be geographic subdivisions of such divisions.

Science Priorities

Emerging scientific fields and the changing importance of established analytical needs necessitate a periodic review to assure proper perspective on scientific priorities, both in regards to analytical, as well as nonanalytical areas. Utilizing staff assistance, the committee prepared the following recommendations for science priorities.

Analytical Areas:

(1) Continue emphasis on the following fields: human food, including meat and nonalcoholic beverages, pesticide formulations and residues, feed, and fertilizer.

(2) Continue existing effort in the following fields: pharmaceuticals, illegal drugs, and wastewater.

(3) Increase activity in the following fields: food microbiology, cosmetics, veterinary toxicology, chemistry, drug and device related microbiology, in vitro mutagenicity tests, industrial waste, medical device materials, and disinfectant analysis.

(4) Put no effort into, but do not disregard entirely: forensic sciences, water/wastewater, air analysis, waste analysis, drinking water analysis, and ground water analysis.

(5) Put no effort into: clinical microbiology—human and veterinary, medical devices and/or tests, human and veterinary biologics, forensics toxicology, small animal toxicology.

Nonanalytical areas:

(1) Continue development and implementation of the international laboratory accreditation program.

(2) Strongly consider involvement in the analytical training area, especially in relationship to international programs and laboratory accreditation. AOAC should not, however, duplicate training services provided by other organizations.

Membership review

On a final item remaining from a comprehensive review of the membership area undertaken during 1981–82, the committee recommended establishment of the following benefits for public sustaining members. Public sustaining members are defined as "states, provinces, municipalities, small national agencies and large national agencies." Increasing benefits were recommended for increasing levels of support as follows:

Below $500—One individual membership; technical assistance; certificate of membership; listing of support in the *Referee,* JAOAC, and annual meeting program; annual report.

$500–$999—Two individual memberships; March issue of JAOAC, other benefits as listed above.

$1000–$2499—Two individual memberships, subscription to JAOAC, other benefits as above.

$2500–$4999—Three individual memberships, 2 subscriptions to JAOAC, other benefits as above.

Over $5000—Benefits to be negotiated with sponsor.

Report of the Committee on Safety

EDWARD H. LOSIEWICZ, *Chairman*
Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857

Other attendees: B. Johnson, R. Hall, R. Bianchi, A. Hofberg, W. Solomon, G. Roach

The Committee met on October 5, 1983, and reviewed and updated its responsibilities by combining the section on the size of the committee with the membership composition. The committee recommends that the chairperson be appointed for not more than 2 consecutive one-year terms; that a member serve for at least one year before being appointed chairperson, and that the committee consist of 9 members representing the various disciplines and organizations (government, industry, and academia) affiliated with AOAC.

The committee proposed for the coming year, 1983–84, to organize a Safety Symposium on Hazardous Chemicals to be presented during the 1984 Annual Meeting and to survey the membership of AOAC on the usage of Chapter 51 in the *Official Methods of Analysis* book.

The committee recommended that **51.043** be deleted from the Book of Methods.

Report of the Committee on State and Provincial Participation

HERSCHEL F. MORRIS, JR, *Chairman*
Louisiana Department of Agriculture, University Station, Box 16390-A, Baton Rouge, LA 70893

Other members: H. B. Bradford, P. C. Brignac, J. Counts, J. Donovan, P. B. Ferrara, A. Gardner, W. Hines, T. L. Jensen, J. Kapish, S. E. Katz, D. Lewis, D. McDaniel, J. Padmore, P. R. Rexroad, M. Rhodes, M. L. Schreiber, R. Speth, V. Thorpe, G. Tischelaar, L. Torma

The Committee on State and Provincial Participation met on October 4, 1983. The following topics were discussed:

(1) Chairman Herschel F. Morris reviewed the primary responsibilities of the committee. They are as follows:

(a) To identify and contact potential AOAC members and sustaining member organizations and make them known to the International Office and AOAC field representatives.

(b) To make recommendations to the AOAC International Office and Board of Directors concerning policies which are of concern to members.

(2) Discussion ensued on the acronym PSM, which stands for public sustaining member, as well as for private sustaining member. It was decided to recommend to the Board of Directors that they change the term "public sustaining member" to "governmental sustaining member" for clarification and to avoid confusion when referring to these terms in the same document.

(3) One committee member suggested that AOAC could be better promoted at the regional section level if the International Office could send information (publications, etc.) and a representative to such meetings.

(4) It was also suggested that a mechanism be established to facilitate the introduction and orientation of first-time attendees to overall AOAC operations and to the Annual International Meeting. This mechanism should allow these individuals to contact and meet with other scientists who share a common interest. Two suggestions were made to implement this mechanism:

(a) Provide a place on the registration form for indicating that one is a first-time attendee at a meeting.

(b) Subdivide the message board into specific subject sections.

(5) An additional concern was expressed by some Canadian representatives present at the meeting that provincial agencies be invoiced for their sustaining memberships on April 1 to coincide with their fiscal year. The committee so recommends.

(6) It was also suggested that the International Office contact sustaining member organizations prior to invoicing to ascertain if the amount of the invoice should (or could) be increased over the amount of the prior year. This could serve as an additional source of income for the Association. The committee also recommends that this action be taken.

(7) At the request of the Executive Director, D. MacLean, the committee also discussed the equalization of the benefits of private and governmental sustaining members.

(8) After lengthy discussion, the following motion was made and adopted:

The Committee on State and Provincial Participation recommends to the Board of Directors that they form a joint subcommittee with representatives from the Long Range Planning Committee, the International Coordination Committee, the Committee on State and Provincial Participation, and industry to formulate a new set of benefits for sustaining members, and that the subscription to the *Journal* be discontinued as a benefit for such membership.

(9) The two representatives from the State and Provincial Participation Committee for the Subcommittee have been named. They are: T. Jensen (Nebraska) and G. Tischelaar (California).

Report of the Committee on Symposia and Special Programs

WARREN R. BONTOYAN, *Chairman*
Environmental Protection Agency, Office of Pesticide Programs, Beltsville, MD 20705

Other members: F. J. Carleton, D. E. Coffin, C. C. Gehrke, L. Hambleton, N. Hardin, A. J. Malanoski, B. McMahon, H. Morris, A. Pohland, D. Stalling

The Committee on Symposia and Special Programs has approved a tentative 1984 symposia program and schedule. The symposium topics approved are: Education in Modern Analytical Chemistry; Quality Assurance/Quality Control for Analytical Laboratories; Environmental Toxicology and Chemical Analysis; Chromatographic Methods in Drug Analysis of Body Fluids; Sampling and Analysis of Fertilizers; Physical-Chemical Methods for Determining Antibiotic Residues in Tissues and Milk of Food-Producing Animals; Analysis of Pesticide Products for Impurities and Degradation Materials; and Safe Handling, Storage, and Disposal of Hazardous and Toxic Laboratory Chemicals.

In addition to these formal symposium topics, there is the possibility of an evening program sponsored by the industry exhibitors. However, it is too early to comment on the specific form this program will take.

The Committee suggested that invitations be extended to appropriate and prominent people for principal centennial speakers. Invitations were sent to Dr. Linus Pauling and Mr. Peter Barton Hutt, and both have accepted. Suggestions from the AOAC membership and staff regarding invitations to other prominent people who may be appropriate to address the 1984 Centennial Meeting are welcomed for consideration by the Committee.

President Bontoyan and President-Elect Gehrke, in conjunction with the staff and the Committee, are in the process of planning a 1984 Banquet Program.

Report of the Twenty Seventh Annual Meeting of the Collaborative International Pesticides Analytical Council (CIPAC)

WARREN R. BONTOYAN, *U.S. Member of CIPAC and AOAC Representative*
Environmental Protection Agency, Office of Pesticides Programs, Beltsville, MD 20705

The 27th meeting of the Collaborative International Pesticides Analytical Council (CIPAC) was held in Brisbane, Australia from July 9 to July 14, 1983. Preceding the CIPAC Expert Witness and Technical Sessions were the Annual Symposium and the Informal FAO (Food Agricultural Organization) meeting. The FAO meeting was held from July 5 to July 7, and the CIPAC Symposium was held on July 8, 1983.

J. Lovett resigned as the United Kingdom (UK) member of CIPAC following his retirement from the UK government service. The CIPAC members elected M. S. Bailey of the UK Ministry of Agriculture, Fisheries, and Food as the United Kingdom member.

W. R. Bontoyan the U.S. member of CIPAC and President of AOAC, at the invitation of the Royal Australian Chemical Institute, gave a presentation on the purpose and organization of AOAC to the Queensland and Northern Territory Branch. President Bontoyan also made contacts with Australian scientists who expressed interest in establishing working relationships as either AOAC collaborators or associate referees.

CIPAC formerly accepted an AOAC invitation to hold its 28th meeting in the U.S. This meeting will be held the week prior to the AOAC Centennial Meeting.

The official CIPAC organization is:

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USDA	J. Plimmer
Food and Agriculture	A. V. Adam

Organization (FAO) — AOAC — W. Bontoyan

World Health Organization (WHO) — G. Quelennec — Oregon Department of Agriculture — J. Launer

Summary of the decisions taken at the 27th meeting of the Technical Committee of CIPAC in Brisbane, 12th and 13th July 1983. Seven AOAC methods for pesticide formulation analysis were adopted by CIPAC.

Code Number	*Chemical*	*Status of the Method*
1	2,4-D	The AOAC HPLC method (6.B09–16.B122) was accepted as the referee method.
2	MCPA	The GLC method, CIPAC/3030 was adopted as *provisional* CIPAC method provided that no objections will be received 3 months after circulation of report CIPAC/3016/R. The HPLC method will be the referee method.
4	HCH	The GLC method presented at the Symposium was accepted as *full* WHO-CIPAC method.
4.a.	lindane	The GLC method for the determination of lindane in solutions presented at the Symposium was accepted as *full* WHO-CIPAC method.
6	2,4,5-T	The GLC method, CIPAC/3030, was adopted as *provisional* CIPAC method provided that no objections were received 3 months after circulation of report CIPAC/3022/R. The HPLC will be the referee method.
32. + 33	pyrethrins + piperonyl-butoxide	The provisional AOAC method 6.C22–6.C25 was adopted as *full* AOAC-CIPAC method (referee method).
35	fenitrothion	The GLC method for fenitrothion technical and formulations, presented at the symposium was adopted as *provisional* WHO-CIPAC method.
38	rotenone	The AOAC HPLC method for rotenone formulations 6.D05–6.D08 was adopted as *provisional* AOAC–CIPAC method.
49	2,3,6-TBA	The provisional CIPAC method, CIPAC/2723 app. A, 7 and 8 was adopted as *full* CIPAC method.
49. + 2	2,3,6-TBA + MCPA	The provisional CIPAC method CIPAC/2723 app. A, 7 and 8, was adopted as *full* CIPAC method.
51	mecoprop	The HPLC method, for mecoprop technical and aqueous salts and solutions, CIPAC/3111/M, was adopted as *full* CIPAC method (referee method).
63. + 43	propham & chlorpropham	The GLC method for the determination of propham and chlorpropham in sprout inhibiting dusts, CIPAC/3094/(M), was adopted as *provisional* CIPAC method.
74	dichlofluanid	The HPLC method for dichlofluanid technical concentrates and formulations, CIPAC/3129/(M) was adopted as *provisional* CIPAC method.
77	phenmedipham	The provisional HPLC and titrimetric methods for phenmedipham technical and emulsifiable concentrates, CIPAC/3064/M, were adopted as *full* CIPAC methods (HPLC referee method).
78	Quintozene	The provisional AOAC GLC method 6.C08–6.C11 was adopted as *full* AOAC-CIPAC method. The last sentence of the method is to be deleted.
84	dichlorprop	The HPLC method for dichlorprop technical and aqueous salts and solutions, CIPAC/3112/M, was adopted as *full* CIPAC method (referee method).
172	chinomethionat	The provisional HPLC method, CIPAC/3040/M, was adopted as *full* CIPAC method.
230	cyanazine	The HPLC method for cyanazine technical and formulations, CIPAC/3089/M, was adopted as *full* CIPAC method.
263	carbendazim	Of the UV and HPLC methods, CIPAC-P81, p. 144–151, the HPLC was selected as the referee method.
284	glyphosate	The AOAC HPLC method for glyphosate technical and formulations, 6.D22–6.D27, was adopted as *provisional* CIPAC method.
303	fensulfothion	The AOAC HPLC method for fensulfothion formulations, 6.D28–6.D32, was adopted as *provisional* AOAC-CIPAC method.
350	pyrazophos	The HPLC method for pyrazophos technical and formulations, CIPAC/3127/(M), was adopted as *provisional* CIPAC method.
352	triadimefon	The HPLC method for triadimefon technical and formulations, CIPAC/3118/(M), was adopted as *provisional* CIPAC method.
358	diclofop-methyl	The provisional method, CIPAC/3044/M, was adopted as *full* CIPAC method.
370	brodifacoum	The AOAC HPLC method for brodifacoum technical and formulations, 6.D01–6.D04 was adopted as *provisional* AOAC-CIPAC method.
Imp. 1	TCDD	The GC-MS method for the determination of 2, 3, 7, 8-TCDD in 2, 4, 5-T acid and esters, CIPAC/3128/(M) was adopted as *provisional* CIPAC method.

230. pp	cyanazine	The method for the preparation of pure cyanazine, CIPAC/3091/M, was adopted as full CIPAC method.
MT-	Spontaneity of sus-suspension	The method for the determination if the spontaneity of suspension of suspension concentrate formulations, CIPAC/3088 app. M, was adopted as *full* CIPAC method.
MT-	Suspensibility	The method for the determination of suspensibility of suspension concentrates, CIPAC/3087, app. E, was adopted as *full* CIPAC method.

Report of the Ways and Means Committee

STANLEY E. KATZ *Chairman*
Rutgers University—Cook College, New Brunswick, NJ 08903

Other members: R. Blinn (deceased), J. Bourke, W. P. Cochrane, C. Gehrke, J. Goleb, M. Malina, L. Perlman, W. Phillips, M. Ready

The committee reviewed the progress and the approach being taken in the solicitation of funds to endow the Wiley Award Fund and support the centennial activities. The committee supported the strategy of soliciting a select (small number) group of foundations and philanthropic funds to endow the Wiley Award Fund.

To aid in the solicitation, a standardized letter was developed as well as the brochure "AOAC—The Next 100 Years." This letter is being and will be sent to foundations, nationally and internationally, with follow up communications from AOAC personnel as well as committee members.

It is the desire of the committee to have the fund raising completed by July 1, 1984, with the realization that fund raising may proceed until October, 1984.

It is the recommendation of the committee that the brochure "AOAC The Next 100 Years" be given wide distribution and be used as a vehicle of advertising the nature and function of AOAC.

In 1984, the Association of Official Analytical Chemists will celebrate its centennial. As AOAC begins its second century of activity, the Association will be rededicating its efforts to the validation and standardization of state-of-the-art analytical methodology in an atmosphere of equal participation by government, industry, and academia. As part of the centennial celebration, it is the intent of the Association to endow the Harvey W. Wiley Award and the educational programs that accompany it in the total amount of $300,000.00.

Every year since 1957, the prestigious Harvey W. Wiley Award has been given to an outstanding scientist for his or her contribution to methodology in an area of interest to AOAC. Using the research areas of awardees as focal points, the objectives of the Wiley Centennial Fund will be:

(1) To continue to honor outstanding analytical scientists for their contributions to analytical methodology.

(2) To foster communication among scientists in areas of mutual interest, need, and importance, by sponsoring symposia on pertinent and timely subjects, and bringing together outstanding analytical scientists from all sectors to participate in these symposia.

(3) To provide scholarship aid to outstanding students.

(4) To provide for continuing training of all scientists through sponsorship of training programs.

The Association is soliciting endowment monies from a few prestigious organizations to support the centennial effort and the philosophy that government and industry as equal partners in the scientific endeavor of analytical standardization is good science. In addition to acknowledgment of their reputations, contributors will accrue far-reaching benefits from the increased cooperation between the private and public sectors, as well as minimizing costs which comes about when scientists in all areas attack mutual problems and share the results.

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D-19: Water: Theodore O. Meiggs (Environmental Protection Agency, National Field Investigations Center, Office of Enforcement, Denver, CO 80225)

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Federation of Oils, Seeds, and Fats Association, Ltd.: David Firestone (Food and Drug Administration, Washington, DC 20204)

Flavor and Extract Manufacturers Association of the U.S.: Roger Middlekauf (900 17th St, NW, Washington, DC 20006)

Health and Welfare Canada: D. Earle Coffin (Health and Welfare Canada, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2)

Institute of Food Technologists: Michael Wehr (State Department of Agriculture, 635 Capital St, NE, Salem, OR 93710)

International Association for Cereal Chemistry: Helmet Glattes (International Association for Cereal Chemistry, Schmidgasse 3-7, A-2320 Schwechat, Austria)

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Office International du Cacao et du Chocolat: Emile Toebosch (OICC, 172 Ave de Cortenberg, B1040, Brussels, Belgium)

Intersociety Committee on Manual of Methods for Air Sampling and Analysis: Bernard E. Saltzman (Kettering Laboratory, University of Cincinnati, Eden and Bethesda Aves, Cincinnati, OH 45267)

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Gas Chromatography Panel: Warren R. Bontoyan

Joint Dimethoate Residues Panel: Robert W. Storherr (Environmental Protection Agency, Beltsville, MD 20705)

Monuron and Diuron Panel: Howard Hammond (State Laboratories Department, North Dakota State Department of Agriculture, Bismarck, ND 58505)

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United States Pharmacopeial Convention: James B. Kottemann (Food and Drug Administration, Washington, DC 20204), *Delegate*

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Amino Acid Analysis in Mixed Feeds
Wayne Stockland, Supersweet Research Farm, Box 117, Courtland, MN 56021

Fat, Crude, in Pet Foods

Fiber, Crude
David O. Holst, University of Missouri, Food Science and Nutrition, Columbia, MO 65211

Fiber, Crude, in Milk Replacers
J. G. Pierce, Pierce Consulting Service, 713 NW Westwood, Ankeny, IA 50021

Infrared Reflectance Techniques in Mixed Feeds

Iodine
Stuart Meridian, West Agro-Chemical, Inc., PO Box 1386, Shawnee Mission, KS 66222

Minerals

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FERTILIZERS

Referee: Robert C. Rund, Purdue University, Department of Biochemistry, West Lafayette, IN 47907

Biuret in Urea and Mixed Fertilizers
Luis F. Corominas, Fertilizantes Mexicanos SA, Piso 06760, Mexico 7 DF, 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, Oregon Department of Agriculture, Laboratory Services Division, Salem, OR 97310

Nitrogen
Paul R. Rexroad, University of Missouri, Experiment Station Chemical Laboratories, Columbia, MO 65201

Phosphorus
Frank J. Johnson, Tennessee Valley Authority, National Fertilizer Development Center, Muscle Shoals, AL 35660

Potash
Peter F. Kane, Purdue University, Department of Biochemistry, West Lafayette, IN 47907

Sampling and Preparation of Sample
Douglas Caine, Estech General Chemicals Corp., 30 N LaSalle St, Chicago, IL 60602

Slow-release Mixed Fertilizers
Stanley E. Katz, Rutgers University, Cook College, Department of Biochemistry and Microbiology, New Brunswick, NJ 08903

Sodium
Luis F. Corominas

Soil and Plant Amendment Ingredients
Clyde E. Jones, Colorado Department of Agriculture, 2331 W 31st Ave, Denver, CO 80211

Sulfur
Virginia A. Thorpe, Michigan Department of Agriculture, Laboratory Division, 1615 S Harrison Rd, East Lansing, MI 48823

Water-Soluble Methylene Ureas
Allan Davidson, OM Scott & Sons, Co., Marysville, OH 43041

Zinc
Dennis G. Jurgens, Department of Agriculture, Laboratory Division, 3703 S 14th St, Lincoln, NE 68502

HAZARDOUS SUBSTANCES

Referee: Dean Hill, Environmental Protection Agency, NEIC, Box 27225, Denver Federal Center, Denver, CO 80225

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

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, Florijrnuwe 111, 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

PESTICIDE FORMULATIONS: CARBAMATE AND SUBSTITUTED UREA INSECTICIDES

Referee: Paul Jung, Environmental Protection Agency, Chemical Laboratory, Beltsville, MD 20705

Aldicarb
William H. McDermott, Union Carbide Corp., Agricultural Products Divisior, Box 12014, Research Triangle Park, NC 27709

Aminocarb
Steven C. Slahck Mobay Chemicals Corp., Agricultural Chemicals Division, Box 4913, Kansas City, MO 64120

Carbaryl
William H. McDermott

Carbofuran and Carbosulfan
E. J. Kikta, FMC Corp., Niagara Chemical Division, 100 Niagara St, Middleport, NY 14105

2,2-Dimethyl-1,3-benzodioxol-4-yl Methylcarbamate (Bendiocarb®)
Peter L. Carter, FBC, Ltd., Agrochemical Division, Hauxton, Cambridge, CB2 5HU, UK

o-Isopropoxyphenyl Methylcarbamate (Propoxur®)
Steven C. Slahck

4-(Methylthio)-3,5-xylyl Methylcarbamate (Methiocarb)
Steven C. Slahck

Methomyl
James E. Conaway, Jr, E. I. du Pont de Nemours & Co., Biochemicals Division, Wilmington, DE 19898

Mexacarbate
William H. McDermott

Oxamyl
Glenn A. Sherwood, Jr, E. I. du Pont de Nemours & Co., Biochemicals Department, Experiment Station, Wilmington, DE 19898

Pirimicarb
Peter D. Bland, ICI Americas, Inc., Biological Research Center, Box 208, Goldsboro, NC 27530

Trimethylphenyl Carbamate Isomers
William H. McDermott

PESTICIDE FORMULATIONS: FUNGICIDES AND DISINFECTANTS

Referee: Thomas Jensen, State Department of Agriculture, 3703 S 14th St, Lincoln, NE 68502

Bayleton

Benomyl

Carboxin and Oxycarboxin
Milton Parkins, Sr, Uniroyal Chemical Co., Crop Protection Analytical Division, Naugatuck, CT 06770

Chlorothalonil
Brian H. Korsch, SDS Biotech, PO Box 348, Painesville, OH 44079

Copper Naphthenate

Dinocap

Dithiocarbamate Fungicides

Pentachloronitrobenzene

o-Phenylphenol

Triphenyltin

PESTICIDE FORMULATIONS: GENERAL METHODS

Referee: Paul D. Jung, Environmental Protection Agency, Office of Pesticide Programs, Beltsville, MD 20705

Dioxins (2,3,7,8-Tetrachlorodibenzo-*p*-dioxin in 2,4,5-T)

Physical Properties of Pesticides

Sampling

Water-Soluble Copper in Water-Insoluble Copper Fungicides

PESTICIDE FORMULATIONS: ORGANOHALOGEN INSECTICIDES

Referee: James Launer, State Department of Agriculture, Laboratory Services, 635 Capitol St, NE, Salem, OR 97310

Benzene Hexachloride and Lindane
James W. Miles, 1228 Bacon Park Dr, Savannah, GA 31406

Chlordane
John E. Forrette, Velsicol Chemical Corp., 341 E Ohio St, Chicago, IL 60611

Chlordimeform
Arthur H. Hofberg, Ciba-Geigy Corp., Analytical Chemistry Division, 410 Swing Rd, Greensboro, NC 27409

Dicofol
Alan M. Rothman, Rohm and Haas Co., Research Laboratories, 727 Norristown Rd, Spring House, PA 19477

Diflubenzuron
A. A. De Reyke, Duphar BV, PO Box 2, 1380 AA Weesp, The Netherlands

Endosulfan
Robert W. Watson, FMC Corp., Agricultural Chemical Division, 2501 Sunland Ave, Fresno, CA 93717

Fenvalerate
R. D. Collins, Shell Development Co., PO Box 4248, Modesto, CA 95352

Heptachlor
John E. Forrette

Methoxychlor

Perthane
Michael Sabbann, Department of Agriculture, Division of Laboratory Services, St Paul, MN 55107

Toxaphene
William H. Clark, Hercules, Inc., Analytical Division, Research Center, Wilmington, DE 19898

Trichlorfon (Dylox®)
Michael Sabbann

PESTICIDE FORMULATIONS: HERBICIDES I

Referee: Marshall Gentry, Florida Department of Agriculture and Consumer Services, Mayo Building, Tallahassee, FL 32301

Chlorophenoxy Herbicides
Robert B. Grorud, North Dakota State Laboratories, Lock Box 937, Bismarck, ND 58501

Dicamba
John Forrette, Velsicol Chemical Corp., 341 E Ohio St, Chicago, IL 60611

Pentachlorophenol
Elmer H. Hayes, Environmental Protection Agency, Chemistry Laboratory, Beltsville, MD 20705

Plant Growth Regulators

2,3,6-Trichlorobenzoic Acid

PESTICIDE FORMULATIONS: HERBICIDES II

Referee: Laszlo Torma, State Department of Agriculture, Montana State University, Bozeman, MT 59715

Alanap
Milton Parkins, Uniroyal Chemical, Crop Protection Chemical Branch, Naugatuck, CT 06770

Barban
John Forrette, Velsicol Chemical Co., 341 E Ohio St, Chicago, IL 60611

Bensulide
William Y. Ja, Stauffer Chemical Co., Richmond Research Center, 1200 S 47th St, Richmond, CA 94804

Benzoylprop-Ethyl

Bromacil and Lenacil
Paul K. Tseng, E. I. du Pont de Nemours & Co., Biochemicals Dept., Wilmington, DE 19898

Dimethyl Tetrachloroterephthalate
Brian Korsch, SDS Biotech, PO Box 348, Painesville, OH 44077

Dinoseb

Diuron
Glenn A. Sherwood, E. I. Dupont de Nemours, Biochemicals Department, Wilmington, DE 19898

***S*-Ethyl Dipropylthiocarbamate**

Fluchloralin, Profluralin, Benefin, Trifluralin, and Penoxalin
Roger Stringham, Purdue University, Department of Biochemistry, West Lafayette, IN 47907

Fluometuron
Arthur H. Hofberg

Linuron
Glenn A. Sherwood

Methazole
John Forrette

Monuron

Oryzalin

Paraquat, HPLC Analysis
Lynn Hageman, Montana Dept. of Agriculture, Montana State University, Bozeman, MT 59717

Siduron
Glenn A. Sherwood

Thiocarbamate Herbicides
William Y. Ja

PESTICIDE FORMULATIONS: HERBICIDES III

Referee: Thomas L. Jensen, State Department of Agriculture, 3703 S 14th St, Lincoln, NE 68502

Alachlor, Butachlor, and Propachlor
David F. Tomkins, Monsanto Agricultural Products Co., 800 N Lindburgh Blvd, St. Louis, MO 63166

Amitrol

Bentazone
Thomas M. Schmitt, BASF Wyandotte Corp., 1609 Biddle Ave, Wyandotte, MI 48192

Bromoxynil
Laurence J. Helfant, UCAPCO Inc., PO Box 12014, Research Triangle Park, NC 27709

Cacodylic Acid

Cyanazine (Bladex®)
Ronald D. Collins, Shell Development Co., PO Box 4248, Modesto, CA 95352

Dalapon
Timothy S. Stevens, Dow Chemical Co., Analytical Laboratories, Midland, MI 48640

Dichlobenil

Disodium Methane Arsenate

Fluazifop-butyl
Peter D. Bland, ICI Americas, Inc., Biological Research Center, Box 208, Goldsboro, NC 27530

Glyphosate (Isopropylamine Salt *N*-(Phosphoromethyl) Glycine)
Arnold J. Burns, Monsanto Agricultural Products Co., PO Box 174, Luling, LA 70070

Metolachlor
Arthur H. Hofberg, Ciba-Geigy Corp., Analytical Section, Box 18300, Greensboro, NC 27419

Metribuzin
William Betker, Mobay Chemical Co., Agricultural Chemicals Division, Box 4913, Kansas City, MO 64120

Monosodium Methane Arsenate

Propanil
Delmas Pennington, Rohm and Haas, PO Box 591, Knoxville, TN 37901

Triazine Herbicides
Arthur H. Hofberg

PESTICIDE FORMULATIONS: INORGANIC PESTICIDES

Referee: Paul D. Jung, Environmental Protection Agency, Office of Pesticide Programs, Beltsville, MD 20705

Aluminum Phosphide

Sodium Chlorate

PESTICIDE FORMULATIONS: OTHER INSECTICIDES, SYNERGISTS, AND INSECT REPELLANTS

Referee: James Launer, State Department of Agriculture, Laboratory Services, Salem, OR 97310

Allethrin
Dean Kassera, McLaughlin Gormley King Co., 8810 Tenth Ave, Minneapolis, MN 55427

2,3:4,5-Bis(2-butylene)tetrahydro-2-furaldehyde (MGK 11®)
Vernon Meinen, McLaughlin Gormley King Co., 8810 Tenth Ave, Minneapolis, MN 55427

Cypromethrin
Peter D. Bland, ICI Americas, Inc., Biological Research Center, Box 208, Goldsboro, NC 27530

Dipropyl Isocinchomeronate (MGK 326®)
Dave Carlson, McLaughlin Gormley King Co., 8810 Tenth Ave, Minneapolis, MN 55427

Fumigants
Dean Yeaman, Dow Chemical Co., Analytical Laboratories, Pittsburgh, CA 94565

Larvadex
Arthur H. Hofberg, Ciba-Geigy Corp., PO Box 18300, Greensboro, NC 27419

Nicotine
Spencer Carrigan. Department of Agriculture, University of Maryland, College Park, MD 20742

Permethrin
Hershel F. Morris, Louisiana Department of Agriculture, Box 16390-A, University Station, Baton Rouge, LA 70893

Piperonyl Butoxide and Pyrethrins
Dean Kassera

Resmethrin
Mark Law, Environmental Protection Agency, TSD-Chemical & Biological Investigation, Beltsville, MD 20705

Rotenone and Other Rotenoids
Rodney J. Bushway, University of Maine, Agricultural Experiment Station, Orono, ME 04469

PESTICIDE FORMULATIONS: OTHER ORGANOPHOSPHORUS INSECTICIDES

Referee: Marshall Gentry, Florida Department of Agriculture and Consumer Services, Division of Chemistry, Tallahassee, FL 32301

Crotoxyphos
Wendy King, Florida Department of Agriculture and Consumer Services, Tallahassee, FL 32304

Crufomate (Ruelene)

Dichlorvos

Mevinphos

Monocrotophos

Naled
A. Aner Carlstrom, Chevron Chemical Co., 940 Hensley St, Richmond, CA 94804

Tetrachlorvinphos

PESTICIDE FORMULATIONS: RODENTICIDES AND MISCELLANEOUS PESTICIDES

Referee: Marshall Gentry, Florida Department of Agriculture and Consumer Services, Division of Chemistry, Tallahassee, FL 32301

Brodifacoum (Talon®)
Peter D. Bland, ICI Americas Inc., Biological Research Center, Box 208, Goldsboro, NC 27530

Chlorophacinone

Diphacinone

α-Naphthylthiourea

***N*-3-Pyridyl-*N'*-*p*-Nitrophenyl Urea (Vacor®)**

Strychnine

Warfarin
Elmer Hayes, Environmental Protection Agency, Chemistry Laboratory, Beltsville, MD 20705

PESTICIDE FORMULATIONS: ORGANOTHIOPHOSPHATE INSECTICIDES

Referee: Edwin R. Jackson, Mississippi State Chemical Laboratory, Box CR, Mississippi State, MS 39762

Acephate
A. Aner Carlstrom, Chevron Chemical Co., 940 Hensley St, Richmond, CA 94804

Azinphos-Methyl

Coumaphos
Linda Ruiz, Bayvet Division of Cutter Labs, Box 390, Shawnee, KS 66201

Demeton

Demeton-*S*-Methyl

Diazinon
Arthur H. Hofberg, Ciba-Geigy Corp., Analytical Chemistry Division, 410 Swing Rd, Greensboro, NC 27409

Dimethoate
Richard S. Wayne, American Cyanamid Co., Agriculture Division, Box 400, Princeton, NJ 08540

Dioxathion
William H. Clark, Hercules, Inc., Analytical Division, Wilmington, DE 19898

Encapsulated Organophosphorus Pesticides
James J. Karr, Pennwalt Technological Center, 900 First Ave, Box C, King of Prussia, PA 19406

EPN
John Forrette, Velsicol Chemical Corp., 341 E Ohio St, Chicago, IL 60611

Ethoprop
Richard W. Smith, Rhone-Poulenc, Inc., Box 352, Mt Pleasant, TN 38474

***O*-Ethyl *O*-(4-Methylthio) Phenyl *S*-Propyl Phosphorothioate**
Willard G. Boyd, Jr, State Chemical Laboratory, Box CR, Mississippi State, MS 39762

Fenitrothion
James W. Miles, 1228 Bacon Park Dr, Savannah, GA 31406

Fensulfothion
Margie Owen, State Chemical Laboratory, Box 329, Auburn, AL 36830

Fenthion
Willard G. Boyd, Jr

Fonophos
William Y. Ja, Stauffer Chemical Co., 1200 S 47th St, Richmond, CA 94804

Malathion
Richard S. Wayne

Methidathion
Thomas Gale, Ciba-Geigy Corp., Box 18300, Greensboro, NC 27409

Oxydemeton

Parathion and Methyl Parathion
Edwin R. Jackson

Phorate (*O,O*-Diethyl *S*-[(Ethylthio)methyl] Phosphorodithioate)
Roman Grypa, Agway Inc., Fertilizer Division, 978 Loucks Hill Rd, York, PA 17402

Sulprofos

Temephos

PLANTS

Referee J. Benton Jones, Jr, University of Georgia, Soil Testing and Plant Laboratory, Athens, GA 30602

Ashing Methods
J. Benton Jones, Jr

Atomic Absorption Methods
Robert A. Isaac, University of Georgia, College of Agriculture, Athens, GA 30602

Boron
James R. Melton, Texas A&M University, Agricultural Analytical Services, College Station, TX 77843

Chromium
Earle E. Cary, U.S. Department of Agriculture, Plant, Soil, and Nutritional Laboratory, Tower Rd, Ithaca, NY 14853

Emission Spectroscopy
Robert A. Isaac

Fluoride
Jay S. Jacobson, Boyce Thompson Institute, Tower Rd, Ithaca, NY 14853

Nitrogen, Nonprotein

Selenium
Oscar E. Olson and Ivan S. Palmer, South Dakota State University, Experiment Station, Biochemistry Department, Brookings, SD 57006

Starch
T. Powell Gaines, University of Georgia, College of Agriculture, Department of Agronomy, Tifton, GA 31797

Sulfa in Plants
Charles W. Gehrke, University of Missouri-Columbia, Columbia, MO 65211
Rose Sweeney, University of Missouri-Columbia, Columbia, MO 65211

Sulfur

REFERENCE MATERIALS AND STANDARD SOLUTIONS

Referee: Robert Alvarez, U.S. Department of Commerce, National Bureau of Standards, Office of Standard Reference Materials, Washington, DC 20234

Stability of Organophosphorus Pesticide Standards

TOBACCO

Referee: Ray Severson, U.S. Dept. of Agriculture, Agricultural Research Service, Athens, GA 30613

Differentiation of Cigar and Cigarette Tobaccos (Sequential Differential Solvent Extraction)
John A. Steele, Department of the Treasury, Bureau of Alcohol, Tobacco and Firearms, 1401 Research Blvd, Rockville, MD 20850

Humectants in Cased Cigarettes

Nicotine, Gas Chromatography
John R. Wagner, Lorillard Corp., 426 English St, Greensboro, NC 27420

Tar and Nicotine in Cigarette Smoke
Harold C. Pillsbury, Federal Trade Commission, 6th and Pennsylvania Ave, NW, Washington, DC 20580

COMMITTEE B

Thomas Layloff (National Center for Drug Analysis, 1114 Market St, St. Louis, MO 63101), *Chairman;* William W. Wright (U.S. Pharmacopeial Convention, 12601 Twinbrook Pkwy, Rockville, MD 20852); Anthony Romano, Jr (Drug Enforcement Administration, Southeast Regional Laboratory, 5205 NW 84th Ave, Miami, FL 33166); John Zarembo (Revlon Health Care, 1 Scarsdale Rd, Tuckahoe, NY 10707); Evelyn Sarnoff (Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232); Charles Bivart (The Upjohn Co., 7171 Portage Rd, Kalamazoo, MI 49001); Sal Fusari (Parke-Davis/Warner Lambert, 170 Tabor Rd, Morris Plains, NJ 07950); Eric Sheinen (Food and Drug Administration, Division of Drug Chemistry, Washington, DC 20204), *Secretary;* Roswitha E. Kelly (Food and Drug Administration, Bureau of Medical Devices, 8757 Georgia Ave, Silver Spring, MD 20853), *Statistical Consultant*

DRUGS, ACIDIC

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

Allopurinol
Donald Shostak, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Amitryptyline HCl in Dosage Forms (HPLC)

Aspirin, Phenacetin, and Caffeine with Other Drugs
Douglas D. Don, Food and Drug Administration, 1521 W Pico Blvd, Los Angeles, CA 90015

Aspirin and Salicylic Acid in Aspirin Products (Semiautomated Analysis)
William E. Juhl, Food and Drug Administration, National Center for Drug Analysis, 1114 Market St, St. Louis, MO 63101

Barbiturates
Loren Gelber, 1804 Beacon St, Brookline, MA 02146

Benzothiazine Derivatives

Benzthiazide by HPLC
Stephen Hauser, Food and Drug Administration, Winchester Engineering and Analytical Center, 109 Holton St, Winchester, MA 01890

Methyldopa
Susan Ting, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Primidone
Stanley E. Roberts, Food and Drug Administration, Winchester Engineering and Analytical Center, 109 Holton St, Winchester, MA 01890

Probenecid
Alexander G. Korzun, Food and Drug Administration, Winchester Engineering and Analytical Center, 109 Holton St, Winchester, MA 01890

Sulfamethoxazole in Tablets (HPLC)
John W. Robinson, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Sulfisoxazoie in Dosage Forms (HPLC)

Sulfonamides (Thin Layer Chromatography)

Thiazide Diuretics, Semiautomated Individual Dosage Unit Analysis
Terry W. Moore, Food and Drug Administration, National Center for Drug Analysis, 1114 Market St, St. Louis, MO 63101

DRUGS, ALKALOIDS AND RELATED BASES

Referee: Edward Smith, Food and Drug Administration, Division of Drug Chemistry, Washington, DC 20204

Aminacrine
Elaine A. Bunch, Food and Drug Administration, 5003 Federal Office Bldg, Seattle, WA 98174

Atropine in Morphine, Atropine Tablets, and Injections
Ira J. Holcomb, Parke Davis, 870 Parkedale Rd, Rochester, MI 48063

Belladonna Alkaloids

Chlorpromazine
Donald J. Smith, Food and Drug Administration, Division of Drug Chemistry, Washington, DC 20204

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
Charles L. Brownell, Food and Drug Administration, 10 W 35th St, Chicago, IL 60616

Epinephrine Lidocaine Combinations
Donald J. Smith

Epinephrine and Related Compounds by HPLC-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

Homatropine Methyl Bromide in Tablets
Duane Hughes, Food and Drug Administration, 1009 Cherry St, Kansas City, MO 64016

Neostigmine
Rita E. Jhangiani, Food and Drug Administration, 2nd and Chestnut Sts, Philadelphia, PA 19106

Phenethylamine Drugs, Semiautomated Individual Unit Analysis
Percy A. McCullen, Food and Drug Administration, National Center For Drug Analysis, 1114 Market St, St. Louis, MO 63101

Pheniramine with Pyrilamine, Phenylpropanolamine, and Phenylephrine
Henry S. Scroggins, Food and Drug Administration, 4298 Elysian Fields Ave, New Orleans, LA 70122

Phenothiazines in Drugs
Edward G. Lovering, Health Protection Branch, Drug Research Lab., Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2

Physostigmine and Its Salts
Norlin W. Tymes, Food and Drug Administration, 900 Madison Ave, Baltimore, MD 21201

Pilocarpine
Irving Wainer, Food and Drug Administration, Division of Drug Chemistry, Washington, DC 20204

***Rauwolfia* Alkaloids**
Susan Barkan, Food and Drug Administration, Division of Drug Chemistry, Washington, DC 20204

Rauwolfia serpentina
Ugo R. Cieri, Food and Drug Administration, 2nd and Chestnut Sts, Philadelphia, PA 19106

TLC Identification of Phenothiazine-type Drugs
Kurt Steinbrecher, Food and Drug Administration, 909 First Ave, Seattle, WA 98174

DRUGS, GENERAL

Referee: Ted M. Hopes, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Ampicillin and Amoxicillan
Michel Margosis, Food and Drug Administration, National Center for Drugs and Biologics, Washington, DC 20204

Betamethasone
Susan Lee, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Bisacodyl
Leonard Valenti, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Disulfiram

Fluoride
John R. Marzilli, Food and Drug Administration, 585 Commercial St, Boston, MA 02109

Halogenated Hydroxyquinoline Drugs
Edward J. Wojtowicz, Food and Drug Administration, 599 Delaware Ave, Buffalo, NY 14202

Hydralazine
Barry Mopper, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Insulin by LC
Donald J. Smith, Food and Drug Administration, Division of Drug Chemistry, Washington, DC 20204

Medicinal Gases
Martin Woodhouse, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Menadiol Sodium Diphosphate Injection
Maurice Y. Alpert, Food and Drug Administration, Winchester Engineering and Analytical Center, 109 Holton St, Winchester, MA 01890

Mercury-Containing Drugs
Walter Holak, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Metals in Drug Bulk Powders
Walter Holak

Microcrystalline Tests
Marshall Rubkin, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Protein Nitrogen Units in Allergenic Extracts
Joan May, Food and Drug Administration, National Center for Drugs and Biologics, 8800 Rockville Pike, Bethesda, MD 20014

Thyroid and Thyroxine Related Compounds
Donald J. Smith, Food and Drug Administration, Division of Drug Chemistry, Washington, DC 20204

Thyroid by Differential Pulse Polarography
Walter Holak

DRUGS, ILLICIT

Referee: Charles C. Clark, Drug Enforcement Administration, 5205 NW 84th Ave, Miami, FL 33166

Amphetamines in Mixtures

Benzodiazepines
Eileen Bargo, Food and Drug Administration, 900 Madison Ave, Baltimore, MD 21201

Chemical Microscopy

Cocaine
Charles C. Clark

Diazepam
Michael Tsougros, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Dimethyltryptamine (DMT), Diethyltryptamine (DET), and Dipropyltryptamine (DPT)

Heroin
Harold F. Hanel, Drug Enforcement Administration, 5205 NW 84th Ave, Miami, FL 33166

Lysergic Acid Diethylamide (LSD)

Marihuana and Synthetic Tetrahydrocannabinol (THC)

Methadone
Eugene McGonigle, Ortho Pharmaceuticals Corp., U.S. Highway 202, Raritan, NJ 08869

Methamphetamine

Methaqualone Hydrochloride
Harold F. Hanel

Methylphenidate Phenidine Hydrochloride

Phencyclidine (PCP)
Charles C. Clark

DRUGS, NEUTRAL

Referee: Thomas G. Alexander, Food and Drug Administration, National Center for Drugs and Biologics, Washington, DC 20204

Automated Methods for Progestins in Tablets
Larry K. Thornton, Food and Drug Administration, National Center for Drug Analysis, 1114 Market St, St. Louis, MO 63101

Digitoxin, Automated Individual Tablet Analysis
Benjamin Westenberger, Food and Drug Administration, National Center for Drug Analysis, 1114 Market St, St. Louis, MO 63101

Estrogens

Conjugated Estrogens
Robert W. Roos, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Ethinyl Estradiol, Automated Individual Tablet Analysis

Methocarbamol

Steroid Acetates

Steroid Phosphates

COMMITTEE C

Harry B. S. Conacher (Health and Welfare Canada, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario, Canada, K1A 0L2), *Chairman;* Arthur E. Waltking (CPC International, Inc., 1120 Commerce Ave, Union, NJ 07083); Raymond Ashworth (U.S. Department of Agriculture, Food Safety and Inspection Service, Beltsville, MD 20705); Odette L. Shotwell (U.S. Department of Agriculture, Northern Regional Research Center, Peoria, IL 61604); Betsy Woodward (State Department of Agriculture and Consumer Services, Tallahassee, FL 32304); Collette Levi (General Foods Corp., White Plains, NY 10602); John McKinney (Ranchers Cotton Oil, 2691 S Cedar, Fresno, CA 93725); Arthur R. Johnson (Food and Drug Administration, Division of Food Technology, Washington, DC 20204), *Secretary;* Michael 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
John M. Newton, Food and Drug Administration, 50 Fulton St, San Francisco, CA 94102

Crude Fiber in Tea

Moisture in Coffee and Tea
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, Canada K1A 0L2

Theophylline in Tea
John M. Newton

Water Extract in Tea
Elpidio de la Teja, Thomas J. Lipton, Inc., Analytical Section, 800 Sylvan Ave, Englewood Cliffs, NJ 07632

DAIRY PRODUCTS

Referee: Robert W. Weik, Food and Drug Administration, Bureau of Foods, Washington, DC 20204

Adulteration of Dairy Products with Vegetable Fat
Graham MacEachern, Agriculture Canada, Plant Products, Ottawa, Ontario, Canada K1A 0C5

Babcock Test and Babcock Glassware
Robert Bradley, University of Wisconsin-Madison, Food and Science Dept., Madison, WI 53706

Casein and Caseinates
Charles Pynes, Stauffer Chemical Co., Technical Sales and Development Department, Westport, CT 06880

Chocolate Milk, Fat Test
James T. Marshall, Frigo Cheese Corp., PO Box 158, Lena, WI 54139

Cryoscopy of Milk
Robert W. Henningson, Clemson University, Office of University Research, Clemson, SC 29631

Fat, Automated Methods
W. Frank Shipe, Cornell University, Department of Dairy and Food Science, Ithaca, NY 14853

Fat in Milk (AutoAnalyzer)
Raymond L. King, University of Maryland, Department of Dairy Science, College Park, MD 20742

Infrared Milk Analyzer (IRMA)
D. A. Biggs, University of Guelph, Department of Food Science, Guelph, Ontario, Canada N1G 2W1

Lactose in Dairy Products (Chromatographic Determination)
Leslie G. West, Kraft Co., 801 Waukegan Rd, Glenview, IL 60025

Lactose in Dairy Products (Enzymatic Determination)
Dick H. Kleyn, Rutgers University, Department of Food Science, New Brunswick, NJ 08903
John W. Sherbon, Cornell University, Department of Dairy and Food Science, Ithaca, NY 14853

Moisture in Cheese
Ronald Case, Kraft Foods, Kraft Court, Glenview, IL 60025

Moisture in Cheese (Karl Fischer Method)
Gary H. Richardson, Utah State University, Department of Nutrition and Food Science, Logan, UT 84322

Nitrates in Cheese
James E. Hamilton, Food and Drug Administration, Division of Drug Labeling—Compliance, 5600 Fishers Lane, Rockville, MD 20857

Phosphatase, Rapid Method
Dick H. Kleyn

Phosphatase, Reactivated
Gopala K. Murthy, Food and Drug Administration, Division of Microbiology, 1090 Tusculum Ave, Cincinnati, OH 45226

Phosphorus
Wallace S. Brammell, Food and Drug Administration, Division of Color Technology, Washington, DC 20204

Protein Constituents in Processed Dairy Products
Frederick W. Douglas, Jr, U.S. Department of Agriculture, Eastern Regional Research Center, Philadelphia, PA 11918

Protein in Milk, Rapid Tests
John W. Sherbon

Protein Reducing Substance Tests
Joseph T. Cardwell, Mississippi State University, Dairy Science Department, Mississippi State, MS 39762

Solids-Not-Fat
John W. Sherbon

Vapor Pressure Osmometry
Gary H. Richardson

DECOMPOSITION AND FILTH IN FOODS (CHEMICAL METHODS)

Referee: Walter F. Staruszkiewicz, Jr, Food and Drug Administration, Division of Food Technology, Washington, DC 20204

Ammonia in Dogfish
Beverly Smith, National Marine Fisheries Service, PO Drawer 1207, Pascagoula, MS 39567

Coprostanol
James G. Stewart, Food and Drug Administration, 3032 Bryan St, Dallas, TX 75204

Crabmeat
Kurt Steinbrecher, Food and Drug Administration, 909 First Ave, Seattle, WA 98104

Diacetyl in Citrus Products
W. S. Hatcher, The Coca-Cola Co., Plymouth, FL 32768

Ethanol in Seafoods
Harold R. Throm, Food and Drug Administration, 909 First Ave, Seattle, WA 98104

Gas and Liquid Chromatography
Walter F. Staruszkiewicz, Jr.

GLC Determination of Volatile Amines—TMA and DMA
Ronald C. Lundstrom, National Marine Fisheries Service, Northeast Fisheries Center, Gloucester, MA 01930

Shellfish

TLC Determination of Amines in Fishery Products
Thomas R. Weber, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Tomatoes
Albert Y. Taira, Food and Drug Administration, 433 W Van Buren St, Chicago, IL 60607

FISH AND OTHER MARINE PRODUCTS

Referee: Louis L. Gershman, Food and Drug Administration, 585 Commercial St, Boston, MA 02109

Crabmeat, Identification
Judith Krzynowek, National Marine Fisheries Service, Northeast Fisheries Center, Gloucester, MA 01930

Drained Weight of Block Frozen Raw, Peeled Shrimp
Frederick J. King, National Marine Fisheries Service, Northeast Fisheries Center, Gloucester, MA 01930

Drip Fluid in Fish Fillets and Fish Fillet Blocks—Quantitation

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Determination of Fish Content in Coated Products (Breaded or in Batter)
Frederick J. King
H. Houwing, TNO, Division of Nutrition and Food Research, Box 183, 1970 AD, Ijmuiden, The Netherlands

Fish Species Identification (Thin Layer Isoelectric Focusing)
Ronald C. Lundstrom, National Marine Fisheries Service, Northeast Fisheries Center, Gloucester, MA 01930

Minced Fish in Fish Fillet Blocks
J. Perry Lane, National Marine Fisheries Service, Northeast Fisheries Center, Gloucester, MA 01930

Nitrites in Smoked Fish

Organometallics in Fish
Walter Holak, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

FOOD ADDITIVES

Referee: Thomas Fazio, Food and Drug Administration, Division of Chemistry and Physics, Washington, DC 20204

Anticaking Agents

Antioxidants
B. Denis Page, Health and Welfare Canada, Food Research Division, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2

Brominated Oils
James F. Lawrence, Health and Welfare Canada, Food Research Division, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2

Chloride Titrator
Alfred H. Free, Ames Co., Technical Services, Elkhart, IN 46514

Chlorobutanol in Milk

Dichlorodifluoromethane in Frozen Foods

Dilauryl Thiodipropionate

Dimethylpolysiloxane

Dressings
Charles R. Warner, Food and Drug Administration, Division of Chemistry and Physics, Washington, DC 20204

EDTA in Food Products
Gracia A. Perfetti, Food and Drug Administration, Division of Chemistry and Physics, Washington, DC 20204

Ethoxyquin in Meats and Eggs

Gums

Indirect Additives from Food Packages
Charles V. Breder, Food and Drug Administration, Division of Chemistry and Physics, Washington, DC 20204

Mineral Oil in Raisins

Nitrates and Nitrites
Jay Fox, U.S. Department 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, Canada K1A 0L2

Polycyclic Aromatic Hydrocarbons in Foods
Frank L. Joe, Food and Drug Administration, Division of Chemistry and Physics, Washington, DC 20204

Polysorbates
Charles F. Smullin, ICI United States Inc., Chemical Research Department, Wilmington, DE 19897

Propylene Chlorohydrin

Sodium Lauryl Sulfate

MEATS, POULTRY, AND MEAT AND POULTRY PRODUCTS

Referee: Richard L. Ellis, U.S. Department of Agriculture, Scientific Services, Food Safety and Inspection Service, Washington, DC 20250

Automated Methods
Jon L. Schermerhorn, Department of Agriculture and Markets, New York State Food Laboratory, Albany, NY 12235

Bioassay Methods for Meat and Poultry Products

Bone Content
Paul Corrao, U.S. Department of Agriculture, Food Safety and Inspection Service, Beltsville, MD 20705

Chemical Antibiotic Methods
Francis B. Suhre, U.S. Department of Agriculture, Food Safety and Inspection Service, Beltsville, MD 20705

Fat in Meat Products
Jon E. McNeal, U.S. Department of Agriculture, Food Safety and Inspection Service, Washington, DC 20250

Fat and Moisture Analysis, Rapid Methods
Julio D. Pettinati, U.S. Department of Agriculture, Eastern Regional Research Center, 600 E Mermaid Lane, Philadelphia, PA 19118

HPLC Methods for Meat and Poultry Products
Douglas Gillard, U.S. Department of Agriculture, Food Safety and Inspection Service, Beltsville, MD 20705

Histologic Identification Methods
Albert M. Carey, U.S. Department of Agriculture, Food Safety and Inspection Service, Beltsville, MD 20705

Identification of Meats, Serological Tests

Nitrates and Nitrites
Francis B. Suhre, U.S. Department of Agriculture, Food Safety and Inspection Service, Beltsville, MD 20705

Nitrosamines in Bacon
Earl L. Greenfield, U.S. Department of Agriculture, Food Safety and Inspection Service, Beltsville, MD 20705

Specific Ion Electrode Applications
Randy Simpson, U.S. Department of Agriculture, Food Safety and Inspection Service, Beltsville, MD 20705

Steroid Analysis
Michael Thomas, U.S. Department of Agriculture, Food Safety and Inspection Service, Beltsville, MD 20705

Sugars and Sugar Alcohol

Temperature, Minimum Processing
James Eye, U.S. Department of Agriculture, Food Safety and Inspection Service, Beltsville, MD 20705

MYCOTOXINS

Referee: Peter M. Scott, Health and Welfare Canada, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2

Aflatoxin M
Robert D. Stubblefield, U.S. Department of Agriculture, Northern Regional Research Center, Peoria, IL 61604

Aflatoxin Methods
Douglas L. Park, Food and Drug Administration, Division of Chemistry and Physics, Washington, DC 20204

Alternaria Toxins
Douglas King, U.S. Department of Agriculture, Western Regional Research Center, 800 Buchanan St, Albany, CA 94710

Citrinin
David Wilson, University of Georgia, Department of Plant Pathology, Tifton, GA 31794

Cyclopiazonic Acids
John A. Landsen, U.S. Department of Agriculture, National Peanut Research Laboratory, Dawson, GA 31742

Ergot Alkaloids
George Ware, Food and Drug Administration, 4298 Elysian Fields Ave, New Orleans, LA 70122

Grains
Odette L. Shotwell, U.S. Department of Agriculture, Northern Regional Research Center, Peoria, IL 61604

Ochratoxins
Stanley Nesheim, Food and Drug Administration, Division of Chemistry and Physics, Washington, DC 20204

Penicillic Acid
Charles W. Thorpe, Food and Drug Administration, Division of Chemistry and Physics, Washington, DC 20204

Secalonic Acids
Kenneth C. Ehrlich, U.S. Department of Agriculture, Food and Feed Safety Research, New Orleans, LA 70179

Sterigmatocystin
Octave J. Francis, Jr, Food and Drug Administration, 4293 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 Chemistry and Physics, Washington, DC 20204

Xanthomegnin
Allen S. Carman, Food and Drug Administration, 4298 Elysian Fields Ave, New Orleans, LA 70122

Zearalenone
Glenn A. Bennett, U.S. Department of Agriculture, Science and Education Administration, Northern Regional Research Center, Peoria, IL 61604

OILS AND FATS

Referee: David Firestone, Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Antioxidants
B. Denis Page, Health and Welfare Canada, Food Research Division, Health Protection Branch, Ottawa, Ontario, Canada K1A 0L2

Emulsifiers
H. Bruschweiler, Laboratoire Federal d'Essai des Materiaux, Industrie, Genie Civil Arts et Metiers, 9001 St. Gallen, Unterstrasse II, Switzerland

Karl Fischer Method for Determination of Water
Raffaele Bernetti, CPC International, Moffett Technical Center, PO Box 345, Argo, IL 60501

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, Canada B3J 2X4

Olive Oil Adulteration
Enzo Fedeli, Exper mental Station for Oils and Fats, via Giuseppe Colompo 79, 20133 Milano, Italy

Oxidized Fats
Arthur E. Waltking, Best Foods, 1120 Commerce Ave, Union, NJ 07083

Pork Fat in Other Fats
Laila El-Sayed Abdel Fattah, King Saud University, PO Box 22452, Riyadh, Saudi Arabia

Sterols and Tocopherols
Hal T. Slover, U.S. Department of Agriculture, Nutrition Institute, Beltsville, MD 20705

PLANT TOXINS

Referee: Samuel W. Page, Food and Drug Administration, Division of Chemistry and Physics, Washington, DC 20204

Glucosinolates

Pyrrolizidine Alkaloids

Solanaceous Alkaloids

PROCESSED VEGETABLE PRODUCTS

Referee: Thomas R. Mulvaney, Food and Drug Administration, Division of Food Technology, Washington, DC 20204

Fibrous Material in Frozen Green Beans
George W. Varseveld, Oregon State University, Department of Food Science and Technology, Corvallis, OR 97331

pH Determination
Frederick E. Boland, Food and Drug Administration, Division of Food Technology, Washington, DC 20204

Sodium Chloride
Wallace S. Brammell, Food and Drug Administration, Division of Color Technology, Washington, DC 20204

Soluble Solids of Tomato Products

Total Solids by Microwave Moisture Analyzer
Henry B. Chin, National Food Processors Association, 1950 Sixth St, Berkeley, CA 94710

Volume of Entrapped Air in Flexible Retort Pouches

Water Activity in Foods
William H. Stroup, Food and Drug Administration, Food Engineering Branch, 1090 Tusculum Ave, Cincinnati, OH 45226

SEAFOOD TOXINS

Referee: Edward P. Ragelis, Food and Drug Administration, Division of Chemistry and Physics, Washington, DC 20204

Ciguatoxins, Biochemical Methods
Yoshitsugi Hokama, University of Hawaii at Manoa, School of Medicine, Honolulu, HI 96844

Paralytic Shellfish Poisoning (Immunoassay Method)
Edward P. Ragelis

Shellfish Poisons
William L. Childress, Food and Drug Administration, 585 Commercial St, Boston, MA 02109

Tetradotoxins
Yururu Shimizu, University of Rhode Island, College of Pharmacy, Kingston, RI 02881

COMMITTEE D

Elmer George (State Department of Agriculture and Markets, State Campus, Albany, NY 12235), *Chairman;* Robert A. Martin (Hershey Food Corp., Hershey Technical Center, 1025 Reese Ave, Hershey, PA 17033); Harry G. Lento (Campbell Soup Co., Campbell Place, Camden, NJ 08151); Laura Zaika (U.S. Department of Agriculture, Eastern Regional Research Center, 600 E Mermaid Lane, Philadelphia, PA 19118); V. William Kadis (Alberta Agriculture, Food Laboratory Service Branch, Edmonton, Alberta, Canada T6H 4P2); Michael Knowles (Ministry of Agriculture, Fisheries and Foods, Food Science Laboratory, Norwich, UK NR2 4SX); Earl F. Richter (Hazleton Raltech, PO Box 7545, Madison, WI 53707); Benjamin Krinitz (Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232), *Secretary;* Dennis Ruggles (Food and Drug Administration, Division of Mathematics, Washington, DC 20204), *Statistical Consultant*

ALCOHOLIC BEVERAGES

Referee: Randolph H. Dyer, Bureau of Alcohol, Tobacco and Firearms, 1401 Research Blvd, Rockville, MD 20850

Acetate in Wines and Fruit Juice (Enzymatic Assay)
Leo P. McCloskey, 126 National St, Santa Cruz, CA 95060

Alcohol Content by Oscillating U-Tube Density Meter
Duane H. Strunk, Joseph E. Seagram & Sons, Inc., Research and Development Department, Box 240, Louisville, KY 40201

Alcohol Content of High Solids Distilled Spirits
Duane H. Strunk

Carbon Dioxide in Wine
Arthur Caputi, Jr, E. & J. Gallo Winery, PO Box 1130, Modesto, CA 95353

Citric Acid in Wine

Color Intensity for Distilled Alcoholic Products
Duane H. Strunk

Ethanol in Wine by GLC
Arthur Caputi, Jr

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
Robert Dowrie, Almaden Vineyards, 1530 Blossom Hill Rd, San Jose, CA 95118

Malt Beverages and Brewing Materials
Anthony J. Cutaia, Stroh Brewing Co., One Stroh Dr, Detroit, MI 48226

Sorbic Acid in Wine
Arthur Caputi, Jr

Sugars in Wine
Guenther Henniger, Boehringer Mannheim GmbH, Bahnhofstrasse 5, D-8132, Tutsing/Obb. Postfach 120, GFR

Sulfur Dioxide in Wine (Ripper Method)
James M. Vahl, Paul Masson Vineyards, PO Box 97, Saratoga, CA 95070

Tartrates in Wine
Masao Ueda, E. & J. Gallo Winery, PO Box 1130, Modesto, CA 95353

Vanillin and Ethyl Vanillin
Felipe Alfonso, Bureau of Alcohol, Tobacco and Firearms, 1401 Research Blvd, Rockville, MD 20850

Volatile Acidity in Wine
Gordon J. Pilone, The Christian Brothers, Mont La Salle Vineyards, PO Box 420, Napa, CA 94558

Volatile Congeners in Alcoholic Beverages
Duane H. Strunk

CACAO PRODUCTS

Referee: H. J. Vos, Populierenlaan, 3735L9 Bosch en Buin, The Netherlands

Caffeine and Theobromine

Carbohydrates in Chocolate Products
William J. Hurst, Hershey Foods Corp., Hershey, PA 17033

Moisture in Cacao Products
Robert A. Martin, Hershey Foods Corp., Hershey, PA 17033

Shell in Cacao Products, Micro Methods

CEREAL FOODS

Referee: Doris Baker, U.S. Department of Agriculture, Nutrition Institute, Beltsville, MD 20705

Iron
James Martin, Food and Drug Administration, 1182 Peachtree St, NW, Atlanta, GA 30309

Phytates
Barbara F. Harland, Food and Drug Administration, Division of Nutrition, 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 and Forbes Co., Third 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

FRUITS AND FRUIT PRODUCTS

Referee: Frederick E. Boland, Food and Drug Administration, Division of Food Technology, Washington, DC 20204

Adulteration of Apple Juice
John Zyren, National Food Processors Association, 1401 New York Ave, Washington, DC 20005

Adulteration of Orange Juice by Pulpwash and Dilution
Donald R. Petrus, Florida Department of Citrus, Box 1088 AREC, Lake Alfred, FL 33850

Fruit Acids
Elia D. Coppola, Ocean Spray Cranberries, Inc., Research and Development, Bridge St, Middleboro, MA 02346

Fruit Juices, Identification and Characterization
Ronald E. Wrolstad, Oregon State University, Department of Food Science and Technology, Corvallis, OR 97331

Orange Juice Content
Carl Vandercook, U.S. Department of Agriculture, Agricultural Research Service, Fruit and Vegetable Chemistry Laboratory, Pasadena, CA 91106

Sodium Benzoate in Orange Juice
James Fisher, Citrus Research and Education Center, 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 Xanthanes in Nonalcoholic Beverages
John M. Newton

Glycyrrhizic Acid Salts in Licorice-Derived Products

Lasiocarpine and Pyrrolizidines in Herbal Beverages

Quinine

PRESERVATIVES AND ARTIFICIAL SWEETENERS

Referee: William S. Adams, Food and Drug Administration, 585 Commercial St, Boston, MA 02109

Aspartame, Benzoates, Saccharin, and Caffeine, High Pressure Liquid Chromatography
Norma G. Webb, Florida Department of Agriculture and Consumer Services, Mayo Bldg, Tallahassee, FL 32304

Formaldehyde
Robert J. Reina, Food and Drug Administration, 585 Commercial St, Boston, MA 02109

Meats, Ground, Screening Methods for Chemical Preservatives
John J. Maxstadt, Department of Agriculture and Markets, New York State Food Laboratory, 120 Washington Ave, Albany, NY 12235

Organic Preservatives (Thin Layer Chromatography)
Colette P. Levi, General Foods Corp., White Plains, NY 10602

Preservatives (Quantitative Methods)

SPICES AND OTHER CONDIMENTS

Referee: Raymond Way, Crescent Manufacturing Co., Box 3985, Seattle, WA 98124

Ash and Pungent Principles in Mustard

Extractable Color in Capsicum Spices and Oleoresins
James E. Woodbury, Cal-Compak Food, Inc., Quality Control, 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

Pungency of Capsicums and Oleoresins
James E. Woodbury

Vinegar

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
Frank G. Carpenter, U.S. Department of Agriculture, Southern Regional Research Laboratory, 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

Honey
Jonathan W. White, Jr, 217 Hillside Dr, Navasota, TX 77868

Lactose Purity Testing
Janice R. Saucerman, Mead Johnson & Co., Environmental Analytical Services, Evansville, IN 47721

Maple Sap and Syrups
Maria Franca Morselli, University of Vermont, Botany Department, Burlington, VT 05405

Stable Carbon Isotope Ratio Analysis
Landis Doner, U.S. Department of Agriculture, Eastern Regional Research Center, 600 E Mermaid Lane, Philadelphia, PA 19118

Standardization of Sugar Methods of Analysis
Margaret A. Clarke, 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 Licorice Products
Raymond M. Tuorto, MacAndrews and Forbes Co., Third St and Jefferson Ave, Camden, NJ 08104

Sugar in Sugar Cane

Weighing, Taring, and Sampling
Melvin Lerner, Department of the Treasury, Bureau of Customs, Washington, DC 20226

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. Department of Agriculture, Eastern Regional Research Center, 600 E Mermaid Ln, Philadelphia, PA 19118

Automated Nutrient Analysis
Jonathan De Vries, General Mills Inc., 9000 Plymouth Ave, Minneapolis, MN 55427

Biotin
Jacob M. Scheiner, Hoffmann-La Roche, Nutley, NJ 07110

Carotenoids
Forrest W. Quackenbush, Purdue University, Department of Biochemistry, West Lafayette, IN 47907

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 Place, Camden, NJ 08151

Folic Acid
Lynn Hoepfinger, Mead Johnson and Co., Nutritional Quality Control, Evansville, IN 47721

HPLC Assay for Total A, D, and E Content in Foods, Feeds, and Pharmaceuticals
James V. Bruno, Waters Associates, 34 Maple St, Milford, MA 01757

Iodine in Foods
Robert A. Moffitt, Carnation Co., 8015 Van Nuys Blvd, Van Nuys, CA 91412

Nutrient Assay of Infant Formula
James J. Tanner, Food and Drug Administration, Division of Nutrition, Washington, DC 20204
Stephan A. Barnett, Mead Johnson and Co., 2404 Pennsylvania Ave, Evansville, IN 47721

Pantothenic Acid, Total Activity in Foods
Raymond Cooke, Laboratory of the Government Chemist, Food Composition and Nutrition, Cornwall House, Stamford St, London, UK SE1 9NQ

Protein Quality, Evaluation in Foods
Philip H. Derse, DS Associates, 979 Jonathan Dr, Madison, WI 53713

Sodium
Edgar R. Elkins, National Food Processors Association, 1133 20th St, NW, Washington, DC 20036

Thiamine Assay, Enzyme and Column Packing Reagents
Wayne Ellefson, Raltech Scientific Services, Box 7545, Madison, WI 53707

Vitamin A in Foods and Feeds
James N. Thompson, Health and Welfare Canada, Health Protection Branch, Ottawa, Ontario, Canada K1A 0L2

Vitamin D
Ellen J. de Vries, Duphar B. V., Research Department 30, PO Box 2, Weesp, The Netherlands

Vitamin E in Foods and Feeds
James P. Clark and Francis Amore, Henkel Corp., 2010 E Hennepin Ave, Minneapolis, MN 55413

Vitamin E in Pharmaceuticals (Gas Chromatography)
Alan J. Sheppard, Food and Drug Administration, Division of Nutrition, Washington, DC 20204

Vitamin K
S. A. Barnett, Mead Johnson & Co., 2404 Pennsylvania Ave, Evansville, IN 47721

COMMITTEE E

Gerald Myrdal (State Department of Agriculture, 4702 University Ave, Madison, WI 53705), *Chairman;* Richard L. Ellis (U.S. Department of Agriculture, Food Safety and Inspection Service, Washington, DC 20250); Kenneth Helrich (Rutgers University—Cook College, Department of Entomology—Economic Zoology, New Brunswick, NJ 08903); William A. Steller (American Cyanamid Co., PO Box 400, Princeton, NJ 08540); Jerry A. Burke (Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204); Richard Schmidt (Environmental Protection Agency, Office of Pesticide Programs, Washington, DC 20460); Wendell F. Phillips (Campbell Soup Co., Campbell Pl, Camden, NJ 08151); Henry F. Enos (Environmental Protection Agency, Pesticide Research Laboratory, Gulf Breeze, FL 32561); Bartholomew Puma (Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204), *Secretary;* Richard H. Albert (Food and Drug Administration, Division of Mathematics, Washington, DC 20204) *Statistical Consultant*

INDUSTRIAL PROCESS WASTE

Referee: David Friedman, Environmental Protection Agency, Office of Solid Waste, Washington, DC 20460

Bioassays

Inorganic Analytes

Organic Analytes
James Poppiti, 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

Sampling

METALS AND OTHER ELEMENTS

Referee: Kenneth Boyer, Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Atomic Absorption
Milan Ihnat, Agriculture Canada, Chemistry and Biology Research Institute, Cttawa, Ontario, Canada, K1A 0C5

Cadmium and Lead in Earthenware
Benjamin Krinitz, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Graphite Furnace—Atomic Absorption Spectrophotometry
Robert W. Dabeka, Health and Welfare Canada, Health Protection Branch, Ottawa, Ontario, Canada K1A 0L2

Emission Spectrochemical Methods
Fred L. Fricke, Food and Drug Administration, 1141 Central Pkwy, Cincinnati, OH 45202

Fluorine
Robert W. Dabeka

Hydride Generating Techniques
Stephen G. Capar, Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Mercury
Walter Holak, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Methyl Mercury in Fish and Shellfish
Susan Hight, Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Multielement Analysis of Infant Food Formulas
Ronald F. Suddendorf, Food and Drug Administration, Division of Nutrition, Washington, DC 20204

Multimetal Residues by Resin Column Separations
Richard A. Baetz, Food and Drug Administration, 3032 Bryan St, Dallas, TX 75204

Multielement Determination after Closed System Digestion
Walter Holak

Polarography
Raymond J. Gajan, Sr, Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Separation Techniques for Trace Elements in Foods
Richard A. Baetz
John Jones, Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Tin
Edgar R. Elkins, Jr, National Food Processors Association, Chemistry Division, 1133 20th St NW, Washington, DC 20036

Voltammetric Methods
Eric Zink, Environmental Sciences Associates, 45 Wiggins Ave, Bedford, MA 01730

MULTIRESIDUE METHODS (INTERLABORATORY STUDIES)

Referee: Paul E. Corneliussen, Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Comprehensive Multiresidue Methodology
Jerry E. Froberg, Food and Drug Administration, 1521 W Pico Blvd, Los Angeles, CA 90015
Leon D. Sawyer, Food and Drug Administration, 240 Hennepin Ave, Minneapolis, MN 55401

Organophosphorus Pesticide Residues
Ronald R. Laski, Food and Drug Administration, 599 Delaware Ave, Buffalo, NY 14202

Pollutant Phenols in Fish
Larry Smith, Fish and Wildlife Service, Columbia Natural Fisheries, Columbia, MO 65201

Whole Blood
Henry M. Stahr, Iowa State University, College of Veterinary Medicine, Ames, IA 50010

ORGANOHALOGEN PESTICIDES

Referee: Bernadette McMahon, Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Chlordane
Wilber 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

Gel Permeation Chromatographic Cleanup
James Ault, ABC Laboratories, 7200 E ABC Lane, Box 1097, Columbia, MO 65205

Chlorobenzilate and Bromopropylate

Chlorophenoxy Alkyl Acids

Ethylene Oxide and its Chlorohydrin
A. R. Stemp, Kraft Co., 801 Waukegan Rd, Glenview, IL 60025

Fenvalerate
Terry D. Spittler, Cornell University, NY State Agricultural Experiment Station, Geneva, NY 14456

Fumigants
James L. Daft, Food and Drug Administration, 1009 Cherry St, Kansas City, MO 64106

Inorganic Bromides in Grains
King T. Zee, Environmental Protection Agency, Benefits and Field Studies Division, Beltsville, MD 20705

Kepone
Francis D. Griffith, Jr, Division of Consolidated Laboratory Services, Richmond, VA 23219

Low Moisture-High Fat Samples (Extraction Procedure)
Leon D. Sawyer, Food and Drug Administration, 240 Hennepin Ave, Minneapolis, MN 55401

Miniaturization of Multiresidue Methods
D. Ronald Erney, Food and Drug Administration, 1560 E Jefferson Ave, Detroit, MI 48207

Pentachlorophenol
George Yip, Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Pentachlorophenol in Animal and Poultry Tissue
Douglas Gillard, U.S. Department of Agriculture, Food Safety and Inspection Service, Beltsville, MD 20705

Permethrin

Polychlorinated Biphenyls (PCBs)
Larry L. Needham, Centers for Disease Control, 1600 Clifton Rd, Atlanta, GA 30333

Resmethrin
Calvin Corbey, Environmental Protection Agency, Benefits and Field Studies Division, Beltsville, MD 20705

Root Absorbed Residues (Extraction Procedure)

Tetradifon, Endosulfan, and Tetrasul
Lawrence R. Mitchell, Food and Drug Administration, 60 Eighth St NE, Atlanta, GA 30309

Toxaphene
Larry G. Lane, Mississippi State Chemical Laboratory, Box CR, Mississippi State, MS 39762

ORGANONITROGEN PESTICIDES

Referee: W. H. Newsome, Health and Welfare Canada, Food Research Division, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2

Anilazine

Benzimidazole-Type Fungicides
Mikio Chiba, Agriculture Canada, Vineland Station, Ontario, Canada L0R 2E0

Captan and Related Fungicides
Dalia Gilvydis, Food and Drug Administration, 1560 E Jefferson Ave, Detroit, MI 48207

Carbamate Insecticides (Gas Chromatography)
Roderick W. Young, Virginia Polytechnic Institute, Department of Biochemistry and Nutrition, Blacksburg, VA 24061

Carbamate Insecticides (Liquid Chromatography)
Richard Krause, Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Carbofuran

Chlorothalonil

Daminozide

Dinitro Compounds
Richard Krause

Diquat and Paraquat
Brian Worobey, Health and Welfare Canada, Food Research Division, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2

Dithiocarbamates, General Residue Methods

Maleic Hydrazide

Organotin in Fungicides

Sodium o-Phenylphenate

Substituted Urea

Thiocarbamate Herbicides

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s-Triazines

Trifluralin

ORGANOPHOSPHORUS PESTICIDES

Referee: Keith A. McCully, Health and Welfare Canada, Field Operations Directorate, Ottawa, Ontario, Canada K1A 1B7

Azinphos-Methyl

Confirmation Procedures
Bill Lee, Inland Waters Directorate, Water Quality Branch, 867 Lakeshore Rd, PO Box 5050 Burlington, Ontario, Canada L7R 4A6

Disulfoton

Extraction Procedures

General Method for Organochlorine and Organophosphorus Pesticides

High Fat Samples
Ronald Scharfe, Agriculture Canada, Pesticide Laboratory, Ottawa, Ontario, Canada K1A 0C5

Methamidophos

Monocrotophos

Phorate

Phosphine
T. Dumas, Research Institute, University Sub Post Office, London, Ontario, Canada N6A 3K0

Soils

Sweep Codistillation
Barry Luke, Australian Government Analytical Laboratory, GPO Box 2809/AA, Melbourne, Victoria 3001, Australia

RADIOACTIVITY

Referee: Edmond J. Baratta, Food and Drug Administration, Northeast Radiological Health Laboratory, Winchester, MA 01890

Cesium-137
Edmond J. Baratta

Iodine-131
Eugene Easterly, Environmental Protection Agency, PO Box 15027, Las Vegas, NV 89114

Neutron Activation Analysis
William Stroube, National Bureau of Standards, Reactor Building 235, Washington, DC 20234

Plutonium

Radium-228
Jacqueline Michel, Research Planning Institute, 925 Gervais St, Columbia, SC 29201

Strontium-89 and -90

Tritium

WATER

Referee: Alfred S. Y. Chau, Canada Centre for Inland Waters, PO Box 5050, Burlington, Ontario, Canada L7R 4A6

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 Dube, State Laboratory of Hygiene, University of Wisconsin, 465 Henry Mall, Madison, WI 53706

Herbicides in Water and Sediment
Bill Lee, Canada Centre for Inland Waters, Box 5050, Burlington, Ontario, Canada L7R 4A6

Major Ions and Nutrients in Water
Larry K. Bailey, Conoco, Inc., PO Box 1267, Ponka City, OK 74603

Organohalogen Pesticides in Water
Marie Siewierski, Rutgers University, Cook College, New Brunswick, NJ 08903

Organophosphorus Pesticides in Water

Triazine Herbicides in Water

COMMITTEE F

Paris M. Brickey (Food and Drug Administration, Division of Microbiology, Washington, DC 20204), *Chairman;* K. Rayman (Health and Welfare Canada, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2); 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 (Wisconsin Dept of Agriculture, 4702 University Ave, Madison, WI 53705); Michael H. Brodsky (Ministry of Health, Laboratory Services Branch, Toronto, Ontario, Canada M5W 1R5); Donald Mastrorocco (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*

ANALYTICAL MYCOLOGY OF FOODS AND DRUGS

Referee: Stanley M. Cichowicz, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Baseline Mold Counts by Blending
Ruth Bandler, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Chemical Methods for Detecting Mold
Ruth Bandler

***Geotrichum candidum* Morphology**
Sylvia Y. Yetts, Food and Drug Administration, 3032 Bryan St, Dallas, TX 75204

Geotrichum Mold in Canned Fruits, Vegetables, and Fruit Juices
Stanley M. Cichowicz

Geotrichum Mold in Frozen Fruits and Vegetables
Jane Kaminski, Food and Drug Administration, United Nations Plaza, San Francisco, CA 94102

Howard Mold Counting, Use of Widefield Eyepiece
Roseamond J. Scott, Superior Laboratory Services, Route 4, Box 245, Portland, IN 47371

Howard to Viable Mold Counts of Frozen Fruits and Vegetables, Comparison
Maria P. Chaput, Food and Drug Administration, 585 Commercial St, Boston, MA 02109

Microscopic Appearance of Mold Hyphae, Effect of Freezing
Charles N. Roderick, Food and Drug Administration, 3032 Bryan St, Dallas, TX 75204

Microscopic Mold Count Methods, Use of Compound Microscope
Don Vail, Jr, Food and Drug Administration, 1182 W Peachtree St, NW, Atlanta, GA 30309

Microscopic Mold Counts, Effects of Interfering Plant Material

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Mold in Spices
Karan L. Repsher, Food and Drug Administration, 900 Madison Ave, Baltimore, MD 21201

Molds and Yeasts in Beverages
Stanley M. Cichowicz

Standardization of Plant Tissue Concentrations for Mold Counting
Stanley M. Cichowicz

Tomato Products, Refractive Index
Jerome La Greca, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Tomato Rot Fragment Count
Gerald E. Russell, Food and Drug Administration, 1560 Jefferson Ave, Detroit, MI 48207

DISINFECTANTS

Referee: Reto Engler, Environmental Protection Agency, Office of Pesticide Programs, Registration Division, Washington, DC 20460

Antimicrobial Agents Used by Laundries on Fabrics and Materials
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

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

Virucide Tests
Dale Fredell, Economics Laboratory, Osborne Bldg, St. Paul, MN 55102

EXTRANEOUS MATERIALS IN FOODS AND DRUGS

Referee: John S. Gecan, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Botanical Drugs, Adulteration by Foreign Plant Materials
Frank D'Amelio, Bio Botanica, 2 Willow Park Center, Farmingdale, NY 11735

Botanicals
Arnold E. Schulze and Marvin Nakashima, Food and Drug Administration, Division of Microbiology, Washington, DC 20204
Joseph A. McDonnell, Food and Drug Administration, 1521 W Pico Blvd, Los Angeles, CA 90015
Harriett R. Gerber, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Brine Extractions, Techniques
Clarence C. Freeman, Food and Drug Administration, 4298 Elysian Fields Ave, New Orleans, LA 70122

Chocolate Products
Donald A. Mastrorocco, Jr, Hershey Chocolate Co., Hershey, PA 17033

Cocoa Powder and Press Cake
C. Robert Graham, Lancaster Laboratories, Inc., 2425 New Holland Pike, Lancaster, PA 17601

Fecal Sterols
Ruth Bandler, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Food Supplement Tablets
Charles E. Highfield, Health and Welfare Canada, Health Protection Branch, 2301 Midland Ave, Toronto, Ontario, Canada M1P 4R7

Grains, Whole, Cracking Flotation Methods
Richard Trauba, Food and Drug Administration, 240 E Hennepin Ave, Minneapolis, MN 55401

Insect Excreta in Flour
Raymond Galacci, Food and Drug Administration, 900 Madison Ave, Baltimore, MD 21201

Isolation of Extraneous Filth from Dehydrated Vegetable Products
Francis J. Farrell, Thomas J. Lipton, Inc., 800 Sylvan Ave, Englewood Cliffs, NJ 07632

Meats, Processed
Phillip Alioto, Wisconsin Department of Agriculture, 4702 University Ave, Madison, WI 53705

Mite Contamination Profiles and Characterization of Damage to Foods
Diane Peace, Health and Welfare Canada, Bureau of Microbiological Hazards, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2

Mites in Stored Foods
Jack L. Boese, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Mushroom Products, Dried
Jack L. Boese
Alan R. Olsen, Food and Drug Administration, 1521 W Pico Blvd, Los Angeles, CA 90015

Mushrooms, Canned
Russell G. Dent, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Particulates in Large-Volume Parenterals
Gordon Oxborrow, Food and Drug Administration, Minneapolis Center for Microbiological Investigations, 240 Hennepin Ave, Minneapolis, MN 55401

Performance Evaluation of Methods for Filth
Jack L. Boese
Russell G. Dent
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
Alan R. Olsen

Soluble Insect and Other Animal Filth
George P. Hoskin and Harriett R. Gerber, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Spices
Susan M. Brown, McCormick & Co., Inc., Hunt Valley, MD 21031

Spirulina
John S. Gecan

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

FOOD MICROBIOLOGY

Referee: Wallace H. Andrews, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Automated Methods for Food and Cosmetics

***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 and Stanley M. Harmon, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Campylobacter Species
Chong Park, Health and Welfare Canada, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2

Canned Foods
Cleve B. Denny, National Food Processors Association, 1401 New York Ave, NW, Washington, DC 20005

***Clostridium botulinum* and its Toxin, Detection**
Donald A. Kautter, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

***Clostridium perfringens*, Isolation and Enumeration**
Stanley M. Harmon

Endotoxins by Limulus Amebocyte Lysate
Christine Twohy, Food and Drug Administration, 240 Hennepin Ave, Minneapolis, MN 55401

Enteropathogenic *Escherichia coli*, Direct Fluorescent Antibody Procedure for Detection

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***Escherichia coli* and Other Coliforms**
Ira J. Mehlman, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Genetic Methods for Detection of Bacterial Pathogens
Walter Hill, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Helium Leaks, Canned Foods
James E. Gilchrist and Ulysses S. Rhea, Food and Drug Administration, Minneapolis Center for Microbiological Investigations, 240 Hennepin Ave, Minneapolis, MN 55401

Hydrophobic Grid Membrane Filter Methods
Phyllis Entis, QA Laboratories, Ltd, 135 The West Mall, Toronto, Ontario, Canada M9C 1C2

Identification of Microorganisms by Biochemical Kits
Nelson Cox, U.S. Department of Agriculture, Southern Regional Research Center, Box 5677, Athens, GA 30613

Parasitology
Richard A. Rude, Food and Drug Administration, Minneapolis Center for Microbiological Investigations, 240 Hennepin Ave, Minneapolis, MN 55401
Robert Barnard, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Salmonella
Paul L. Poelma, Food and Drug Administration, Division of Microbiology, Washington, DC 20204
Dean Wagner, Food and Drug Administration, Minneapolis Center for Microbiological Investigations, 240 Hennepin Ave, Minneapolis, MN 55401

Somatic Cell, Automated Optical Counting Method
Wesley N. Kelley, University of South Dakota, State Chemical Laboratory, Vermillion, SD 57609

Somatic Cell, Fossomatic Counting Method
R. D. Mochrie, North Carolina State University, Animal Science Department, Raleigh, NC 27650

Spore-Formers and Non-Spore-Formers in Low Acid Foods
Mary L. Schafer, Food and Drug Administration, 1090 Tusculum Ave, Cincinnati, OH 45226

Staphylococcus
Gayle Lancette

***Staphylococcus* Toxin**
Reginald W. Bennett

Sugars
Cleve B. Denny

***Vibrio cholerae* and Detection of its Toxins**

Vibrio parahaemolyticus
Eugene H. Peterson, Food and Drug Administration, Minneapolis Center for Microbiological Investigations, 240 Hennepin Ave, Minneapolis, MN 55401

Virology and Animal Oncology
Edward P. Larkin, Food and Drug Administration, Division of Microbiology, 1090 Tusculum Ave, Cincinnati, OH 45226

Yeast, Molds, and Actinomycetes
Philip B. Mislivec, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Yersinia enterocolitica
James S. Cholensley and Sallie McLaughlin, Food and Drug Administration, 240 Hennepin Ave, Minneapolis, MN 55401

DRUG AND DEVICE RELATED MICROBIOLOGY

Referee: Gordon Oxborrow, Food and Drug Administration, Minneapolis Center for Microbiological Investigations, 240 Hennepin Ave, Minneapolis, MN 55401

Limulus Amebocyte Lysate Tests for Endotoxin

Testing and Standardization of Biological Indicators
Robert Berube, Medical Products Labs, 3M Center, St. Paul, MN 55144

Testing Biological Sterility Indicators
Gordon Oxborrow

Sterility Testing of Medical Devices
Daniel A. Quagliaro, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

COMMITTEE G

Rodney J. Noel (Purdue University, Department of Biochemistry, West Lafayette, IN 47907), *Chairman;* Glenn M. George (Salsbury Laboratories, 2000 Rockford Rd, Charles City, IA 50616); Alexander MacDonald (Hoffmann-La Roche Co., 340 Kingsland St, Nutley, NJ 07110); Howard Casper (North Dakota State University, Veterinary Diagnostic Laboratory, Fargo, ND 58102); Harold Thompson (National Center for Toxicological Research, Jefferson, AR 72079); Paul B. Ferrara (Division of Consolidated Laboratory Services, Bureau of Product Testing, Richmond, VA 23219); Wilbert Shimoda (Food and Drug Administration, U.S. Customhouse, Denver, CO 80202); Patricia Bulhack (Food and Drug Administration, Division of Color Technology, Washington, DC 20204); *Secretary;* Ruey Chi (Food and Drug Administration, Division of Mathematics, Washington, DC 20204); *Statistical Consultant*

ANTIBIOTICS

Referee: Stanley E. Katz, Rutgers University, Department of Biochemistry and Microbiology, New Brunswick, NJ 08903

Affinity Quantitative Determination of Penicillin in Milk
Stanley E. Charm, Tufts Medical School, Enzyme Center, 136 Harrison Ave, Boston MA 02111

Bacitracin in Feeds
Carol Harpster, AL Laboratories, 185 LeGrand Ave, Northvale, NJ 07647
John B. Gallagher, International Minerals & Chemicals Corp., 1331 S First St, Terre Haute, IN 47808

Bacitracin in Premixes and Foods (Chemical Method)
John B. Gallagher

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 Medicine, Beltsville, MD 20705

Chlortetracycline in Feeds

Cup Plate System for Antibiotic Analysis
Virginia A. Thorpe, State Department of Agriculture, 1615 S Harrison Rd, East Lansing, MI 48823

Design and Computerization of Microbiological Assays
Peter Kahn, Rutgers University, Cook College, Department of Biochemistry and Microbiology, New Brunswick, NJ 08903

Erythromycins

Lasalocid in Feeds (LC Method)
Edward Waysek, Hoffmann-La Roche, Inc., 340 Kingsland St, Nutley, NJ 07110

Lasalocid Sodium in Feeds (Microbiological Assay)
Jacob M. Scheiner, Hoffmann-La Roche, Inc, Food and Agricultural Products, 340 Kingsland St, Nutley, NJ 07110

Lincomycin in Feeds
A. William Neff, The Upjohn Co., Agricultural Division, Kalamazoo, MI 49001

Monensin
Robert E. Scroggs, Elanco Products Co., Box 1750, Indianapolis, IN 46206

Oxytetracycline
Dorothy M. Brennecke, 3981 Dover Pl, St. Louis, MO 63116

Qualitative Delvo-test for β-Lactam Residues in Milk
Wesley N. Kelley, State Chemical Laboratory, University of South Dakota, Vermillion, SD 57609

Qualitative Determination of β-Lactam Antibiotic Residues in Milk
James Messer, Food and Drug Administration, 1090 Tusculum Ave, Cincinnati, OH 45226

Quantitative Determination of β-Lactam Antibiotic Residues in Milk
Ronald Case, Kraft Foods, Kraft Ct, Glenview, IL 60025
Roy Ginn, Dairy Quality Control Institute, Inc., 2353 N Rice St, St. Paul, MN 55113

Screening Procedures for Antibiotics in Feeds
Mary L. Hasselberger, Deprtment of Agriculture, Laboratory Division, 3703 S 14th St, Lincoln, NE 68502

Statistics of Microbiological Assay
John R. Murphy, Elanco Products Co., PO Box 1750, Indianapolis, IN 46206

Tetracyclines in Tissues (Chromatographic Assay)
Ray B. Ashworth, U.S. Department of Agriculture, Food Safety and Inspection Service, Beltsville, MD 20705

Tetracyclines in Tissues (Microbiological Assay)
Stanley E. Katz

Turbidimetric Virginiamycin Assay
Dorothy M. Brennecke

Tylosin
Paul Handy, Eli Lilly & Co., Box 708, Greenfield, IN 46140

BIOCHEMICAL METHODS

Referee: John O'Rangers, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857

Aminoglycosides in Animal Tissue

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17 β-Estradiol and Diethylstilbestrol in Tissues (Immunochemical Methods)

Hormones in Tissues (Immunospecific Affinity Chromatography)

Hybridoma-Monoclonal Antibodies
Richard Meyer, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Immunochemical Species Identification of Meat
David Berkowitz, U.S. Department of Agriculture, Serology Branch, Beltsville, MD 20705

Performance Evaluation Methods for Non-RIA Procedures Measuring Human Chorionicgonadatropin
Lillian Gill, Food and Drug Administration, 900 Madison Ave, Baltimore, MD 21201

Performance Evaluation Protocols for Clinical, Chemical and Immunochemical Diagnostic Products

---

Steroid Quantitation (Enzymatic Methods)

Sulfa Drugs in Animal Tissues (Immunoassay Procedures)

COLOR ADDITIVES

Referee: Keith S. Heine, Food and Drug Administration, Division of Color Technology, Washington, DC 20204

Arsenic and Heavy Metals
Catherine Bailey, Food and Drug Administration, Division of Color Technololgy, Washington, DC 20204

Atomic Absorption in Color Analysis
Lueangier Moten, Food and Drug Administration, Division of Color Technology, Washington, DC 20204

Cosmetics
Sandra Bell, Food and Drug Administration, Division of Color Technology, Washington, DC 20204

Color in Candy and Beverages
Mary Young, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Color in Drugs
Edward Woznicki, Colorcon Inc., Moyer Blvd, West Point, PA 19486

Color in Nonfrozen Dairy Desserts
Desire L. Massart, Virje Universiteit Brussel, Pharmaceutical Institute, Laarbeeklaan 103, B-1090 Brussels, Belgium

Color in Other Foods
Nicholas Adamo, Food and Drug Administration, Division of Color Technology, Washington, DC 20204

FD&C Red No. 4 in Maraschino Cherries
Ronald E. Draper, Food and Drug Administration, 50 United Nations Plaza, San Francisco, CA 94102

Liquid Chromatography
Elizabeth A. Cox, Food and Drug Administration, Division of Color Technology, Washington, DC 20204

Inorganic Salts
Wallace S. Brammell, Food and Drug Administration, Division of Color Technology, Washington, DC 20204

Intermediates, Uncombined, in Certifiable Triphenylmethane Colors

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Intermediates, Uncombined, in Certifiable Water-Soluble Azo Colors
Daniel M. Marmion, Allied Chemical Corp., 1051 S Park Ave, Buffalo, NY 14240

Subsidiary Colors in Certifiable Color Additives
John E. Bailey, Food and Drug Administration, Division of Color Technology, Washington, DC 20204

X-Ray Fluorescence Spectroscopy
Catherine Bailey

COSMETICS

Referee: Ronald L. Yates, Food and Drug Administration, Division of Cosmetics Technology, Washington, DC 20204

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

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DRUG RESIDUES IN ANIMAL TISSUES

Referee: Charlie J. Barnes, Food and Drug Administration, Division of Chemistry and Physics, Washington, DC 20204

Benzimidazole
Leon LeVan, Hazleton Raltech, 3301 Kinsman Blvd, Madison, WI 53704

Screening Methods
Henry R. Cook, U.S. Department of Agriculture, Food Safety and Inspection Service, Beltsville, MD 20705

Steroids

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Sulfa Drugs

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Sulfonamide

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Tiamulin

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DRUGS IN FEEDS

Referee: Mary L. Hasselberger, Department of Agriculture, Laboratory Division, Lincoln, NE 68502

Amprolium

———

Arsanilic Acid

———

Carbadox
Mark A. Litchman, Pfizer Inc., Agricultural Division, 1107 S Missouri St, Lee's Summit, MO 64063

1,2-Dimethyl-5-nitroimidazole (Dimetridazole)
Larry J. Frahm, Salsbury Laboratories, Research Division, Charles City, IA 50616

Ethopabate

———

Ethylenediamine Dihydroiodide
Gary Ross, North Dakota State Laboratories, 2635 E Main St, Bismarck, ND 58501

Furazolidone and Nitrofurazone
Robert E. Smallidge, Purdue University, Department of Biochemistry, West Lafayette, IN 47907

Melengestrol Acetate
Raymond Davis, The Upjohn Co., Henrietta St Labs, Kalamazoo, MI 49001

Morantel Tartrate
James E. Peters, Pfizer, Inc., 1107 S Missouri 291, Lee's Summit, MO 64063

Nifursol
Glenn M. George

Ormetoprim
Edward Waysek

Phenothiazine
Chris Foret, West Argo Chemical, 501 Santa Fe, Kansas City, MO 64015

Pyrantel Tartrate
James A. Braswell, Pfizer, Inc., Agriculture Division, 1107 S Missouri St, Lee's Summit, MO 64063

Roxarsone
Glenn M. George

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 Department of Agriculture, 4000 Reedy Creek Rd, Raleigh, NC 27607

FORENSIC SCIENCES

Referee: ———

ABO Blood Typing
Henry C. Lee, State Police Forensic Science Laboratory, Box A-D, Amity Station, New Haven, CT 06516

Biological Fluids (Immunoelectrophoresis)
James D. Hauncher, Michigan State Police, Scientific Laboratory, 42145 W Seven Mile Rd, Northville, MI 48167

Blood
Ralph Plackenhorn, Pennsylvania State Police, Laboratory Division, PO Box 38, Greensburg, PA 15601

Blood Stains, ABH Typing

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Blood Stains, Species Determination

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Bomb Residues
William Kinard, Department of the Treasury, Bureau of Alcohol, Tobacco and Firearms, 1401 Research Blvd, Rockville, MD 20850

Documents

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Fingerprints
Charles M. Conner, Department of the Treasury, Bureau of Alcohol, Tobacco and Firearms, 550 Main St, Cincinnati, OH 45202

Firearms
James Booker, State Crime Laboratory, 591 Hathaway Bldg, PO Box 1895, Cheyenne, WY 82001

Flammable Fluids
Phillip Wineman, Department of the Treasury, Bureau of Alcohol, Tobacco and Firearms, 1401 Research Blvd, Rockville, MD 20850

Gunshot Residue
William Kinard

Gunshot Residue by Atomic Absorption Spectroscopy
Kent A. Oakes, State Regional Crime Laboratory, 15725 W Ryerson Rd, New Berlin, WI 53151

Hair Examination
Walter C. McCrone, Walter C. McCrone Associates, 2820 S Michigan Ave, Chicago, IL 60616

Infrared Spectroscopy
Kent A. Oakes

Microscopic Methods and Glass Products
Walter C. McCrone

Paints, Pyrolysis-Gas Chromatographic Methods

Safe Insulation

Serial Number Restoration (Chemical Etching Techniques)

Soils, Geological Analysis
R. C. Murray, University of Montana, Office of the Associate Vice-President for Research and Dean of the Graduate School, Missoula, MT 59801

Voice Print Identification
Lonnie L. Smrkovski, Michigan State Police, 714 S Harrison Rd, East Lansing, MI 48823

MICROBIAL MUTAGENICITY TESTING

Referee: Frederick Deserres, National Institute of Environmental Protection, Box 12233, Research Triangle Park, NC 27709

Prophage Induction
John H. S. Chen, Environmental Protection Agency, Beltsville, MD 20705

VETERINARY ANALYTICAL TOXICOLOGY

Referee: P. Frank Ross, U.S. Department of Agriculture, National Veterinary Services Laboratory, Ames, IA 50010

Arsenic in Animal Tissue
Tracy Hunter, Division of Consolidated Laboratory Services, 1 N 14th St, Richmond, VA 23219

Cholinesterase
Paula Martin, Iowa State University, Veterinary Diagnostic Laboratory, Ames, IA 50010

Copper in Animal Tissue
David Osheim, U.S. Department of Agriculture, National Veterinary Services Laboratory, Ames, IA 50010

Fluoride in Animal Tissue
David Osheim

Lead in Animal Tissue
R. J. Everson, Purdue University, School of Veterinary Medicine, West Lafayette, IN 47907

Molybdenum
Tracy Hunter

Monensin
Ronda A. Moore, Iowa State University, College of Veterinary Medicine, Ames, IA 50010

Multiple Anticoagulant Screening
John D. Reynolds, Animal Disease Laboratory, 235 N Walnut St, Centralia, IL 62801

Multielement Analysis by ICP
Emmett Beazelton, Michigan State University, Department of Pharmacology and Toxicology, East Lansing, MI 48824

Nitrates and Nitrites
Norman R. Schneider and Michael P. Carlson, Veterinary Diagnostic Center, Department of Veterinary Science, Lincoln, NE 68583

Poisonous Plants
George Rottinghaus, University of Missouri, College of Veterinary Medicine, Columbia, MO 65211

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

CHANGES IN OFFICIAL METHODS OF ANALYSIS

This report gives the newly adopted methods by abbreviated titles, lists those methods in which revisions have been made, and summarizes other pertinent official actions. Details of the new methods and revisions will appear in the 14th edition of *Official Methods of Analysis,* to be published in October 1984. In some cases the details of revisions are given in the reports of the appropriate Committees, A, B, C, D, E, F, and G, and General Referee reports in this issue of the *Journal,* and in Associate Referee reports in this and prior and subsequent issues. Where revisions do not appear, the Association will supply details of specific methods on request up to the time of publication of the 14th edition of *Official Methods of Analysis.*

The changes given below in the Methods of the Association, made at the 97th Annual International Meeting, October 3–6, 1983, become effective, as provided in Article VII, Section 6, of the Bylaws, on the thirtieth day from the date of publication of this *Journal* issue. Section numbers refer to the 13th edition, 1980, and its supplements, unless otherwise noted.

1. AGRICULTURAL LIMING MATERIALS

No additions, deletions, or changes.

2. FERTILIZERS

The official first action liquid chromatographic method for the determination of urea and water-soluble methyleneureas in fertilizers, **2.D01–2.D05,** was adopted official final action.

3. PLANTS

The official first action gas chromatographic method for nicotine on Cambridge filter pads, **3.158–3.161,** was adopted official final action.

4. DISINFECTANTS

No additions, deletions, or changes.

5. HAZARDOUS SUBSTANCES

No additions, deletions, or changes.

6. PESTICIDE FORMULATIONS

(1) The following official first action methods were adopted official final action:

(a) CIPAC–AOAC liquid chromatographic method for diflubenzuron formulations, **6.D09–6.D15,** JAOAC **66,** 312(1983).

(b) CIPAC-AOAC gas chromatographic method for endosulfan formulations, **6.D16–6.D21,** JAOAC **66,** 999(1983).

(c) Liquid chromatographic method for glyphosate technical and formulations, **6.D22–6.D27,** JAOAC **66,** 1214(1983).

(d) Liquid chromatographic method for fensulfothion formulations, **6.D28–6.D32,** JAOAC **66,** 810(1983).

(2) The following methods were adopted official first action:

(a) Official final action procedures for sampling fertilizers, **2.001–2.002,** adopted for fertilizer–pesticide mixtures.

(b) Gas chromatographic method for γ-benzene hexachloride (lindane) in technical formulations and in shampoos and lotions.

(c) Liquid chromatographic method for combinations of 2,4-D, dicamba, and MCPP amine salts.

(d) Interim liquid chromatographic method for dalapon formulations, JAOAC **66,** 1390(1983).

(e) Gas chromatographic method for fluazifop-butyl formulations.

(f) Liquid chromatographic method for benomyl formulations (method determines benomyl equivalent of benomyl and methyl-2-benzimidazole carbamate present).

(g) Liquid chromatographic method for methiocarb formulations.

(h) Liquid chromatographic method for propoxur formulations.

(i) Gas chromatographic method for metribuzin formulations (limited to glass columns and flame ionization detectors).

(j) Interim gas chromatographic method for triphenyltin formulations.

(3) The following methods were declared surplus:

(a) Official first action AOAC–CIPAC gas chromatographic method for captan formulations, **6.215–6.219.**

(b) Official first action volumetric, **6.388–6.394,** and colorimetric, **6.395–6.399,** methods for parathion formulations.

7. ANIMAL FEED

A copper catalyst method for determining crude protein was adopted official first action.

8. BAKING POWDERS AND BAKING CHEMICALS

No additions, deletions, or changes.

9. BEVERAGES: DISTILLED LIQUORS

No additions, deletions, or changes.

10. BEVERAGES: MALT BEVERAGES AND BREWING MATERIALS

The interim gas chromatographic method for the determination of ethanol in beer (ASBC method) was adopted official first action.

11. BEVERAGES: WINES

(1) The following official first action methods were adopted official final action:

(a) Gas chromatographic method for coumarin in wine, **11.079–11.080.**

(b) Gas chromatographic method for β-asarone in wine, **11.081–11.083.**

(2) The official first action gas chromatographic method for the determination of ethanol in wine, **11.D01–11.D02,** was revised to specify the use of only one column and to change the internal standard solution.

12. BEVERAGES: NONALCOHOLIC AND CONCENTRATES

The official first action liquid chromatographic method for the determination of benzoate, caffeine, and saccharin in soda beverages, **12.050–12.253,** was adopted official final action.

13. CACAO BEAN AND ITS PRODUCTS

No additions, deletions, or changes.

14. CEREAL FOODS

The official first action liquid chromatographic method for the determination of fructose, glucose, sucrose, and maltose in pre-sweetened cereal, **14.C01–14.C04,** was editorially revised to eliminate sodium chloride interference, JAOAC **66,** 197(1983).

15. COFFEE AND TEA

No additions, deletions, or changes.

16. DAIRY PRODUCTS

(1) The following official first action methods were adopted official final action:

(a) Phosphatase, Method V, **16.125–16.126.**

(b) Reactivated and residual phosphatase, differential test, **16.129–16.130.**

(c) Residual phosphatase in casein, **16.B01–16.B03.**

(d) Affinity quantitative determination of penicillin in milk, **16.C01–16.C05.**

(e) *Bacillus stearothermophilus* qualitative disc method II for penicillins in milk, **16.C06–16.C11.**

(f) IDF–ISO–AOAC potentiometric method for chloride in cheese, **16.D01–16.D04.**

(2) The following methods were adopted official first action:

(a) Interim method for the determination of *N*-nitrosodimethylamine in nonfat dry milk (Method I).

(b) Enzymatic ultraviolet method for measuring lactose in milk.

(3) The official final action IDF–ISO–AOAC method for total chlorides in cheese, **16.242–16.243** (repealed 1983), was deleted.

17. EGGS AND EGG PRODUCTS

No additions, deletions, or changes.

18. FISH AND OTHER MARINE PRODUCTS

The official first action potentiometric method for the determination of salt in fish, **18.036,** was adopted official final action.

19. FLAVORS

A liquid chromatographic method for the determination of sugars in licorice extracts was adopted official first action.

20. FOOD ADDITIVES: DIRECT

(1) The official first action IUPAC–AOAC liquid chromatographic method for the determination of antioxidants in fats and oils, **20.D01–20.D04,** was adopted official final action.

(2) The official final action differential pulse polarographic method for the determination of saccharin, **20.A06–20.A10,** was editorially revised to correct the first formula in **20.A10.**

21. FOOD ADDITIVES: INDIRECT

No additions, deletions, or changes.

22. FRUITS AND FRUIT PRODUCTS

No additions, deletions, or changes.

23. GELATIN, DESSERT PREPARATIONS, AND MIXES

No additions, deletions, or changes.

24. MEAT AND MEAT PRODUCTS

(1) The official first action mineral oil vacuum distillation–thermal energy analyzer method for the determination of nitrosamines in fried bacon, **24.C01–24.C07,** was adopted official final action.

(2) A dry column–thermal energy analyzer method for the determination of *N*-nitrosopyrrolidine in fried bacon was adopted official first action.

(3) The official first action titrimetric method for the determination of calcium in mechanically separated poultry and beef, **24.D01–24.D02,** was editorially revised in **24.D02** to replace the terms "broilers" and "fryers" with the terms "young chickens" and "chickens."

25. METALS AND OTHER ELEMENTS

(1) The official first action anodic stripping voltammetric method for the determination of lead in evaporated milk and fruit juice, **25.080–25.082,** was adopted official final action.

(2) The interim WHO–AOAC hot leach atomic absorption method for cadmium and lead in cookware, **25.D11–25.D17,** was adopted official first action.

26. NATURAL POISONS

No additions, deletions, or changes.

27. NUTS AND NUT PRODUCTS

No additions, deletions, or changes.

28. OILS AND FATS

(1) The following official first action methods were adopted official final action:

(a) Preparation of methyl esters—boron trifluoride method, **28.053–28.056.**

(b) AOAC–IUPAC gas chromatographic method for the determination of methyl esters of fatty acids, **28.057–28.065.**

(c) Gas chromatographic method for docosenoic acid, **28.066–28.069.**

(d) Polymers and oxidation products of heated vegetable oils, gas chromatographic method for non-elution materials, **28.070.**

(e) *cis, cis*-Methylene interrupted polyunsaturated fatty acids, **28.071–28.074.**

(f) IUPAC–AOAC method for the determination of polar components in frying fats, **28.C01–28.C08.**

(2) The interim Karl Fischer method for the determination of water in oils and fats was adopted official first action.

29. PESTICIDE RESIDUES

(1) The interim gel permeation chromatographic multiresidue method for the determination of organochlorine pesticides in poultry fat was adopted official first action.

(2) The micro-scale saponification procedure for organochlorine and organophosphorus pesticides, **29.017,** was replaced, official first action, with the micro-scale saponification procedure, **29.D06,** in the miniaturized multiresidue method, **29.D01–29.D07.** Sec. **29.D06** was then revised to refer to **29.017** (as revised).

30. SPICES AND OTHER CONDIMENTS

(1) The surplus official first action method for the determination of sucrose in prepared mustard, **30.039,** was deleted.

(2) The official first action method for the determination of extractable color in capsicums and oleoresin paprika, **30.002–30.004,** was editorially revised to delete **30.002(b),** standard color solution, and references to that section.

31. SUGARS AND SUGAR PRODUCTS

The following methods were adopted official first action:

(a) Liquid chromatographic method for the determination of lactose purity.

(b) Interim electrometric titration method for titratable acidity in corn syrups (method limited to high fructose corn syrups).

(c) Interim mass spectrometric carbon isotope ratio method for the determination of corn and cane sugars in maple syrup.

32. VEGETABLE PRODUCTS, PROCESSED

(1) The official first action method for the determination of water activity, **32.004–32.009,** was adopted official final action.

(2) A method for the determination of loss of mass on drying of quick frozen french fried potatoes was adopted official first action.

33. WATERS; AND SALT

The official first action atomic absorption method for the determination of cadmium, chromium, copper, iron, lead, magnesium, manganese, silver, and zinc in water, **33.089–33.094,** was adopted official final action. The statement on glassware in **33.090** was revised to include other than Pyrex ware and to change the cleaning procedure.

34. COLOR ADDITIVES

No additions, deletions, or changes.

35. COSMETICS

No additions, deletions, or changes.

36. DRUGS: GENERAL

(1) An ion-specific electrode method for the determination of fluoride in sodium fluoride preparations was adopted official first action.

(2) A revised sampling procedure was adopted to replace **36.003–36.005.**

37. DRUGS: ACIDIC

The following interim methods were adopted official first action:

(a) Liquid chromatographic method for amitriptyline in tablets and injectables, **37.D01–37.D05.**

(b) Liquid chromatographic method for sulfisoxazole in tablets, solutions, and ointments, **37.D06–37.D11.**

(c) Liquid chromatographic method for methocarbamol in pharmaceutical dosage forms.

38. DRUGS: ALKALOID AND RELATED BASES

A liquid chromatographic method for the simultaneous determination of pilocarpine, isopilocarpine, and pilocarpic acid was adopted official first action.

39. DRUGS: NEUTRAL

(1) The official first action differential pulse polarographic method for the determination of iodine in thyroid tablets, **39.C04–39.C07,** was adopted official final action.

(2) The interim first action liquid chromatographic method for the determination of hydrocortisone in drug substance and tablets was adopted official first action.

40. DRUGS: ILLICIT

No additions, deletions, or changes.

41. DRUGS AND FEED ADDITIVES IN ANIMAL TISSUES

The following official first action methods were adopted official final action:

(a) Gas chromatographic–mass spectrometric determination of sulfamethazine in swine tissue, **41.C01–41.C08.**

(b) Gas chromatographic method for sulfamethazine in swine tissues, **41.C09–41.C12.**

(c) Thin layer chromatographic screening method for sulfonamides in swine, turkey, and duck tissues, **41.D01–41.D06.**

42. DRUGS IN FEEDS

The official first action spectrophotometric method for the determination of nifursol in feed, **42.098–42.104,** was adopted official final action.

43. VITAMINS AND OTHER NUTRIENTS

(1) The official first action chloroform–methanol extraction method for the determination of fat in foods, **43.D01–43.D02,** was adopted official final action.

(2) The following methods were adopted official first action:

(a) Interim semiautomated fluorometric method for the determination of vitamin C in food products.

(b) Inductively coupled plasma emission spectroscopic method for calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc in infant formulas.

44. EXTRANEOUS MATERIALS: ISOLATION

No additions, deletions, or changes.

45. FORENSIC SCIENCES

No additions, deletions, or changes.

46. MICROBIOLOGICAL METHODS

(1) The following official first action methods were adopted official final action:

(a) Surface plating method for the isolation and enumeration of *Staphylococcus aureus* in food, **46.075–46.076.**

(b) Diffferentiation of members of *Bacillus cereus* group, **46.D01–46.D03.**

(2) The following methods were adopted official first action:

(a) Biological methods for detecting heat-stabile and heat-labile enterotoxins of *Escherichia coli* by mouse adrenal cell and suckling mouse assays.

(b) DNA colony hybridization method for detection of *Escherichia coli* producing the heat-labile enterotoxin.

(c) Helium leak test for detection of bacterial contamination in low-acid canned foods.

(d) Hydrophobic grid membrane filter method for detecting total coliforms, fecal coliforms, and *E. coli* in foods.

(3) The official final action method for *Salmonella,* **46.054–46.067,** was revised official first action to incorporate a modification of method for nonfat dry milk.

(4) The official final action method for *Salmonella,* **46.054–46.067,** was editorially revised to change the incubation period of lysine decarboxylase broth.

(5) In the official final action method for fecal coliforms, **46.117–46.119,** the applicability statement was editorially revised to include information on the use of medium A-1.

47. MICROCHEMICAL METHODS

No additions, deletions, or changes.

48. RADIOACTIVITY

No additions, deletions, or changes.

49. SPECTROSCOPIC METHODS

No additions, deletions, or changes.

50. STANDARD SOLUTIONS AND MATERIALS

No additions, deletions, or changes.

51. LABORATORY SAFETY

In **51.043,** delete the last sentence.

52. REFERENCE TABLES

No additions, deletions, or changes.

ERRATA AND EMENDATIONS, OFFICIAL METHODS OF ANALYSIS, AOAC

The following changes should be made in the 13th edition:

Section	*Page*	
28.093	454	Change applicability statement to read: ". . . β-sitosterol/100 g butter oil)"
46.120	850	Title: Change to read "Official First Action." The method has not been adopted final action; the status is incorrect on p. 850.

The following corrections should be made in "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **65,** March 1982:

Section	*Page*	
6.C04	452	Method title, lines 1 and 2, change to read: **"Pirimicarb (2-(Dimethylamino)-5,6-dimethyl-4-pyrimidinyl dimethylcarbamate)"**
	512	Right column, lines 33 and 34, change to read: **"2-(Dimethylamino)-5,6-dimethyl-4-pyrimidinyl dimethylcarbamate, . . ."**
39.C02	486	Change to read: "*Phosphotungstic acid (PTA) precipitating solution.*—15% PTA in 10% HCl. Dissolve 15.0 g PTA in ca 70 mL H_2O. Add 22.2 mL HCl (sp. gr. 1.19 g/mL, 37.8% HCl) and dil. to 100 mL with H_2O."
	499	**44. EXTRANEOUS MATERIALS: ISOLATION,** (1): Reinstate **44.037** for grains other than wheat.

The following corrections should be made in "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **66,** March 1983:

Section	*Page*	
2.D06	513	Right column, line 6, change 761 nm to read "671 nm."
	554	Right column, lines 24 and 25, change to read: **"2-(Dimethylamino)-5,6-dimethyl-4-pyrimidinyl dimethylcarbamate, . . ."**

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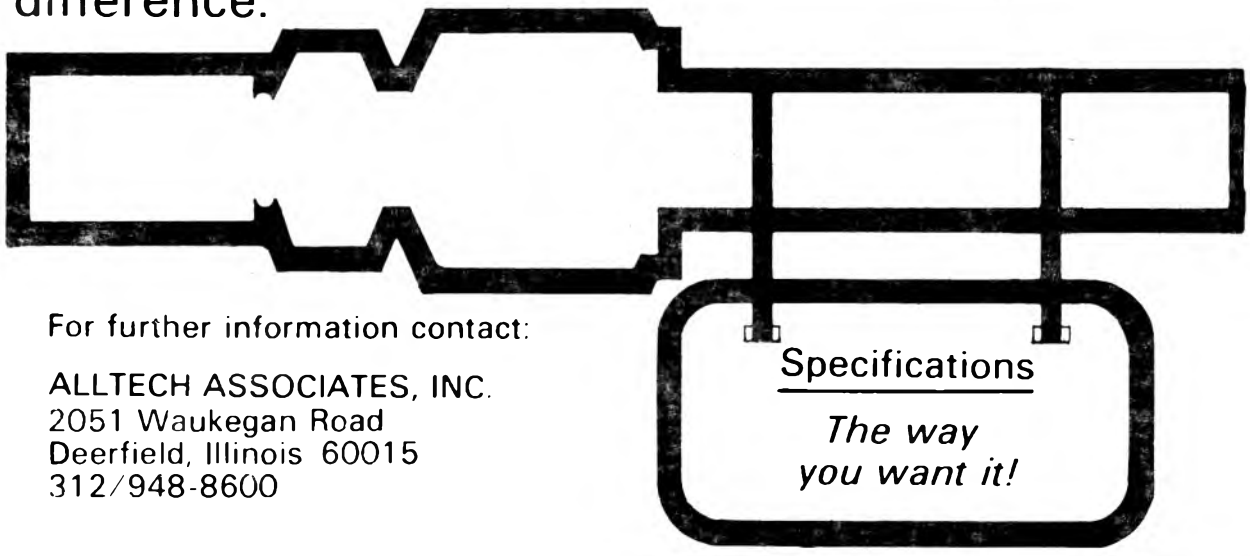

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