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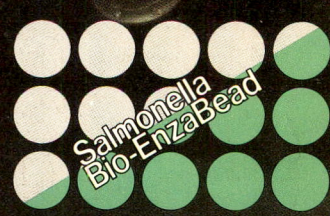

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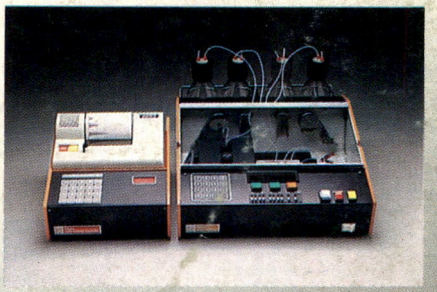

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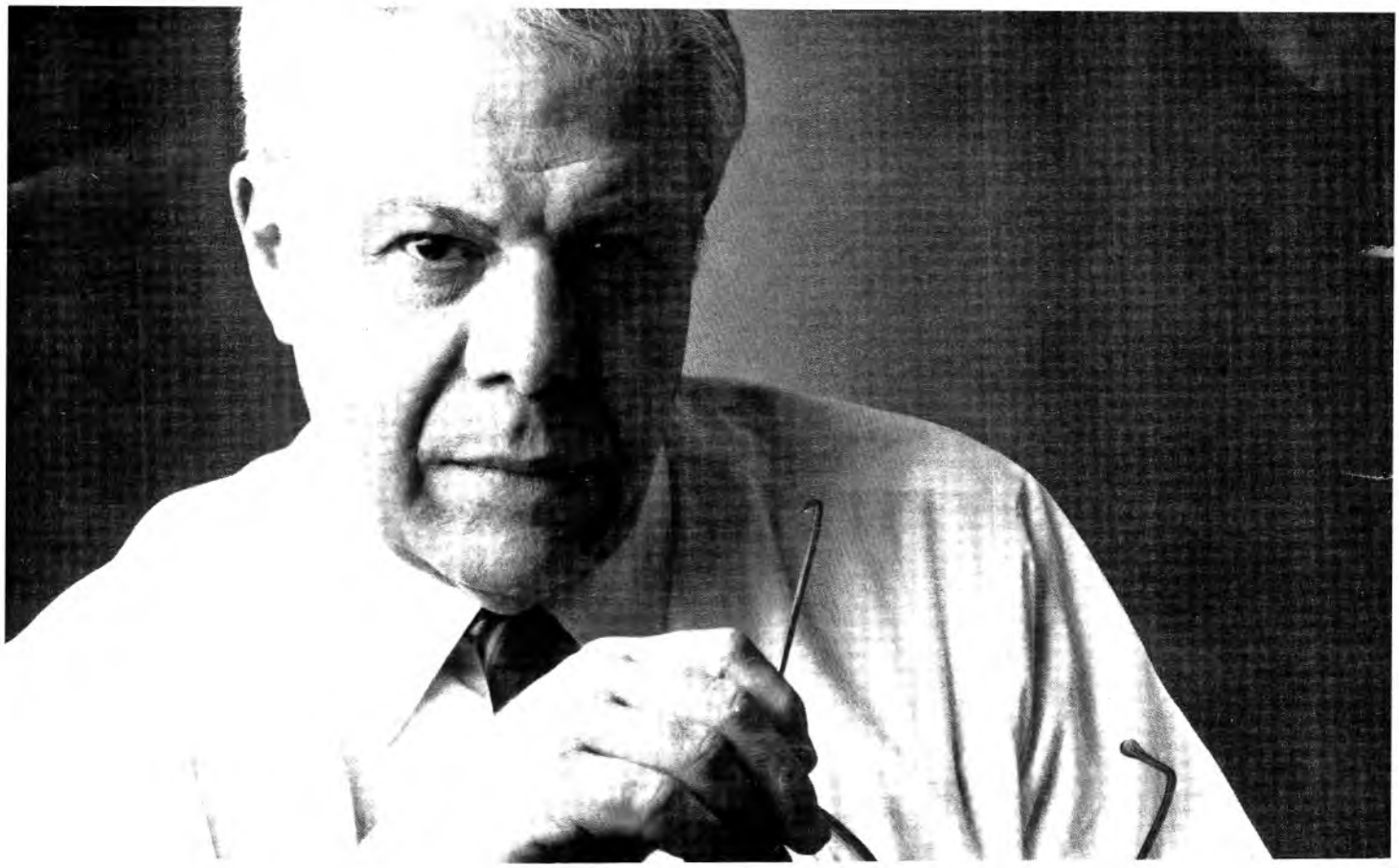

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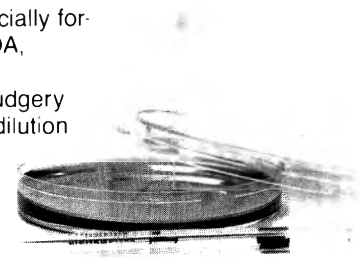

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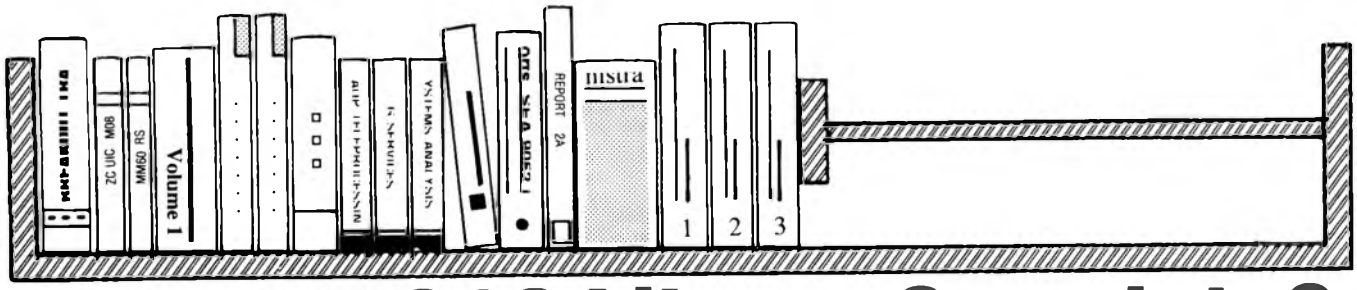

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AOAC 99th Annual International Meeting and Exposition

Approximately 1038 analytical scientists attended the AOAC 99th Annual International Meeting held October 27–31, 1985, at the Shoreham Hotel in Washington, DC.

The welcome reception Sunday evening in the Shoreham's Palladian Room featured a "preview of Arizona"—the site of the AOAC 100th annual meeting.

The meeting officially began Monday morning at the General Session with some opening remarks from the outgoing president, Richard J. Ronk (FDA, Washington, DC). AOAC Executive Director David B. MacLean followed with some brief announcements.

Wiley Award Winner Daniel P. Schwartz gave his address, "Improved Methods for Analysis of Trace Constituents in Natural Products." This was followed by the presentation of awards by Richard J. Ronk.

The Associate Referee of the Year Award went to Leon D. Sawyer (FDA, Minneapolis, MN), Committee on Residues, for his report, "Ethylene Dibromide in Grains and Grain-Based Products."

The General Referee (GR) of the Year Award went to Bernadette McMahon (FDA, Washington, DC), GR on Organohalogen Pesticides, Committee on Residues.

Fellow of the AOAC Awards went to Charlotte Brunner (FDA, Washington, DC), Edwin M. Glocker (Glenelg, MD), Prince G. Harrill (FDA, Washington, DC), Paul D. Jung (EPA, Beltsville, MD), Berhard Larsen (Fairfax, VA), John M. Newton (FDA, San Francisco, CA), Rodney J. Noel (Purdue University, West Lafayette, IN), Richard J. Ronk (FDA, Washington, DC), Leon D. Sawyer (FDA, Minneapolis, MN), Arnold E. Schulze (FDA, Washington, DC), and Donald J. Smith (FDA, Washington, DC).

Marianne Martling, Longwood, FL, was announced recipient of the 1985 AOAC Scholarship Award. Staff service awards were presented to Cecilia Cassidy (10 years service), Richard Blakely (5 years service), and Marilyn Taub (5 years service).

The following Committee members were presented certificates for outstanding services to AOAC in planning and conducting the 1985 Spring Training Workshop and Exhibition: Molly A. Ready (Co-Chairman), Virginia Gipson (Co-Chairman), James Stewart (Program Chairman), and Danny Dunn (Program Chairman). AOAC also recognized a representative for each of the regional sections.

Incoming President D. Earle Coffin (Director of the Bureau of Nutritional Sciences, Health and Welfare Canada) presented Richard Ronk with the presidential plaque and Ronk then gave his presidential address.

The 4 day meeting which followed featured various committee meetings, papers, and poster sessions, and 5 symposia: Immunotoxicity (Parts I and II), Nutritional Analyses, Safety and Training of Laboratory Analysts and Managers, Chromatography of Amino Acids and Determination of Amino Acid Composition of Nutritionally Important Protein Sources (Parts I and II), and Quality Assurance (Parts I and II).

The Exhibit Hall, which featured more than 46 exhibitors of analytical apparatus and equipment, was visited by many meeting attendees.

The Harvey W. Wiley Award Banquet was held Tuesday evening in the Palladian Room in honor of the award recipient, Daniel P. Schwartz. Keynote speaker Paul Hile (Associate Commissioner of Regulatory Affairs, FDA, Washington, DC), discussed the role of AOAC in fostering government/industry communication and cooperation in analysis and methods validation.

The 2 quality assurance short courses that were held at the Shoreham, one immediately preceding the meeting and one immediately following the meeting, were a success.

Leon D. Sawyer Winner of the 1985 Associate Referee of the Year Award

Leon D. Sawyer (Food and Drug Administration, Minneapolis, MN), won the 1985 award for the best Associate Referee Report of the Year. Official Methods Board Chairman, H. Michael Wehr, presented the award at the AOAC 99th Annual International Meeting, October 28 in Washington, DC.

Sawyer was nominated by the Committee on Residues for his report, "Ethylene Dibromide in Grains and Grain-Based Products."

Every year, each Methods Committee is asked to nominate one Associate Referee for this award. Each nominee receives an award for the best report from that Committee. Other nominees this year were:

Committee on Pesticide Formulations and Disinfectants—Stephen C. Slahck, Mobay Chemical Corp., Kansas City, MO.

Committee on Foods I—Robert M. Eppley, FDA, Washington, DC.

Committee on Microbiology—Walter Hill, FDA, Washington, DC.

Committee on Feeds, Fertilizers, and Related Materials—Michael P. Carlson, Veterinary Diagnostic Center, University of Nebraska, Lincoln, NE.

Three committees has no nominees: Drugs and Related Topics, Foods II, and Hazardous Substances in Water and the Environment.

Short Courses

The American Oil Chemists' Society (AOCS) will host a short course on the biochemistry and dietary implications of fish oils on May 11–14, 1986, at the Sheraton Royal Waikoloa Hotel, Hawaii. Registration forms and further information are available from the meeting manager AOCS, 508 S 6th St, Champaign, IL 61820; 217/359-2344.

The American Association of Cereal Chemists (AACC) announces 2 short courses: (*1*) Introduction to Cereal Chemistry and Technology: May 12–15, 1986, Holiday Inn, Minneapolis, MN. This course provides an overview of production, processing, and usage of cereal grains. It is directed to those who have limited formal training in cereal chemistry or are new to the industry. (*2*) Computers in the Lab: May 21–22, 1986, Holiday Inn O'Hare Kennedy, Chicago, IL. This course is designed to present practical applications for the use of microcomputers in the laboratory. The course is cosponsored by the American Institute of Chemists.

Descriptive brochures and registration forms for both AACC short courses can be obtained from Dotty Ginsburg, AACC Short Course Coordinator, 3340 Pilot Knob Rd, St. Paul, MN 55121; 612/454-7250.

Update of Mass Spectral Database

The mass spectral database, which has been assembled over a number of years through a cooperative effort between the National Bureau of Standards (NBS), the Environmental Protection Agency (EPA), the National Institutes of Health (NIH), and the Mass Spectrometry Data Centre (MSDC) in Nottingham, England, has been recently updated. A large number of other laboratories worldwide have also contributed to this effort. The database now contains 42 261 electron impact mass spectra, an increase of 2434 over the present version. The mass spectral database is leased to industrial, aca-

demic, and government organizations who use it primarily for rapid chemical identification. In some cases the database is used by an individual laboratory, in others it is disseminated as part of a dedicated library look-up system on mass spectrometers. It is also leased by a number of on-line dissemination systems. For further information contact Sherman Fivozinsky, Office of Standard Reference Data, National Bureau of Standards, Gaithersburg, MD 20899; 301/921-2104.

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 American National Standards Institute, Inc., 1430 Broadway, New York, NY 10018; 212/354-3300.

ISO 7586-1985 Butter—Determination of water dispersion value—$12.00

ISO 729-1985 Oilseeds—Determination of acidity of oils—$12.00

ISO 6564-1985 Sensory analysis—Methodology—Flavour profile methods—$14.00

ISO 6754-1985 Whole thyme (*Thymus vulgaris* Linnaeus)—Specification—$12.00

ISO 7402-1985 Microbiology—General guidance for the enumeration of Enterobacteriaceae without resuscitation—MPN technique and colony count technique—$16.00

ISO 6658-1985 Sensory analysis—Methodology—General guidance—$12.00

ISO 7925-1985 Dried oregano (*Origanum vulgare* Linnaeus)—Whole or ground leaves—Specification—$12.00

ISO 6820-1985 Wheat flour and rye flour—General guidance on the drafting of bread making tests—$12.00

ISO 1737-1985 Evaporated milk and sweetened condensed milk—Determination of fat content—Gravimetric method (Reference method)—$16.00

New Sustaining Members

AOAC welcomes the following sustaining members to the growing list of organizations aware of the need to support an independent methods validation association. *Private:* Welch Foods, Inc., Concord, MA; Beech-Nut Nutrition Corp., Canajoharie, NY. *Government:* Instituto Di Tecnica E Sperimentazione Lattiero-Casearia Di Thiene, Thiene, Italy.

New International Journal

Chemometrics and Intelligent Laboratory Systems is an international journal that publishes articles about new developments on laboratory techniques on chemistry and related disciplines which are characterized by the applications of statistical and computer methods.

One of the main aims of the journal is to be an interdisciplinary journal; more

particularly, it intends to build bridges among chemists, statisticians, and designers for laboratory systems.

The journal deals with the following topics: (1) Chemometrics—the chemical discipline that uses mathematical and statistical methods to design or select optimal procedures and experiments, and to provide maximum chemical information by analyzing chemical data, (2) computerized acquisition, processing, and evaluation of data, (3) developments in statistical theory destined to be used in chemistry, (4) intelligent laboratory systems including self-optimizing instruments, and the application of expert systems and robotics in the laboratory, (5) techniques for the modeling of chemical processes such as environmental models and industrial processes including quality control, and (6) new software to implement the methods described above.

Volume 1 (4 issues) will cover 1986–1987. The subscription price is 242 Dutch guilders or $83.50 (U.S.).

Requests for further information and orders may be sent to Elsevier Science Publishers, PO Box 330, 1000 AH Amsterdam, The Netherlands.

Standard Reference Materials

The National Bureau of Standards (NBS), Office of Standard Reference Materials announces the availability of the following Standard Reference Materials (SRM): SRM 1614, Dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) in Isooctane, consists of 3 vials of unlabeled and carbon-13-labeled 2,3,7,8-TCDD for concentrations at the ppb level. Besides its use in calibrating analytical instruments, this SRM can also be used to "spike" research samples with known amounts of dioxin. Price: $162.00 per unit of 6 vials.

SRM 2034, Holmium Oxide in Perchloric Acid Solutions, is intended for use as a reference standard for verifying the accuracy of wavelength scale of spectrophotometers in the ultraviolet and visible spectral region (240–650 nm). SRM 2034 consists of a solution of 4% holmium oxide in 10% perchloric acid in a fused silica cuvette of a normal light path of 10 mm that fits the sample compartment of conventional spectrophotometers. Price: $315.00 per unit.

SRM 1856, Acoustic Emission Transducer, is intended to be used to determine the size and character of surface vibrations in the frequency range of 0.1 to 1 MHz. It may also be used as a standard against which other transducers may be calibrated. Price: $1856.00 (this also includes an amplifier).

SRMs may be obtained from the Office of Standard Reference Materials, Room B311, Chemistry Bldg, National Bureau of Standards, Gaithersburg, MD 20899; 301/921-3181.

Meetings

April 27–30, 1986: 11th Annual AOAC Spring Training Workshop and Exposition, Madison Hotel, Seattle, WA. Contact: H. Michael Wehr, Oregon Department of Agriculture, Laboratory

REGIONAL AOAC MEETINGS

April 27-30, 1986 — **Northwest Regional Section Meeting**
Madison Hotel, Seattle, Washington
to be held in conjunction with the 11th Annual Spring Training Workshop
Contact: H. Michael Wehr, Oregon Department of Agriculture,
Laboratory Services Division
635 Capitol Street, NE, Salem, OR 97310, (503) 378-3793

May 12, 1986 — **AOAC Europe**
Florence, Italy
Contact: Margreet Tuinstra
6721 SR Bennekom, The Netherlands, 011 + 31-8389-18725

June 16-18, 1986 — **Midwest Regional Section Meeting**
Lincoln, Nebraska
Contact: Tom Jensen, Nebraska Department of Agriculture
3703 S. 14th Street, Lincoln, NE 68502, (402) 471-2176

June 24-25, 1986 — **Northeast Regional Section Meeting**
Canisius College, Buffalo, NY
Contact: Gerald L. Roach, Food and Drug Administration
599 Delaware Avenue, Buffalo, NY 14202, (716) 846-4494

Services Division, 635 Capital St, NE, Salem, OR 97310; 503/378-3793.

April 27–30, 1986: AOAC Northwest Regional Section Meeting, in conjunction with the 11th Annual Spring Training Workshop, Madison Hotel, Seattle WA. Contact: H. Michael Wehr, Oregon Department of Agriculture, Laboratory Services Division, 635 Capitol St, NE, Salem, OR 97310; 503/378-3793.

April 29–May 1, 1986: BIO EXPO '86, World Trade Exposition Center, Boston, MA. Contact: Cahners Exposition Group, 999 Summer St, Stamford, CT 06905; 203/964-0000.

May 5–6, 1986: Analytichem International's 3rd Annual Symposium, "Sample Preparation and Isolation Using Bonded Silicas," Shearaton Post Inn, Cherry Hill, NJ. Contact: Analytichem International, Inc., 24201 Frampton Ave, Harbor City, CA 90710; 213/539-6490 or 800/421-2825.

May 7–8, 1986: 16th Symposium on Advances in Applied Analytical Chemistry, Airport Hilton, New Orleans, LA. Contact: Michael Legendre, PO Box 19687, New Orleans, LA 70179; 504/589-7038.

May 12, 1986: AOAC Europe, Milano, Italy. Contact: Margreet Tuinstra, AOAC European Representative, 6721 SR Bennekom, The Netherlands; 011+31-8389-8725.

May 14–18, 1986: American Oil Chemists' Society (AOCS) 77th Annual Meeting, Hilton Hawaiian Hotel, Honolulu, Hawaii. Contact: Meetings Manager, AOCS, 508 S 6th St, Champaigne, IL 61820; 217/359-2344.

June 16–18, 1986: AOAC Midwest Regional Section Meeting, Lincoln, NE. Contact: Thomas Jensen, Nebraska Department of Agriculture, 3703 S 14th St, Lincoln, NE 68502; 402/471-2176.

June 24–25, 1986: AOAC Northeast Regional Section Meeting, Canisius College, Buffalo, NY. Contact: Gerald L. Roach, Food and Drug Administration, 599 Delaware Ave, Buffalo, NY 14202; 716/846-4494.

September 8–10, 1986: American Chemical Society (ACS) Exposition, Anaheim Convention Center, Anaheim, CA. Contact: Evalyn M. Fuller, ACS, Office of Expositions, 1155 16th St, NW, Washington, DC 20036; 202/872-4485.

September 15–18, 1986: AOAC 100th Annual International Meeting and Exposition, The Registry, Scottsdale, AZ. Contact: Margaret Ridgell, AOAC, 1111 N 19th St, Suite 210, Arlington, VA 22209; 703/522-3032.

September 14–17, 1987: AOAC 101st Annual International Meeting and Exposition, The Cathedral Hill Hotel, San Francisco, CA. Contact: Margaret Ridgell, AOAC, 1111 N 19th St, Suite 210, Arlington, VA 22209; 703/522-3032.

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

Bruce Poundstone (1906–1985)

Bruce Poundstone, who worked for 42 years at the University of Kentucky and who had a tremendous influence on the development of fertilizer and feed regulatory programs, died at his home in Lexington, Kentucky on February 24, 1985. In addition to his wife, Myra, he is survived by two sons, three sisters, and six grandchildren.

Bruce was born July 29, 1906, and was reared on a farm near Grand Ridge, Illinois. He majored in mathematics for 2 years at Knox College, Galesburg, Illinois. He received a BS in economics from Colorado State University in 1928.

After a year's teaching and research at Colorado State, he joined the staff of the University of Kentucky as an assistant in farm management. During the mid-1930s, he served as Land Planning Consultant for the Kentucky State Planning Board and the National Resources Board. In 1936, he joined the Kentucky Cooperative Extension Service for 10 years of agricultural extension work. He was director of the Division of Regulatory Services from 1946 until he retired in 1971.

Bruce was active in several professional associations. He held membership in the Association of American Feed Control Officials; Association of American Plant Food Control Officials; Association of Official Analytical Chemists; Association of Southern Feed, Fertilizer, and Pesticide Control Officials; Association of Feed Microscopists (which he helped found); Association of American Pesticide Control Officials; Association of American Seed Control Officials; Animal Nutrition Research Council; Creative Education Foundation; American Management Association; and American Society of Association Executives.

After his retirement, he shared his love of nature by presenting slide lectures at Natural Bridge State Park. In 1983, he received the Naturalist of the Year Award given by the Kentucky Society of Natural History. His life philosophy was expressed in the words of the poet Sam Walter Foss: "Let me live in my house by the side of the road and be a friend to man."

THANKS TO REVIEWERS OF JOURNAL MANUSCRIPTS

The Association is greatly indebted to those scientists who have contributed their time and expertise as reviewers to assist in the selection and revision of manuscripts for publication in the 1985 *Journal*. Reviewers are asked to critically evaluate manuscripts on the basis of scientific quality, originality, and relevance to the *Journal*. Comments and recommendations made by reviewers greatly affect the editors' decisions to accept or reject a paper for publication. This time consuming task is one that reviewers are asked to fit into their already busy schedules so that the publication process can proceed as rapidly as possible. Again, the Association extends its appreciation to all reviewers of *Journal* manuscripts, who help to produce and maintain a high quality scientific publication.

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INSTRUCTIONS TO AUTHORS

Scope of Articles

The Journal of the AOAC will publish articles that present, within the fields of interest of the Association *(a)* unpublished original research; *(b)* new methods; *(c)* further studies of previously published methods; *(d)* background work leading to development of methods; *(e)* compilations of authentic data; *(f)* technical communications, cautionary notes, and comments on techniques, apparatus, and reagents; *(g)* invited reviews of methodology in special fields. The scope broadly encompasses the development and validation of analytical procedures pertaining to both the physical and biological sciences related to agriculture, public health and safety, consumer protection, and quality of the environment. Emphasis is focused on research and development to test and adopt precise, accurate, and sensitive methods for the analysis of foods, food additives, supplements and contaminants, cosmetics, drugs, toxins, hazardous substances, pesticides, feeds, fertilizers, and the environment. Compilations of authentic data include monitoring data of pesticide, metal, and industrial chemical residues in food, tissues, and the environment. All articles are reviewed for scientific content and appropriateness.

Preparation of Manuscript

Authors must submit 3 copies of the complete manuscript, including tables and illustrations, to AOAC, 1111 N 19th St, Suite 210, Arlington, VA 22209. The manuscript is to be typewritten on one side of white bond paper, 8½ × 11 inches, with page margins of 1 inch, and **double-spaced** throughout (i.e., title, authors' names and addresses, footnotes, tables, references, captions for illustrations, and the text itself). Tables are to be typed on separate sheets, *not* interspersed through the manuscript. Drawings and photographs should be mounted apart from the text or submitted as separate items.

Style and Format

The text should be written in clear, concise, grammatical English. Unusual abbreviations should be employed as little as possible and must always be defined the first time they appear. Titles of articles should be specific and descriptive. Full first names, middle initial (if any), and last names of authors should be given. The address of the institution (including zip code) from which the paper is submitted should be given and should be in a form to which inquiries, proofs, and requests for reprints can be sent. Information supplementing the title and names and addresses should be given as footnotes.

Methods, Results and/or Discussion, Acknowledgments, and Recommendations (applicable to reports of General and Associate Referees) should be placed in sections under appropriate headings.

Abstracts: Each manuscript should be accompanied by a concise abstract (not more than 200 words). The abstract should provide specific information rather than generalized statements.

Introduction: Each article should include a statement on why the work was done, the previous work done, and the use of the compound being studied.

Methods: Methods should be written in imperative style, i.e., "Add 10 mL . . . Heat to boiling . . . Read in spectrophotometer." Special reagents and apparatus should be separated from the details of the procedure and placed in sections with appropriate headings; however, common reagents and apparatus or those which require no special preparation or assembly, need not be listed separately. Reagents and apparatus listed should be described in generic terms and in terms of performance; use of brand names should be avoided. Hazardous and/or carcinogenic chemicals should be noted. Any very long, detailed operation can be given in a separate section with an appropriate heading (e.g., Preparation of Sample; Extraction and Cleanup; Preparation of Standard Curve). Any necessary calculations should be included; number of significant figures must reflect the accuracy of the method. Wherever possible, metric units should be used for measurements or quantities.

Tables: All tables must be cited in the text consecutively. Tables are numbered by arabic numbers, and every table must have a descriptive title, sufficient so that the table can stand by itself without reference to the text. Every vertical column in the table should have a heading; abbreviations may be used freely in the headings to save space, but should be self-evident or must be explained in footnotes. Footnotes to both the headings and the body of the table are indicated by lower case letters in alphabetical order; these letters should be underscored and raised above the line of type. Horizontal rules should be used sparingly; however, they are used to bound the table at top and bottom and to divide the heads from the columns. Authors should refer to recent issues of the Journal for acceptable format of tables.

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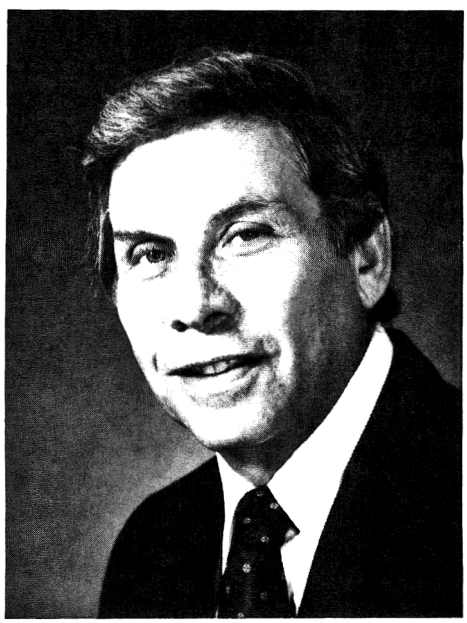
Wiley Award Address

Improved Methods for the Analysis of Trace Constituents in Natural Products

DANIEL P. SCHWARTZ
U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Philadelphia, PA 19118

One of the rewards of research is to develop new and improved methods that will not only help solve your problem, but will also be used in laboratories throughout the world to expedite analysis. Another reward, perhaps equally as satisfying, is to have your work recognized by your peers, and I am indeed grateful to receive the AOAC Harvey W. Wiley Award. I am also grateful to my former colleagues who worked alongside me in the old Dairy Products Laboratory in the South Agriculture Building in Washington, DC—Ron Brewington and John Weihrauch. I am especially indebted to Owen Parks, a long-time coworker in Washington, DC, who now occupies a laboratory adjacent to mine at the Eastern Regional Research Center in Philadelphia, PA; it was he who applied a number of the methods to isolate, identify, and quantitate several novel classes of compounds and individual components occurring in dairy products.



Our methods development program began 25 years ago in an effort to isolate and identify flavor compounds and, over the years, expanded into fats and oils and then into drug residues in biological fluids, tissues, and feeds. A special effort was made in the development of all methods to make them simple, rapid, and economical regarding both laboratory space and equipment.

A number of what we call column reactions evolved as needed in the overall development of major quantitative methods. In all column reactions, one of the reactants is present in large excess compared with the other. Usually this excess reactant is impregnated onto an inert support in water or is ground dry with the dried support. The other reactant, which is present in micro amounts and in very dilute solution in a water immiscible solvent, is then brought in contact with the support by either passing the solution over the bed or less often by permitting a small volume to reside on the support for a given time. In a few reactions, the trace component is deposited on the "naked" support and the other reactant, usually a vapor, is brought in contact with the support.

Two-phase column systems are extremely efficient for reacting pico- to micromole quantities of specific chemical classes. Besides requiring minimal manipulation, these reactions are carried out at ambient temperatures and are almost always faster than the same reaction carried out in a true solution. Reactions tend to go to completion because of the large molar ratio of one reactant to the other as well as because of the continuous removal of the reaction product(s) from the reaction sphere.

In our early development of column reactions, relatively large beds of supports were used. As detection methods became more sensitive, we made the beds and columns smaller, finally using only a few mg of supported reagents, contained in melting point capillaries and μL syringes, to apply and remove solutions. A partial list of 2-phase column reactions that were developed is shown below:

Methyl esters with diazomethane
Reductions with $NaBH_4$ and $LiBH_4$
Oxidation of alcohols with chromic acid
Location of double bond position with chromic acid
Acylation; transesterification; hydrogenation; saponification
Oxidations with periodic acid
Digitonin complexation of 3 β-sterols
Adduction of carbonyl compounds with bisulfite
Isopropylidene formation
Derivatization with 2,4-dinitrophenylhydrazine
Isolation of saturated compound from a mixture with $PdCl_2$

Most column reactions have been carried out on a Celite support, but other supports, including silicic acid, magnesium and calcium sulfates, and polymeric resins, have also been used.

Presented at the 99th Annual International Meeting of the AOAC, Oct. 27–31, 1985, at Washington, DC.

Practically all column reactions have been used at one time or another in the isolation, fractionation, or identification of individual constituents or classes of compounds present in natural products. A number of column reaction applications are given below:

2,4-Dinitrophenylhydrazine:
- Isolation of ketoglycerides from lipids
- Recognition of glyceryl-1-alkenyl ethers
- Isolation of ketosteroids from lipids
- Quantitation of β-ketoacids in butteroil
- Quantitation and identification of fatty aldehydes in plasmalogens

Bisulfite adduction:
- Fatty aldehyde isolation from butteroil

Digitonin complexation:
- Detection of adulteration of fats and oils

Oxidation with periodic acid:
- Isolation of glyceryl-1-alkyl ethers

Each column reaction application was supplemented by the development of numerous methods for fractionation of classes (e.g., aldehydes and ketones), separation of saturated from unsaturated compounds, resolution of individual members, and, finally, identification of most of the compounds in a given class. More than 100 compounds derived from the above applications have been shown to exist in the glycerides of milkfat in addition to the fatty acids.

The successful application of the 2,4-dinitrophenylhydrazine reaction column to the isolation and quantitation of the classes of carbonyl compounds described above, led us to synthesize a somewhat analogous reagent for alcohols, amines, and thiols in natural products whose derivatives can be easily isolated. This reagent, pyruvic acid chloride 2,6-dinitrophenylhydrazone (Figure 1), reacts very rapidly with primary, secondary, and tertiary alcohols, primary and secondary amines, and thiols when the acylation is catalyzed by the di-tert-cyclic amine, triethylenediamine. The highly colored derivatives like the 2,4-dinitrophenylhydrazones are acidic and can be isolated and purified using a basic adsorbent and/or an anion exchange resin. Methods were developed using

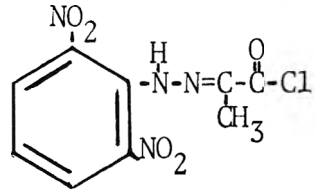


Figure 1. Structure of pyruvic acid chloride 2,6-dinitrophenylhydrazone.

this reagent for directly isolating sterols, fatty and triterpene alcohols, and hydroxy fatty acids from lipids, both qualitatively and quantitatively. Column and thin layer chromatographic (TLC) separations of the 3 aliphatic alcohol classes were developed, as well as similar methods for primary and secondary amines. Also column and TLC methods were perfected for separating the alcohol, amine, and thiol derivatives from each other.

Finally, some mention should be made of our more recent endeavors to develop new and improved methods for screening biological fluids and feeds for drug residues. These methods have been developed for sulfa drugs in swine and poultry feeds, swine urine and blood, and honey. A screen for the antibiotic, chloramphenicol, in milk has been published. All methods were designed to be run in the field using disposable plastic tubes, very small volumes of or no organic solvents, and either anion exchange and/or non-ionic polymeric resins to trap the drugs. Colorimetric detection of the drug is used; sensitivity of all the methods is in the range 50–100 ppb for small samples, and usually 40–50 samples/day/analyst can be run.

In summary, a number of microcolumn reactions have been developed and incorporated into schemes for isolating, purifying, fractionating, and identifying a number of classes of compounds occurring in natural products. Following successful application of these procedures, a new acid chloride, pyruvic acid chloride 2,6-dinitrophenylhydrazone, was synthesized and used in the development of methods designed to directly isolate, fractionate, and quantitate hydroxyl components from natural products. Experience gained from these endeavors facilitated the development of sensitive screening methods for drug residues in animal products.

DRUGS

Simplified Automated Procedure for Measurement of Certain Catecholamine Drugs Delivered from Aerosol Inhalation Units

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An automated colorimetric method is described for the measurement of certain catecholamine drugs, such as isoproterenol and epinephrine, in sample solutions derived from 2 metered doses delivered from the mouthpiece of aerosol inhalation units. The procedure, applicable to the expressed product, involves oxidizing the catecholamine with potassium ferricyanide at pH 6 to produce an aminochrome. This method is similar to the well known trihydroxyindole reaction procedure but differs in that the formed aminochrome is measured spectrophotometrically at 495 nm instead of being further derivatized by alkaline rearrangement to the trihydroxyindole species followed by either spectrophotometric or fluorometric measurement.

A variety of drugs are administered by way of metered aerosol inhalation units. Considering that they are mass-produced, these delivery systems are remarkable devices capable of delivering doses of drugs with a high degree of precision and accuracy. Nevertheless, confirmatory quality control testing of these products is necessary and required. For optimum clinical results and safety, these units must deliver a proper and uniform quantity of product each time the container valve is activated. Quantitative analysis of a single metered dose provides the most meaningful measure of unit spray content reproducibility. Assurance of uniformity of dosage is particularly important in the control of highly potent bronchodilator drugs (1).

In a previous publication we presented a procedure for the quantitative measurement of certain bronchodilator drugs delivered through the valve of metered aerosol containers (2). The automated procedure provided a simple means of determining the content of drug delivered through the valve after a single actuation.

Obtaining a sample expelled through the metering valve, as described in our previous work, is a relatively simple process. The product from one or 2 actuations can be collected directly from the valve-orifice in a beaker containing a small volume of solvent (2 mL). Generally this will provide a sufficiently concentrated sample solution for quantitative measurement of the catecholamine. When the aerosol units are fitted with a mouthpiece-actuator, sample preparation is more complicated than for the determination of drug delivered through the valve-orifice and requires a more elaborate approach.

This paper describes a procedure for making automated measurements of isoproterenol HCl and epinephrine bitartrate in sample solutions theoretically containing 2.5 or 5.4 μg/mL, respectively, derived from 2 metered doses delivered through the mouthpiece-actuator using the unit spray sampling apparatus described in USP XXI (3).

Samples collected through a mouthpiece-actuator using the unit spray content sampling apparatus require a minimum of 20 mL solvent to trap the expressed sample. As a result, unit spray content determinations of samples collected through the mouthpiece-actuator require an analytical procedure with more sensitivity than is needed for measurements made on samples collected directly at the valve-orifice.

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USP XXI procedures for the determination of unit spray content of aerosol products containing isoproterenol and epinephrine specify the expression of from 4 to 12 actuations through the mouthpiece-actuator for each sample preparation (4, 5). The procedure reported here describes a method sufficiently sensitive to make unit spray determinations on sample preparations derived from 2 actuations through the mouthpiece-actuator. Since products of this type recommend one or 2 doses, this method provides a more meaningful assessment of dosage delivery to the patient.

The chemical oxidation of catecholamines resulting in the production of highly colored quinone-indole derivatives, sometimes called aminochromes, has been widely used and studied (6). Catecholamines are often measured by the well known trihydroxyindole reaction described by Leow and modified by Ehrlen (7–9). It consists of oxidizing the catecholamine with potassium ferricyanide solution at pH 6 to produce the colored aminochrome which is further derivatized by alkaline rearrangement to the fluorescent trihydroxyindole derivative. Hellberg used the trihydroxyindole procedure to measure epinephrine in combination with local anesthetics (10). More recently, Tarlin et al. presented an automated procedure for the determination of epinephrine in lidocaine injection, measuring the fluorescence of the trihydroxyindole derivative (11). Craig et al. automated a fluorometric procedure, based on the trihydroxyindole reaction to measure dopamine in brain tissue (12). The analytical reaction used in the work reported in this paper is similar to the trihydroxyindole procedure but differs in that the formed quinone-indole derivative is measured spectrophotometrically at 495 nm instead of being further derivatized by alkaline rearrangement for fluorescence measurement. The chemical reaction used in this procedure is shown in Figure 1.

METHOD

Apparatus

(a) *Unit spray sampling apparatus.*—USP XXI, p. 1220.

(b) *Automated analyzer.*—Technicon AutoAnalyzer II system has been satisfactorily used for this determination. Modules required for this procedure are sampler IV, proportioning pump III, cartridge assembly, and UV/Vis spectrophotometer with 15 mm flow cell and wavelength capacity of 495 nm.

Assemble coils, tubing, and fittings used with automatic analyzer as illustrated in Figure 2.

Reagents

Use analytical grade reagents and distilled water throughout.

(a) *acetate buffer.*—pH 6. Dissolve 273 g sodium acetate ($NaC_2H_3O_2 \cdot 3H_2O$) in 500 mL water in 1 L volumetric flask. Add 6.2 mL acetic acid, dilute to volume with water, and mix. Check pH of prepared buffer potentiometrically and adjust to pH 6 ± 0.1 pH unit, using ca 1N solutions of acetic acid or NaOH as required.

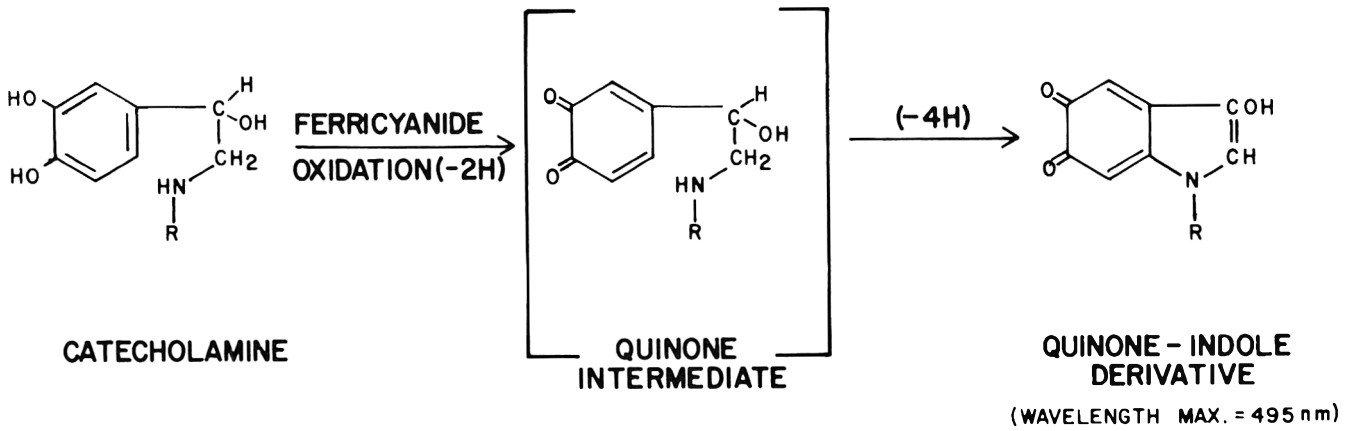

Figure 1. Reaction scheme.

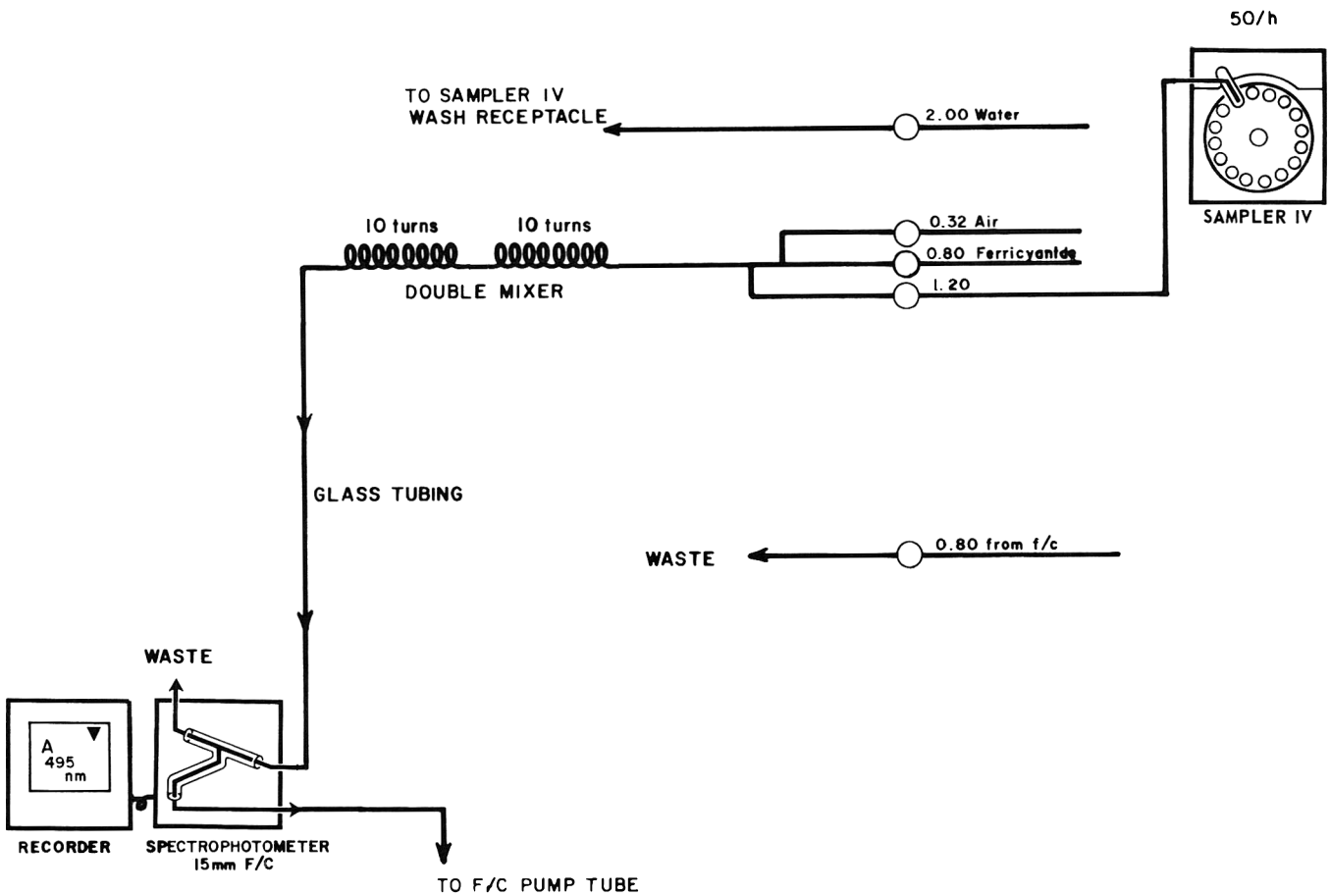

Figure 2. Flow diagram of automated system.

(b) *Potassium ferricyanide–acetate reagent.*—Dissolve 500 mg potassium ferricyanide ($K_3Fe(CN)_6$) in 500 mL pH 6 acetate buffer. Add 1 mL Brij 35 (Fisher CS-285) and mix. Prepare fresh daily.

(c) *Isoproterenol hydrochloride standard.*—For samples which deliver ca 125 μg drug per actuation. Transfer 100.0 mg isoproterenol hydrochloride reference standard to 100 mL volumetric flask. Add ca 80 mL water and 1 drop of 6N HCl and mix. Dilute to volume with water and mix. Further dilute 10.0 mL to 100.0 mL with water containing 1 drop of 6N HCl/100 mL. From this second dilution, further dilute 2.0, 3.0, and 4.0 mL each to 100.0 mL with water containing 1 drop of 6N HCl/100 mL to provide reference standard solutions representing 2.0, 3.0, and 4.0 μg isoproterenol HCl/mL.

(d) *Epinephrine bitartrate standard.*—For samples which deliver ca 270 μg drug per actuation. Transfer 91.0 mg epinephrine bitartrate reference standard to 50 mL volumetric flask. Dilute to volume with water containing 1 drop of 6N HCl/100 mL and mix. Further dilute 10.0 mL to 100.0 mL with the same solvent. From this second dilution, further dilute 3.0, 5.0, and 8.0 mL each to 100.0 mL with water containing 1 drop of 6N HCl/100 mL to provide reference standard solutions representing 3.0, 5.0, and 8.0 μg epinephrine base/mL.

Recovery Experiments

Sample solutions for recovery experiments were prepared as follows: Appropriate amounts of the particular drug under

Table 1. Recovery of isoproterenol from a commercial formula matrix

Isoproterenol HCl added, μg	Isoproterenol HCl found, μg			
	Run 1	Run 2	Run 3	Run 4
0	—[a]	—	—	—
0	—	—	—	—
200	200	198	202	198
200	201	203	199	202
300	300	305	303	301
300	303	300	305	302
350	352	353	346	352
350	353	351	352	351
Av. recovery of run, %	100	101	100	100
Rel. std dev. of run, %	0.4	1.0	1.0	0.7

[a]No response above noise level.

Table 2. Recovery of epinephrine from a commercial formula matrix

Epinephrine added, μg	Epinephrine found, μg				
	Run 1 (day 1)	Run 2 (day 1)	Run 3 (day 1)	Run 4 (day 1)	Run 5 (day 2)
0	—[a]	—	—	—	—
0	—	—	—	—	—
400	402	400	396	399	392
400	403	401	399	403	400
500	501	501	504	502	498
500	505	501	502	501	498
600	601	603	601	601	598
600	604	602	605	601	600
Av. recovery of run, %	101	100	100	100	99.5
Rel. std dev. of run, %	0.3	0.2	0.7	0.3	0.8

[a]No response above noise level.

consideration as listed in Tables 1 and 2 were placed directly into the collection chamber of the USP unit spray sampling apparatus. Following the procedure described under *Sample Preparation,* 2 actuations of a prepared aerosol blank preparation (containing all formula ingredients except the drug) were made into the apparatus through a mouthpiece-actuator. The collected blank solution containing the added drug was assayed as described for the sample preparations.

Sample Preparation

Collect 2 actuations from aerosol sample container being tested, in water, according to appropriate sampling protocol. (An example of a typical sample collection procedure is described on p. 570 of USP XXI for isoproterenol sulfate inhalation aerosol.) Quantitatively transfer collected solution from unit spray sampling apparatus to 100 mL volumetric flask, using water to complete transfer. Add 1 drop of 6N HCl and mix. Dilute to volume with water and mix.

Assay Procedure

Pump reagents and sampler rinse until steady baseline is achieved using flow system shown in Figure 2. Transfer portion of each reference standard and sample preparation to series of 2 mL sample cups and position cups appropriately on sampler tray. Cycle standards and sample solutions at rate of 50 per hour. Use sampling-to-wash ratio, for isoproterenol and epinephrine, of 6:1 and 3:1, respectively. Sample solutions derived from each of the named drugs should be processed separately by the automated system along with their respective reference standard solutions.

From recorded peaks, read absorbance of standard and sample preparations. Plot absorbance of standards against their known concentrations. From standard curve, read concentration, C_u, in μg/mL, of isoproterenol HCl or epinephrine base in sample preparations.

Calculate μg drug delivered per spray as follows:

$$\text{Drug, mg/spray} = C_u \times 100/\text{ number of actuations}$$

Table 3. Carryover study: determination of intersample effects (at 50 samples per hour)

Ref. std[a]	Measured concn
A, A, A,	3.03, 3.01, 2.99
C, C, C	7.93, 7.98, 7.99
B, B, B	5.02, 5.03, 5.05
A, A, A	3.01, 2.99, 2.98
B, B, B	4.98, 5.00, 5.02
C, C, C*	7.96, 7.98, 7.95
A, A, A**	3.04, 2.99, 2.98
B, C, B	5.02, 8.05, 5.05
C, B, C	8.08, 5.07, 8.06
A, A, A	3.06, 3.03, 3.01
	Overall RSD = 0.7%

[a]Reference standards shown in the order they were assayed. Epinephrine concentrations were A, 3.0 μg/mL; B, 5.0 μg/mL; C, 8.0 μg/mL.
*Highest concentration.
**Lowest concentration.

Results and Discussion

Processed reference standard solutions of the named drugs produced absorbance values which, when plotted against their known concentrations, indicated linearity in the concentration ranges described in this paper. The residual standard deviation of the responses for isoproterenol and epinephrine are 0.163 and 0.032, respectively. The correlation coefficient for each response curve is 0.999.

Reference standard solutions prepared and maintained at ambient temperature for 24 h produced standard curves similar to those derived from freshly prepared standard solutions, indicating no significant degradation.

The system standard deviation of the proposed automated system was determined by processing, according to the prescribed procedure, 10 portions each of common reference standard solutions of isoproterenol and epinephrine. The coefficients of variation were 0.4 and 0.3%, respectively.

Aminochrome derivatives of catecholamines are unstable. Using conditions similar to those described here, we have demonstrated that time is a critical parameter when the procedure is performed manually. Experiments done on epinephrine demonstrated that maximum absorbance is produced at about 2 min. After about 8 min the epinephrine aminochrome absorbance decreased with time (internal files). Experiments performed with the proposed automated procedure with stop-flow technique indicated that at 8 min an approximate 5% loss of absorbance was observed. This is not a critical factor for the automated procedure due to the precise nature of the common treatment of reference standards and sample solutions.

The proposed automated system is capable of measuring isoproterenol or epinephrine in solution at the rate of 50 samples per hour with an overall standard deviation of less than 1%. Suitability of the methodology is shown by the data in Tables 1 and 2.

Intersample effect (carryover) was shown to be insignificant for the described automated procedure at 50 samples per hour for the concentration ratios prescribed. The order of processing of the 3 concentration levels of reference standard solutions is shown in Table 3 along with concentration values.

Table 4. Unit spray content of typical commercial sample

Detn	Isoproterenol HCL, % of label claim found[a]
1	101
2	102
3	102
4	95
5	109
6	92
7	112
8	101
9	102
10	110
11	103
12	97
Av. (n = 12)	102
RSD	5.9%

[a]Average of 2 actuations for each determination.

The proposed automated method has been applied to sample preparations derived from 2 actuations of aerosol inhalation units containing either isoproterenol or epinephrine. A summary of the analysis of 12 determinations made on a typical commercial sample of isoproterenol inhalation aerosol is shown in Table 4.

A simple modification of this procedure would provide the capacity to make measurements on sample assay solutions derived from a single actuation of an aerosol unit. This could be accomplished by simply reducing the final sample preparation volume by one half.

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Simple Colorimetric Method for Determination of Terbutaline Sulfate from Pharmaceutical Preparations

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A simple colorimetric method is described for the determination of terbutaline sulfate. The method is based on measurement of a colored species formed when terbutaline sulfate is treated with diazotized dapsone and *p*-nitroaniline at room temperature. Compounds such as starch, talc, and common excipients do not interfere in the reaction. Statistical validation showed that the method was highly precise and accurate. The results agree well with those obtained by other methods reported in the literature.

Terbutaline sulfate is a sympathomimetic agent used as a bronchodilator. The *U.S. Pharmacopeia* (1) describes nonaqueous titrimetry for its assay whereas a spectrophotometric assay is recommended for terbutaline sulfate tablets and injections. Kamalpurkar and Priolkar (2) and Shingbal and Agni (3) have described colorimetric methods.

In the proposed method, dapsone and *p*-nitroaniline were diazotized in glacial acetic acid medium and quantitatively coupled with terbutaline sulfate. The method has several advantages over those cited in the literature.

METHOD

Apparatus and Reagents

(a) *Instrument.*—CZ Spekol colorimeter with 1 cm matched glass cells.

(b) *Dapsone and p-nitroaniline working solutions.*—Dissolve 250 mg reagent in separate 250 mL volumetric flasks by adding 50 mL alcohol and 25 mL glacial acetic acid; dilute to volume with water.

(c) *Sodium nitrite solution.*—2% aqueous.

(d) *Ethyl alcohol.*—96% v/v.

(e) *Terbutaline sulfate standard solution.*—Dissolve 25 mg standard in 250 mL water (100 ppm).

Preparation of Assay Solution

Tablets.—Weigh 20 tablets and transfer powder equivalent to 25 mg terbutaline sulfate to beaker. Add 25 mL water and filter through Whatman No. 1 paper into 250 mL volumetric flask. Wash beaker and filter paper with 3 or 4 portions of water and add washings to flask. Dilute to volume with water.

Syrup.—Weigh syrup equivalent to 25 mg terbutaline sulfate from thoroughly shaken formulation into 250 mL volumetric flask; dilute to volume with water.

Standard Curve

Dilute standard solution to give concentrations of 1 to 8 μg/mL. Develop color and read absorbance of reaction product as described under *Assay*. Plot absorbance against concentration of terbutaline sulfate to give straight line passing through origin. Beer's law is obeyed between 1 and 8 μg/mL. Molar absorptivities are 4.21×10^{-4} and 1.11×10^{-4} L/mol./cm for dapsone and *p*-nitroaniline, respectively.

Assay

(a) *Dapsone.*—Pipet 2.5 mL dapsone solution into series of 25 mL volumetric flasks. Add 2 mL sodium nitrite solution and aliquots of terbutaline sulfate standard solution (0.25 to 2.00 mL). Simultaneously prepare reagent blank. Dilute mixed solutions to volume with water after 10 min and measure absorbances at 420 nm against reagent blank. Determine concentration in test solution from standard curve using same procedure detailed earlier.

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Table 1. Colorimetric determination of terbutaline sulfate from formulations[a]

Product	Amount declared	Amount found		Recovery, % (addition method)		Std dev.		Coeff. of var., %	
		A	B	A	B	A	B	A	B
Terbutaline sulfate tab. 1	5 mg/tab.	4.98	5.03	100.60	99.85	0.11	0.12	1.39	1.43
Terbutaline sulfate tab. 2	2.5 mg/tab.	2.48	2.52	100.49	99.45	0.09	0.05	2.59	1.39
Terbutaline sulfate syrup	0.3 mg/mL	0.31	0.302	99.27	101.26	0.01	0.02	2.35	1.98

[a]A, with dapsone; B, with *p*-nitroaniline.

Table 2. Results of recovery experiments of terbutaline sulfate from formulations[a]

Product	Amount declared	Recovery, %					
		A			B		
		1st level	2nd level	3rd level	1st level	2nd level	3rd level
Terbutaline sulfate tab. 1	5 mg/tab.	99.49	99.81	102.21	100.02	99.57	100.01
Terbutaline sulfate tab. 2	2.5 mg/tab.	101.00	99.43	100.30	100.01	99.28	100.16
Terbutaline sulfate syrup	0.3 mg/mL	99.75	99.81	100.60	100.76	100.42	99.36

[a]A, with dapsone; B, with *p*-nitroaniline.

(b) *p-Nitroaniline.*—Carry out same procedure as detailed under *Dapsone* (λ max. = 420 nm).

Recovery Experiment

To study reliability and recovery of the proposed method, we used a method of standard addition. A fixed weight of sample was taken and standard drug was added at 3 different levels. Each level was repeated 7 times. The total amount of drug was determined by the proposed method. Percent recovery was calculated as follows:

$$\text{Recovery},\% = \frac{N(\Sigma XY) - (\Sigma X)(\Sigma Y)}{N(\Sigma X^2) - (\Sigma X)^2} \times 100$$

where X = amount of drug added/mg sample; Y = amount of drug found/mg sample; N = total number of observations.

First level addition.—A portion of sample equivalent to 20 mg terbutaline sulfate was added to a series of 100 mL volumetric flasks. To that, 10 mg standard drug was added, and the contents were dissolved in distilled water, filtered, and diluted to 100 mL. A 0.25 mL aliquot of the solution was pipetted for color development as described under *Assay*.

Second and third level additions.—For the second and third level additions, the amount of the sample was kept constant, and 20 and 30 mg standard drug was added, respectively. The remaining procedure was the same as that for the first level. The percentage recoveries at each level are summarized in Table 2.

Results and Discussion

Terbutaline sulfate reacts with diazotized dapsone and *p*-nitroaniline according to the well known and stoichiometric azo dye formation reaction (Figure 1). The color development has been successfully used to determine terbutaline sulfate in formulations. The USP volumetric titration method requires larger quantities of the drug. The present method uses a smaller amount of drug, and is direct, fast, simple, sensitive, and accurate. The diazotization and coupling reactions are carried out at room temperature, excess nitrite ions need not be removed by addition of ammonium sulfamate, and the color development and absorbance measurements are complete within 15 min. Percentage recoveries are in the range 98–102%, indicating noninterference from other ingredients and excipients (Table 1). The high values of molar absorptivities indicate high sensitivity of method. The method is also reproducible and precise as shown by results in Table 2. Therefore, we recommend this method for routine quality control analysis of terbutaline sulfate in formulations.

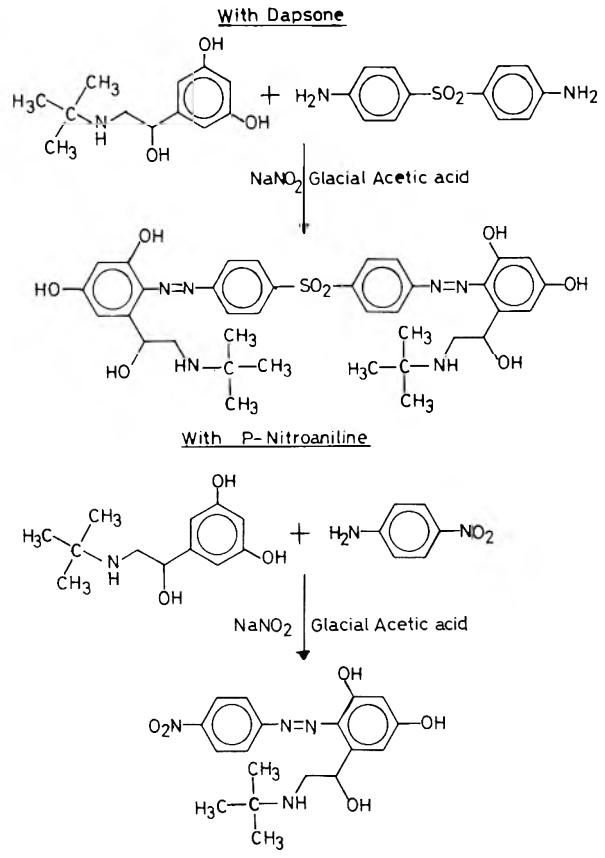

Figure 1. Reaction of terbutaline sulfate with diazotized dapsone and *p*-nitroaniline.

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The authors are grateful to S. V. Sawant for helpful discussions and to UGC, New Delhi, for a research grant to R. T. Sane.

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Indirect Spectrophotometric Method for Determining Epicillin

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A new UV spectrophotometric assay for determination of epicillin in capsules and plasma is reported. The method is based on the absorptivity of a degradation product obtained from the acidic hydrolysis of the antibiotic. The isolation, identification, individual capsule assays, composite assay, and recovery studies are described. Also, the proposed method is compared with a dc polarographic assay in plasma.

Epicillin is a semisynthetic penicillin that exhibits an excellent therapeutic activity when administered orally or subcutaneously against a variety of pathogenic microorganisms (1). Several methods for the determination of epicillin in aqueous solution and blood samples have been described (2–4). Recently, a polarographic method was proposed by the authors (5), based on the electroactivity of a degradation product obtained from the acidic hydrolysis of the antibiotic. The precise identity of this degradation product was not reported; however, a strong UV absorption was noted.

This paper reports the isolation and identification of the degradation product and proposes a new UV spectrophotometric analytical method that can be applied to capsules and plasma samples.

METHOD

Apparatus and Reagents

(a) *Spectrophotometers.*—Karl Zeiss Model PMR-21, with 1 cm quartz cells; Bausch and Lomb Spectronic 20 (Shimadzu Seisakusko Ltd, Kyoto, Japan)

(b) *Nuclear magnetic resonance spectrometer.*—Varian EM; TMS external standard.

(c) *Infrared spectrophotometer.*—Leitz G III, KBr disk.

(d) *Polarograph.*—Tacussel assembly operated in dc mode, essentially the same as described earlier (6). Polarographic conditions: current range, 25–50 μamp for full scale of recorder; potential scan rate, 2 mV/s; capillary characteristics $m^{2/3}t^{1/6} = 1.88\ mg^{2/3}s^{1/6}$ at − 0.70 vs SCE and temperature, 25 ± 1°C.

(e) *Polarographic cell.*—Tacussel CPRA measuring cell with dropping mercury electrode, platinum wire counter electrode, and saturated calomel reference electrode (SCE).

(f) *Standard solutions.*—Accurately weigh 10 mg epicillin standard (Squibb & Sons Inc., Santiago, Chile); dissolve and dilute to 100 mL with 1.0N HCl plus 1% formaldehyde. Spectrophotometric determination: Dilute solution to obtain final concentrations between 0.5 and 20 μg/mL. Polarographic assay: Prepare solutions as indicated in *Spectrophotometry* to obtain concentrations between 0.60 and 65mM.

Samples

Preparation of synethetic samples.—Prepare excipient powders for recovery studies according to manufactuer's batch formulas for 500 mg epicillin capsules, adding 500 mg epicillin standard. Follow same procedure used for standard solutions and adequately dilute to obtain final concentrations about 10 μg/mL.

Preparation of composite samples.—Weigh and finely grind powder from 30 epicillin capsules. Accurately weigh and transfer portions of powdered capsules equivalent to 500 mg epicillin; dissolve and dilute with 1.0N HCl + 1% HCHO to obtain final concentrations about 10 μg/mL.

Table 1. Recovery of epicillin from synthetic capsules[a]

	Found, mg	Rec., %
	498.0	99.6
	494.0	98.8
	501.0	100.2
	495.0	99.0
	499.0	99.8
	497.0	99.4
	496.0	99.2
Av.	497.1	99.4
SD	2.4	0.5
CV, %	0.5	0.5

[a]Each synthetic mix represents a formulation which is available commercially, containing 500 mg epicillin.

Table 2. Multiple analyses of epicillin samples

	Epicillin, mg	
	Individual assay[a]	Composite[b]
	495.0	498.0
	493.0	497.0
	494.0	497.0
	499.0	495.0
	491.0	496.0
	492.0	499.0
	490.0	491.0
Av.	493.4	496.1
SD	3.0	2.6
CV,%	0.6	0.5

[a]Dexacilina capsules (Squibb & Sons Laboratories, Santiago, Chile), declared amount 500 mg epicillin. Individual capsule assay is expressed as mg averaged capsule weight.
[b]575 mg taken, equivalent to 500 mg epicillin.

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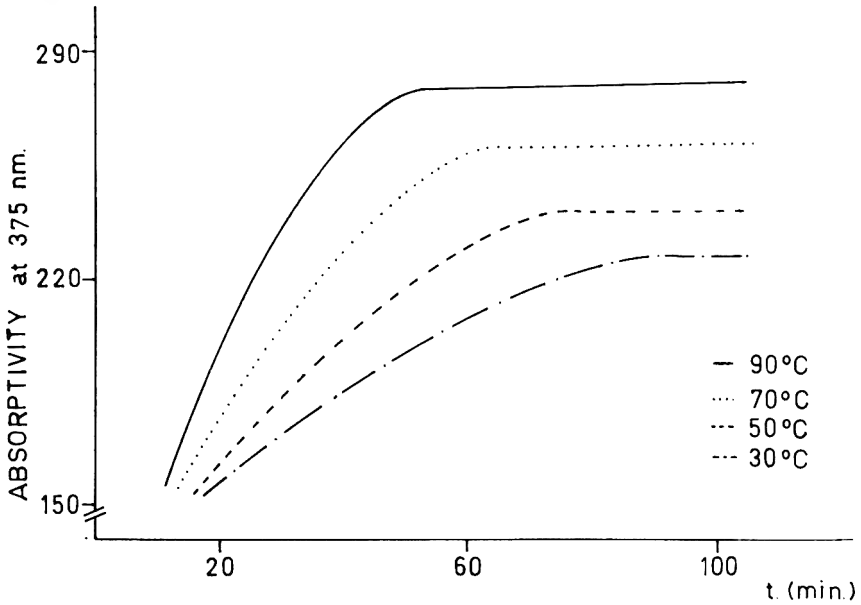

Figure 1. Temperature dependence of degradation product absorptivity vs hydrolysis time.

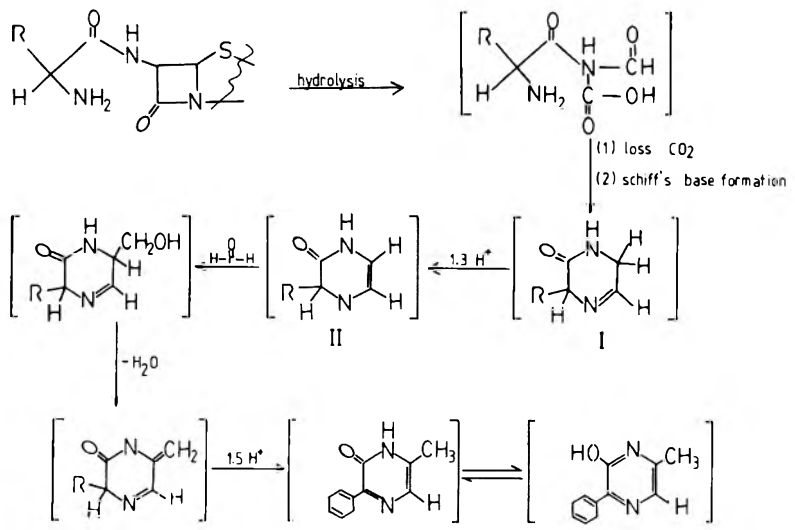

Figure 2. Scheme of degradation of epicillin to 2-hydroxy-3-(1,4-cyclohexadien-1-yl)-6-methyl pyrazine.

Preparation of plasma samples.—Prepare samples of plasma with concentrations between 6.0×10^{-4}M and 6.5×10^{-2}M epicillin. Then proceed with hydrolysis procedure, using 1 mL aliquots.

Hydrolysis Procedure

Heat standard and sample solutions at 90 ± 0.5°C in constant temperature bath for 60 min and proceed with spectrophotometric and polarographic determinations, respectively.

Spectrophotometry

Measure absorptivity of acidic solution at 375 nm, using 1.0N HCl + 1% HCHO as blank. Calculate μg epicillin/mL from standard calibration curve.

Polarography

Transfer 10 mL of each solution to dry polarographic cell, and degas by bubbling nitrogen through solution 10 min. Scan each sample solution at least twice from −0.4 to −0.9 V. Calculate mg epicillin in sample solution from prepared standard calibration curve.

Isolation of Degradation Product

Dissolve and dilute 3 g epicillin with 1% HCHO in 1.0N HCl to 100 mL. Heat solution 60 min, and let cool at room temperature. Adjust to pH 5.0 with NaOH and buffer with 100 mL Sorensen citrate buffer, pH 5.0. Extract 5 times with 50 mL portions of ethyl acetate–diethyl ether (50 + 50). Evaporate combined organic layers; crystallize and recrystallize crude product from ethyl acetate.

Results and Discussion

This paper proposes an indirect UV spectrophotometric assay for the quantitative determination of epicillin in capsules and plasma.

To obtain a useful spectrophotometric response, we studied different hydrolysis conditions. Hydrolyses were carried out at several acidities, formaldehyde percentages, and temperatures. The amount of degradation product was greater with 1% HCHO in 1.0N HCl; Figure 1 shows the effect of temperature on the hydrolysis of epicillin. The optimal hydrolysis conditions were 1% HCHO in 1.0N HCl, 60 min, and 90°C.

Table 3. Correlation of UV assay results with polarographic assay results for epicillin in plasma

		UV assay				Polarographic assay			
Samples	N	Added, mM	Mean found, mM	Rec.,%	CV, %	Added, mM	Mean found, mM	Rec.,%	CV, %
1	7	0.60	0.59	98.3	4.2	0.60	0.63	105.0	6.8
2	7	0.95	0.96	101.1	3.1	0.95	0.91	95.8	6.3
3	7	2.50	2.48	99.2	2.9	2.50	2.39	95.6	5.1
4	7	9.50	9.38	98.7	4.0	9.50	9.44	99.4	12.1
5	7	30.9	30.7	99.4	5.0	30.9	31.2	101.0	7.5
6	7	65.0	64.0	98.5	5.0	65.0	62.0	95.4	8.1

Correlation coefficient: 0.999.

The presence of formaldehyde, which was originally proposed by Jusko for ampicillin hydrolysis (7), enhances both the rate and extent of the epicillin hydrolysis. The role of HCHO on the hydrolysis mechanism of β-lactam antibiotics has been elucidated by Indelicato et al. (8) using ^{13}C-labeled formaldehyde, demonstrating the incorporation of formaldehyde carbon in the methyl group of the degradation product.

In our laboratory it has been demonstrated that acidic hydrolysis of several aminopenicillins (9–11) yields pyrazine derivatives. Since the formaldehyde carbon becomes the 6-methyl carbon of the pyrazine derivative, the scheme in Figure 2 is proposed for the degradation pathway of epicillin hydrolysis.

This scheme is completely consistent with the finding that the degradation product is 2-hydroxy-3-(1,4 cyclohexadien-1-yl)-6-methyl pyrazine. Such a structure is in accordance with the elemental analysis and spectral characteristics reported here: uncorrected mp 207–211°C; elemental composition, found: C, 70.4; H, 6.3; N, 14.6; O, 8.6; calcd for $C_{11}H_{12}N_2O$: C, 70.2; H, 6.43; N, 14.88; O,8.50; characteristics: λmax. (CH_3OH) 245 nm; λmax. (1N, HCl) 375 nm; δ ($CDCl_3$, TMS external standard) 2.38 (3H,S), 3.03 (2H,m), 3.17 (2H,m), 5.17 (1H,m), 5.87 (1H,m), 7.27 (1H,s), 7.69 (1H, broad S), 12.50 (1H,s). Such spectroscopic characteristics indicate that the degradation product responsible for the strong UV absorption and the electroactivity is the proposed compound.

For analytical purposes, the UV peak at 375 nm was used. A linear relation between absorptivity and concentration of the antibiotic is obtained for levels between 0.5 and 30 μg/mL:

$$A_{375} = 0.029\,C\ (\mu g/mL) + 0.01$$

(corr. coeff. = 0.996 for 15 data points)

Table 1 shows the recovery for synthetic samples. The average recovery of 99.4 (SD 0.5) indicates satisfactory accuracy for the developed assay. Individual capsule assays and composite assays are shown in Table 2. No significant differences between these samples were found.

Correlations of the results for plasma samples obtained by polarographic assay are shown in Table 3. The correlation coefficient was 0.999. The average coefficient of variation for the UV spectrophotometric assay as 5.4% while that of the polarographic technique was 7.7%.

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Rapid and Simple Determination of Oxyphenbutazone in Dosage Forms by Biamperometric Titration

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Oxyphenbutazone was determined in the presence of glacial acetic acid, hydrochloric acid, and potassium bromide by bromometric titration with biamperometric end point indication. The course of the titration was followed with double platinum electrode with 100 mV of polarizing voltage.

Oxyphenbutazone (4-butyl-2-(4-hydroxyphenyl)-1-phenylpyrazolidine-3,5-dione monohydrate) is a pyrazolidine derivative used for years as an anti-inflammatory drug in various dosage forms. Oxyphenbutazone can be determined by different methods, e.g., alkalimetry in nonaqueous solvents (1), colorimetry (2), liquid chromatography (3, 4), and voltammetry (5, 6). This paper describes a simple direct titrimetric method applicable to both in-process and final control of the dosage forms of oxyphenbutazone.

METHOD

Apparatus and Reagent

(a) *Apparatus.*—Potentiograph Metrohm E 436; double platinum electrode Metrohm EA 240.

(b) *Potassium bromate.*—Volumetric solution, 0.0167 mol/L (0.1N) (1).

Procedure

Accurately weigh amount of finely powdered tablets or well mixed ophthalmic ointment corresponding to 50 mg oxyphenbutazone into titration vessel, add 25 mL 98% acetic acid, and mix under gentle heating (do not exceed 60°C) for 10 min. Cool, add 5 mL 25% HCl and 5 mL 20% potassium bromide solution and titrate with potassium bromate volumetric solution. Follow course of titration by using double platinum electrode with 100 mV polarizing voltage and 10 μA current sensitivity. Register change of current during titration by Potentiograph Metrohm.

Calculation: 1 mL 0.0167 mol/L of potassium bromate solution is equivalent to 0.01712 g oxyphenbutazone.

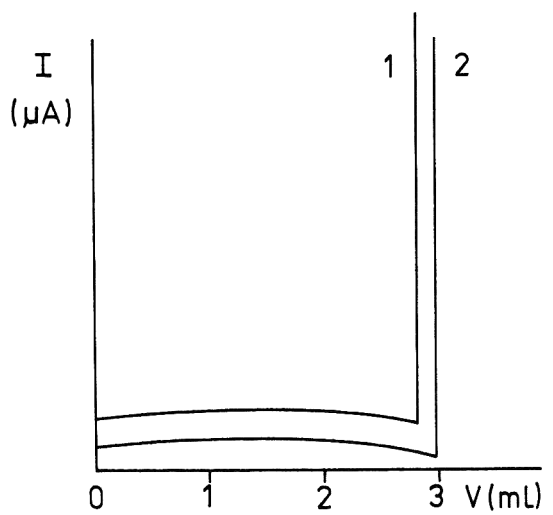

Figure 1. Biamperometric curve of oxyphenbutazone determination in commercial coated tablets (1) and eye ointment (2).

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Results and Discussion

The bromometric determination of oxyphenbutazone is based on the substitution of hydrogen of the enole form by bromine. The bromination takes place on the 4-position of the pyrazolidinedione ring which has an electrophilic character due to the ketoenole tautomerism. This type of reaction was described for phenylbutazone by Jančík et al. (7). The reaction mechanism of some pyrazolidinedione derivatives including oxyphenbutazone was later studied by Amer at al. (8) who confirmed that the bromination concerns only the 4-position and not the phenyl ring. From the shape of the biamperometric curve (Figure 1) it is evident that the current is almost equal to zero during the titration. In the equivalence, the free bromine appears in the solution and forms a reversible system with bromide ions. At this moment the electrodes are depolarized and the current rapidly increases, giving a stable end point.

The results obtained for oxyphenbutazone content in samples of the substance, coated tablets, and eye ointment are given in Tables 1 and 2. In Table 3, the results obtained by the newly developed method are compared with results from the official method (1). Both sets of results were evaluated statistically using Student's *t*-test. There is no significant difference, which means that the proposed method is as accurate and precise as the official method.

The advantage of our method is the possibility of direct determination of oxyphenbutazone content in its dosage forms, e.g., coated tablets and ophthalmic ointment. The excipients do not interfere in the reaction. Therefore, the analytical procedure is short and gives accurate results. To improve the

Table 1. Biamperometric determination of oxyphenbutazone substance with 0.0167 mol/L solution of potassium bromate

Taken, mg	Found mg	Found %
55.8	55.7	99.76
53.3	52.9	99.32
54.9	54.6	99.42
56.7	56.5	99.61
55.0	54.5	99.10
Mean		99.44
CV,%		0.29

Table 2. Biamperometric determination of oxyphenbutazone content in some pharmaceutical preparations by proposed method

Preparation	Found mg	Found %
Coated tab., 100 mg/tab.	96.8	96.8
	97.1	97.1
	96.2	96.2
Mean		96.7
CV,%		0.55
Eye ointment, 100 mg/g	97.2	97.2
	97.9	97.9
	97.7	97.7
Mean		97.6
CV,%		0.42

Table 3. Determination of oxyphenbutazone content in pure substance and commercial tablets: comparison of biamperometric and official (1) methods

Preparation	Biamperometric method					Official method				
	Taken, mg	Found, mg	Found, %	Mean, %	SD, %	Taken, mg	Found, mg	Found, %	Mean, %	SD, %
Oxyphenbutazone[a]	55.8	55.6	99.7			521.4	517.8	99.3		
	53.3	52.9	99.3	99.4	0.30	504.5	502.5	99.6	99.6	0.41
	54.9	54.5	99.2			512.6	512.1	99.9		
Coated tab.,	103.8	96.7	96.7			1009.9	97.9	97.9		
100 mg/tab.	100.6	97.4	97.4	96.9	0.53	1003.8	96.9	96.9	97.3	0.59
	105.2	96.5	96.5			1026.2	97.1	97.1		

[a]Oxyphenbutazone monohydrate, Ciba-Geigy, Switzerland.
Student's *t*-test: oxyphenbutazone monohyd: $t = 0.557$; coated tablets: $t = 0.709$.
Critical value $t = 2.776$ (at $\alpha = 0.05$ and 4 degrees of freedom).

precision we give special attention to cleaning the electrodes. After each titration we mechanically clean the electrodes with a paper tissue, then rinse with dichromate–sulfuric acid cleaning solution, 65% nitric acid, and finally distilled water. The biamperometric method is applicable for routine determination of oxyphenbutazone content in coated tablets and eye ointments for both in-process and final quality control.

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Polarographic Determination of Antihistamines by Complexation with Cd(II)[1]

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The polarographic behavior of the complexes formed by Cd(II) ion with ethanolamine derivative antihistamines such as diphenhydramine hydrochloride, dimenhydrinate, and chlorphenoxamine hydrochloride was studied. Antihistamines form spontaneous complexes with Cd(II) ion in the presence of KNO_3. In addition, pH 8.00 borate buffer was added to increase the differential pulse polarogram peak height, and tetraalkyl ammonium salts were added to increase the linear range. The method of determination developed has been applied to commercial tablet, capsule, elixir, and injection forms of ethanolamine derivative drugs and has been compared with official methods.

Diphenhydramine hydrochloride (DPHM-HCl), dimenhydrinate (DMHR), and chlorphenoxamine hydrochloride (CLPO-HCl) are found in various combinations in commercially available pharmaceutical preparations and well known antihistamine drugs which have been used clinically for many years. Several methods are available for the determination of these drugs. USP XX (1), BP (2), and TF (3) monographs describe titrimetric and UV assays for ethanolamine derivative antihistamines. Various chromatographic methods have also been described thin layer chromatography (4–7), gas chromatography (8, 9), and liquid chromatography (10) have proved to be valuable tools for the separation and identification of antihistamine compounds. Fluorometric (11) and UV-Vis spectroscopic (12–15) determinations have been also reported.

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[1]Based on experimental material from a dissertation presented by N. Özaltin in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Since antihistamines are combined with a wide variety of other medicinal agents in multicomponent products, their isolation and determination from such mixtures is frequently time consuming. They do not show inherent polarographic activity. For this reason there is no record of polarographic determination of antihistamine compounds. However, a variety of derivatization procedures can be used to develop an indirect method of analysis.

The purpose of the present paper is to use complexation procedures in an indirect polarographic determination of ethanolamine derivative antihistamines in pure and pharmaceutical forms such as tablet, capsule, elixir, and injection.

Experimental

Apparatus.—The polarographic experiments were performed using an EG&G Princeton Applied Research Corp. (PARC) Model 174 A polarographic analyzer with a Houston Omnigraphic Model 2000 X-Y recorder (16, 17). The PARC 174 A instrument was equipped with a PARC Model 174/70 drop timer for experiments at the dropping mercury electrode (DME). The cell used has a 3-electrode system. A platinum wire auxiliary electrode (British Drug Houses, 0.46 mm) and saturated Calomel reference (SCE) electrode (Corning) were used in conjunction with a tapered DME. A Luggin capillary system was used to decrease internal resistance drop. All solutions were degassed with nitrogen for at least 10 min before experiments were run. Electrode characteristics of the tapered DME glass capillary were $m = 1.557$ mg/s and $t = 1$ s at a mercury column height of 90.0 cm measured in pH 8.00

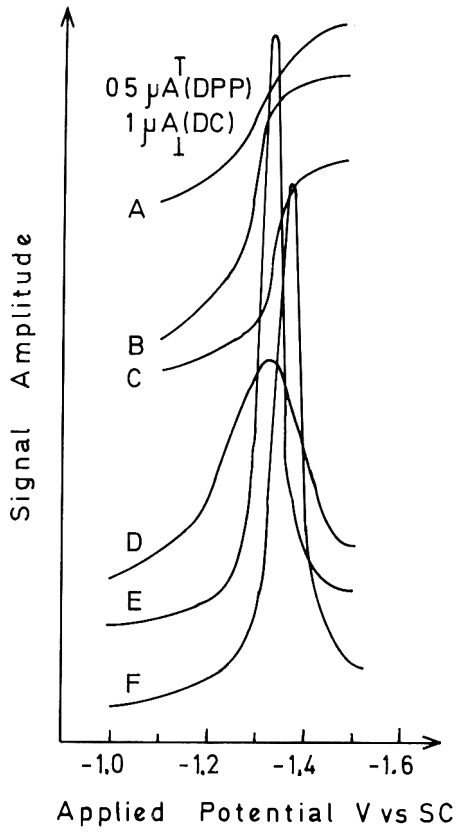
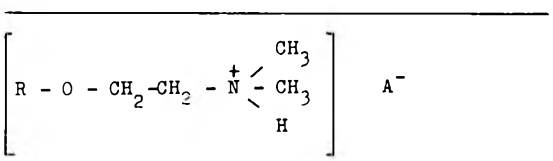


Figure 1. DC and DP polarograms of complexes in mixture of borate buffer pH 8.00 + 0.3F KNO_3 + 0.01F Me_4NBF_4 + 0.001F Cd(II). t_D = 1 s, scan rate = 5 mV/s, ΔE = 50 mV, $C = 2.5 \times 10^{-4}$F. (A) DCP of Cd(II)-CLPO, (B) DCP of Cd(II)-DMHR, (C)DCP of Cd(II)-DPHM, (D) DPP of Cd(II)-CLPO, (E) DPP of Cd(II)-DMHR, (F) DPP of Cd(II)-DPHM complexes.

Table 1. Structures of antihistamines studied



Compound	R	A
DPHM-HCl	$(C_6H_5)_2CH$	Cl
DMHR	$(C_6H_5)_2CH$	theophyllinate
CLPO-HCl	$Cl(C_6H_4)C_6H_5CCH_3$	Cl

borate buffer together with 0.3F KNO_3, 0.01F Me_4NBF_4, and 8.8×10^{-3}F Cd(II) at 0.0 V vs SCE at room temperature (20 + 0.1 °C).

For polarographic analysis, the Model 174 A instrument controls were set as follows: current range 5 μA, modulation amplitude 50 mV, drop time 1 s, initial potential −1.1 V vs SCE, potential scan rate 2 mV/s, and mercury column height 90.0 cm. The differential pulse operation mode was used throughout this work unless otherwise specified. The potential of the system was measured by using Heath Universal Digital Instrument Model EU 805.

Reagents and solutions.—Standard samples of antihistamines were obtained from the Central Institute of Hygiene of Turkey. These substances were tested for purity by melting points, UV and IR spectra, and thin layer chromatography (18). These analyses showed no impurities and drugs were used as supplied without further purification. "Pro analysi" quality chemicals from Merck were H_3BO_3, H_3PO_4, CH_3COOH, NaOH, KNO_3, $Cd(NO_3)_2.4H_2O$, Me_4NI, Me_4NOH, Me_4NCl, Me_4NBF_4, Et_4NOH, and Bu_4NI. Triple distilled mercury was used throughout the experiments.

Ethanolamine derivative antihistamines spontaneously form complexes with Cd(II) ion in the presence of KNO_3. The polarographic peak is highest at pH 8.00 borate buffer and range of linearity is widest in the presence of Me_4NBF_4. Therefore, a mixture of these substances was the supporting electrolyte for all of antihistamines studied.

Preparation of pharmaceuticals.—Ten tablets or 10 capsules were weighed, tablets were finely powdered, and an amount of powder corresponding to the mass of one tablet or one capsule was accurately weighed into a 100 mL volumetric flask. Five ampules or 10 mL elixir were transferred to a 100 mL volumetric flask, 50 mL water (deionized first and then triple distilled) was added, and samples were stirred magnetically 15 min. The solution was then diluted with water to the mark. A small amount of excipients settled at the bottom of the flask for tablet and capsule forms. A 100 μL portion of the upper, clear supernate was transferred to the polarographic cell containing 3 mL supporting electrolyte.

Polarographic experiments.—Three mL of the mixture of pH 8.00 borate buffer, 0.3F KNO_3, 0.010F Me_4NBF_4, and 8.8×10^{-3}F Cd(II) was deoxygenated with prepurified nitrogen for 15 min; nitrogen was also directed above the solution during the scan. After the polarogram of the supporting electrolyte was recorded, antihistamines were added by microliter pipet. A nitrogen stream was directed into the solution for 1 min and the polarograms of the complex were recorded. This procedure was repeated until peak height no longer increased. A calibration curve was drawn for pure antihistamines and the amount of active ingredient in pharmaceutical preparations was calculated by using this curve. No matrix effect was observed.

Results and Discussion

A thermodynamic analysis of the complexes of a series of commercial antihistamines with Cu(II), Ni(II), and Co(II) has been evaluated (19). Stepwise formation constants for the Be(II) (20) and Cd(II) (21) complexes with various antihistamine substances have been reported. Results show that all the antihistamines form stable compounds with metal ions, thereby demonstrating the validity of the nitrogen-metal ion coordination bond. The spontaneous reaction between metal ions and antihistamines, it has been suggested, forms chelate structures as a consequence of nitrogen coordination. The coordinate bond formation of Cd(II) with antihistamine and some related molecules likely occurs between the side chain amine nitrogen and the nitrogen of the imidazole ring, resulting in a 6-membered chelate.

Figure 1 shows the DC and DP polarograms of Cd(II)-CLPO, Cd(II)-DMHR, and Cd(II)-DPHM complexes. The DP polarograms produced by the Cd(II)-ethanolamine derivative antihistamines are well defined and sensitive enough so that the present method can be used for the indirect determination of those antihistamines. Chemical structures of their basic forms are similar, as seen in Table 1. The complex formation reaction proceeds via the nitrogen atom. For these

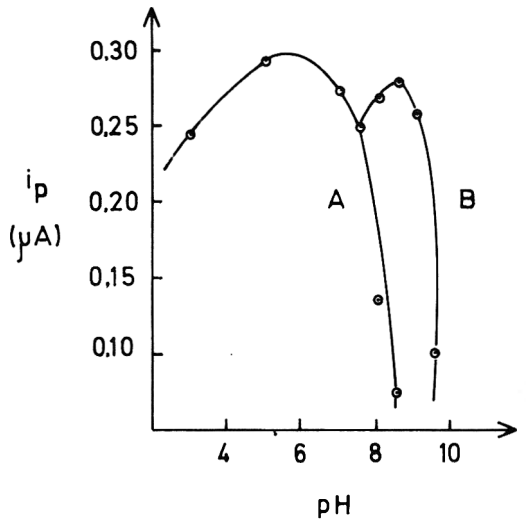


Figure 2. Variation of peak currents with pH of Cd(II)-DPHM complex: (A) Britton-Robinson buffer, (B) borate buffer.

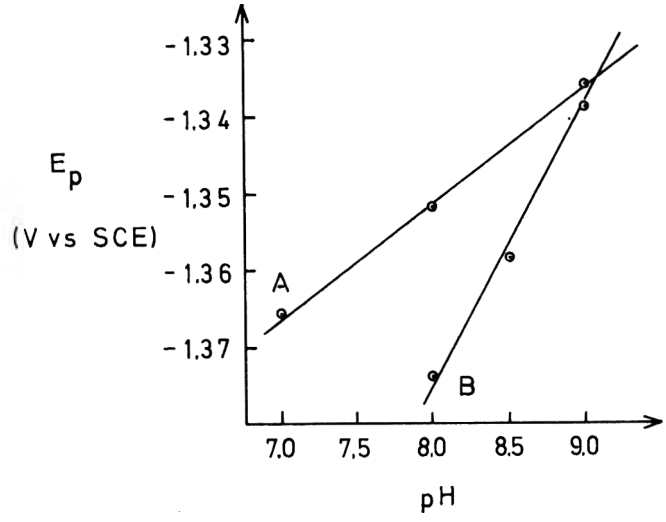


Figure 3. Variation of peak potentials with pH of Cd(II)-DPHM complex: (A) Britton-Robinson buffer, (B) borate buffer.

reasons discussion will be mainly based on Cd(II)-DPHM complex.

During the complex formation reaction, salts of bases were preferred as supporting electrolytes, to maintain the same ionic strength. Salt concentration, which varies over a wide range, affects the peak height and position of the metal ion-ligand complex (22). Different salts such as NaCl, NaI, Na_2CO_3, $NaHCO_3$, KCl, and KNO_3 in various concentrations (0.01–1.0F) were tried, and 0.30F KNO_3 gave the highest peak current.

Antihistamines form stable complexes with Be(II) ion between pH 4.0 and 7.5 (20) and with Cd(II) ion between pH 4.0 and 10.0 as seen in Figure 2 and Figure 3. The shift of peak potential and peak current shows us that H^+ transfer takes part in the reduction mechanism. The peak potential shifted to a negative direction as much as 30–40 mV with increasing concentration of antihistamines. The highest value for the peak current was observed around pH 8.00 for borate buffer. A thermodynamic analysis of Cd(II) complexes of antihistamines show that *pK* formation values were about 8.00 (21). For these reasons, pH 8.00 borate buffer was included in the supporting electrolyte mixture.

A quaternary ammonium salt was added to increase the linear range of calibration. The analysis can also be performed without it but with a narrower range of calibration. We assumed that the presence of quaternary ammonium salt prevents, to some degree, the adsorption of antihistamines to the surface of the mercury droplet. The highest peak current was observed in the presence of Me_4NBF_4.

There is no record of DP polarographic indirect determination of antihistamine compounds although some related compounds have been subjected to DC polarographic examination. The literature indicates the possibility of 2 centers of polarographic activity in the compounds having nitrogen heterocycle nucleus and the tertiary or quaternary amine group (23). DC polarographic study of antihistamine compounds based on the quaternary amine group showed that the reduction curves were not indicative of concentration nor were they reproducible (24). Table 2 gives the results obtained by linear regression analysis of several calibration curves of 10 points each obtained using DP polarography. Results indicate that Cd(II)-DPHM, Cd(II)-DMHR, and Cd(II)-CLPO complexes give linear calibration curves. The quantities of ethanolamine derivative antihistamines studied were easily determined by this method. Elixir and capsule forms of DPHM-HCl, tablet and injection forms of DMHR, and injection forms of CLPO-HCl were analyzed. Table 3 shows some results for pharmaceutical forms compared with analysis of similar material from the same batch by the UV spectroscopic method described in USP XX (1) and BP (2). The similarity of results indicates that indirect polarographic analysis of antihistamines can be used instead as an alternative to the official method.

Conclusion

A new method for the polarographic determination of ethanolamine derivative antihistamines is proposed. Antihistamines form complexes with Cd(II) ion in the presence of KNO_3 and the reduction of this complex polarographically can be used for quantitative analysis. Active ingredients in elixir, capsule, tablet, and injection forms of pharmaceutical preparations having these antihistamines can be analyzed easily by this method. The official methods require very long, tedious sample handling, which is not usually amenable to

Table 2. Characteristics of linear regression of calibration graphs for ethanolamine derivative antihistamines[a]

Compound	Slope	CI	CC	SEE	LL	UL	*N*
Cd(II)-DPHM	1.77	−2.32	0.997	0.029	0.04	0.09	10
Cd(II)-DMHR	1.62	−2.27	0.999	0.018	0.07	0.14	10
Cd(II)-CLPO	1.04	−1.27	0.988	0.008	0.05	0.07	10

[a]Conditions were the same as in Figure 1.
Abbreviations: CI = concentration intercept
CC = correlation coefficient
SEE = standard error of estimate
LL = lower limit of detection (mg/mL)
UL = upper limit of detection (mg/mL)
N = number of data points

Table 3. Assay of antihistamine preparations[a]

Compound	Theoretical	Polarographic	Official method
DPHM-HCl (elixir)	10.00 mg/ 4 mL	$\bar{x}$ = 10.083 ± 0.115	$\bar{x}$ = 10.010 ± 0.13
DPHM-HCl (capsule)	25.00 mg/ capsule	$\bar{x}$ = 25.405 ± 0.227	$\bar{x}$ = 24.390 ± 0.25
DMHR (tablet)	50.0 mg/ tablet	$\bar{x}$ = 50.431 ± 0.392	$\bar{x}$ = 48.5 ± 0.43
DMHR (ampule)	50.0 mg/ ampule	$\bar{x}$ = 50.15 ± 0.237	$\bar{x}$ = 48.9 ± 0.26
CLPO-HCl (ampule)	10.0 mg/ ampule	$\bar{x}$ = 11.27 ± 0.421	$\bar{x}$ = 10.74 ± 0.46

[a]Results are means of 10 separate measurements ± standard deviations.

routine analysis. No extraction is required before polarographic analysis and no excipients present in the tablet, elixir, capsule, and injection forms interfere with the analysis. Ethylenediamine and alkylamine derivative antihistamines will be the subject of our further study.

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VETERINARY ANALYTICAL TOXICOLOGY

Determination of Nitrate in Forages by Using Selective Ion Electrode: Collaborative Study

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Each of 10 collaborating laboratories analyzed 4 blind duplicate pairs of forage samples for nitrate, by using a potentiometric method. Two forage controls and a 100 000 mg KNO_3/L standard were also provided. Nitrate was extracted into an aqueous $Al_2(SO_4)_3$ solution containing 70 mg KNO_3/L and quantitated with a nitrate-selective electrode. Standards were prepared using extracting solution as diluent. Nitrate concentrations in forage samples ranged from <0.50 to 4.35% KNO_3. Repeatability coefficients of variation (CV_o) ranged from 1.74 to 3.61%, and reproducibility coefficients of variation (CV_R) ranged from 6.92 to 7.66%. Mean recovery of a 0.55% KNO_3 spike was 94.5%. The method has been adopted official first action.

Nitrate toxicoses in livestock have been recognized for a number of years (1). Ingested forages are the most common source of excessive nitrate exposure (2). Acute poisonings may be expected when forage nitrate concentrations exceed 1.6% KNO_3 (2). A rapid, accurate, and precise analytical method for determination of nitrate in forages is needed in prevention and diagnosis of nitrate toxicoses.

Several analytical methods are available for quantitation of nitrate in forages (3–7). McMahon and Casper (8) found that electrode methods gave results as reliable as other analytical methods. The method submitted to collaborative study was a modification of the method of Baker and Smith (4) and was selected because of its simple and rapid execution and its applicability to a wide range of concentrations.

Collaborative Study

The method, 4 blind duplicate pairs of forage samples, 2 control samples obtained from McMahon and Casper (8), and a 100 000 mg KNO_3/L standard were sent to 10 laboratories. Laboratories were requested to analyze each sample once. Samples quantitated at <0.50% KNO_3 were to be reported as <0.50% KNO_3.

Preparation of Samples

Collaborative samples were made by pooling and mixing ground forages of similar nitrate concentrations. Nitrate concentrations chosen were of diagnostic interest. One pair contained less than 0.50% KNO_3, one pair contained potentially hazardous concentrations (1–2% KNO_3), and one pair contained hazardous concentrations (> 3% KNO_3). The spiked sample was prepared by mixing 0.83 g oven-dried KNO_3 dissolved in enough water to make a slurry with 150.00 g milled forage sample. The slurry was dried (with occasional stirring) in a forced-air oven for 72 h at 60°C and, after cooling to room temperature, remilled through a 2 mm screen. All samples were submitted to collaborators as blind duplicates.

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The recommendation of the Associate Referees was approved by the General Referee and the Committee on Feeds, Fertilizers, and Related Materials and was adopoted by the Association. See the General Referee and Committee reports, *J. Asoc. Off. Anal Chem* (1986) **69**, March issue.
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Nitrate in Forages
Potentiometric Method
First Action

Principle

Nitrate is extd from sample into aq. $Al_2(SO_4)_3$ soln and detd potentiometrically. Lower limit of detection is 0.50% KNO_3.

Apparatus

(a) *Drying oven.*—Forced air, capable of heating to 100°.

(b) *Nitrate-specific ion electrode.*—Model 93-07, Orion Research, Inc., 840 Memorial Dr, Cambridge, MA 02139, or equiv. Monitor performance by assuring absolute value of std curve per decade slope ≥ 54 mV.

(c) *Reference electrode.*—Double junction (Model 90-02, Orion Research, Inc., or equiv.). Use extg soln as outer filling soln.

(d) *pH meter.*—Capable of measuring electrode potentials to nearest mV.

Reagents

(a) *Preservation soln.*—Dissolve 0.1 g phenylmercuric acetate in 20 mL dioxane (Caution: May form dangerous peroxides; see **51.070**) and dil. to 100 mL with H_2O.

(b) *Extracting soln.*—Dissolve 15.76 g $Al_2(SO_4)_3 \cdot 18\ H_2O$, 70.0 mg oven-dried KNO_3 (dry 2 h at 100°), and 1.0 mL preservation soln, **(a)**, in 500 mL H_2O. Dil. to 1 L with H_2O.

(c) *Nitrate std solns.—(1) Stock std soln.*—100 000 mg KNO_3/L. Weigh 20.00 g KNO_3 (dried 2 h at 100°) into 200 mL vol. flask, dissolve in 100 mL H_2O, and dil. to vol. with H_2O. *(2) Intermediate std soln.*—10 000 mg KNO_3/L. Dil. 20.0 mL soln *(1)* to 200 mL with extg soln. *(3) Working std solns.*—Dil. 1.00, 2.00, 4.00, and 10.0 mL soln *(2)* to 200 mL with extg soln to make 120, 170, 270, and 570 mg KNO_3/L solns. (Caution: Prep. all std solns from same lot of extg soln.)

Preparation of Standard Curve

Det. potential of blank (use extg soln as blank, equiv. to 70 mg KNO_3/L) and each working std soln while mag. stirring. Plot potential against nitrate concn (mg KNO_3/L) on semilog paper with concn on log scale. Det. std curve per decade slope. Per decade slope specification of electrode manuf. should be met or exceeded. (Note: Measure potential only after sample and stds are at same temp.)

Preparation of Sample

Dry sample in 60° forced air oven to constant weight. Grind dried sample to pass 2 mm screen and thoroly mix. Ext 1.00 g mixed ground sample with 100 mL extg soln by shaking 15 min. Keep ext sealed in container until potential is measured.

Quality Control

Analyze one control sample (if available) or spiked sample (at least one for every 10 samples or fraction thereof). Results should indicate acceptable accuracy.

Table 1. Collaborative data for potentiometric determination of forage nitrate (% KNO_3 on dry weight basis)

	Sample pairs (blind duplicates)[a]							
Lab.	11	17	4	9	3	7	6	14
1[b]	—	—	—	—	—	—	—	—
2	1.70	1.70	4.90	4.50	<0.50	<0.50	2.20	2.20
3	1.50	1.45	4.10	4.10	<0.50	<0.50	2.00	1.90
4	1.51	1.54	4.25	4.30	<0.50	<0.50	1.98	2.10
5	1.47	1.52	4.35	4.35	<0.50	<0.50	1.83	1.98
6	1.68	1.68	4.70	4.70	<0.50	<0.50	2.30	2.20
7	1.50	1.50	4.10	4.10	<0.50	<0.50	2.10	2.10
8[c]	—	—	—	—	—	—	—	—
9	1.59	1.59	4.01	4.21	<0.50	<0.50	2.00	2.00
10	1.80	1.72	4.67	5.12	<0.50	<0.50	2.17	2.33

[a]Sample pairs: 11/17; 4/9; 3/7; 6/14.
[b]Results rejected—QC indicators unsatisfactory.
[c]Results not available—electrode malfunction.

Hold suspect any analyses with unacceptable external control results and unacceptable std curve per decade slopes.

Determination

Det. potential of unfiltered ext at same temp. that std potentials are measured, stirring ext at same rate used for stds. Record potential after reading becomes stable or 1 min after insertion, whichever comes first.

Calculation

Calc. forage nitrate concn (% KNO_3 on dry wt basis):

$$\% \ KNO_3 = C_u = (C_g - 70) \times 0.010$$

where C_u = forage nitrate concn and C_g = nitrate concn (mg KNO_3/L) of ext obtained from std curve. If desired, convert nitrate concn expressed as % KNO_3 to ppm NO_3-N and ppm NO_3^- by multiplying by 1386 and 6133, resp.

Results and Discussion

Complete results were received from 9 laboratories (Table 1). One laboratory was unable to provide results because of electrode malfunction. Results from another laboratory were rejected because of quality control indicators.

Quality control was conducted by evaluating standard curve per decade slopes and control sample results. Per decade slopes had to meet or exceed electrode response specifications of electrode manufacturer. All laboratories reported slopes between −60 and −54 mV. No data were rejected on the basis of this criterion.

Data sets with control sample results deviating from accepted means of control samples by more than ± 20% were considered for rejection.

Statistical Data for Forage Controls (8)

x	=	3.60% KNO_3	1.16% KNO_3
S	=	0.37	0.22
CV	=	10%	19%
n	=	9	9

Data from one set, with control sample results deviating by >40%, were rejected. Another set with control sample results deviating between 20 and 30% was not rejected because the 1.16% KNO_3 control result was within 2 standard deviations of accepted mean. Statistical data for forage controls were calculated using only specific ion electrode results for samples from the study by McMahon and Casper (8).

Laboratories 2 and 5 made slight modifications of the procedure. One laboratory dried samples in a convection oven, which should not have affected results because collaborative samples were dried before shipping. However, drying time for samples obtained from the field may increase in a convection oven. Relatively small (<300 g) and dry (<15% moisture) samples usually dry overnight in a forced air oven, but larger and wetter samples may take up to 48 h to dry.

Table 2. Statistical data from potentiometric forage nitrate collaborative study

	Sample pairs (blind duplicates)[a]							
Statistic	11[b]	17[b]	4	9	6[b]	14[b]	3	7
Mean (% KNO_3 dry wt)	1.57		4.35		2.09		<0.50	
Repeatability								
S_o	0.28		0.16		0.72		—	
CV_o, %	1.74		3.61		3.44		—	
Reproducibility								
S_x	0.11		0.34		0.14		—	
CV_x, %	6.98		7.66		6.92		—	

[a]Sample pairs: 11/17; 4/9; 6/14; 3/7.
[b]Sample pair 6/14 were blind duplicates of a 0.55% KNO_3 spike of sample pair 11/17.

Another laboratory used a single junction reference electrode (SJE) instead of the specified double junction reference electrode (DJE). Data were not rejected. Most electrode manufacturers recommend using the DJE to eliminate possible chloride interference from the SJE. Care should be taken when using any SJE in this method.

Statistical analysis was performed according to the AOAC statistical manual (9), and results are listed in Table 2. No data were rejected on the basis of statistical analysis.

Results show that the analytical procedure is adequate for its intended purpose: analysis of forages for nitrate in diagnosis and prevention of acute nitrate toxicoses. No false positives or false negatives were reported. As indicated by 95% confidence intervals calculated from results in Table 2, the method can detect forage nitrate concentration between 0.50 and 4.35% KNO_3 to ± 0.2% KNO_3 with $CV_o \leq 3.6\%$ and $CV_x \leq 7.7\%$. This method has 2 advantages over method **7.046–7.052** (10): Execution is more rapid and the method is applicable to a wider range of concentrations without dilution.

The variation of this data is higher than found by Horwitz et al. (11) for collaborative studies with analyte concentrations of 0.5–5%. But comparison with the Horwitz curve is difficult: No indication of scatter about his curve was given, and the position on the concentration axis can be changed by almost an order of magnitude if concentration units of %NO_3-N are used instead of %KNO_3.

The CV_o/CV_x ratios for sample pairs 4/9 and 6/14 approximate those found by Horwitz et al. (11). The ratio for sample pair 11/17 is considerably less. A possible explanation is that

sample homogeneity within laboratories was much better than sample homogeneity between laboratories for this sample pair.

Accuracy of the method was *estimated* using the 0.55% KNO_3 spike. Mean recovery was 94.5%.

Lower limit of detection for the method is 0.50% KNO_3, based on the standards used. Quantitation of nitrate concentrations as low as 0.10% KNO_3 may be possible with the use of appropriate standards. All standard curves submitted were of such quality that direct concentration readout could be used on pH meters so equipped.

The authors acknowledge that there are several different units used in reporting nitrate concentrations (2, 12). Certain scientific disciplines prefer reporting nitrate concentrations as % KNO_3, and others as ppm NO_3-N or ppm NO_3^-. The method is easily adapted to individual laboratory preferences in expressing nitrate concentrations. Conversion to other units is trivial and will not affect method performance.

Recommendation

The Associate Referees recommend that this method be adopted official first action.

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FRUIT AND FRUIT PRODUCTS

Cranberry Juice Composition

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Eight samples of cranberries *(Vaccinium macrocarpon)* representing the major varieties and principal commercial growing regions in the United States were processed into juice. Four of the 8 samples were concentrated to 50° Brix. Liquid chromatography (LC) was used to determine nonvolatile organic acid, anthocyanidin pigment, and sugar profiles. Ultraviolet-visible spectral methods were used to determine anthocyanin concentration, polymeric color, and percent polymeric color. Other data presented include stable isotope carbon ratios, degree Brix, pH, and Hunter color parameters. These data are presented to serve as an authentic data base for use in detection of adulterated cranberry juice.

Cranberries *(Vaccinium macrocarpon)* and cranberry juice products are popular because of their attractive red color and mildly astringent flavor (1). Unfortunately, as it is with many higher priced commodities, the problem of adulteration in these products has become of concern to the fruit juice industry. Much work has been done in the area of establishing compositional data bases for popular fruit juices such as apple juice (2–4) and orange juice (4, 5), but the literature on cranberry juice is incomplete. The purpose of this paper, therefore, is to establish a compositional data base for authentic cranberry juice. Emphasis will be given to those techniques which are useful in detection of adulteration in cranberry juice drinks and cranberry juice concentrates.

METHODS

Samples

Frozen cranberries (10–14 lb per sample) were obtained from Ocean Spray Cranberries, Inc. (Middleborough, MA) and Minot Food Packers, Inc. (Bridgeton, NJ). All samples arrived frozen and were stored at −12°C until pressed. The samples had the following varietal make up, geographic origin, and seasonal history: Early Blacks (MA, 1982); Early Blacks (MA, 1983); Early Blacks (NJ); Howes (MA, 1982); Howes (MA, 1983); McFarlin (WA); Massachusetts Mixed (Howes and Early Blacks); Wisconsin Mixed (McFarlin, Howes, Stevens, and Searles).

Apparatus

(a) *Liquid chromatograph (LC).*—Varian Model 5000 equipped with column heater and either UV-50 variable wavelength detector or refractive index detector (Varian Instrument Group, Walnut Creek, CA).

(b) *Integrator.*—Model HP 3380A (Hewlett-Packard Corp., Avondale, PA).

(c) *LC Columns.*—*(1)* Micropak MCH-10 C18 column, (10 μm particle size), 4 mm id × 30 cm (Varian) fitted with RP-18, 3 cm × 4.6 mm id guard column (Rainin Instrument Corp., Woburn, MA). *(2)* Interaction CHO-620 carbohydrate column (Los Altos, CA) fitted with 3 cm × 4.6 mm id carbohydrate guard column (Rainin Instrument Corp.).

(d) *UV-visible spectrophotometer.*—Model DMS 100 interfaced with DS-15 data station (Varian).

(e) *Color difference meter.*—Model DP-25P-2 (Hunter Instruments, Reston, VA).

(f) *C18 Mini column.*—C18 Sep-Pak (Waters Associates, Milford, MA). To activate, pass 5 mL methanol through cartridge followed by 5 mL water.

Reagents

(a) *Mobile phase for LC organic acid analysis (phosphate buffer, pH 2.4).*—To 1 L glass-distilled, deionized water, add 20 g KH_2PO_4 and 20 g NaCl. Dissolve salts and adjust pH to 2.4 with concentrated phosphoric acid. Filter through 0.45 μm filter, type HA (Millipore Corp., Bedford, MA) and degas.

(b) *Mobile phase for LC of anthocyanidins (water–acetic acid–methanol–acetonitrile, 70+10+10+10).*—To 700 mL glass-distilled, deionized water, add 100 mL LC grade glacial acetic acid, 100 mL LC grade methanol, and 100 mL LC grade acetonitrile. Mix, filter through 0.45 μm Millipore filter, and degas. Make fresh solvent daily.

(c) *Mobile phase for LC determination of sugars (distilled water).*—Filter 1 L glass-distilled, deionized water through 0.45 μm type HA Millipore filter and degas. Make fresh solvent daily.

(d) *Organic acid standards 1.*—Add 400 mg each of reagent grade malic, citric, and quinic acids to 100 mL volumetric flask and dilute to volume with water.

(e) *Organic acid standards 2.*—Add 10 mg each of reagent grade shikimic acid and fumaric acid to 100 mL volumetric flask and dilute to volume with water.

(f) *Sugar standards.*—Add 2 g each of reagent grade glucose, sucrose, fructose, and sorbitol to 100 mL volumetric flask, dissolve, and dilute to volume with water.

(g) *Sugar internal standard.*—Add 700 mg reagent grade mannitol to 100 mL volumetric flask, dissolve, and dilute to volume with water.

(h) *0.01% Methanolic HCl.*—Add 0.1 mL concentrated HCl to 1 L reagent grade methanol.

(i) *0.01% HCl.*—Add 0.1 mL concentrated HCl to 1 L water.

(j) *25% Juice solution.*—Add 25 mL juice to 100 mL volumetric flask and dilute to volume with water.

Table 1. General properties of single strength cranberry juice

Variety	Degree Brix	Specific gravity	pH	$\delta^{13}C$, ‰ (PDB)
Howes '82	7.9	1.0327	2.53	−24.7
Howes '83	8.3	1.0339	2.54	−25.1
Early Blacks '82	7.8	1.0308	2.58	−24.1
Early Blacks '83	7.8	1.0326	2.58	−24.2
Early Blacks NJ	8.7	1.0364	2.64	−24.7
McFarlin	8.1	1.0336	2.57	−25.4
Mass. Mixed	7.8	1.0339	2.57	−25.0
Wisc. Mixed	6.5	1.0289	2.64	−25.5
Range: Min.:	6.5	1.029	2.5	−25.5
Max.:	8.7	1.036	2.6	−24.1
Average	7.9	1.033	2.6	−24.8
SD	0.6	0.002	0.041	0.513
CV, %	8.1	0.2	1.6	2.1

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Table 2. Nonvolatile organic acid composition of cranberry juice (expressed as single strength juice normalized to 8.0° Brix)

	Organic acid composition, g/100 g					Percentage of total acids, %				Acid ratios		
Sample	Quinic	Malic	Shikimic	Citric	Total	Quinic	Malic	Shikimic	Citric	Quinic/ malic	Quinic/ citric	Citric/ malic
Howes '82	1.39	0.78	0.03	0.86	3.06	45.4	25.5	1.0	28.1	1.8	1.6	1.1
Howes '83	1.25	0.70	0.03	0.80	2.78	45.0	25.2	1.1	28.8	1.8	1.6	1.1
Early Blacks '82	1.04	0.74	0.02	1.02	2.82	36.9	26.3	0.5	36.2	1.4	1.0	1.4
Early Blacks '83	1.08	0.64	0.01	0.95	2.68	40.3	23.9	0.4	35.4	1.7	1.1	1.5
Early Blacks NJ	0.90	0.60	0.01	0.77	2.28	39.5	26.3	0.4	33.8	1.5	1.2	1.3
McFarlin	0.83	0.71	0.05	0.66	2.25	36.9	31.6	2.2	29.3	1.2	1.3	0.9
Mass. Mixed	0.93	0.78	0.02	0.84	2.57	36.2	30.4	0.8	32.7	1.2	1.1	1.1
Wisc. Mixed	0.95	0.82	0.09	0.98	2.84	33.5	28.9	3.2	34.5	1.2	1.0	1.2
Range Min.:	0.83	0.60	0.01	0.66	2.25	33.5	23.9	0.4	28.1	1.2	1.0	0.9
Max.:	1.39	0.82	0.09	1.02	3.06	45.4	31.6	3.2	36.2	1.8	1.6	1.5
Mean	1.05	0.72	0.03	0.86	2.66	39.2	27.2	1.2	32.4	1.5	1.2	1.2
SD	0.19	0.07	0.03	0.12	0.28	4.2	2.7	1.0	3.2	0.27	0.24	0.18
CV, %	18.1	10.3	85.2	13.9	10.6	10.8	10.0	83.3	9.9	18.5	19.4	14.8

Table 3. Compilation of nonvolatile organic acid composition and titratable acidity values of cranberry juice

		Percentage of total acids, %			Total acid composition, g/100 g			Titratable acidity (as citrate), g/100 g		
Ref.	No. of samples	Quinic	Malic	Citric	Min.	Max.	Av.	Min.	Max.	Av.
—[a]	8	39.2	27.2	32.3	2.3	3.1	2.7	1.9	2.1	2.0
25	27	39.8	27.7	32.5			3.3			
26	1	39.5	28.5	32.0			2.5[b]			
21	12							1.9	2.3	2.1
27	17							2.1	2.9	2.3

[a]Results from this paper. Expressed as single strength juice normalized to 8.0° Brix.
[b]Original published value was for cranberry juice drink. Value converted to single strength juice by multiplying by 4 (assuming that cranberry juice drink analyzed contained 25% juice).

Table 4. Anthocyanin content of single strength cranberry juice as determined by LC

	Anthocyanin concn,[a] mg/100 g (as cyd-3-glu)			Percentage of total anthocyanins, %		
Sample	[cyd]	[pnd]	Total	[cyd]	[pnd]	[cyd/pnd] Ratio
Howes '82	8.6	7.5	16.0	53	47	1.1
Howes '83	10.3	6.5	16.8	61	39	1.6
Early Blacks '82	20.5	11.3	31.8	65	35	1.8
Early Blacks '83	17.9	10.4	28.3	63	37	1.7
Early Blacks NJ	8.8	7.6	16.4	54	46	1.2
McFarlin	11.0	9.9	20.9	53	47	1.1
Mass. Mixed	7.6	6.2	13.9	55	45	1.2
Wisc. Mixed	13.9	12.1	26.0	54	46	1.2
Range: Min.:	7.6	6.2	13.9	53	35	1.1
Max.:	20.5	12.1	31.8	65	47	1.8
Average	12.3	8.9	21.2	57	43	1.4
SD	4.7	2.3	6.6	5	5	0.3
CV, %	38	25	31	9	12	22

[a]Results normalized to 8.0° Brix. Key: cyd-3-glu = cyanidin-3-glucoside; [cyd] = cyanidin; [pnd] = peonidin.

Production of Cranberry Juice

Remove cranberries from frozen storage and thaw overnight at ambient temperature (ca 25°C). Grind berries through hammer mill (Model D Comminuting Machine, W. J. Fitzpatrick Co.) equipped with ½ in. diameter circular pore mesh at speed which will produce mainly ¼ berries. Add press aid (rice hulls) at the rate of 3%. Press in Willmes bag press (60 Type, Moffet Co., San Jose, CA), using the following pressure program: 0 psi for 5 min followed by 11 psi for 5 min, 0 psi for 5 min, 22 psi for 5 min, 0 psi for 5 min, 58 psi for 5 min, 0 psi for 5 min, and finally 66 psi for 5 min.

Filter juice through nylon mesh and store frozen.

Concentration of Samples

Concentrate ca 200 mL single strength juice on rotary evaporator (water bath, 30°C) to 50° Brix. For spectral analyses, redilute with water to original Brix value.

Determination of Specific Gravity and Brix

Measure specific gravity using the pycnometer technique, AOAC **9.017–9.019** (6). Determine Brix values with refractometer.

Determination of Titratable Acidity

Determine titratable acidity according to glass electrode method, AOAC **22.059** (6). Express results in terms of g anhydrous citric acid/100 g juice.

Spectral Analyses

Filter juice solutions through Whatman No. 1 paper in Buchner funnel. For scans in visible region, take 25% juice solution and make ⅛ dilution with 0.1N HCl. Scan from 700 to 300 nm. For scans in UV region, take 25% juice solution and make ⅛₈ dilution with 0.1N HCl and scan from 400 to 200 nm. Use 0.1N HCl for blank. Conditions: 1.0 cm quartz

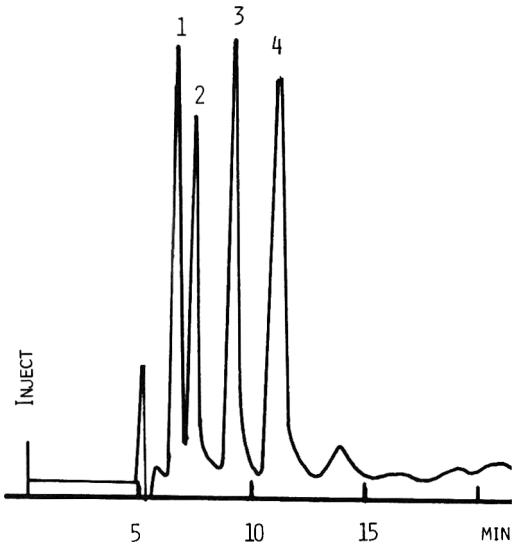

Figure 1. LC separation of cranberry organic acids: peak identification: 1, quinic; 2, malic; 3, shikimic; 4, citric.

cells, 1 nm slit width, 50 nm/min scan rate. Derivative spectra can be re-computed from original zero-order absorbance spectra.

Measure anthocyanin concentration, color density, and polymeric color by pH differential method and bisulfite bleaching method as described by Wrolstad (7). Calculate anthocyanin concentration from formula and extinction coefficient for cranberry pigments as reported by Fuleki and Francis (8).

Determination of Hunter Parameters

Set up instrument to read transmitted color, spectral component included (Arrangement III). Calibrate instrument as described by manufacturer. Take 25 mL 25% juice solution and acidify with 1.0 mL 6N HCl. Read Hunter parameters for 1.0 cm pathlength cell.

Determination of $^{13}C/^{12}C$ Stable Isotope Ratio Analysis

Concentrated samples (ca 50° Brix) were sent to Coastal Science Laboratories (5321 Industrial Oaks Blvd, Suite 103, Austin, TX 78735).

Preparation of Samples for LC of Nonvolatile Organic Acids

Take 1.0 mL 25% juice solution and pass through activated Sep-Pak cartridge into 10 mL graduated cylinder. Wash cartridge with pH 2.4 buffer until final volume in cylinder is 4.0 mL. Filter through 0.45 μm Millipore filter and inject onto LC column.

LC Determination of Nonvolatile Organic Acids

Operate LC apparatus under following conditions: column, Micropak MCH-10 C18; mobile phase, pH 2.4 phosphate buffer; flow rate, isocratic, 0.5 mL/min; temperature, 25°C; detector, UV at 227 nm, 0.1 absorbance unit full scale (AUFS); injection volume, 50 μL.

Calculate organic acid composition by external standard method. Prepare standard curve from ¼, ⅛, and ½ dilutions of organic acid standards 1 and 2. (Cranberry juice is typically high in acid; when other products are analyzed, different dilutions may need to be made.) Determine percent recovery of acids by comparing peak areas of standard acid mixture to peak areas of same solution subjected to cartridge cleanup procedure. Calculate percent recovery for each acid as follows:

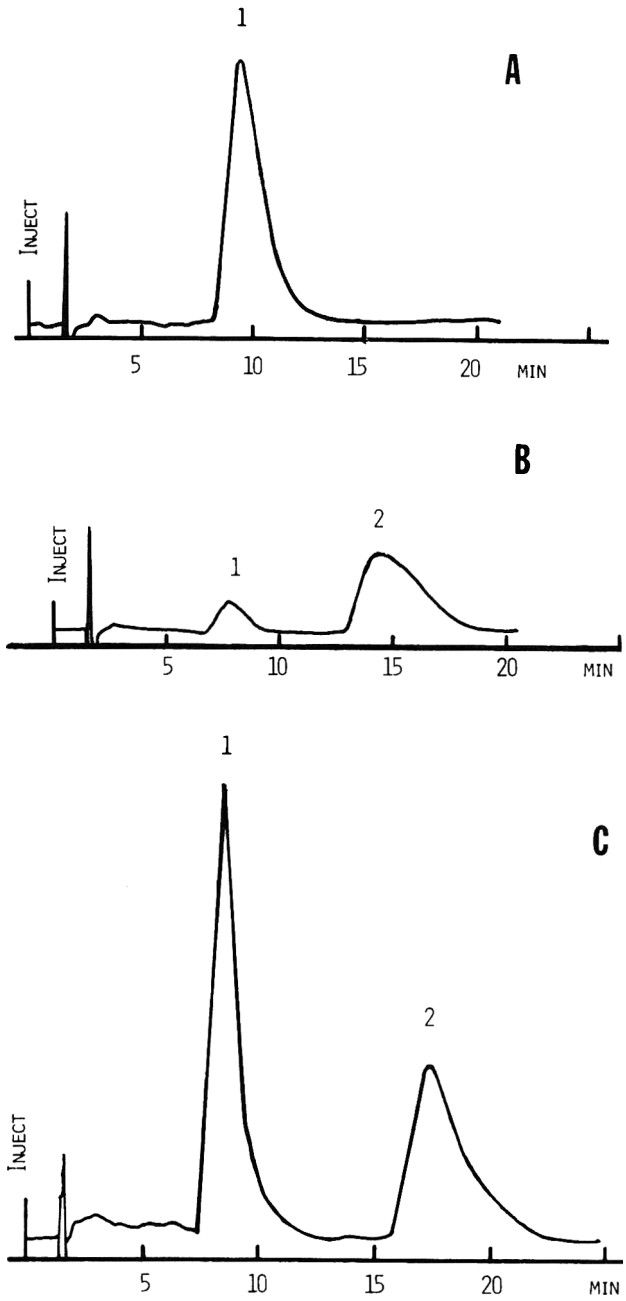

Figure 2. LC separation of anthocyanidins from A, blackberries; B, mangos; C, cranberries: peak identification: 1, cyanidin; 2, peonidin.

$$\%R = (A_2/A_1) \times 100$$

where A_1 is peak area of untreated acid standard and A_2 is peak area of treated acid standard.

Reagent grade malic acid invariably contains trace quantities of fumaric acid (9); make corrections by subtracting weight of fumaric acid from weight of malic acid to determine actual amount of malic acid present.

Calculate detector response factor (k) for each individual acid by using following formula:

$$k = C/A$$

where A is peak area and C is acid concentration in mg/mL.

Compute acid concentrations in samples by using appropriate values in following equation:

$$\text{Acid concentration (mg/mL)} = (A_s \times k \times 100 \times \text{DF})/\%R$$

where DF = dilution factor, A_s = peak area of acid in sample.

Table 5. Sugar composition of cranberry juice

Sample	Sugar concn,[a] g/100 g				Percentage of total sugars, %		Glucose/ fructose ratio
	Glucose	Fructose	Sorbitol	Total	Glucose	Fructose	
Howes '82	4.0	1.0	0.00	5.0	80.9	19.1	4.2
Howes '83	4.3	1.0	0.00	5.3	81.9	18.1	4.5
Early Blacks '82	3.7	1.2	trace[b]	4.8	75.7	24.3	3.1
Early Blacks '83	3.9	1.1	0.00	5.0	78.3	21.7	3.6
Early Blacks NJ	5.0	1.4	0.00	6.4	78.4	21.6	3.6
McFarlin	4.8	1.5	0.00	6.3	76.7	23.3	3.3
Mass. Mixed	4.1	1.2	0.00	5.3	77.3	22.7	3.4
Wisc. Mixed	4.3	0.9	trace	5.1	82.8	17.2	4.8
Range: Min.:	3.7	0.9		4.8	75.7	17.2	3.1
Max.:	5.0	1.5		6.4	82.8	24.3	4.8
Average	4.3	1.1		5.4	79.0	21.0	3.8
SD	0.5	0.2		0.6	2.6	2.6	0.6
CV, %	10.7	18.3		11.1	3.2	12.2	16.1

[a]Expressed as single strength juice normalized to 8.0° Brix.
[b]Trace levels at less than 0.005 g/100 g.

Preparation of Samples for LC Determination of Sugars

Take 5 mL 25% juice solution and pass through activated C18 Sep-Pak cartridge. Discard first 2 mL and collect clear eluate. Dilute ½ with mannitol sugar internal standard solution, filter through 0.45 μm Millipore filter, and inject onto LC column.

LC Determination of Sugars

Operate LC under following conditions: column, Interaction CHO-620 carbohydrate column; mobile phase, 100% water; flow rate, isocratic, 0.5 mL/min; temperature, 90°C; detector, refractive index, 4 × attenuation; injection volume, 10 μL.

Quantitate sugars by internal standard method. To determine detector response factors, take 1, ½, and ¼ dilutions of sugar standard solution and treat as described above. Calculate detector response factor *(k)* for individual sugars from following formula:

$$k = (A_{is}/A) \times (C/C_{is})$$

where A_{is} is peak area of internal standard, A is area of sugar in question, C is concentration (mg/mL) of sugar, and C_{is} is concentration (mg/mL) of internal standard.

Calculate concentration of individual sugars in samples, substituting appropriate values into following formula:

$$\text{Concn (mg/mL)} = A \times C_{is} \times \text{DF} \times k/A_{is}$$

where A is peak area of sugar in question and DF is dilution factor.

Sample Preparation for LC Determination of Anthocyanidins

Take 20 g 25% juice solution, and add 1 g NaCl and 20 mL 0.01% HCl. Mix to dissolve and cool solution in ice bath. Take 20 mL aliquot of cooled solution and adsorb pigment into activated Sep-Pak cartridge. Wash cartridge by injecting ca 5 mL 0.01% HCl; then elute the red pigment from column with 0.01% methanolic HCl and collect in 250 mL round-bottom flask. Repeat procedure with remaining juice solution, and combine eluates.

Concentrate to ca 1 mL on rotary evaporator (water bath, 27°C) and add 15 mL 2N HCl. Transfer mixture to Kimax screw-top test tube, flush with nitrogen, and seal with Teflon-lined cap. Immerse tube in rapidly boiling water bath and hydrolyze for 30 min.

Quickly cool hydrolysate in ice bath. Pass half of the solution into activated Sep-Pak cartridge, wash with ca 5 mL 0.01% HCl, and elute purple pigments with 0.01% methanolic HCl into 100 mL round-bottom flask until color has been removed from cartridge. Repeat adsorption and elution process with remaining solution. Combine eluates and concentrate to ca 1 mL on rotary evaporator. Measure volume, filter through 0.45 μm Millipore filter (type HV), and inject immediately onto LC column. Note: Due to instability of these anthocyanidin pigments, all of the above procedures should be carried out in the dark or in subdued light. Inject samples onto LC column immediately after sample preparation. Between injections, store samples in the dark in an ice bath.

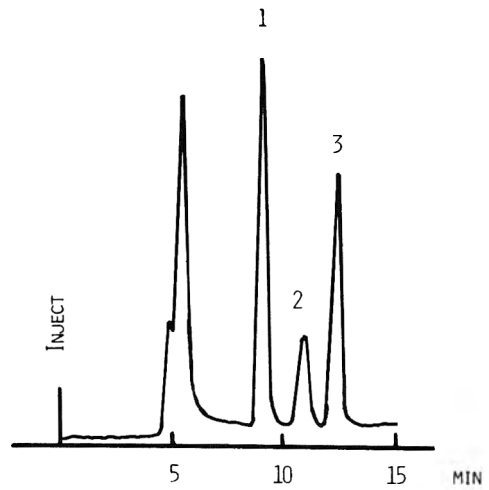

Figure 3. LC separation of cranberry sugars. Peak identification: 1, glucose; 2, fructose; 3, mannitol (internal standard). Retention times (min): sucrose, 7.0; glucose, 8.9; fructose, 10.8; mannitol, 12.3; sorbitol, 14.4.

LC Determination of Anthocyanidins

Operate LC apparatus under following conditions: column, MCH-10 C18 Micropak (10 μm); mobile phase, water–acetic acid–methanol–acetonitrile (70 + 10 + 10 + 10); flow rate, isocratic, 1.8 mL/min; temperature, 27°C; detector, visible at 530 nm; injection volume, 50 μL.

Determine retention times of the 6 major anthocyanidins by injecting known standards. If known standards are not available, commercial concord grape juice is an excellent source of 5 (all but pelargonidin) of the 6 major anthocyanidins (10, 11). The sixth anthocyanidin, pelargonidin, can be found in strawberries. The elution order is as follows: delphinidin, cyanidin, petunidin, pelargonidin, peonidin, and malvidin (11–13). Calculate detector response factor *(k)* for cyanidin and peonidin by injecting reference pigments and

Table 6. UV-Visible spectral characteristics for 8 authentic cranberry juice samples

Mode	Wavelength range, nm	
	Maxima	Minima
Absorbance	511–512	
	277–279	
	202–203	
1st derivative	472–474	537–542
	343–346	374–379
	261–264	327–329
	213–214	232–239
2nd derivative	550–551	502–503
	330–336	348–350
	331–332	268–272
	282–287	218–220
	232–236	206–208

using the following formula (if reference pigments are not available, see below):

$$k = C/A$$

where C is concentration of pigment (moles/L) and A is peak area.

Calculate concentrations of individual pigments in samples by using following formula:

$$\text{Pigment concn(moles/L)} = A_s \times k \times \text{DF}$$

where A_s is peak area of appropriate sample peak and DF is dilution factor.

In this study, detector response factors were estimated using semipurified anthocyanin extracts. Mangos *(Mangifera indica)*, reported to contain only peonidin-3-galactoside (14) were used as a source of peonidin. Blackberries *(Rubus fruticosus)*, reported to contain cyanidin-3-glucoside as a major pigment with only traces of cyanidin-3-rutinoside (15–17), were used as a source of cyanidin. To isolate pigments, blend ca 50–100 g fruit (for mangos, use skins only) in 0.01% methanolic HCl and extract overnight in refrigerator. Filter solution through Whatman No. 1 paper, using Buchner funnel. Remove methanol with rotary evaporator (water bath, 27°C) and dissolve pigments in ca 10 mL 0.01% HCl. Pass pigment solution through activated Sep-Pak cartridge, wash with ca 5 mL 0.01% HCl, and elute pigments into round-bottom flask with 0.01% methanolic HCl. Evaporate on a rotary evaporator until final volume is ca 1 mL. Determine pigment concentration by using published procedures and extinction coefficients (7, 18–20); molar absorbance for cyanidin-3-glucoside = 2.574×10^4; $E^{1\%}$ for cyanidin = 1134, peonidin = 1150, cyanidin-3-galactoside = 920, peonidin-3-galactoside = 936, cyanidin-3-arabinoside = 941, peonidin-3-arabinoside = 947.

Prepare sample for LC analysis, using 0.5 mL pigment concentrate.

Results and Discussion

General Properties

Table 1 lists the physical properties of the 8 cranberry samples analyzed. The pH values are in agreement with those reported by Schmid (21) who found pH values in the range 2.43–2.77 for 12 different varieties.

Degree Brix values range from 6.5 to 8.7 with a mean of 7.9. These values are well below the 10.5° Brix standard for cranberries as specified by the USDA jelly standard.

The $\delta^{13}C$ values exhibit a narrow range and lower %CV values than have been found for apples (22) and oranges (23). The value is in close agreement with the −25.0 value previously reported by Parker (24).

Nonvolatile Organic Acid Analyses

A major characteristic of cranberry juice is its high acidity. The organic acid profile of cranberries is highly unusual in that the major acid present is quinic acid. Table 2 lists the results of the organic acid analyses while Table 3 lists titrat-

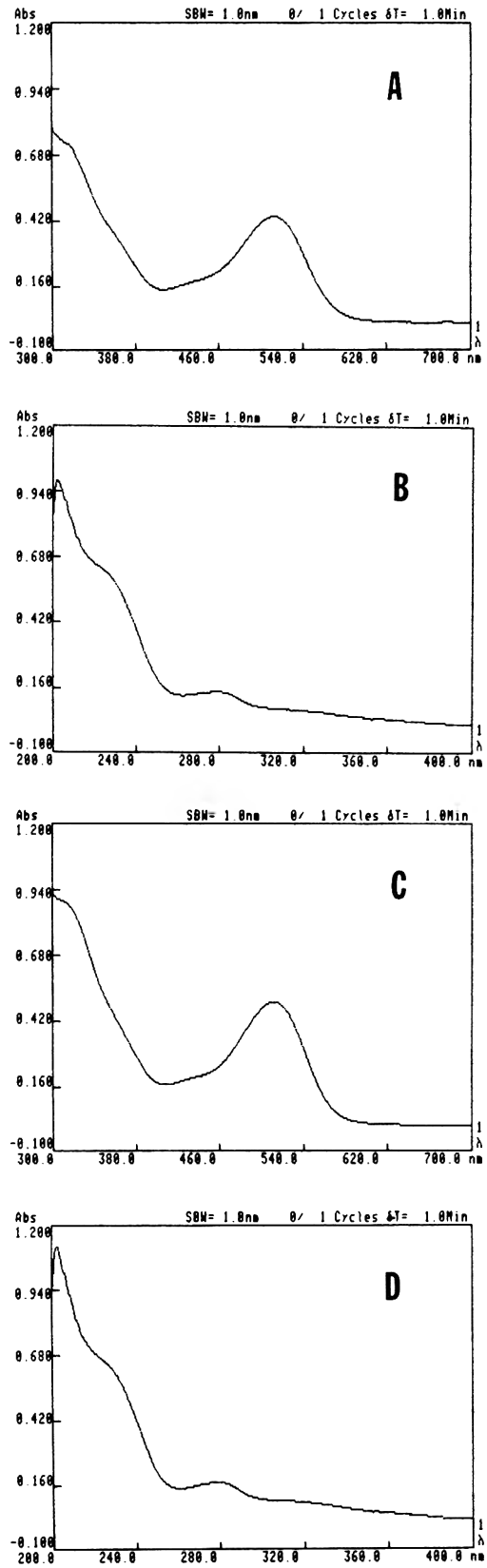

Figure 4. Absorbance spectra for 2 major varieties of cranberry juice: A, B, Howes (MA, 1982); C, D, Early Blacks (MA, 1983). Visible spectra, 1/32 dilution, A, Howes, C, Early Blacks. UV spectra, 1/352 dilution, B, Howes, D, Early Blacks.

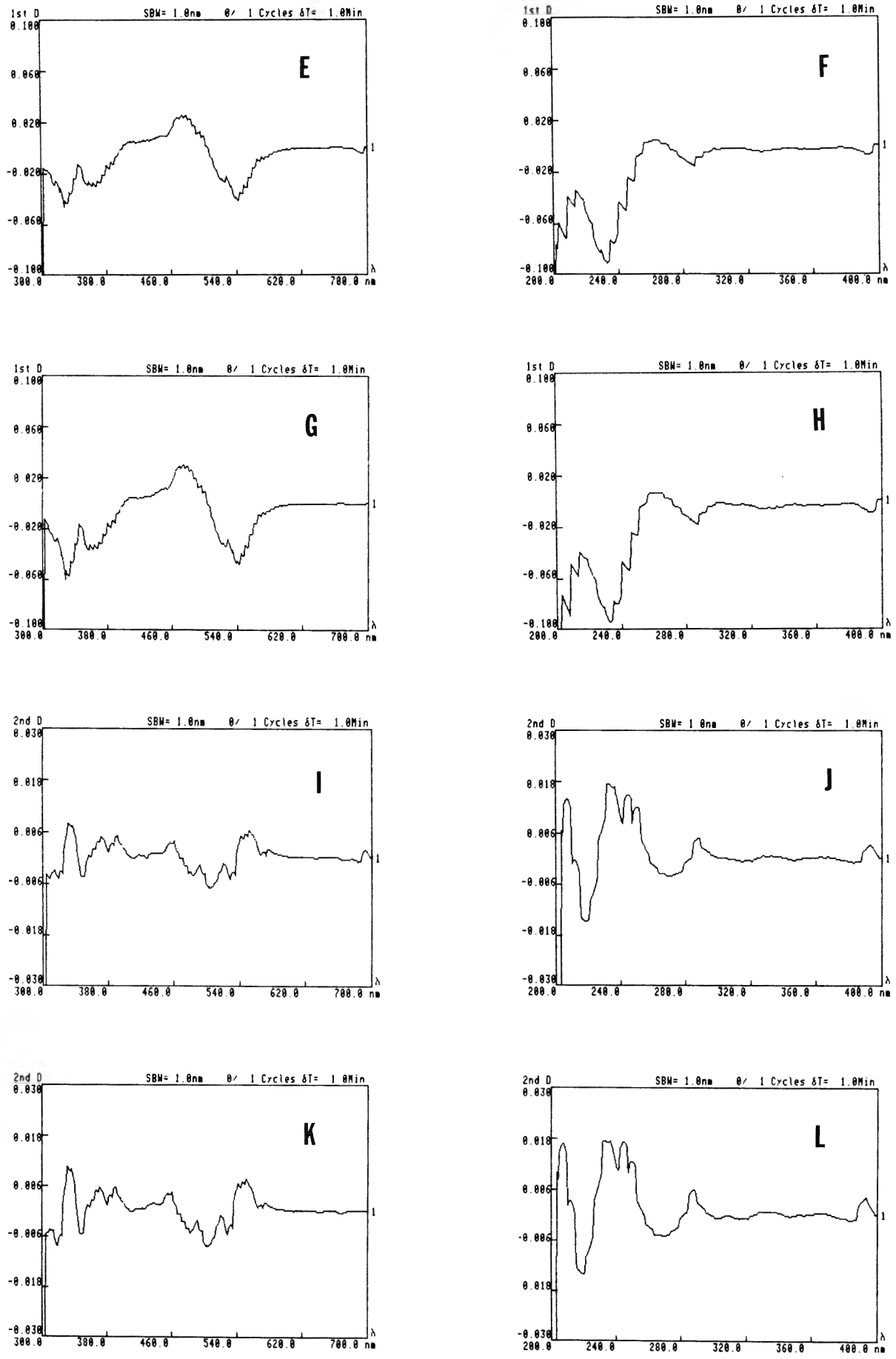

Figure 5. First and second derivative spectra for 2 major varieties of cranberry juice: E,F,I,J, Howes (MA, 1982); G,H,K,L, Early Blacks (MA, 1983). First derivative spectra: visible (1/32 dilution), E, Howes, G, Early Blacks; UV (1/352 dilution), F, Howes, H, Early Blacks. Second derivative spectra: visible (1/32 dilution), I, Howes, K, Early Blacks; UV (1/352 dilution), J, Howes, L, Early Blacks.

Table 7. Anthocyanin concentration, color density, and percent polymeric color of cranberry juice

Variety	Anthocyanin concn,[a] mg/100 g	Color density	Polymeric color	Percent polymeric color, %
Howes '82	28.4	10.5	1.0	9.1
Howes '83	30.7	9.2	0.9	9.9
Early Blacks '82	47.0	14.0	1.1	7.6
Early Blacks '83	34.8	10.6	1.2	11.7
Early Blacks NJ	29.6	8.9	1.2	13.9
McFarlin	38.7	15.7	1.9	11.9
Mass. Mixed	19.0	6.0	0.8	13.3
Wisc. Mixed	47.2	11.9	0.8	6.5
Howes '82 C[b]	29.9	10.6	1.0	9.4
Howes '83 C	30.8	10.2	1.4	14.0
Early Blacks '82 C	53.4	16.0	1.1	6.7
Early Blacks '83 C	36.5	11.1	1.2	11.3
Range: Min.:	19.0	6.0	0.8	6.5
Max.:	53.4	16.0	1.9	14.0
Mean	35.5	11.2	1.1	10.4
SD	9.7	2.8	0.3	2.6
CV, %	27.3	25.4	26.9	25.4

[a]Expressed in terms of cranberry anthocyanins (ref. 8). Results normalized to 8.0° Brix.
[b]C denotes samples that have been concentrated and re-diluted.

able acidity values and organic acid values, and compares these results with compositional cranberry acid data from the literature. A typical chromatogram of the cranberry acids is shown in Figure 1. The quantitative data were normalized to an 8.0° Brix single strength standard to facilitate comparison with commercial cranberry juice drinks and concentrates. The values can be readily converted to the original numbers by reference to the degree Brix values for the individual samples (Table 1). The quantities and proportions of quinic, malic, and citric acids are in close agreement with those reported by Coppola et al. (25). The total acidity (by summation) is lower than that reported by Coppola et al. but higher than that reported by Ryan and Dupont (26). The total titratable acidity is in close agreement with values reported by Schmid (21) and Boland et al. (27). The relative percentages of the acids are remarkably constant and in very close agreement with other workers. This illustrates the importance of using ratios as well as absolute quantities in compositional analyses. Nonvolatile organic acid profiles have proved to be very useful in determining the authenticity of cranberry juice (28).

Trace quantities of shikimic acid were found in all samples. This acid has not been previously reported in cranberries. Identification was made by comparison of retention time, production of a single symmetrical peak after spiking with shikimic acid standard, and comparison of relative extinction coefficients at several different wavelengths. Since shikimic acid and quinic acid are metabolically related (29), its presence in cranberry juice is not surprising.

Anthocyanidin Analyses

The anthocyanins of cranberries have been thoroughly investigated (8, 19, 20, 30–36). Four pigments have been identified, the 2 major pigments being cyanidin-3-galactoside and peonidin-3-galactoside, and the 2 minor pigments being cyanidin-3-arabinoside and peonidin-3-arabinoside (19, 20).

The use of LC to separate cranberry anthocyanins has been reported (37); however, the time required for analysis is long and the identification of peaks is difficult because of the many different anthocyanins found in nature. Hydrolysis of the anthocyanin to the aglycone (anthocyanidin) simplifies peak identification as only 6 major anthocyanidins occur in nature. Several LC systems have been developed to quickly separate these 6 compounds (11, 12). Listed in Table 4 are the results of our anthocyanidin analyses. The quantities of the individual anthocyanidins show more variation than their relative percentages. The percentages are relatively constant and in close agreement with the work of Fuleki and Francis (35) who reported 55% cyanidin and 45% peonidin by using thin layer chromatography (TLC)/transmission densitometry. The total anthocyanin pigment concentration as estimated by LC is considerably lower (18–47%) than that determined by spectral methods. This could be due to cumulative losses during the hydrolysis, isolation, and sample preparation steps.

Typical chromatograms of cranberry, blackberry, and mango anthocyanidins are shown in Figure 2. Mango was found to contain about 15% cyanidin, this pigment has not been previously reported for this fruit. When the amount of peonidin was calculated, the amount of cyanidin was corrected for, using the detector response factor for cyanidin. The detector response factors for both cyanidin and peonidin were found to differ by only 8%.

The use of anthocyanin and anthocyanidin profiles in verification of authenticity of a fruit can be very helpful when adulteration with another fruit is suspected, because the anthocyanin composition of many fruits is quite distinctive (38). The consistent anthocyanidin ratios for cranberry fruit makes anthocyanidin profiles a potentially very useful index for use in verification of authentic cranberry juice.

Sugar Analyses

The results of LC analyses for the 8 samples is shown in Table 5 and a typical chromatogram is shown in Figure 3. Glucose and fructose are the major sugars with much more glucose accumulating than fructose. Sucrose was not found and a sorbitol peak was detected in 2 samples, the quantity of sorbitol in those samples being less than 0.005 g/100 g. The relative proportion of glucose and fructose shows less variation than concentration on a g/100 g basis. The total sugar content is similar to that reported by Schmid (21)—an average of 6.10 g/100 g fruit and a range of 5.25–7.13 g/100 g whole berries for 12 samples. Schmid found a wider range in glucose/fructose ratios (2.27–5.20, average of 3.09). He attributed the variance in sugar content and sugar ratios to the ripeness of the fruits because it is difficult to determine the maturity of the fruit by visual means. Sugar profiles have limited use in detecting adulteration in commercial cranberry juice drinks because of added sweeteners. Sugar analyses however can be of value in checking cranberry juice concentrates for authenticity. The high glucose-fructose ratio is unusual and would be altered by adulteration with most other juice concentrates. Detection of significant quantities of sorbitol would be indicative of adulteration with less expensive sorbitol containing fruits such as plum or pear (28, 38, 39).

Spectral Characteristics

The attractive red color of cranberries is one of the most important attributes of cranberry products. It is not surprising, therefore, that much attention has been given to measurement of cranberry anthocyanins (8, 30, 32). Shown in Table 6 are the spectral characteristics of the samples analyzed. Figures 4 and 5 show typical absorbance, first derivative, and second derivative plots. The use of derivative techniques helps to resolve the fine structure of the spectra not normally seen in zero order plots (40). Spectral curves have been used in the past for detection of adulteration in orange juice (41). The relative simplicity of taking spectra along with the presence of common spectral patterns obtained

Table 8. Hunter and CIE parameters for authentic cranberry juice (transmission spectra, spectral component included)

	Hunter parameters			CIE parameters					
Sample	L	a	b	Y	X	Z	Hue	SI[a]	Hue/SI
Howes '82	49.1	66.9	29.0	24.0	41.8	4.5	66.6	72.9	0.91
Howes '83	49.7	66.2	29.4	24.7	42.5	4.6	66.0	72.4	0.91
Early Blacks '82	46.3	67.6	30.7	21.4	38.3	1.4	65.6	74.2	0.88
Early Blacks '83	48.5	66.2	30	23.5	40.8	3.3	65.6	72.7	0.90
Early Blacks NJ	47.9	63.2	29.5	22.9	39.3	3.3	65.0	69.7	0.93
McFarlin	43.4	67.6	27.9	18.8	34.7	1.8	67.6	73.1	0.92
Mass. Mixed	56.8	61.5	25.9	32.2	50.9	13.3	67.2	66.7	1.01
Wisc. Mixed	48.9	66.8	30	23.8	41.4	3.5	65.8	73.2	0.90
Range: Min.:	43.4	61.5	25.9	18.8	34.7	1.4	65.0	66.7	0.88
Max.:	56.8	67.6	30.7	32.2	50.9	13.3	67.6	74.2	1.01
Average	48.8	65.8	29.1	23.9	41.2	4.5	66.2	71.9	0.92
SD	3.8	2.2	1.5	3.8	4.6	3.7	0.9	2.5	0.04
CV, %	7.8	3.4	5.2	16.0	11.3	83.9	1.3	3.4	4.1

[a]SI = Saturation Index

from cranberry juice may allow these methods to be useful as a screening technique.

Anthocyanin concentration, color density, polymeric color, and percent polymeric color (Table 7) show that the juices contain a relatively low amount of polymerized pigments. Anthocyanin concentration is highly dependent on variety, harvesting year, and geographic origin. Previous studies reported anthocyanin content ranging from 26 to 119 mg/100 g whole berries (21).

Hunter Parameters

Much work has been done using tristimulus colorimetry for measurement of cranberry color (42–46). Johnson et al. (46) showed that Hunter L, a, and chroma values $([a^2 + b^2]^{1/2})$ exhibited excellent correlation with anthocyanin concentration. Shown in Table 8 are the Hunter parameters for the 8 samples analyzed. All 8 of the samples were found to exhibit similar Hunter transmittance values.

Summary

The chemical composition of 8 authentic samples of cranberry juice is reported. Distinctive patterns in acids, pigments, sugars, stable isotopic carbon ratios, and tristimulus transmittance values were found for the samples analyzed. The principal characteristics of an authentic single strength cranberry juice were as follows: total organic acids, 2.2–3.3 g/100 g; relative percentages of organic acids, quinic-39%, citric-32%, and malic-27%; total anthocyanins by pH differential, 19.0–53.3 mg/100 g; relative percentages of anthocyanidins, cyanidin-57%, peonidin-43%; total sugars, 3.6–5.0 g/100 g; relative percentages of sugars, glucose-79%, fructose-21%; and $\delta^{13}C$ (PDB), −24.1 to −25.5‰. These data are consistent with previous reports and should prove useful as a reference base for detecting adulteration in cranberry juice drinks and concentrates.

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FOOD ADULTERATION

Detection of Adulteration in Commercial Cranberry Juice Drinks and Concentrates

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Thirty-one samples of commercial cranberry juice drink and one sample of commercial cranberry juice concentrate were analyzed for nonvolatile acids and anthocyanidin profiles by liquid chromatography (LC). Ultraviolet-visible spectral measurements were used to measure pigment concentration, polymeric color, and percent polymeric color. Nineteen of the 31 samples analyzed were found to be adulterated. The adulterated samples exhibited nonvolatile organic acid profiles indicative of added malic and/or citric acid. Anthocyanidin profiles of the adulterated samples showed the presence of substantial quantities of delphinidin and malvidin, neither of which are present in cranberries in significant amounts. Grape skin extract is believed to be the added colorant.

Because of their attractive color and distinctive flavor, cranberry juice products have become very popular in the United States. Recently, concern has been growing over the presence of suspect cranberry juice cocktail on the market. These suspicions led to the beginning of this investigation, which was to develop methods to detect adulteration in cranberry juice drinks and cranberry juice concentrates.

In today's highly competitive market, considerable economic incentive exists to manufacture and sell diluted and/or adulterated goods. The high cost of the fruit along with the potential for high earnings are incentive for adulteration by unscrupulous manufacturers. Such practices allow companies to easily gain an economic advantage over any competitor. Considerable profit can be realized if only 10 or 15% juice is used instead of the 25% juice normally used in formulation of cranberry juice cocktail. Detection of these fraudulent products is therefore important not only to those who manufacture the authentic product, but also the farmers who grow the berries, to those who are involved in the regulatory aspects, and finally to those consumers who purchase the product.

Cranberry juice cocktail commonly consists of 25% single strength cranberry juice, water, sweeteners (usually high fructose corn syrup), and ascorbic acid. To fabricate a cranberry juice cocktail with considerably less than 25% cranberry juice but still retain the color and taste characteristics of a 25% juice drink, color and acid will need to be added. Detection of added colorants or acidulants would therefore be indicative of adulteration provided they were not declared on the label. In this study, emphasis will be given to development and use of methods to detect the presence of these compounds that are added by manufacturers to disguise an adulterated juice drink.

METHODS

Samples

Samples a–e were supplied by Minot Foot Packers, Inc., a manufacturer of cranberry juice. These juice drinks were manufactured by Minot with the following specifications: Sample a contained 10% juice, added citric acid, and added grape skin extract; Sample b contained 20% juice and added citric acid; Sample c contained 20% juice and added citric acid; Sample d contained 25% juice; Sample e contained 27% juice. Samples f–i were commercial samples sent by Minot with the labels removed.

Samples 1–22 were commercial samples, purchased in markets by A. Rosenzweig Associates, Inc., an independent third party contracted on behalf of the sponsor of the project. All labels were removed; samples were coded and delivered "blind" to the OSU Department of Food Science for analysis. Sample 11 was received in the form of a juice concentrate. The identities of these 22 samples were not revealed until all analyses and data interpretation were completed. We were informed that the samples did not represent a cross section of the marketplace, but we were not given any information as to who produced the samples or which of the samples (or how many of them) were suspected of being adulterated. All 22 blind samples were claimed by the manufacturers as containing only cranberry juice and/or cranberry juice concentrate, water, sweeteners, and ascorbic acid as ingredients.

Procedure

The following determinations were made as described in a previous publication (1): LC determination of nonvolatile organic acids and anthocyanidins; spectral determination of total anthocyanin and polymeric color; Hunter L, a, b, hue, and Saturation Index by tristimulus colorimetry. Cranberry juice cocktail is equivalent to 25% single strength juice solution. To make a single strength juice from a concentrate, the concentrate is diluted with distilled water to 8.0° Brix.

In the determination of anthocyanidin profiles, the detector response factors of all the anthocyanidins were assumed to be the same.

Results and Discussion

The results of the nonvolatile organic acid analyses are shown in Table 1. Chromatograms of both an authentic juice and an adulterated juice are shown in Figure 1. Previous work on the major cranberry nonvolatile acids show that the acid profile for cranberries is quite constant; 1.11–1.62 g/100 g (mean, 1.32 g/100 g) quinic acid, 0.75–1.14 g/100 g (mean, 0.92 g/100 g) malic acid, and 0.94–1.30 g/100 g (mean, 1.08 g/100 g) citric acid (2). A more recent study shows similar results, 0.83–1.39 g/100 g (mean, 1.05 g/100 g) quinic acid, 0.60–0.82 g/100 g (mean, 0.72 g/100 g) malic acid, and 0.66–1.02 g/100 g (mean, 0.86 g/100 g) citric acid (1). Three independent studies show extremely close agreement for the mean percentages of each acid: 40% quinic, 28% malic, and 32% citric (1–3). Cranberry juice cocktail commonly contains 25% single strength cranberry juice, hence we would expect an authentic cranberry juice drink to contain at least 0.21–0.41 g/100 g quinic, 0.15–0.29 g/100 g malic, and 0.17–0.33 g/100 g citric acids. Major deviations from these values are therefore considered indicative of adulteration with an acidulant.

Samples b, c, d, e, 1–6, 8, and 9 exhibit typical cranberry organic acid profiles. Samples h, i, 14, 15, 17, 18, and 22 all contain more malic acid than quinic acid, indicating that malic

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Table 1. LC nonvolatile organic acid profiles for 31 commercial cranberry juice drinks

Sample	Acid composition, g/100 g				Percent of total acids, %			Acid ratios		
	Quinic	Malic	Citric	Total	Quinic	Malic	Citric	Quinic/ malic	Quinic/ citric	Citric/ malic
a	0.18	0.11	0.31	0.60	30.0	18.3	51.7	1.6	0.6	2.8
b	0.27	0.17	0.26	0.70	38.9	24.2	36.9	1.6	1.1	1.5
c	0.26	0.18	0.26	0.70	37.2	26.2	36.6	1.4	1.0	1.4
d	0.34	0.22	0.30	0.85	39.6	25.5	34.9	1.6	1.1	1.4
e	0.34	0.24	0.34	0.93	37.1	26.0	36.8	1.4	1.0	1.4
f	0.08	0.04	0.48	0.60	13.7	5.8	80.5	2.3	0.2	13.8
g	0.07	0.02	0.58	0.68	10.5	3.4	86.1	3.1	0.1	25.3
h[a]	0.36	0.40	0.13	0.90	40.3	44.8	14.9	0.9	2.7	0.3
i[a]	0.42	0.43	0.13	0.98	42.6	44.3	13.1	1.0	3.2	0.3
1[a]	0.34	0.23	0.22	0.80	43.2	28.8	28.0	1.5	1.5	1.0
2	0.28	0.23	0.24	0.76	37.1	30.9	32.1	1.2	1.2	1.0
3	0.32	0.23	0.31	0.86	37.6	26.2	36.1	1.4	1.0	1.4
4	0.37	0.30	0.30	0.97	38.0	30.8	31.2	1.2	1.2	1.0
5	0.34	0.30	0.32	0.95	35.4	31.5	33.1	1.1	1.1	1.1
6	0.37	0.29	0.33	0.99	37.1	29.3	33.6	1.3	1.1	1.1
7	0.02	0.03	0.53	0.58	3.8	4.5	91.7	0.8	0.0	20.3
8	0.38	0.32	0.28	0.98	38.7	32.9	28.4	1.2	1.4	0.9
9	0.34	0.18	0.35	0.87	39.3	20.5	40.1	1.9	1.0	2.0
10	0.09	0.03	0.49	0.62	15.3	5.1	79.6	3.0	0.2	15.6
11[b]	0.16	0.18	2.11	2.44	6.5	7.3	86.1	0.9	0.1	11.8
12	0.13	0.07	0.53	0.72	17.4	10.1	72.5	1.7	0.2	7.2
13	0.08	0.03	0.61	0.72	10.8	4.6	84.6	2.3	0.1	18.4
14[c]	0.23	0.31	0.48	1.02	22.7	30.2	47.1	0.8	0.5	1.6
15[c]	0.20	0.35	0.49	1.03	19.1	33.6	47.2	0.6	0.4	1.4
16	0.14	0.04	0.47	0.64	21.1	6.1	72.8	3.5	0.3	12.0
17[c]	0.12	0.12	0.47	0.70	16.5	16.5	67.0	1.0	0.2	4.1
18[c]	0.02	0.04	0.40	0.46	3.5	9.4	87.1	0.4	0.0	9.3
19	0.12	0.05	0.33	0.50	23.3	9.9	66.8	2.4	0.3	6.8
20	0.17	0.08	0.56	0.82	21.3	10.0	68.7	2.1	0.3	6.9
21	0.19	0.06	0.31	0.55	34.0	10.1	55.9	3.4	0.6	5.5
22[c]	0.15	0.20	0.29	0.64	23.0	31.2	45.8	0.7	0.5	1.5

[a]Fumaric acid present at greater than 1%.
[b]Sample 11 is reported in terms of single strength juice.
[c]Fumaric acid present at levels between 0.05 and 1.0%.

Table 2. LC anthocyanidin profiles for 31 commercial cranberry juice drinks

Sample	Percentage of total pigments,[a] %					Cyd/Pnd ratio
	Dpd	Cyd	Ptd	Pnd	Mvd	
a		49	12	36	4	1.4
b		54	tr	46		1.2
c		53	tr	47		1.1
d		59	tr	41		1.4
e		52	tr	48		1.1
f	4	38		31	27	1.2
g	3	51	tr	27	19	1.9
h	2	51	10	27	10	1.9
i	2	50	14	26	7	1.9
1	5	64	9	16	5	4.0
2	tr	57		42		1.4
3	tr	53		47		1.1
4	tr	59		41		1.4
5	tr	53		47		1.1
6	tr	61		39		1.6
7	2	45	9	26	18	1.7
8	tr	55		45		1.2
9	tr	62		38		1.6
10	tr	48		31	20	1.5
11	tr	86		14		6.1
12	9	37	7	29	18	1.3
13	8	39	11	26	16	1.5
14	6	46	6	30	11	1.5
15	12	54	8	20	6	2.7
16	10	63		22	5	2.9
17	13	63		17	7	3.7
18	6	58	6	17	13	3.4
19	3	50	4	33	9	1.5
20	13	59		22	5	2.7
21	2	61	5	26	6	2.3
22	12	68		19	tr	3.6

[a]Key: Dpd = delphinidin; Cyd = cyanidin; Pnd = peonidin; Ptd = pentunidin; Mvd = malvidin; tr = trace levels at less than 1%.

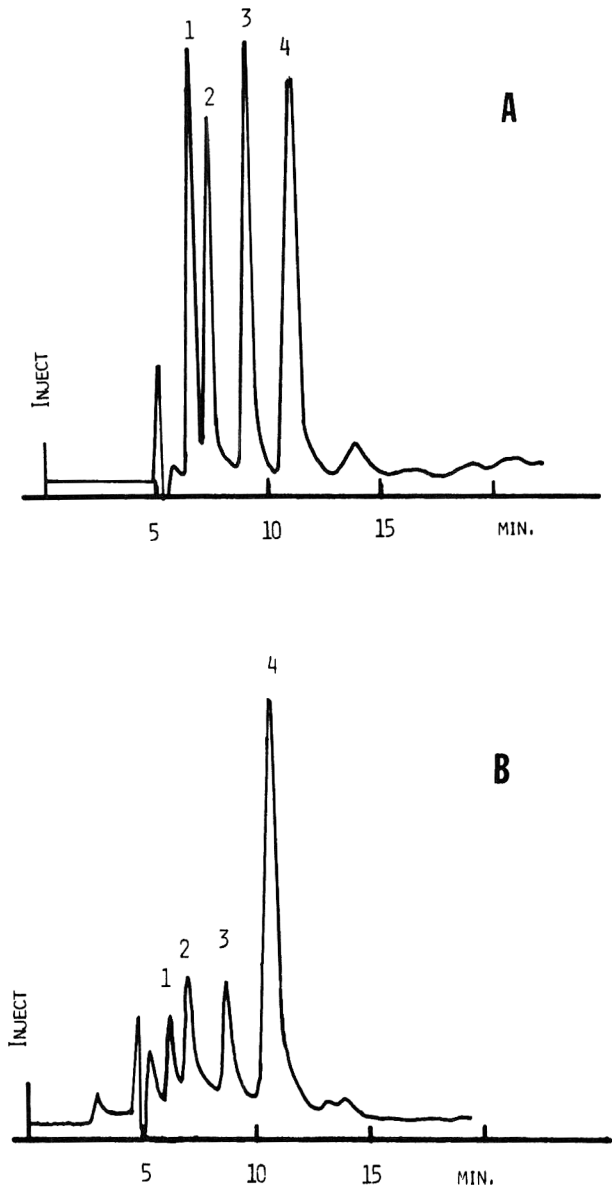

Figure 1. LC organic acid chromatograms of A, authentic cranberry juice; B, adulterated commercial cranberry juice drink. Peak identification: 1, quinic acid; 2, malic acid; 3, shikimic acid; 4, citric acid.

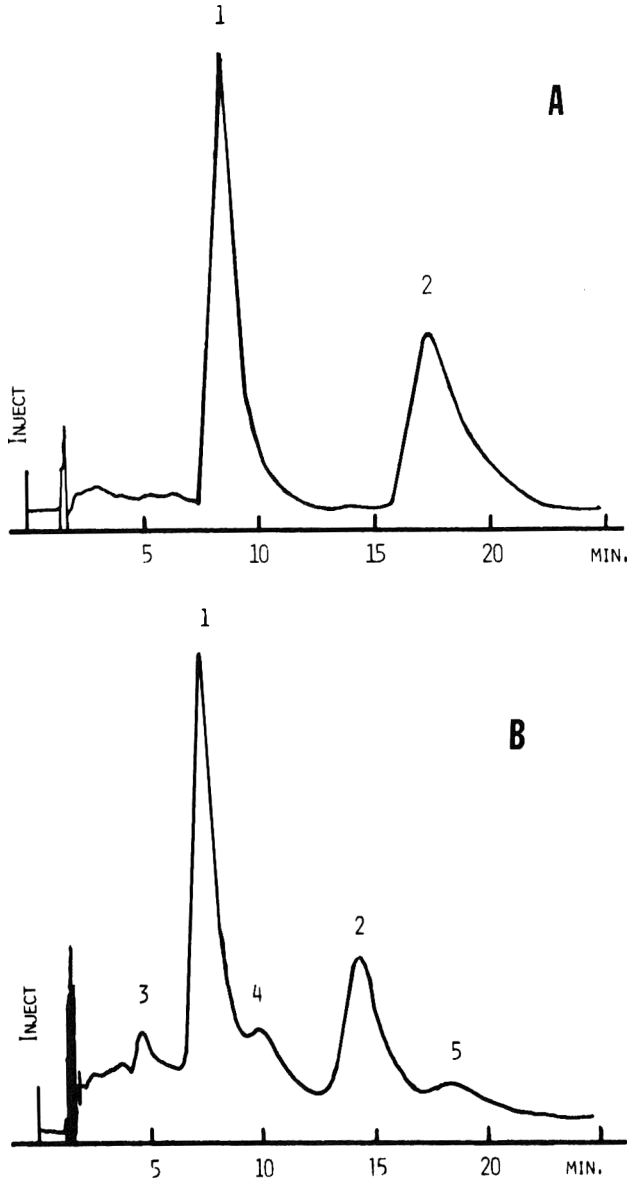

Figure 2. LC anthocyanidin chromatograms of A, authentic cranberry juice; B, adulterated commercial cranberry juice drink. Peak identification: 1, cyanidin; 2, peonidin; 3, delphinidin; 4, petunidin; 5-malvidin.

acid has been added. Previous studies have established that quinic acid is always present in greater concentrations than malic acid in authentic cranberry juice (1–3). Samples h, i, 14, 15, and 22 contain malic acid in concentrations greater than 0.28 g/100 g; Sample i contains 0.44 g/100 g malic acid. Significant quantities (greater than 0.1% of total acids) of fumaric acid were also found in Samples h, i, 14, and 22. Commercial malic acid invariably contains trace quantities of fumaric acid, an acid that is not known to accumulate in fruits in any significant quantity (4). The presence of fumaric acid in excess of 3 mg/L of apple juice has been used in the past to detect adulteration with malic acid (4). The presence of fumaric acid in Samples h, i, 14, and 22 is additional evidence that malic acid has been added.

Samples a, f, g, 7, and 10–22 all exhibit unusually high percentages of citric acid, ranging from 45.7% (Sample 22) to 91.5% (Sample 7) of total acids. The citric content of these adulterated samples ranges from 0.30 g/100 g (Sample 22) to 0.58 g/100 g (Sample g). Samples f, g, 7, 10–18, and 20 greatly exceed 0.33 g/100 g, the upper range limit reported for authentic 25% cranberry juice drink. Quinic acid levels were present ranging from 0.23 g/100 g (Sample 14) to as low as 0.016 g/100 g (Sample 18), well under the 0.33 g/100 g average for quinic acid in authentic 25% cranberry juice drink.

From the organic acid data, we can conclude that the following samples have undeclared added ingredients: Samples h and i, added malic acid; Samples a, f, g, 7, 10–13, and 16–21, added citric acid; and Samples 14, 15, and 22, both added citric and malic acids.

The percentage of cranberry juice (Table 5) in the adulterated samples was estimated by assuming that an authentic 25% cranberry juice drink has a mean value for quinic of 0.33 g/100 g (2, 5):

$$\text{Percent juice} = 25\% \times Q/0.33$$

where Q is the concentration of quinic acid in g/100 g. This calculation is justified by the fact that quinic acid is not currently commercially available as a food grade acidulant. This formula gave reasonably accurate estimates (Table 5) for the juice percentages of Samples a–e whose actual juice content was known. Because of the natural variability of

Table 3. Spectral data for 31 commercial cranberry juice drinks

Sample	Anthocyanin Concn,[a] mg/100 mL	Color density	Polymeric color	Polymeric color, %
a	3.31	1.59	0.31	19.4
b	8.38	2.90	0.34	11.9
c	8.03	2.87	0.34	11.7
d	5.63	2.30	0.39	16.7
e	8.09	3.24	0.35	10.8
f	2.83	2.14	0.80	37.4
g	0.72	1.35	0.90	66.4
h	1.61	1.48	0.70	47.3
i	1.31	1.49	0.76	50.9
1	1.59	1.34	0.69	51.5
2	3.27	1.33	0.31	23.3
3	5.24	2.24	0.34	15.2
4	6.21	1.98	0.16	8.1
5	3.21	1.38	0.33	23.9
6	4.54	1.62	0.22	13.6
7	1.15	0.77	0.28	36.4
8	6.66	2.18	0.14	6.4
9	4.78	2.19	0.44	20.1
10	0.64	0.57	0.30	52.6
11[b]	6.34	1.60	0.31	19.4
12	1.77	0.78	0.19	24.4
13	1.55	0.55	0.32	58.2
14	3.44	1.78	0.33	18.5
15	1.73	1.00	0.27	27.0
16	1.31	0.48	0.23	47.9
17	0.93	0.60	0.23	38.3
18	1.77	0.85	0.13	15.3
19	2.57	1.26	0.21	16.7
20	1.48	0.46	0.24	52.2
21	1.70	0.82	0.18	22.0
22	2.40	1.30	0.40	31.0

[a]Expressed in terms of cranberry anthocyanins (reference 6).
[b]Sample 11 is reported in terms of a 25% single strength juice solution.

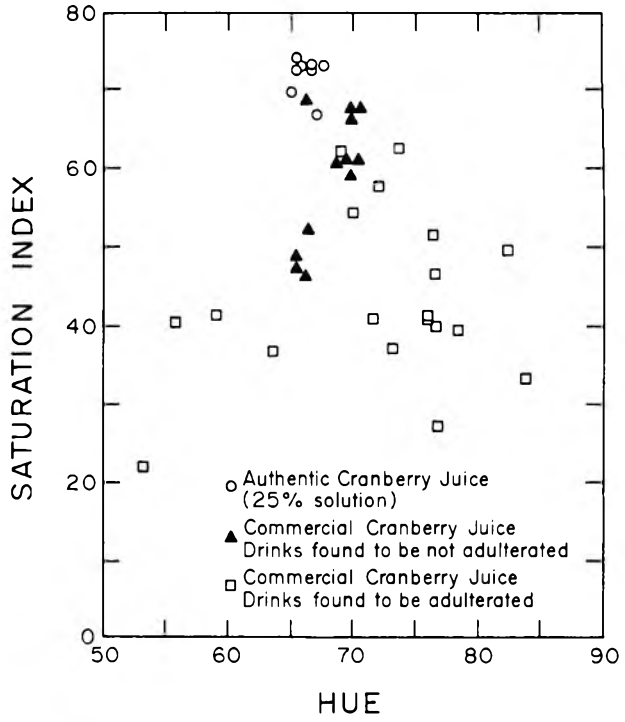

Figure 3. Scatter diagram of Hunter hue vs Hunter Saturation Index.

quinic acid content, we would assign an accuracy of ± 5% to our estimates.

The anthocyanins of cranberries have been thoroughly investigated (6–16). Four pigments have been identified, cyanidin-3-galactoside, cyanidin-3-arabinoside, peonidin-3-galactoside, and peonidin-3-arabinoside. Subsequent work (1, 14, 16) established that cyanidin and peonidin are present in ratios of 60:40 (cyanidin:peonidin). Shown on Table 2 are the results of the anthocyanidin analyses; Figure 2 shows chromatograms of both authentic and adulterated juices. Samples b, c, d, e, 2–6, 8, and 9 exhibited typical cranberry anthocyanidin profiles. While several of these samples did contain trace (less than 1%) amounts of delphinidin, we are hesitant to conclude that this is, by itself, sufficient evidence for adulteration. It is possible that delphinidin glycosides are true constituents of cranberries in small quantities but have not yet been identified; also, delphinidin may possibly be generated in trace amounts from leucoanthocyanins during sample preparation. The presence of leucoanthocyanins in cranberry fruit has been reported, but the compounds have not been identified (17).

Samples a, g, h, i, 7, 10, and 12–21 showed atypical anthocyanidin profiles. All contained at least 4% malvidin and all but Samples a and 10 contained 2% or more delphinidin. Neither of these pigments has been reported in authentic cranberries. Naturally occurring delphinidin and malvidin in such percentages would have been detected in the authentic cranberry juices that we previously analyzed (1). One can conclude that adulteration has occurred with a natural anthocyanin-containing extract or juice. The presence of malvidin and delphinidin in the suspect samples lead us to believe that grape skin extract is the added colorant. It is well known that grape contains large quantities of malvidin and delphinidin (18–22).

Sample 11 is unusual in that the cyanidin:peonidin ratio is atypical. An average value of 60% cyanidin and 40% peonidin has been reported by previous workers (1, 14, 16). Sample 11 contains 86% cyanidin and 14% peonidin. Cyanidin is widely distributed in plants; adulteration with a cyanidin-containing source such as blackberry or elderberry would result in a high cyanidin to peonidin ratio.

Lingonberries, *Vaccinium vitis-ideae*, which are of commercial importance in Europe, are sometimes confused with the American cranberry, *Vaccinium macrocarpon*. The fruits are different species and substitution of one for the other would be fraudulent practice. Previous reports reveal that lingonberries contain only cyanidin-3-galactoside (23, 24). These 2 fruits should therefore be distinguishable through pigment analysis.

Spectral measurements (Table 3) were made using the pH differential method which measures only nondegraded, monomeric pigment. Total pigment content in cranberries varies greatly depending on growing season and variety (17). The following values have been reported in the literature, 4.76–5.96 mg/100 mL Ocean Spray cranberry juice cocktail (13); 26–119 mg/100 mL fresh fruit (17); 9.88 mg/100 mL Ocean Spray cranberry juice cocktail (6); 19–53 mg/100 g single strength juice (1). As expected, Minot sample a (10% juice) exhibited a low pigment concentration at 3.31 mg/100 mL, whereas the Minot 20–27% group exhibited pigment concentrations ranging from 5.63 to 8.38 mg/100 mL. Several of the commercial samples however (1, 7, 10, 12, 13, 15–18, 20, and 21), exhibited anthocyanin concentrations below 2.0 mg/100 mL. Very low levels of pigments may be indicative of a diluted cranberry juice drink.

Percent polymeric color values are used as an index for the amount of polymerized pigments present (25). Samples 1, 10, 13, 16, and 20 showed very high percent polymeric color values. Abnormally high percent polymeric color values (greater than 50%) suggest the possibility of pigment degradation and polymerization during processing or storage (26). Alternatively, the addition of commercial grape skin extract

Table 4. Hunter L, a, b, hue, chroma, and hue/chroma values for 31 commercial cranberry juice drinks

Sample	L	a	b	Hue	SI[a]	Hue/SI	L/a	L/b
a	65.9	38.6	12.8	71.7	40.7	1.8	1.7	5.1
b	51.7	55.6	20.5	69.8	59.3	1.2	0.9	2.5
c	52.3	56.6	22.1	68.7	60.8	1.1	0.9	2.4
d	49.7	48.1	21.0	66.4	52.5	1.3	1.0	2.4
e	50.7	57.2	21.4	69.5	61.1	1.1	0.9	2.4
f	49.1	51.1	18.5	70.1	54.3	1.3	1.0	2.7
g	64.8	29.8	18.1	58.7	34.9	1.7	2.2	3.6
h	57.7	35.6	21.3	59.0	41.5	1.4	1.6	2.7
i	56.9	33.4	22.7	55.8	40.4	1.4	1.7	2.5
1	56.3	44.3	20.2	65.5	48.7	1.3	1.3	2.8
2	62.2	42.5	18.7	66.3	46.4	1.4	1.5	3.3
3	50.0	63.8	23.4	69.9	68.0	1.0	0.8	2.1
4	52.3	64.1	22.5	70.7	67.9	1.0	0.8	2.3
5	61.7	43.0	19.5	65.6	47.2	1.4	1.4	3.2
6	56.9	57.5	20.4	70.5	61.0	1.2	1.0	2.8
7	65.7	39.8	9.9	76.0	41.0	1.9	1.7	6.6
8	58.0	58.1	22.2	69.1	62.2	1.1	1.0	2.6
9	48.3	62.2	22.9	69.8	66.3	1.1	0.8	2.1
10	77.3	17.5	13.1	53.2	21.9	2.4	4.4	5.9
11[b]	43.7	63.0	27.6	66.3	68.8	1.0	0.7	1.6
12	69.2	35.4	10.6	73.3	37.0	2.0	2.0	6.5
13	59.1	49.9	12.1	76.4	51.3	1.5	1.2	4.9
14	52.2	60.0	17.7	73.6	62.6	1.2	0.9	2.9
15	68.0	38.8	7.9	78.5	39.6	2.0	1.8	8.6
16	68.3	38.9	9.2	76.7	40.0	1.9	1.8	7.4
17	76.1	26.5	6.2	76.8	27.2	2.8	2.9	12.3
18	74.2	32.9	3.6	83.8	33.1	2.5	2.3	20.6
19	64.2	48.9	6.4	82.5	49.3	1.7	1.3	10.0
20	64.5	45.3	10.8	76.6	46.6	1.6	1.4	6.0
21	70.4	32.7	16.2	63.7	36.5	1.7	2.2	4.3
22	53.0	55.0	17.8	72.1	57.8	1.2	1.0	3.0

[a]SI = Saturation Index.
[b]Sample 11 (concentrate) measured as a 25% single.

Table 5. Summary of results for spectral, organic acid, and anthocyanidin assay for 31 samples of commercial cranberry juice drinks

Sample	Spectral values	Acid profile	Pigment profile	Conclusion	Comments	Estimated juice content,[a]%
a	OK	atypical	atypical	adulterated	added citric and color	14
b	OK	OK	OK	OK		20
c	OK	OK	OK	OK		20
d	OK	OK	OK	OK		26
e	OK	OK	OK	OK		26
f	OK	atypical	atypical	adulterated	added citric and color	6
g	atypical	atypical	atypical	adulterated	added citric and color	5
h	atypical	atypical	atypical	adulterated	added malic and color	27
i	atypical	atypical	atypical	adulterated	added malic and color	32
1	atypical	atypical	atypical	adulterated	added color, malic (?)	26
2	OK	OK	OK	OK		21
3	OK	OK	OK	OK		24
4	OK	OK	OK	OK		28
5	OK	OK	OK	OK		26
6	OK	OK	OK	OK		28
7	OK	atypical	atypical	adulterated	added citric and color	1
8	OK	OK	OK	OK		29
9	OK	OK	OK	OK		26
10	atypical	atypical	atypical	adulterated	added citric and color	7
11	OK	atypical	atypical	adulterated	high citric, atypical acy ratios	12
12	OK	atypical	atypical	adulterated	added citric and color	10
13	atypical	atypical	atypical	adulterated	added citric and color	6
14	OK	atypical	atypical	adulterated	added citric and color	17
15	OK	atypical	atypical	adulterated	added citric and color	15
16	OK	atypical	atypical	adulterated	added citric and color	11
17	OK	atypical	atypical	adulterated	added citric and color	9
18	OK	atypical	atypical	adulterated	added citric and color	2
19	OK	atypical	atypical	adulterated	added citric and color	9
20	atypical	atypical	atypical	adulterated	added citric and color	13
21	OK	atypical	atypical	adulterated	added citric and color	14
22	OK	atypical	atypical	adulterated	added citric and color	11

[a]Estimated on the basis of quinic acid concentration.

can also increase the percent polymeric color value because it is high in polymerized pigments.

Given in Table 4 are the Hunter color parameters for the samples. Although there are considerable differences in the L, a, b values for these samples, very little can be concluded from these results because the data base for Hunter L, a, b values for cranberry juice is limited. A scatter diagram of hue vs Saturation Index for both authentic and adulterated samples (Figure 3) shows that the authentic juices and the juices determined to be adulterated fall into different groups. With further work, it may be possible to use Hunter measurements as a quick screening method.

A summary of our findings and conclusions regarding the 31 samples is found in Table 5. Only those samples exhibiting

both atypical acid and pigment profiles are reported as being adulterated. Fifteen of the 22 samples supplied by A. Rosenzweig Associates, Inc., were found to be adulterated. Included in that set were controls believed by the supplier to be authentic; none of those samples were found by our criteria to be adulterated. This is important from an analytical standpoint in that no errors were made in reporting false-positives.

Summary

Adulteration of commercial cranberry juice beverages was detected by LC analysis of organic acids and anthocyanidins. Both percentages and ratios of compounds were useful in making comparisons with authentic data bases.

Nineteen of the 31 samples analyzed were found to be adulterated. The adulterated samples exhibited atypical organic acid and anthocyanidin pigment profiles, indicating that citric acid and/or malic acid and a natural colorant, most likely grape-skin extract, were added.

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PESTICIDE AND INDUSTRIAL CHEMICAL RESIDUES

Identification of *O,O*-Dialkyl-*S*-Methylphosphorodithioate Residues in Fish

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O,O-Dialkyl-S-methylphosphorodithioates were found in Mississippi River buffalo fish caught near several chemical plants and oil refineries in Hartford and Wood River, IL. These chemicals, which have not been previously recognized as environmental or food contaminants, were identified and quantitated by a procedure similar to the AOAC multiresidue method for organochlorine and organophosphorus pesticides, using gas chromatography (GC) with flame photometric detection (FPD). The key to their identification was a GC/FPD retention time pattern that was virtually the same as that for the diazomethane reaction products of a commercial zinc dialkyl dithiophosphate motor oil additive. GC/mass spectrometry (MS) showed that the compound producing the largest GC/FPD peak contained butoxy groups. The identification of this compound as O,O-di(2-methylpropyl)-S-methylphosphorodithioate (Compound C) was confirmed by GC/MS analysis by comparison with the authentic material. The buffalo fish contained 0.15 ppm Compound C and 0.5 ppm total O,O-dialkyl-S-methylphosphorodithioates. Subsequent analyses of fish from other areas showed that these contaminants were not limited to the Hartford-Wood River area. Lower residue levels of Compound C, ranging from 0.01 to 0.05 ppm, were found in fish from the Mississippi River at Sauget, IL, and from the Delaware River and Newark Bay in NJ.

O,O-Dialkyl-*S*-methylphosphorodithioates (Figure 1A) have not previously been reported as environmental or food contaminants. The investigation which led to their identification as contaminants in fish started when the Division of Chemical Technology was requested by the Minneapolis laboratory of the Food and Drug Administration (FDA) to characterize a series of unidentified analytical responses from a composite of Mississippi River buffalo fish caught near gasoline refineries and other chemical operations near Hartford and Wood River, IL. The Minneapolis laboratory had examined the buffalo fish, using a method based on the AOAC multiresidue method for organochlorine and organophosphorus pesticides (1). The unidentified materials were found in the ethyl ether (EE)–petroleum ether (PE) (6 + 94) Florisil eluate (6% eluate) that was analyzed by gas chromatography (GC) with flame photometric detection (FPD) in the phosphorus (P)-selective mode.

The composite was analyzed again in our laboratory by using a cleanup procedure (1) similar to that used in the first analysis. Appropriate reference materials, leading to the identification of the residues, were found by reviewing work previously performed in our laboratory on methodology for the detection of zinc dialkyl dithiophosphates (Figure 1B), which are used as lubricating oil additives (2). The identities of the residues were confirmed by full spectrum electron ionization (EI)/GC/mass spectrometry (MS) as the *O,O*-dialkyl-*S*-methylphosphorodithioates (Figure 1A) that are produced in the reaction of diazomethane with Amoco 198, a commercial zinc dialkyl dithiophosphate. The major product of this reaction, *O,O*-di(2-methylpropyl)-*S*-methylphosphorodithioate (Compound C) (Figure 1C), which was synthesized independently for use as a reference material, accounted for the major residue peak.

No references to Compound C were found in the chemical literature, but mass spectra similar to that of Compound C have been reported (3) for unidentified compounds found in methylated polar extracts of waste oil.

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Experimental

Reagents

(a) *General reagents.*—See sec. **29.002** (1).

(b) *Amoco 198.*—Division of Chemical Technology Repository, No. 1240, Division of Chemical Technology, FDA, 200 C St, SW, Washington, DC 20204.

(c) *O,O,O-Tri-n-butylphosphorothioate.*—Division of Chemical Technology Repository, No. 1255.

(d) *Solution of diazomethane in ethyl ether.*—Prepared by the method of deBoer and Backer (4). See CAUTIONS, sec. 221.12c (5), before preparing or using diazomethane.

(e) *Methylated Amoco 198.*—Synthesized by slow addition of ca 1 mL ethereal solution of diazomethane to 3 mL isooctane containing ca 100 mg Amoco 198. After 10 min, solution was placed on steam bath in fume hood until yellow color of diazomethane disappeared. Solution was then placed on Florisil column (1, sec. **29.015**) and eluted with 200 mL PE, followed by 50 mL EE–PE (6 + 94). The 6% eluate contained the *O,O*-dialkyl-*S*-methylphosphorodithioates referred to as methylated Amoco 198.

(f) *O,O-Di(2-methylpropyl)-S-methylphosphorodithioate (Compound C).*—Synthesized by slow addition of ethereal solution of diazomethane to 1 mL benzene solution of *O,O*-di(2-methylpropyl)phosphorodithioic acid prepared as described by Hoegberg and Cassaday (6). This solution was allowed to stand for 10 min and then placed on a steam bath in a fume hood to remove unreacted diazomethane. The solution was then cleaned up by Florisil column chromatography, using 200 mL PE as the first eluant, followed by 50 mL EE–PE (6 + 94), which eluted Compound C. The 6% eluate was concentrated to 0.2 mL for GC analysis on an OV-101 column operated at 130°C. A 7.5 mg portion of Compound C was isolated from the 6% eluate by trapping the compound eluting at 0.50 relative to *O,O,O*-tri-*n*-butylphosphorothioate (see *Apparatus* (**d**)). The structure of Compound C was verified by proton nuclear magnetic resonance (NMR) spectroscopy.

Apparatus

(a) *General apparatus.*—See sec. **29.005** (1).

(b) *Gas chromatograph.*—Tracor Model 560 equipped with FPD and 6 ft × 4 mm id glass tube packed with 5% OV-101 on 80–100 mesh Chromosorb W(HP). Operating conditions: flow rates (mL/min)—nitrogen (carrier) 50, hydrogen (flame gas) 100, air (flame gas) 120; photomultiplier voltage 65; temperatures (°C)—column 130 or 200, inlet 210, detector 200; attenuator 32; range 10; current 1.6×10^{-9} A. At a column temperature of 200°C, these conditions give ½ full scale deflection (FSD) for 2 ng parathion (P mode). The attenuator was adjusted to give ½ FSD for 20 ng parathion in the sulfur (S) mode. At a column temperature of 130°C, ½ FSD was obtained for 2 ng *O,O,O*-tri-*n*-butylphosphorothioate (P mode) by adjusting the attenuator.

(c) *Gas chromatograph/mass spectrometer/data system.*—Finnigan 4023 GC/MS system. The gas chromatograph

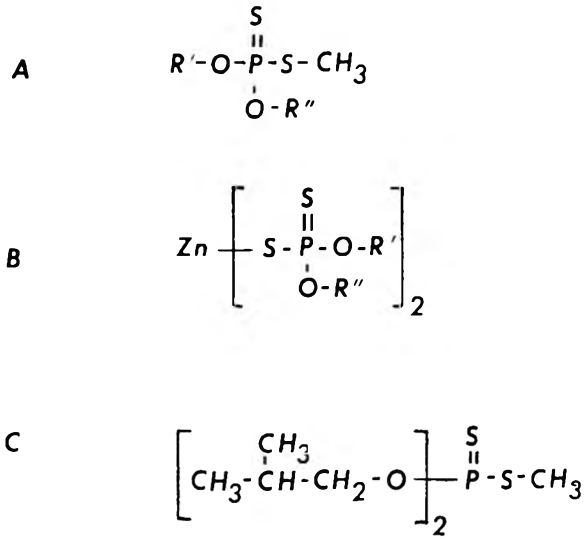

Figure 1. Structure of A, *O,O*-dialkyl-*S*-methylphosphorodithioates; B, zinc dialkyl dithiophosphates; C, *O,O*-di(2-methylpropyl)-*S*-methylphosphorodithioate (Compound C).

was directly coupled to the mass spectrometer through a 37 m × 0.22 mm methyl silicone fused silica capillary column. Splitless injections (7) were made by using the following operating conditions: helium set at 100 psi at the inlet port to give a linear velocity of 27 cm/s for carrier gas; temperatures (°C)—mass spectrometer manifold 90, ion source 250, transfer line region 270, column 70 for 1 min, then increased 20°/min to 170, followed by increase of 8°/min to 270, and held at 270 for 10 min. Scans were taken in the electron ionization (EI) mode with a 70 eV source from m/z 65 to 465 every 2.10 s beginning 5 min after injection. Chemical ionization (CI) was performed by using methane reactant gas at a source pressure of 0.5 torr.

(d) *Gas chromatograph (for isolation of Compound C).*—Varian Model 3700 equipped with flame ionization detector, Packard Model 850 fraction collector, glass-lined adjustable splitter, and 1.8 m × 2 mm id glass column packed with 3% OV-101 on 80–100 mesh Chromosorb W(HP). Operating conditions: flow rates (mL/min)—nitrogen (carrier) 30, hydrogen (flame gas) 30, air (flame gas) 300; temperatures (°C)—column 130, injector 200, detector 300, splitter transfer line 200; attenuator 512, range 10^{-12} A. Adjustable splitter was set 40:1 (fraction collector:flame). Five 20 μL injections from 0.2 mL solution were trapped for 6 min beginning 2 min after injections were made. Fractions were collected on glass beads in tray cooled with dry ice. Compound C was washed from glass beads with PE into a 15 mL centrifuge tube by using a sintered glass funnel to remove beads. PE was evaporated on a steam bath. A 7.5 mg portion of Compound C was isolated.

Analysis of Fish

Fish were prepared for analysis by the AOAC general method for organochlorine and organophosphorus pesticides (1) as described in the FDA *Pesticide Analytical Manual,* Vol. I (5). Ground fillet of ocean perch was used for recovery studies. Portions of thoroughly ground and mixed edible tissues were extracted by using the official AOAC fatty food extraction procedure for fish (1, sec. **29.012**(e)). To avoid the loss of volatile compounds, fat was determined from an aliquot of each extract. The extracts were cleaned up for GC analysis by acetonitrile partitioning (1, sec. **29.014**) and Florisil column chromatography (1, sec. **29.015**). The Florisil

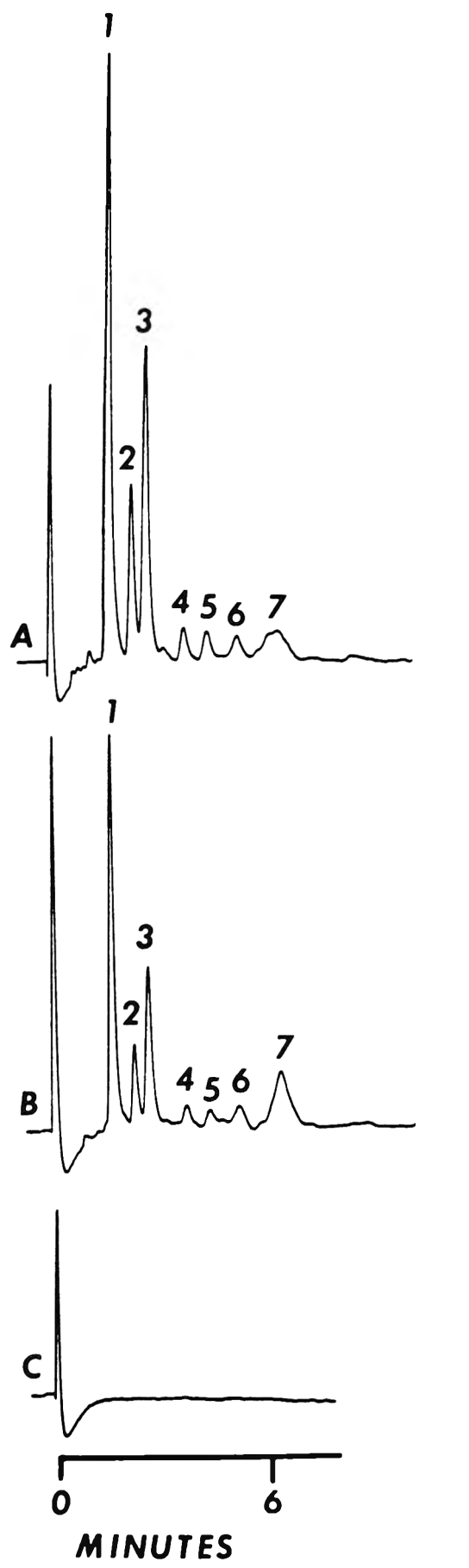

Figure 2. GC/FPD (P mode) chromatograms: A, methylated Amoco 198; B, 6% Florisil eluate from buffalo fish extract; C, 6% Florisil eluate from ocean perch extract. 5% OV-101 column at 200°C and GC parameters in *Apparatus* (b).

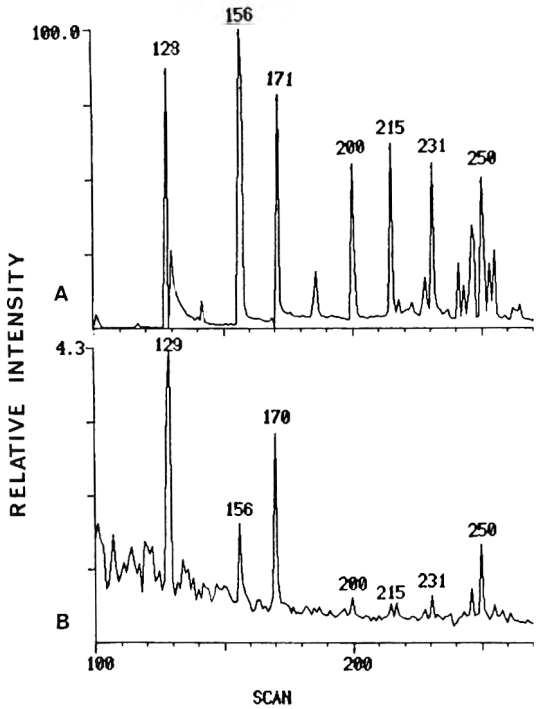

Figure 3. Reconstructed mass chromatograms using m/z 145 as the ion detected: A, methylated Amoco 198; B, 6% Florisil eluate from buffalo fish extract. The response at scan number 129 in A is saturated but reads 0 relative intensity as a result of the data system design.

cleanup procedure was modified to elute the Florisil column with 250 mL PE before the usual 6 and 15% eluates were collected. Compound C was determined by GC/FPD (P mode) at a column temperature of 130°C to minimize solvent-quenching effects associated with FPD.

Results and Discussion

Figure 2B shows the GC/FPD chromatogram obtained in the P mode for the 6% Florisil eluate of the Mississippi River buffalo fish composite. The GC peaks (retention times relative to parathion) identified in Figure 2B as 1 (0.22), 2 (0.31), 3 (0.37), 4 (0.52), 5 (0.61), 6 (0.72), and 7 (0.87) were also produced in a virtually identical pattern when the S mode was used. The relative retention times and the Florisil column data obtained for the residues did not match those of any previously encountered pesticide or other industrial chemical pollutant for which published data were available (5, Appendix; 8). Initial examination of this 6% eluate by EI/GC/MS analysis did not produce specific mass spectral data for the residues, because residues undetected by GC/FPD were not distinguishable from the compounds of interest. These other residues were subsequently identified as alkylnaphthalenes by using a previously published technique (9).

At this stage of the investigation, emphasis was on the early eluting residues (Figure 2B, peaks 1–3), because they appeared to be present at the highest levels. We obtained GC data from an OV-101 column operated at 130°C, and we used *O,O,O*-tri-*n*-butylphosphorothioate as a reference standard because it contains both S and P and produces a peak at about the same retention time as peak 3 (Figure 2). The possibility that the residues might be thionates was considered, but there was no supporting evidence from the MS data (10). Besides thionates, the only reference materials we had that contained both S and P were zinc dialkyl dithiophosphates. Previous (unpublished) work in our laboratory showed that these compounds would not produce GC peaks with retention times in the region of interest. However, these compounds are used as motor oil additives; because the fish were caught near oil refineries, we decided to examine the zinc dialkyl dithiophosphates for impurities or minor components that might help to identify the residues. Minor components were found in several of the zinc dialkyl dithiophosphates that were analyzed by GC/FPD. These minor components eluted from the GC and Florisil columns in the same retention region as the residues. In no instance, however, did we find that both the retention time and the mass spectrum of a specific minor component of a zinc dialkyl dithiophosphate matched those of a residue found in the buffalo fish extract.

Because of the similarities between the minor components of the zinc dialkyl dithiophosphates and the major components of the residues, we reviewed our previous work on the zinc dialkyl dithiophosphates. We learned from the methodology used to determine the presence of a zinc dialkyl dithiophosphate that a series of *O,O*-dialkyl-*S*-methylphosphorodithioates was produced by the reaction of zinc dialkyl dithiophosphates with an ethereal solution of diazomethane. These *O,O*-dialkyl-*S*-methylphosphorodithioates were also recovered by the AOAC method (1) in the 6% eluate. A review of chromatograms indicated that the retention pattern of the Amoco 198 reaction products was similar to the pattern of the residues in the buffalo fish extract. Amoco 198 was again methylated and the reaction products were cleaned up by Florisil column chromatography. Figure 2A shows a chromatogram of methylated Amoco 198 obtained in the P mode. The retention times were identical to those of the buffalo fish residues shown in Figure 2B. Figure 2C is a chromatogram of an ocean perch extract used as a method control.

EI/GC/MS data for the methylated Amoco 198 and the buffalo fish residues are compared in Figure 3, which shows reconstructed mass chromatograms obtained by using m/z 145 as the ion detected. Scans using m/z 127 as the ion detected gave the same relative intensities. In all spectra examined m/z 145 was the base peak, Ions corresponding to $M^{+\cdot} - [R - 2H]$ for R = butyl, amyl, and octyl were the only ions higher than m/z 145 observed in the data for both the buffalo fish extract and the methylated Amoco 198. The ions observed were m/z 201 (scans 128, 156, 231, 250), m/z 215 (scans 156, 171, 200, 215), and m/z 257 (scans 231, 250). The compositions of R′ and R″ (Figure 1A) are consistent with the following:

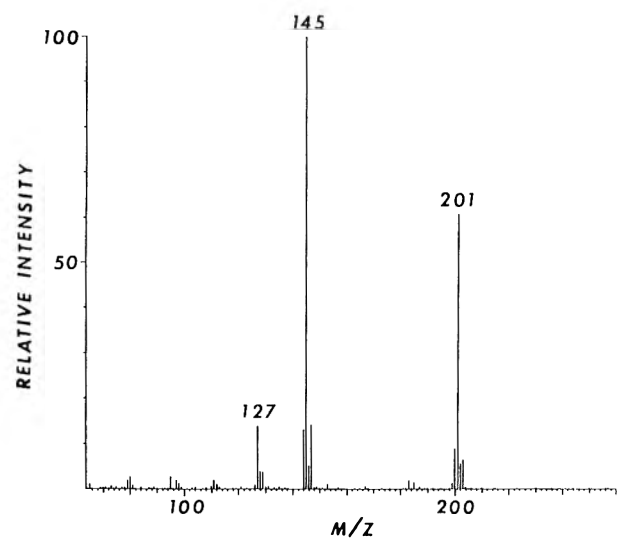

Figure 4. EI mass spectrum of *O,O*-di(2-methylpropyl)-S-methylphosphorodithioate (Compound C).

Table 1. Findings of *O,O*-di(2-methylpropyl)-*S*-methylphosphorodithioate residues in fish

Location	Species	Found, ppm
Mississippi River at Hartford/Wood River, IL	buffalo fish	0.15
Mississippi River at Sauget, IL	carp, sucker	0.01
Newark Bay at Bayonne, NJ	carp	0.05
Delaware River at Gloucester, NJ	carp	0.01

scan 128 (R′ = R″ = butyl); scan 156 (R′ = butyl; R″ = amyl); scans 171, 200, 215 (R′ = R″ = amyl); scans 231, 250 (R′ = butyl, R″ = octyl). No parent ions were detected by using pulsed positive/negative methane CI/GC/MS. Interpretation of this GC/MS data as well as examination of retention times obtained for compounds of known identity in previous work allowed us to identify the major component (peak 1 in Figure 2, A and B) as Compound C.

Compound C was synthesized for confirmation of identification (see *Reagents* (**f**)). The mass spectrum of Compound C (Figure 4) differs from the mass spectra of most pesticide and pollutant organophosphorus compounds in that no parent ion was seen by using either EI or pulsed positive/negative methane CI/GC/MS. This is mainly due to the fact that the isobutyl oxygen substituents are much more easily removed than the more commonly encountered methyl and ethyl substituents (10).

Compound C and methylated Amoco 198 were each added to ocean perch, which was then analyzed by the method described. The recovery of Compound C added at the 0.067 ppm level was 86%. By using Compound C as a GC/FPD peak area reference standard, the concentration of methylated Amoco 198 that was used as a spike was calculated to be 0.29 ppm. Recoveries of the methylated Amoco 198 spike were 95 and 84% as determined in the 6% Florisil eluate of the method. By using Compound C as the sole reference material (peak area, P mode), the concentration of *O,O*-dialkyl-*S*-methylphosphorodithioates found in the buffalo fish was calculated as 0.5 ppm, which included 0.15 ppm of the major component, Compound C.

To determine the uniqueness of these findings, we re-examined chromatograms of fish extracts found to contain unidentified residues when analyzed by GC with nitrogen-P-selective detection. Fish from Newark Bay near Bayonne, NJ, from the Delaware River near Gloucester, NJ, and from the Mississippi River near Sauget, IL, contained residues similar to those found in the buffalo fish. These identifications were confirmed by GC/FPD (P mode) reanalysis of reserved portions of these fish. The concentrations of Compound C that were found are shown in Table 1. Although the GC pattern of methylated Amoco 198 was discernible in these 3 chromatograms, other S/P responses were also seen. We did not estimate the total concentration of *O,O*-dialkyl-*S*-methylphosphorodithioates present. The unidentified responses may have been due to *O,O*-dialkyl-*S*-methylphosphorodithioates that are not associated with methylated Amoco 198.

Conclusions

O,O-Dialkyl-*S*-methylphosphorodithioates have been found in freshwater fish caught in widely different locations. A literature search for Compound C produced no references to these compounds. From the data presented here, it appears that the residues may be related to Amoco 198. Further work would be necessary to determine whether other *O,O*-dialkyl-*S*-methylphosphorodithioates are present in fish, or whether residues of Amoco 198 or other commercially produced dithiophosphate salts are also present as residues in freshwater fish.

Acknowledgments

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Liquid Chromatographic Determination of Basic Nitrogen-Containing Polynuclear Aromatic Hydrocarbons in Smoked Foods

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Several smoked foods were analyzed for basic nitrogen-containing polynuclear aromatic hydrocarbon (NPAH) content by a relatively rapid liquid chromatographic (LC) technique. The analyzed products included both domestic and imported market basket commodities. Nanogram quantities of NPAH standards were detected by UV and fluorescence detectors connected in series. The NPAHs were extracted from basic aqueous ethanolic solution into cyclohexane, extracted from cyclohexane into 6N HCl, and extracted back into cyclohexane after neutralization of the acid. The NPAHs were then purified by filtering the extract through deactivated basic alumina. The eluate from this step was concentrated to dryness, and the residue was dissolved in 95% ethanol and analyzed by LC, using a Vydac C-18 column and acetonitrile–water (9 + 1) as the mobile phase. Recoveries of 3 NPAHs, 5,7-dimethylbenz(a)acridine, dibenz(a,j)acridine, and dibenz(a,h)acridine, each added to salmon and sausage at the 5 ppb level, ranged from 62 to 101% by fluorescence measurement and from 64 to 106% by UV measurement. None of the NPAHs used as standards were found by either fluorescence or UV detection at levels ≥5 ppb in any of the foods analyzed.

As the availability of U.S. domestic oil has decreased, interest in the production of petroleum substitutes from shale oil and coal has increased (1). The increasing use of coal and synthetic fuels may significantly increase the levels of heterocyclic nitrogen compounds in the environment because of the characteristically higher level of organic nitrogen in coal and its by-products, compared to other fossil fuels (2). Many nitrogen bases, thought to be products of incomplete combustion of organic matter that contains nitrogen or to be derived from hydrolytic processes similar to those producing polynuclear aromatic hydrocarbons, have been shown by various types of skin tests to be toxic, carcinogenic, and mutagenic to terrestrial and aquatic organisms (3–7).

Many azaarenes have been identified in sources such as tobacco (8), automobile exhaust (9), high boiling petroleum distillates (10), crude oil (11), shale oil (12), and lake sediments (13). However, few papers dealing with analytical methodology for the determination of nitrogen-containing polynuclear aromatic hydrocarbons (NPAHs) in foods are to be found in the published literature. Vassilaros et al. (14) used a rather elaborate scheme to characterize the neutral and basic NPAHs in vertebrate fish by capillary gas chromatography/mass spectrometry (GC/MS). Grimmer et al. (15) employed a complex scheme for analysis of smoked herring to determine neutral and basic NPAHs, and a similarly complex scheme (G. Grimmer, Biochemisches Institut für Umweltcarcinogene, Ahrensburg, 1980) was used for analysis of other smoked foods to determine basic NPAHs only; both methods use capillary GC as the determinative technique.

Although capillary GC is a very powerful technique because of its high efficiency, liquid chromatography (LC) is also a very attractive tool for studying azaarenes in complex mixtures because it can be used at 3 levels, i.e., for analytical, preparative, or cleanup purposes (16).

The purpose of our investigation was to develop an analytical method for the extraction, cleanup, and LC analysis of the basic NPAH fractions obtained from some smoked foods. The foods analyzed included both domestic and imported products.

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METHOD

Apparatus

Clean all glassware with chromic acid/sulfuric acid solution, rinse thoroughly with distilled water, oven-dry at 120°C, and solvent-rinse before use to minimize interferences.

(a) *Meat chopper.*—Launders, Frary and Clark Co., New Britain, CT, or equivalent.

(b) *Digestion apparatus.*—*(1) Heating mantle.*—500 mL (Series 0, hemispherical, K-721000, Kontes Glass Co., Vineland, NJ 08360, or equivalent). Use with variable auto transformer heat control (E 2101-2T, American Scientific Products Div., American Hospital Supply Corp., McGaw Park, IL 60085). *(2) Friedrich condenser.*—With inclined Ŧ 24/40 outer joint and Ŧ 24/40 inner, drip joint at bottom (Kontes K-437000, or equivalent). *(3) Boiling flask.*—Short-neck, round-bottom, 500 mL with Ŧ 24/40 outer joint.

(c) *Separatory funnels.*—Three 1 L, each equipped with Teflon stopcock.

(d) *Flasks.*—Pear-shape, 300 mL with Ŧ 24/40 joint (Kontes K-294260, or equivalent).

(e) *Flash evaporator.*—Spiral condenser, with temperature-controlled water bath (Buchler Instruments Inc., Fort Lee, NJ 07024, or equivalent).

(f) *Filter funnel.*—Buchner, 60 mL with coarse porosity fritted disk.

(g) *Sample clarification kit.*—Parts include 2 mL Luer-Lok syringe and 13 mm Swinny stainless steel disk filter holder (XX30-012-00, Millipore Corp., Bedford, MA 01730, or equivalent) with 0.2 μm filters (FGLP 01300, Millipore Corp.).

(h) *Liquid chromatograph.*—Model 332 programmable gradient system (Beckman Instruments, Inc., Altex Scientific Operations, Berkeley, CA 94710). *Column.*—4.6 mm × 25 cm, packed with 5μm C-18 particles (Vydac 201TP54, The Separations Group, Hesperia, CA 92345), or equivalent C-18 column. *Injector.*—Rheodyne Model 7125 fitted with a 20 μL loop (Rheodyne, Inc., Cotati, CA 94928). *Detectors.*—Model SF 770 UV-Vis detector (Schoeffel Instruments Div., Kratos, Inc., Westwood, NJ 07675) set at 289 nm; and Model FS 970 fluorometer (Schoeffel) set at 333 nm (excitation filter 7-54; emission filter KV-370) in series with UV detector. (Note: The 7-54 excitation filter has a broad transmission range with a maximum at 310 nm; the KV-370 emission filter has 10^{-3}% transmission at 340 nm and 99% transmission at 390 nm.)

(i) *Recording data processors.*—Altex Model C-R1A integrator/recorder (manufactured by Shimadzu Seisakusho Ltd., Kyoto, Japan; distributed by Beckman Instruments, Inc.), or equivalent, used with fluorescence detector; Model 3390A integrator/recorder (Hewlett-Packard Co., Palo Alto, CA 94304), or equivalent, used with UV detector.

Reagents

(a) *Solvents.*—Ethanol, 190 proof, distill before use; cyclohexane, distilled-in-glass; acetonitrile, LC grade (Fisher Scientific Co., Pittsburgh, PA 15219); double-distilled water.

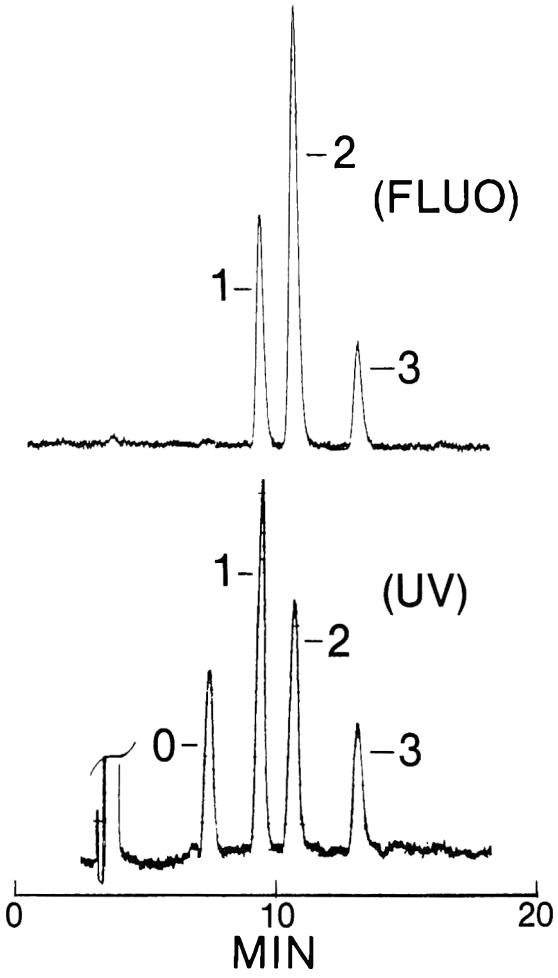

Figure 1. Typical fluorescence and UV chromatograms for 5 ng of each NPAH standard. Column: 4.6 mm × 25 cm, packed with 5 μm C-18 particles (Vydac 201TP54). Mobile phase: acetonitrile–water (9 + 1). Flow rate: 1 mL/min. Fluorescence detector: excitation, 333 nm; emission, >340 nm. UV detector: 289 nm. 0 = phthalate contamination; 1 = DMBaAc; 2 = DBajAc; 3 = DBahAc.

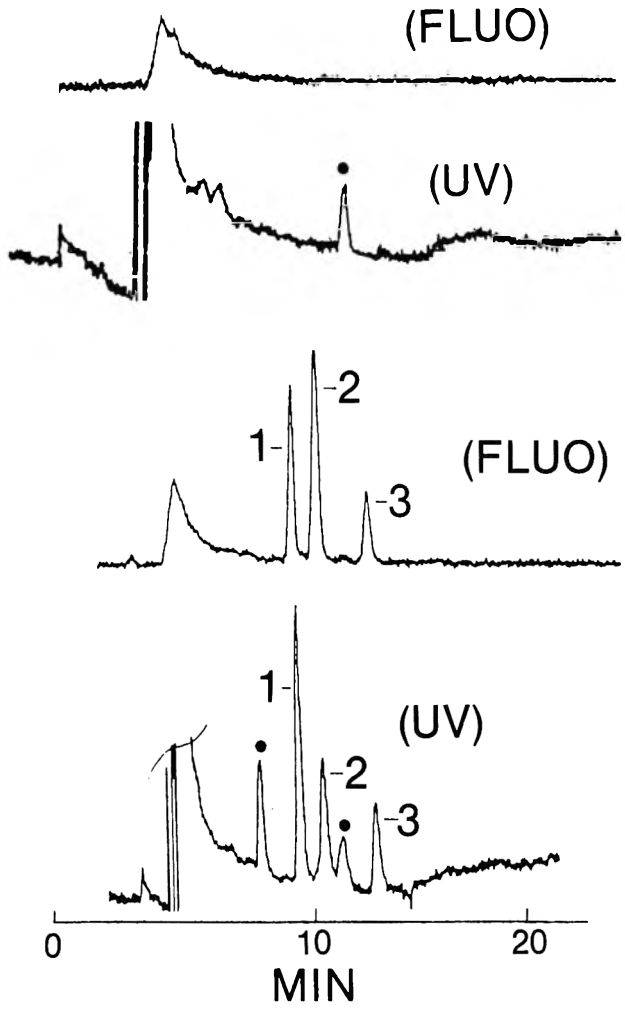

Figure 2. Fluorescence and UV chromatograms for spiked (5 ppb level) and unspiked salmon. • = phthalate contamination; 1 = DMBaAc; 2 = DBajAc; 3 = DBahAc. Conditions are the same as in Figure 1.

(b) *NPAH standards.*—5,7-Dimethylbenz(a)acridine (DMBaAc), dibenz(a,j)acridine (DBajAc), and dibenz(a,h)acridine (DBahAc), obtained from National Bureau of Standards, Washington, DC 20234. Each standard was purified by reverse phase LC, and the identity of each was verified by GC/MS (electron ionization using solid probe on Finnigan Model 3300 EI with low resolution full scan) before use in this study.

(c) *Standard solutions.—(1) Stock solutions.*—0.1 mg/mL. Dissolve 1 mg of each NPAH standard in 10 mL ethanol. *(2) Working standard solution.*—0.25 μg/mL. Pipet 0.25 mL of each stock solution into 100 mL volumetric flask; dilute to volume with ethanol.

(d) *Alumina.*—Aluminum oxide 90, active basic, activity stage 1; particle size 0.063–0.200 mm (E. Merck, Darmstadt, W. Germany; obtained from Millipore Corp.).

(e) *Deactivated alumina.*—Prepared from alumina *(d)* above. Determine apparent weight loss by heating 10 g alumina in weighed platinum or porcelain dish to red heat for 15 min over Bunsen burner. Cover dish immediately and place in desiccator to cool before weighing. (Weighings should be made rapidly because alumina readily adsorbs atmospheric water.) Calculate water content that was present before heating. Discard heated alumina. Add enough double-distilled water to fresh alumina so that total added water accounts for 10% by weight of the final deactivated alumina. Vigorously shake alumina 15 min. Store in brown glass bottle for at least 4 h before use.

(f) *Solutions.*—6N HCl, 10N KOH, ethanol–methylene chloride (2 + 98).

Extraction

Place 25 g homogenized smoked food into 500 mL boiling flask. Add 100 mL ethanol, 4 g KOH, and boiling chips. Insert Friedrich condenser and reflux at rapid rate for 2 h. Let digest cool for ca 20 min, remove condenser, and filter contents of flask through ethanol-rinsed glass wool pad into 1 L separatory funnel. Sequentially rinse boiling flask with 150 mL 0.1N KOH, 50 mL ethanol, and 50 mL cyclohexane; filter each rinse through pad into funnel. Shake funnel vigorously for 2 min. Let layers separate; then draw lower aqueous layer into second 1 L separatory funnel. Repeat extraction with 50 mL cyclohexane. Let layers separate; then draw off lower aqueous layer into third 1 L separatory funnel and repeat extraction with 50 mL cyclohexane. Discard lower aqueous layer. Combine cyclohexane extracts. Rinse third and second funnels in tandem twice with 10 mL portions of cyclohexane and combine rinses with extract in first funnel. Wash combined cyclohexane extracts with three 100 mL portions of warm (>50°C) 0.1N KOH by gently swirling funnel. Draw off and discard lower aqueous layer after each wash. If slight emulsion persists, wash once with ≥50°C double-distilled water.

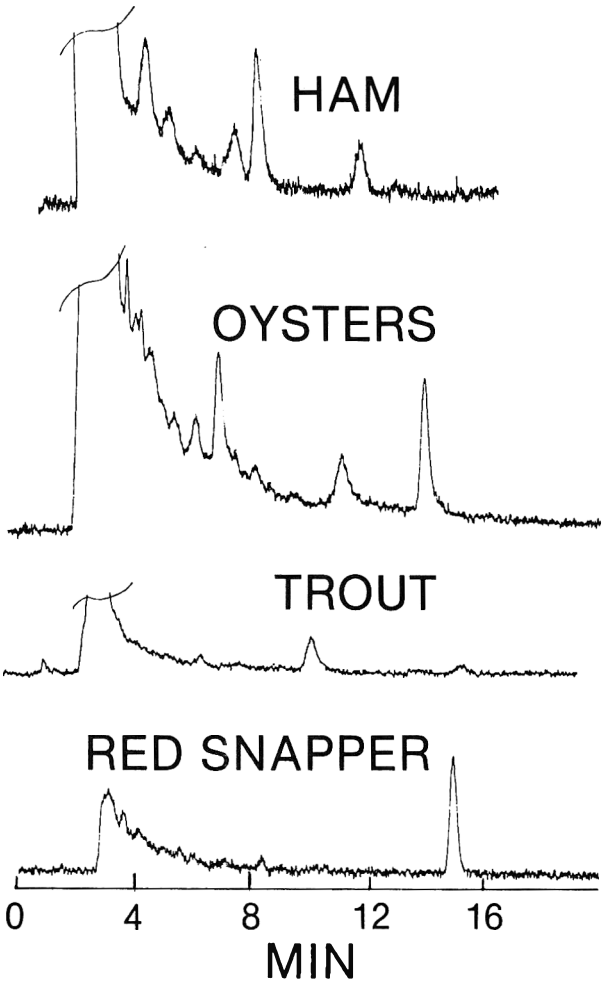

Figure 3. Fluorescence chromatograms for several unspiked smoked foods. Conditions are the same as in Figure 1.

Add 20 mL 6N HCl and shake funnel for 2 min. Let layers separate and draw off bottom acidic layer into second 1 L funnel. Repeat extraction in first funnel with two 20 mL portions of 6N HCl, and combine each extract with first extract in second funnel. Discard cyclohexane layer remaining in first funnel (unless analysis for polynuclear aromatic hydrocarbons is to be conducted).

Carefully add 50 mL 10N KOH to acidic extracts while cooling funnel under cold tap water. Add 50 mL cyclohexane and shake funnel for 2 min. Draw off lower aqueous layer into second funnel and repeat extraction with 50 mL cyclohexane. Discard lower aqueous layer. Combine cyclohexane extracts in first funnel. Rinse second funnel with 10 mL cyclohexane and add rinse to combined extracts. Wash combined cyclohexane extracts with 100 mL double-distilled water. Let layers separate completely (ca 15 min). Discard lower aqueous layer.

Place 10 g deactivated basic alumina (water content 10%) and 40 g Na_2SO_4 in 60 mL Buchner funnel. Wash with 50 mL cyclohexane and discard wash. Filter cyclohexane extract through Buchner funnel into 500 mL Erlenmeyer flask. Rinse separatory funnel with 25 mL cyclohexane, and pass rinse through Buchner funnel. After cyclohexane has completely passed through, pass 150 mL ethanol–methylene chloride (2 + 98) through Buchner funnel and combine with cyclohexane filtrate. Concentrate solvents to ≤4 mL by rotary evaporation; do not evaporate to dryness. Quantitatively transfer residue with total of 6 mL cyclohexane to 13 mL sample tube and concentrate just to dryness at ca 30°C under gentle stream of nitrogen. Add 0.5 mL ethanol and subject to ultrasonic vibration for 5 min. Save concentrate for LC analysis.

Liquid Chromatography

Inject 20 μL concentrate or standard solution onto Vydac 201TP54 column and run mobile phase consisting of acetonitrile–water (9 + 1) at flow rate of 1 mL/min. Between injections, flush injection loop and port passages with ca 3 mL acetonitrile–water (9 + 1) to prevent cross-contamination. For tentative identification, compare retention times of any peaks observed with those of known NPAH standards chromatographed under same conditions. Inject standards after every third sample. Calculate concentration of each NPAH as indicated in calculations section.

Recovery Studies

Conduct recovery studies by spiking 25 g smoked food with 0.5 mL working standard solution, which contains each NPAH at a concentration of 0.25 μg/mL. This level is equivalent to a concentration of 5 ppb for each NPAH. Dilute final residue to 0.5 mL with ethanol. Calculate recovery of each NPAH as indicated in calculations section.

Calculations

Calculate concentration of each NPAH in smoked food as follows:

$$\text{ppb} = (PH_1 \times C_2 \times V_D)/(PH_2 \times W_S)$$

where PH_1 = height of NPAH peak from chromatogram of concentrate, PH_2 = height of corresponding peak from chromatogram of standard solution, C_2 = concentration of standard solution injected (ng/μL), V_D = volume of final concentrate (μL), and W_S = weight of smoked food taken for analysis (g).

Calculate recovery of each NPAH from smoked food as follows:

$$\text{Recovery (\%)} = (PH_1 \times 100)/PH_2$$

where PH_1 and PH_2 are as defined above, and the standard solution used to produce PH_2 is the same as that used for spiking.

Results and Discussion

All of the domestic smoked foods analyzed in this study were previously analyzed for their polynuclear aromatic hydrocarbon content (17); ham, bacon, sausage, whiting, salmon, and trout represent this group. Some imported smoked foods were also analyzed; oysters, clams, sardines, red snapper, and brislings represent this group. The 3 NPAHs used as standards for this study, DMBaAc, DBahAc, and DBajAc, were chosen primarily because they were readily available and easily purified. These compounds represent some of the carcinogenic basic NPAHs which might be found in contaminated smoked foods. The NPAHs were measured with UV and fluorescence detectors connected in series, with the former set at 289 nm and latter set at an excitation wavelength of 333 nm. The use of these wavelengths provided the sensitivity and the degree of selectivity needed for the determination of individual NPAHs. The calculations were based on peak height rather than peak area because we obtained better precision with peak heights.

Typical fluorescence and UV chromatograms for approximately 5 ng of each standard are shown in Figure 1. The order of elution is DMBaAc, DBajAc, and DBahAc. As indicated in these chromatograms, DBajAc shows the highest

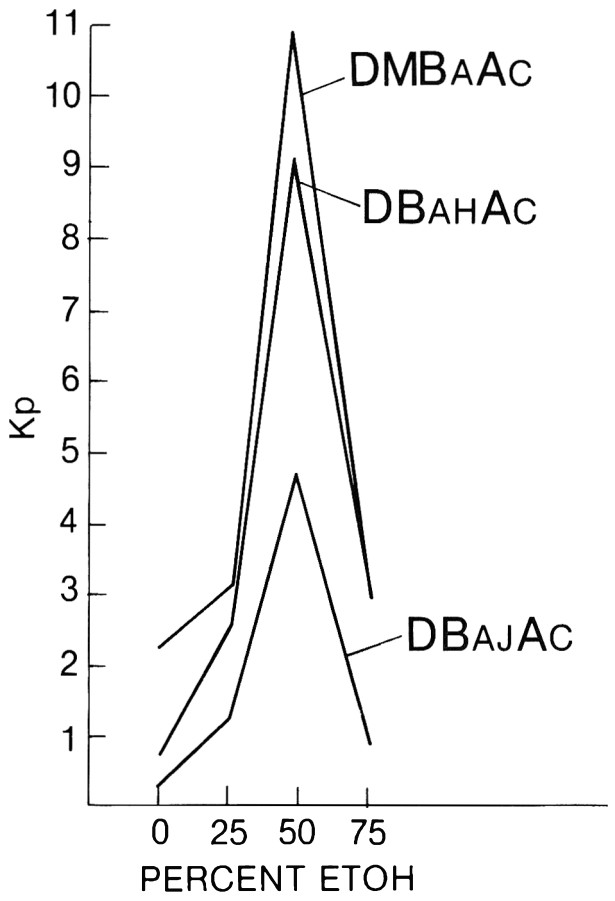

Figure 4. Plot for each NPAH of partition coefficient, *Kp*, between cyclohexane and aqueous ethanol vs percent ethanol.

Table 1. Recoveries (%)[a] calculated from fluorescence and UV data[b] for NPAHs added to smoked foods[c]

	NPAH					
	DMBaAc		DBajAc		DBahAc	
Food	F	UV	F	UV	F	UV
Sausage[d]	97	93	66	64	94	96
	96	94	66	68	88	86
Salmon[d]	97	98	62	64	90	94
	101	104	64	68	96	106
Av.	97.8	97.2	64.5	66.0	92.0	95.5
SD	2.2	5.0	1.9	2.3	3.6	8.2
CV, %	2.2	5.1	2.9	3.5	3.9	8.6

[a]Each value is the average of at least 2 analyses.
[b]F = fluorescence, excitation wavelength = 333 nm; UV wavelength = 289 nm.
[c]5 ppb spiking level for each NPAH.
[d]Different lots from the same manufacturer.

fluorescence response, whereas DMBaAc shows the highest UV response. Along with retention times for each compound, the ratio of fluorescence response:UV response was useful in indicating whether peaks observed in the chromatograms of the smoked food extracts were also present in those of the NPAH standards used in this study, or whether they were due to other compounds.

Typical fluorescence and UV chromatograms for an unfortified smoked food and the same smoked food fortified at the 5 ppb level are shown in Figure 2. The fluorescence chromatogram for the unspiked domestic product is free of any peaks, although the UV chromatogram does show one peak at a retention time of approximately 11 min. This same peak also appears in the UV recovery chromatogram for the spiked product. Analysis of the unidentified component by GC/MS indicated that it was a phthalate, a ubiquitous compound, whose source in this method has not been absolutely confirmed. Fluorescence chromatograms for several additional unfortified smoked foods (oysters, ham, red snapper, and trout) are shown in Figure 3. Similar chromatograms were obtained for the remaining smoked food products analyzed. Capillary GC/MS analyses were also performed for all extracts for which LC peaks were obtained. The GC/MS results indicated the presence of additional phthalate and adipate esters and some free sterols, but the spectra did not resemble those of NPAH standards.

Recovery studies were carried out with a high fat food (sausage) and a high protein food (salmon). Each of the foods analyzed was spiked at the 5 ppb level with a standard solution of 3 NPAHs. The results of these recovery studies are shown in Table 1, in which each value is the average of at least 2 analyses. Recoveries from sausage and salmon that were calculated from fluorescence data ranged from 62% for DBajAc to 101% for DMBaAc, whereas those calculated from UV data ranged from 64% for DBajAc to 106% for DBahAc.

As Table 1 shows, the recoveries for DBajAc are somewhat lower than those obtained for DBahAc and DMBaAc. A probable explanation is suggested from work of previous investigators (18). These investigators showed that the compound benzo(h)quinoline differs in chemical properties from its isomers, e.g., benzo(c)quinoline and acridine, because the benzo group in the (h) position completely blocks access to the lone pair of electrons on the nitrogen atom. Thus steric factors account for the differences in basicity and retention of these compounds, which are comparable to the NPAHs in this study. Similarly, the benzo group of DBahAc and the methyl group of DMBaAc block the nitrogen electrons, but this does not occur with DBajAc. This difference probably accounts for the lower recoveries for DBajAc.

A study of the partition coefficient, *Kp*, was made to determine the optimum ratio of ethanol:water needed to extract the NPAHs. The optimum ratio was determined by varying the ratio of ethanol:water but leaving its total volume and the cyclohexane volume constant, extracting each NPAH into cyclohexane, and determining recoveries as follows:

$$Kp = A/(B - A)$$

where A = peak height of NPAH in cyclohexane layer, and B = peak height of NPAH in standard solution.

Table 2 lists the average values for the *Kp* determinations, and the results are plotted in Figure 4, which shows that at 0% ethanol (100% water) the NPAHs are somewhat soluble and thus slightly polar. Further study of the solubility showed that the NPAHs used in this study are soluble in water in concentrations ≥ 1.2 μg/mL. When reasons for less than total recovery were explored, this solubility and the type of matrix examined were thought to be responsible. The matrix type was thought to play a role in affecting recoveries because, as indicated by Thompson and Duval (19), products with high fat content produce the largest amount of soap in aqueous ethanol solution after saponification. The soaps make the aqueous ethanol solvent more like an organic solvent, and partitioning between it and the cyclohexane becomes impaired. Figure 4 also shows that maximum amounts of the NPAHs are partitioned into the cyclohexane layer at a concentration of 50% ethanol. Because the ethanol–water (1 + 1) solution was found to give maximum NPAH recoveries, it was used in this method for extracting the azaarenes from the various smoked food matrices.

Table 2. Partition coefficients (*Kp*)[a,b] for NPAHs

	DMBaAc		DBajAc		DBahAc	
EtOH, %	F	UV	F	UV	F	UV
0	0.7	0.7	0.3	0.3	2.2	2.2
25	2.4	2.5	1.2	1.1	3.0	5.5
50	10.8	11.1	4.6	5.1	9.1	7.6
75	2.8	4.0	0.8	0.8	2.8	2.9

[a]See definition in text; peak heights obtained by fluorescence (F) detection (excitation wavelength = 333 nm) and by UV detection (289 nm).
[b]Values are averages of 4 determinations.

Conclusions

A relatively short, straightforward LC method has been developed for the analysis of several smoked foods for NPAHs. Although no NPAHs were isolated from any of the smoked foods analyzed, the recovery data indicate that the method is reliable. A study of the distribution ratios of DMBaAc, DBajAc, and DBahAc between cyclohexane and the aqueous ethanolic saponification digest indicates that the best recovery of the azaarenes occurred when the water–ethanol ratio was 1:1. Future studies will include oil-contaminated foods.

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GENERAL REFEREE REPORTS: COMMITTEE ON PESTICIDE FORMULATIONS AND DISINFECTANTS

Disinfectants

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Use-Dilution Test.—During the past year a considerable number of studies were done on parts of the test. S&L steel cylinders, **4.008(d),** "polished" and "dull" were compared with Fisher "polished" and "dull" stainless steel cylinders by test results. S&L cylinders are specified in the test, but during periods of unavailability, Fisher cylinders have been used. Results show that both cylinder sources can produce positive results in the test; Fisher showed the most positives as purchased. S&L "polished" compared with Fisher "dull," and S&L "dull" compared with Fisher "polished" showed significantly more positives with Fisher. Inquiries addressed to S&L as to reasons for apparent variations in the surface polish of cylinders revealed that a subcontractor produced the cylinders without quality controls. If the problem cannot be corrected, S&L will resume producing cylinders itself but at much higher cost. The co-Associate Referee advises that S&L cylinders should not be purchased until production problems are resolved.

Removal of *Ps. aeruginosa* pellicle as described (**4.011**) was compared with vacuuming with a pipet and filtration. Evaluation of broth variability after pellicle removal was based on resistance against phenol, bacterial numbers on cylinders, and conducting the complete test. Results showed no significant differences between the 3 methods of pellicle removal. For ease and practicality, the co-Associate Referee recommends pipet suctioning followed by decantation.

Studies were conducted to replace loop sampling, **4.002(d),** with micropipets in determining phenol resistance of test bacteria. The specified bacteria were used. No increase in precision or accuracy could be demonstrated for a micropipet in place of a loop. Several tests were needed to confirm phenol resistance. *Pseudomonas* exhibited the greatest variability. The co-Associate Referee states that if no correlation can be shown between resistance to phenol and failures in the test, consideration should be given to eliminating phenol resistance from the test.

Bacterial wash-off from steel cylinders after drying of bacteria in an incubator was investigated. Phosphate-buffered water under test conditions was used and the number of bacteria on a cylinder after drying and after transfer to subculture media was determined. Mean wash-off was as follows: *S. aureus,* 39%; *Ps. aeruginosa,* 49%; *S. choleraesuis,* 90%. The co-Associate Referee states that since the numbers of bacteria remaining on cylinders can vary with each test bacterium, this could be a source of variation.

Studies were conducted on the ability of commercially prepared letheen broth (Difco) to effectively neutralize disinfectant carryover into subculture media. This medium was chosen because of its wide use among testers of disinfectants. Five quaternary ammonium chlorides and 5 substituted phenolics were studied using the 3 bacteria in the test. Increasing volumes of disinfectant were added (0.1–1.0 mL) to a series of tubes of letheen broth. To each of these, 5–10 cells of test bacterium were added, followed by incubation for 48 h. All disinfectants tested showed effective neutralization for all 3 bacteria, the exception being *S. aureus* tested against the quaternary ammonium compounds. Neutralization was shown only through 0.2 mL added quaternary, indicating that careless transfer of cylinders can cause variable results. The usual amount of disinfectant carried over into subculture medium is 0.01 mL, so the commercial medium tested is an adequate neutralizer. The co-Associate Referee recommends that Difco letheen broth be adopted in the test. We intend to amend the test to include positive and negative controls and instructions to pretest each batch of purchased medium for neutralization efficacy before use in the test.

The co-Associate Referee is conducting a collaborative test to assess interlaboratory error in the present test and to serve as a basis to measure if the test can be made less variable when all amendments and modifications are incorporated. Statistical analyses are also being done to define true pass-fail. Seven technical papers are submitted or are being prepared on the work done so far in updating this test. Five amendments were recommended by the co-Associate Referee. We believe that such amendments should be held in abeyance until the whole method is rewritten and published on a first-action basis. It is recommended that work continue on this topic.

Tuberculocidal Test.—Based on recommendations from the statistician of the Committee on Pesticide Formulations and Disinfectants, the Associate Referee will do a ruggedness statistical test before proceeding with a minimum collaborative test. Continued study is recommended.

Virucidal Test.—No report was received from the Associate Referee. It is recommended that this topic be discontinued.

Sporicidal Test.—No report was received from the Associate Referee. Continued study is recommended.

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Pesticide Formulations and Disinfectants and adopted by the Association. See the report of the committee and "Changes in Methods," this issue.

Pesticide Formulations: Carbamate and Substituted Urea Insecticides; General Methods

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This year has not been as productive as recent years. Most of the methods considered for action or collaboration are based on internal standard LC procedures. 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 reports no additional work on this topic.

Bendiocarb.—Peter L. Carter has completed a collaborative study of an internal standard LC method. Results indicate that the method is applicable to materials in the 20–95% range with acceptable variation; the Associate Referee recommends adoption as first action.

Carbofuran and Carbosulfan.—Edward J. Kikta, Jr, has completed a carbofuran collaborative study of an internal standard reverse phase LC method applicable to most formulations. Adoption as first action is recommended. A collaborative study of carbosulfan is planned for the new year.

Dioxins in Pentachlorophenol.—Jo-Anne Campbell is considering the best GC/MS method for collaboration.

Methiocarb.—Stephen C. Slahck reports no additional work on this topic.

Methomyl.—James E. Conaway, Jr, is further revising the collaborative study report of a reverse phase internal standard LC method presented earlier.

Mexacarbate.—William H. McDermott is considering methods for collaborative study.

Oxamyl.—Glenn A. Sherwood, Jr, is further revising a collaborative study of a reverse phase internal standard LC method presented earlier.

Pirimicarb.—John E. Bagness reports no additional work on this topic.

Propoxur.—Stephen C. Slahck reports no additional work on this topic.

3,4,5- and 2,3,5-Isomers of Trimethylphenyl Carbamate.—William H. McDermott has completed collaborative study of an internal standard LC method and a recommendation for first action is anticipated.

Recommendations

(1) Continue as first action the LC method for aminocarb, to allow more time for comments.

(2) Adopt as official first action the methods for bendiocarb and carbofuran.

(3) Continue study on all other topics.

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Pesticide Formulations and Disinfectants and adopted by the Association. See the report of the committee and "Changes in Methods," this issue.

Pesticide Formulations: Fungicides and Disinfectants

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In the past, the areas of fungicides and disinfectants has been an active section. Again this year, much progress has been reported by Associate Referees in the area. The results of one collaborative study have been reported. Also, another collaborative study is in progress as of this writing. Another Associate Referee has been awaiting publication of a paper, and has plans to conduct a collaborative study following publication. Work is also being done by CIPAC on one compound (thiram), and results were reported at their 29th annual meeting. Several other Associate Referees have reported progress on methods development.

The following is a status report of selected topics within this section.

Anilazine.—Associate Referee Steve Slahck reports that a collaborative study on this compound is currently in progress.

Benomyl.—Associate Referee Mikio Chiba reports that a method to determine this compound will be ready for collaboration following publication of the method.

Dithiocarbamate Fungicides.—Associate Referee Warren Bontoyan reports that further work is being done on an LC method for these compounds.

Oxythioquinox.—Associate Referee Steve Slahck reports the results of a collaborative study on this compound with his recommendation to adopt the method as official first action.

Pentachloronitrobenzene (PCNB).—Associate Referee Alan Hanks recommends this topic be inactivated.

Thiram.—CIPAC reports adoption of a method for this compound during their 29th annual meeting.

Recommendations

(1) Adopt as official first action the LC method for oxythiquionox as described by the Associate Referee.

(2) Inactivate the topic Pentachloronitrobenzene.

(3) Continue study in all other areas.

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Pesticide Formulations and Disinfectants and adopted by the Association. See the report of the committee and "Changes in Methods," this issue.

Pesticide Formulations: Herbicides I; Other Organophosphorus Insecticides; Rodenticides and Miscellaneous Pesticides

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The following is a summation of activities this past year:

Herbicides I:

Chlorophenoxy Herbicides.—Robert Grorud's method was adopted first action in 1984 for dicamba, 2,4-D, and MCPP.

Dicamba.—(Ben Belkind) The method being studied has shown some problems with silylation. These problems should be corrected and a collaborative study should be conducted during the next year.

Pentachlorophenol.—Elmer H. Hayes has found it necessary to be relieved as Associate Referee. He is willing to share the data he has accumulated with his successor.

Other Organophosphorus Insecticides.

Crotoxyphos.—Wendy King is continuing to study a method.

Fenamiphos.—Carl Gregg is studying a method utilizing gas chromatography with thermal conductivity detection. A collaborative study is anticipated within the next year.

Naled.—A. A. Carlstrom is continuing to study a method.

Rodenticides and Miscellaneous Pesticides:

Brodifacoum.—Peter D. Bland is studying wax baits containing this compound. Problems have been encountered with the extraction of aged bait.

Warfarin.—Elmer H. Hayes has asked to be relieved of the duties of Associate Referee for this compound.

Recommendation

Continue study on all topics.

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Pesticide Formulations and Disinfectants and adopted by the Association. See the report of the committee and "Changes in Methods," this issue.

Pesticide Formulations: Herbicides II

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The past year has not been very productive. Changes in responsibilities, shifting priorities, and generally heavy workloads have created a difficult environment for Associate Referees to initiate and complete collaborative studies. No collaborative studies have been completed in the last year.

A new Associate Referee, Glenn Sherwood of du Pont, has been appointed for the group of allied compounds, metsulfuron-methyl, sulfometuron-methyl, and chlorsulfuron. He has relinquished his refereeships with respect to the substituted urea herbicides, diuron, linuron, and siduron.

Ben Belkind of Velsicol has replaced John Forrette (retired) as Associate Referee for methazole and barban. Fluchloralin and profluralin have been dropped from Rodger Stringham's Associate Refereeships for substituted nitroanilines, and ethafluralin has been added.

An internal standard gas chromatographic method for benzoylprop-ethyl has been recently adopted by CIPAC and is currently being rewritten for submittal to the Committee on Pesticide Formulations, assuming the collaborative data are complete and adequate.

Associate Referees are needed for the substituted urea herbicides (including monuron and monuron TCA) and oryzalin and dinoseb.

The following reflect the current status of other projects:

Barban.—B. Belkind is continuing method development.

Benefin, Trifluralin, Pendimethalin, and Ethafluralin.—R. Stringham is concentrating his efforts toward initiating a collaborative study involving a uniform method.

Bensulide.—W. Ja is working on developing uniform methodology for different formulations.

Benzoylprop-ethyl.—J. Launer is rewriting a CIPAC method for AOAC submittal.

Bromacil and Lenacil.—P. Tseng has developed an internal standard LC procedure for each compound and plans to initiate collaborative studies later this year.

Dimethyl Tetrachloroterephthalate.—B. Korsch has a project on hold, pending company authorization to proceed with a collaborative study.

Fluometuron.—A. Hofberg is continuing ruggedness testing of an internal standard LC procedure.

Methazole.—B. Belkind has a good internal standard LC procedure ready for collaboration.

Naptalam (Alanap).—P. Parkins has developed an internal standard LC procedure for mixtures with dinoseb and plans to initiate a collaborative study later this year.

Recommendation

Continue study on all topics.

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Pesticide Formulations and Disinfectants and adopted by the Association. See the report of the committee and "Changes in Methods," this issue.

Pesticide Formulations: Herbicides III

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Again this year, method development continues in this section, with several Associate Referees reporting progress on collaborative study preparations. In addition, one Associate Referee, Dave Tomkins, has reported the results of 2 collaborative studies, along with recommendations for adoption.

Results from 2 CIPAC studies were received and evaluated. One of these studies (cyanazine) was adopted as a full CIPAC method during the 27th annual meeting. Another study (bentazon) was adopted as a provisional method by CIPAC at the 29th annual meeting.

The following is a status report of selected topics within this section.

Alachlor, Butachlor, and Propachlor.—Associate Referee David F. Tomkins has reported the results of 2 collaborative studies, one on propachlor and the other on butachlor. He recommends adoption of both methods as official first action. He further reports a modification to the official first action method for alachlor, to include a sample preparation for microencapsulated product. Also, he reports much activity concerning the official first action gas chromatographic method, with many copies of the method being sent to interested parties. Having received no negative feedback, he recommends that the method be adopted official final action.

Bentazon.—Associate Referee Thomas M. Schmitt reports the results of a collaborative study done by CIPAC on this compound. The LC method has been accepted as a provisional CIPAC method.

Bromoxynil.—Associate Referee Laurence Helfant continues to work on an LC method for various formulations of this compound. A collaborative study is planned for presentation in 1986.

Cyanazine.—The General Referee has received from CIPAC the results of an LC study run on this compound. The statistics have been evaluated.

Dalapon.—Associate Referee Tim Stevens has submitted a paper to the AOAC *Journal* entitled "Comparative Assay of Dalapon Products Using the AOAC Method and an Independent Method."

Fluazifop-butyl.—Associate Referee Peter Bland reports work being done in-house necessitated by changes in the formulation for this compound. The current method used by the company does not lend itself to a collaborative study.

Pesticides in Fertilizers.—Associate Referee Paul Korger reports work on a method he intends to present to the AOAC *Journal* for publication.

Recommendations

(1) Adopt as official first action the gas chromatographic method for propachlor described by the Associate Referee.

(2) Adopt as official first action the gas chromatographic method for butachlor described by the Associate Referee.

(3) Adopt as official final action the official first action gas chromatographic method for alachlor described by the Associate Referee.

(4) Adopt as official first action the CIPAC LC method for cyanazine.

(5) Adopt as official final action the official first action gas chromatographic method for metribuzin (**6.553–6.559**).

(6) Continue study in all other areas.

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Pesticide Formulations and Disinfectants and adopted by the Association, except as follows: the method for alachlor was continued official first action, and the method for cyanazine was not adopted. See the report of the committee and "Changes in Methods," this issue.

Pesticide Formulations: Halogenated; Other Insecticides, Synergists, and Insect Repellents

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Due to time restrictions, no AOAC collaborative studies were conducted under these topics; however, the Collaborative International Pesticides Analytical Council (CIPAC) in 1984 adopted as full CIPAC methods 2 GC methods for determination of total permethrin and cypermethrin, respectively, in technical material and formulations. The General Referee submitted these methods for adoption by AOAC. Three Associate Referees were appointed on 5 topics this past year: Benjamin Belkind on Chlordane and Heptachlor, Dianne Bradway on Ethylan and Trichlorfon, and Fred Churchill on Benzene Hexachloride (and Lindane).

The following is the present status of selected topics assigned to Associate Referees:

Halogenated Insecticides:

Benzene Hexachloride.—F. Churchill recommends adoption as official final action the official first action CIPAC-AOAC gas chromatographic method (**6.221–6.226**). The General Referee concurs. The Associate Referee is also evaluating a gas chromatographic method for lindane in maneb-lindane liquid formulations.

Chlordane.—B. Belkind is investigating GC methods.

Dicofol.—A. Rothman clarified the data from the 1982 collaborative study of the LC method and recommends adoption as official first action. The General Referee concurs.

Ethylan (Perthane).—D. Bradway is investigating GC and LC methods.

Fenvalerate.—R. Collins is cooperating with CIPAC on a GC method for technical material.

Trichlorfon.—D. Bradway is investigating GC and LC methods.

Other Insecticides, Synergists, and Insect Repellents:

Allethrin.—Since the present official first action titrimetric method (**6.165–6.170**) provides useful information on impurities, D. Kassera recommends adoption as official final action. The General Referee concurs.

Cyhexatin (Plictran).—S. Lupan has completed a collaborative study of an LC method and recommends adoption by CIPAC as a full method. The General Referee will investigate this action.

Cypermethrin.—P. Bland recommends adoption as official final action the official first action capillary gas chromatographic method (**6.A01–6.A04**). The General Referee recommends delay of this action until next year. The General Referee recommends adoption as official first action the CIPAC gas chromatographic method.

Cyromazine (Larvadex).—A. Hofberg is evaluating GC and LC methods.

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Pesticide Formulations and Disinfectants and adopted by the Association, except the method for allethrin was continued official first action. See the report of the committee and "Changes in Methods," this issue.

Fumigants.—D. Yeaman plans a collaborative study of a GC method for sulfuryl fluoride.

Nicotine.—R. Bushway is investigating LC methods.

Permethrin.—The General Referee recommends adoption as official first action the CIPAC gas chromatographic method.

Rotenone and Other Rotenoids.—R. Bushway plans a collaborative study of modifications to the official first action liquid chromatographic method (**6.182–6.186**).

Recommendations

(1) Continue first action status of the following methods: (a) GC method for chlordimeform (**6.A09–6.A13**); (b) capillary GC method for cypermethrin (**6.A01–6.A04**); (c) hydrolyzable chloride method for dicofol (**6.332–6.337**); (d) infrared method for rotenone (**6.179–6.180**); (e) LC method for rotenone (**6.182–6.186**).

(2) Adopt as official first action the following methods: (a) full official CIPAC GC method for cypermethrin; (b) LC method for dicofol; (c) full official CIPAC GC method for permethrin.

(3) Adopt as official final action the following first action methods: (a) titrimetric method for technical allethrin (**6.165–6.170**); (b) GC method for the gamma-isomer of benzene hexachloride.

(4) Continue study of piperonyl butoxide and pyrethrins, including low levels and mixed formulations.

(5) Continue study on all other topics.

Pesticide Formulations: Organothiophosphorus Pesticides

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Two collaborative studies were completed during the year and 3 new Associate Referees accepted topics in this area of work. The following is a summation of activities and recommendations of various Associate Referees.

Acephate.—A. Aner Carlstrom now has an LC column which appears satisfactory for use in the analysis of acephate, but he feels some additional ruggedness data are necessary before the method is submitted for study.

Azinphos-methyl.—Associate Referee Stephen Slahck hopes to get a standard evaluated next year so that he can begin work on a method for this pesticide.

Dimethoate.—Richard S. Wayne has developed a reverse phase LC method and hopes to study it collaboratively next year.

EPN.—Ben Belkind accepted appointment as Associate Referee and plans to evaluate GC methodology as soon as time will permit.

Ethoprop.—Associate Referee Richard W. Smith plans to do a GC collaborative study during the coming year.

Fenitrothion.—Newly appointed Associate Referee Dwight L. Mount plans to do a mini-collaborative study of the first action GC method, utilizing a different stationary phase and internal standard.

Fensulfothion.—Co-Associate Referee William Betker completed a collaborative study of a GC method and recommends adoption as official first action.

Fenthion.—Bill Boyd plans to complete an evaluation of the capillary GC approach during the coming year.

Isofenphos.—Daniel Terry completed a collaborative study of a GC method and recommends that it be adopted official first action.

Methamidophos.—Associate Referee Jim Baird's investigation of the normal phase LC approach has not produced satisfactory data. He plans to look at capillary GC.

Methidathion.—Tom Gale has an LC method ready for study as soon as time is available.

Oxydemeton-methyl.—Stephen Slahck plans to complete method development and do a collaborative study as soon as he completes a study in another area.

Pirimiphos-methyl.—Newly appointed Associate Referee Peter Bland submitted, in AOAC format, a CIPAC GC study of pirimiphos-methyl and recommends that it be adopted official first action as a joint AOAC-CIPAC method.

S,S,S,-Tributyl Phosphorotrithioate.—William Bekter has a GC method which he plans to submit for collaborative study during the coming year.

No further work has been done on coumaphos, diazinon, dioxathion, encapsulated organophosphate pesticides, fonofos, malathion, parathion and methyl parathion, or phorate.

Recommendations

(1) Adopt as official first action the GC methods for fensulfothion and isofenphos.

(2) Adopt as official first action the CIPAC GC method for pirimiphos-methyl.

(3) Continue study on other topics.

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Pesticide Formulations and Disinfectants and adopted by the Association, except as follows: the methods for isofenphos and for pirimiphos-methyl were not adopted. See the report of the committee and "Changes in Methods," this issue.

GENERAL REFEREE REPORTS: COMMITTEE ON DRUGS AND RELATED TOPICS

Biochemical Methods; Diagnostics and Test Kits

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One of the problems in regulatory application of immunoassay is that no well developed data base is available on the performance of immunoassay procedures. It is not sufficient to extrapolate theoretical arguments or data developed in clinical laboratory medicine to assess suitability of an immunoassay for nonclinical regulatory use. For example, in many cases, antibodies are not monospecific. Therefore unambiguous results suitable for forensic or regulatory use depend heavily on the details of the assay method and on the techniques of analyte separation and purification.

The necessary data base can be developed by positioning immunochemical procedures including immunoassay at the "front end" of a more definitive analytical technique. Initially, immunoassays would be used primarily as screening tools, with follow-up analysis by other techniques such as gas chromatography or liquid chromatography/mass spectrometry. Immunochemical techniques can also be applied as affinity chromatographic procedures, which could help in simplifying and speeding analyses. Screening methodology would focus on using existing, established procedures such as simple colorimetric or new hemoagglutination techniques using immunochemical or other biochemical reagents. The incorporation of aspects of immunochemical techniques into an overall analytical scheme is a practical way of developing a data base on the performance of immuno-techniques in regulatory or forensic analysis.

The use of immuno-affinity chromatography is an example of how immunochemistry can be applied to sample cleanup schemes. Also, screening procedures based on immunological or biochemical principles, which are designed as independent, stand-alone techniques, can be very effectively used as monitoring tools to assess the performance of critical steps in a complex cleanup scheme. Used in this way, immunochemical techniques can have an immediate and useful application in regulatory or forensic analysis, while their performance is also being assessed.

While immunochemical techniques can have an immediate use as part of an overall analytical scheme in regulatory analysis, the collaborative study of an immunochemical or other rapid screening techniques intended for stand-alone use, may require new procedures in collaborative study design.

In many cases, screening techniques are intended to be used in nonlaboratory settings by individuals not necessarily trained in chemical analysis. In these cases, a collaborative study must measure the performance of the method under the intended conditions of use as applied by an intended user. This will often be in a field test setting with relatively inexperienced personnel. A useful collaborative study requires that the methods be tested in part under conditions that closely emulate if not exactly duplicate those under which the test will be routinely used. It is also equally important that the study design test the soundness of scientific principles of the test and the workability of the technology used. This aspect of the collaborative study is best done under controlled laboratory conditions by personnel trained in the technology used in the test. The information derived from this aspect of the study will serve as a reference point whereby field test performance biases can be measured.

It is recommended that collaborative studies for immunochemical or other biochemical screening tests intended for field use incorporate the following features:

Phase	*Location and Analyst*
I. Test of scientific principles and technology	In an experienced lab. by experienced personnel
Ia. Intralaboratory collaborative study	In an experienced lab. by inexperienced personnel
II. Field test	On-site by intended end users

It is further recommended that the validation strategies for screening methods be designed to show method performance under the above conditions. At a minimum, the following points should be addressed in a validation design: *(1)* optimization of method performance; *(2)* identification of critical steps; *(3)* recognition and solution of interferences; *(4)* assessment of method performance using authentic samples under authentic conditions; and *(5)* comparison with other techniques, if appropriate.

Specific Topics

Determination of oxalate.—Associate Referee Quincy E. Crider (Sigma Chemical Co., St. Louis, MO). Methodology has been developed for the rapid measurement of oxalate in urine. The technique has been applied with success to various foods and works well except with certain wines. The method is being reviewed for collaborative study.

Quantitation of heparin.—Associate Referee Lillian Gill (Food and Drug Administration, Baltimore District Laboratory). The current USP XXI assay for heparin is based on extent of clotting of sheep blood plasma judged to have occurred at the end of 1 h. Heparin unitage is assigned by a comparison of the clotting performance of the test preparation relative to a standard heparin reference preparation. This method is highly technique dependent and is cumbersome to apply to a large number of samples such as would be encountered in a manufacturing or regulatory situation.

The method under development is based on the esterolytic activity of thrombin on a colorimetric substrate and on the effect of heparin on thrombin activity. The latter effect plays a central role in the anticoagulant effect of heparin.

The method under development will provide a better quantitative tool and will be more easily automated for analysis of a large number of samples. Work currently involves assessment of the effect of heparin on the linearity of the quantitative reaction. The method, which will be correlated with the USP procedure before final assessment for collaborative study, is in the final stages of development.

Monoclonal antibodies for use in detecting staphylococcal enterotoxin.—Associate Referee Richard Meyer (Food and Drug Administration, New York Regional Laboratory). Monoclonal antibodies which interact with all 5 *S. aureus* serotypes have been developed. Presumably, the antibodies react with an epitope common to all 5 serotypes. Because the antibodies react with a common epitope on all *S. aureus* toxins, they are not useful as a specific toxin speciation reagent. However, recently developed monoclonals appear to react only with serotype A or with A and D serotype toxin. The complete characterization of this effect is under way.

The planned work consists of antibody characterization as to specificity and utilization in a double sandwich enzyme-

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Drugs and Related Topics and adopted by the Association. See the report of the committee, this issue.

linked immunoassay. Work on this project has been slowed by new laboratory construction for biotechnology operations at the New York regional laboratory. A collaborative study on a monoclonal-based immunoassay for salmonella in foods has been performed by R. S. Flowers et al. (Silliker Laboratories, Chicago, IL). Details are available from the General Referee on Food Microbiology. Work is continuing on methodology development.

Immunochemical methods for animal species identification.—Associate Referee David Berkowitz (U.S. Dept of Agriculture, Washington, DC). No methods for species identification have been developed sufficiently to warrant collaborative study at this time. However, this is an area of substantial interest especially in the application of immunoassay techniques to both tissue speciation and adulteration problems. Work on this topic will continue in 1986.

Drugs I

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Acetaminophen and Codeine by LC.—Norlin W. Tymes reported on the completion of his collaborative study. Seven collaborators independently analyzed 3 tablet, 3 capsule, and 2 elixir samples, and 1 elixir authentic sample in blind duplicate for a total of 126 determinations. With the authentic elixir formulation, the collaborators obtained a mean recovery of 100.5% for acetaminophen. Preliminary statistical evaluation indicates that coefficients of variation ranged from 2.31 to 4.10% for the drugs in the various formulations. The Associate Referee will present detailed information on the liquid chromatographic method and the results of the collaborative study at the 1985 Annual International Meeting.

Benzothiazine Derivatives.—The topic has been discontinued, but work will continue under Benzthiazides by LC under the direction of Stephen T. Hauser.

Phenothiazines and Tricyclic Antidepressants in Formulations by LC.—Edward G. Lovering has completed his collaborative study in which 11 collaborators participated. The method was found suitable for the liquid chromatographic determination of trifluoperazine, chlorpromazine, and imipramine in tablets. The method uses a CN column, with detection at 254 nm.

We are also discontinuing the topics Barbiturates, Chlorpromazine, Probenecid, Sulfonamides, and Thiazide Diuretics due to the lack of Associate Referees who have time to work on the projects. Work on other topics is continuing.

Recommendations

(1) Adopt as official final action the first action liquid chromatographic method for determination of amitriptyline in tablets and injectables, **36.207–36.211.**

(2) Adopt as official final action the first action liquid chromatographic method for determination of sulfisoxazole in tablets, solutions, and ointments, **37.166–37.172.**

(3) Adopt as official first action the interim first action liquid chromatographic method for determination of primidone in tablets, **37.A17–37.A22.**

(4) Discontinue the topic Benzothiazine Derivatives and continue study on that topic under Benzthiazides by LC.

(5) Discontinue the topics Barbiturates; Chlorpromazine; Probenecid; Sulfonamides; and Thiazide Diuretics, Semiautomated Individual Dosage Unit Analysis.

(6) Continue study on all other topics.

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Drugs and Related Topics and adopted by the Association. See the report of the committee and "Changes in Methods," this issue.

Section numbers refer to *Official Methods of Analysis* (1984) 14th edition, and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* (1985) **68**, 369–411.

Drugs II

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Aminacrine.—Associate Referee Elaine Bunch reported the results of the collaborative interlaboratory study of her proposed method for spectrophotometric determination of aminacrine HCl in creams, jellies, and suppositories (1) and fluorometric determination-TLC identification of aminacrine HCl in drug preparations (2). The Associate Referee recommends that the visible spectrophotometric method for aminacrine HCl in creams, jellies, and molded and gelatin-encapsulated suppositories be adopted official first action (3).

Atropine in Morphine and Atropine Tablets and Injections.—Associate Referee I. J. Holcomb reported that he is continuing his investigation of chromatographic methods for the separation and determination of the atropine and morphine content of these pharmaceuticals.

Belladonna Alkaloids.—A replacement for this vacant Associate Refereeship is being sought. Barkan et al. (4) developed a liquid chromatographic (LC) method for the individual determination of *d*- and *l*-hyoscyamine when they are present together. This method would make it possible to determine *l*-hyoscyamine and atropine (*d,l*-hyoscyamine) individually when they are combined in a pharmaceutical. This study would also involve the determination of the other belladonna alkaloids that might be present, as well as ensure the suitability of the method for detecting any decomposition product present.

Colchicine in Tablets.—Associate Referee Richard D. Thompson reported the successful completion of the collaborative study of his proposed liquid chromatographic method for the determination of colchicine (5). A subsequent study will be made on dosage forms containing colchicine in combination with probenecid. One decomposition product, colchiceine, does not chromatograph with the proposed LC method. Either a TLC limits test or an alternative chromatographic method will have to be developed for that determination.

Curare Alkaloids.—Associate Referee J. R. Hohmann reported no further progress on revision of his LC method

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Drugs and Related Topics and adopted by the Association. See the report of the Committee, this issue.

for the determination of tubocurarine chloride in tubocurarine chloride injection. The results indicate that additional work is needed to establish a suitable stable internal standard.

Dicyclomine Capsules.—A new Associate Referee is needed for this topic. A GC method for dicyclomine in capsules and other pharmaceuticals was proposed by the former Associate Referee.

Epinephrine-Lidocaine Combinations.—The Associate Referee reported that he did no work on this project. A new Associate Referee is being sought for this topic.

Epinephrine and Related Compounds by Liquid Chromatography-Electrochemical Detectors.—Associate Referee John Newton reported that he is continuing his investigation of the electrochemical properties of epinephrine and its degradation products after their separation by LC.

Ergot Alkaloids.—Associate Referee T. C. Knott reported that he is investigating the cause of the low recoveries obtained with the samples used in the last collaborative study. Sample decomposition may be a cause of some low results from samples stored in certain containers. Once the source of the low assays is confirmed and overcome, a collaborative study will be conducted.

Homatropine Methyl Bromide in Tablets.—A new Associate Referee is being sought to continue work on separating homatropine methyl bromide from the other active ingredients before a chromatographic determinative step.

Morphine Sulfate in Morphine Sulfate Injection.—Rita Kling Jhangiani and Ada C. Bello reported their proposed LC determination of morphine sulfate and some contaminants in injections and bulk drug material (6). Ada Bello has accepted the Associate Refereeship and is planning to initiate a collaborative study of this proposed method, which has been demonstrated to be more specific than the current official method.

Neostigmine.—Associate Referee Rita 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 colorimetric semiautomated method.

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 after the validation is completed.

Physostigmine and Its Salts.—Associate Referee N. W. Tymes reported no further work on this topic.

Pilocarpine.—Associate Referee I. W. Wainer reported no further work on this project.

Rauwolfia Alkaloids (Reserpine and Rescinnamine).—Associate Referee Susan Barkan reported that no work was done on this topic.

Rauwolfia serpentina.—Associate Referee U. Cieri reported that he is continuing the investigation of the development of a quantitative LC method for determining the reserpine-rescinnamine content of *Rauwolfia serpentina* tablets. A report on his progress was presented at the 1985 AOAC meeting (7).

Recommendations

(1) Continue the following methods in official first action status: LC method, **38.A01–38.A06,** for colchicine in tablets (5); physostigmine alkaloids, **38.074–38.080,** LC method for the salicylate solution and sulfate ointment (8, 9); LC method, **38.144–38.150,** for pilocarpine, isopilocarpine, and pilocarpic acid in drugs (10).

(2) Reject the method for aminacrine proposed for first action adoption because of the limitations found in the collaborative study.

(3) Continue study on all topics.

(4) Declare open any topic that has become inactive for an extended period (more than 2 years). New Associate Referees are needed for the following topics: Belladonna Alkaloids, Epinephrine-Lidocaine Combinations, Rauwolfia Alkaloids, Neostigmine, and Homatropine Methyl Bromide.

References

(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) Bunch, E. A. (1985) Abstract No. 249, 99th Annual AOAC International Meeting, Washington, DC
(4) Barkan, S. A., Schill, G., & Wainer, I. W. (1985) Abstract No. P-2, Pharmaceutical Analysis and Control Section, American Pharmaceutical Association Academy of Pharmaceutical Sciences, 39th National Meeting, Minneapolis, MN
(5) Thompson, R. D. (1985) *J. Assoc. Off. Anal. Chem.* **68,** 1051–1055
(6) Jhangiani, R. K., & Bello, A. C. (1984) *J. Assoc. Off. Anal. Chem.* **68,** 523–527
(7) Cieri, U. R. (1985) Abstract No. 245, 99th Annual AOAC International Meeting, Washington, DC
(8) Tymes, N. (1982) *J. Assoc. Off. Anal. Chem.* **65,** 132–137
(9) Smith, E. (1983) *J. Assoc. Off. Anal. Chem.* **66,** 339
(10) Van Ackeren, J. L., Venable, R. M., & Wainer, I. M. (1984) *J. Assoc. Off. Anal. Chem.* **67,** 924–926

Drugs III

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Ampicillin and Amoxicillin.—A liquid chromatographic (LC) procedure has been developed. Further study is recommended.

Bisacodyl.—A collaborative study of a reverse phase LC method (*J. Assoc. Off. Anal. Chem.* (1985) **68,** 529–532) has been completed. Results of this study are being evaluated.

Coumarin Anticoagulants.—A collaborative study of the method presented by the Associate Referee at the 1983 Annual International Meeting has been completed. The results of the study will be presented at the 1985 Annual International Meeting. The Associate Referee, Ella Moore of the New York Regional Laboratory, FDA, recommends that the method be adopted official first action. The General Referee concurs.

Flucytosine.—Associate Referee Donald Shostak (New York Regional Laboratory, FDA) has developed an LC procedure for this compound and a collaborative study is currently in process. A presentation of the method will be given at the 1985 Annual International Meeting.

Fluoride.—A method developed by Associate Referee John Marzilli (Boston District, FDA) was adopted official first action at the 1983 Annual International Meeting. The method has been used successfully over the past 2 years without any reported difficulties. The General Referee concurs with the recommendation of the Associate Referee that this method be adopted official final action.

Halogenated Hydroxyquinoline Drugs.—A collaborative study of the method described in *J. Pharm. Sci.* **73,** 1430–1433 (1984) has been completed. The results of this study will be presented at the 1985 Annual International Meeting. The Associate Referee, Edward J. Wojtowicz (Buffalo District Laboratory, FDA) recommends that the method be adopted official first action. The General Referee concurs.

Hydralazine.—A collaborative study of the method presented at the October 1984 meeting of the American Pharmaceutical Association is currently in process.

Insulin by Liquid Chromatography.—An improved method over the one submitted to the *Journal of Chromatography* was presented at the Pittsburgh Conference and will be scheduled for collaborative study in 1986.

Levodopa.—A collaborative study of the method presented at the 1984 Annual International Meeting has been completed. Results of this study are currently being evaluated. A presentation will be made at the 1985 Annual International Meeting by Associate Referee Susan Ting (New York Regional Laboratory, FDA) on the LC determination of levodopa and levodopa-carbidopa combinations in dosage forms.

Medicinal Gases.—Collaborators are being sought in order to perform a study of the method presented at the 1982 Annual International Meeting.

Mercury-Containing Drugs.—No new work was done on this topic.

Metals in Bulk Drug Powders.—A collaborative study of the method presented at the 1984 Annual International Meeting is anticipated in 1986.

Microchemical Tests.—The microcrystalline identification method for perphenazine, promethazine, thiethylperazine, and triflupromazine pure drug substances was adopted official first action, **36.A07–36.A10.** Associate Referee Marshall Rabkin (New York Regional Laboratory, FDA) is currently working on identification tests for commercial dosage forms of the 4 compounds described above and other pure drug substances.

Penicillins.—A collaborative study of the method presented at the 1984 Annual International Meeting is being prepared for issuance.

Protein Nitrogen Units in Allergenic Extracts.—Make editorial changes in the official final action method as recommended by the Associate Referee to specify the Kjeldahl procedure to be used. The General Referee concurs with this recommendation.

Salts of Organic Nitrogenous Bases.—An LC method developed by Associate Referee Samuel T. Walker (New York Regional Laboratory, FDA) has been published (*J. Assoc. Off. Anal. Chem.* (1985) **68,** 539–542). A collaborative study scheme is currently being developed.

Thyroid and Thyroxine Related Compounds.—The Associate Referee on this topic does not have time to continue this study. The General Referee recommends that this topic be discontinued.

Trimethobenzamide.—No additional work was performed on this topic.

Recommendations

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

(2) Adopt as official final action the first action method for fluoride, **36.063–36.069.**

(3) Adopt as official first action the method for halogenated hydroxyquinoline drugs.

(4) Amend official final action method **38.205–38.207** to specify use of the Kjeldahl method for determination of nitrogen as shown in *J. Assoc. Off. Anal. Chem.* (1981) **64,** 1436–1437.

(5) Discontinue the topic Thyroid and Thyroxine Related Compounds.

(6) Continue study on all other topics.

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Drugs and Related Topics, except recommendations 1 and 3, 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* (1984) 14th edition.

Drugs IV

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A new Associate Referee, Irving Wainer, has been appointed on the topic D- and L-Amphetamines—LC Separation. The previous Associate Referee, John L. Van Ackeren, was unable to continue work on this topic. A collaborative study is in progress.

The Associate Referee on Heroin, Harold F. Hanel, states that he plans no further work in this area. The topic should be declared open.

No reports were received on the topics Benzodiazepines and Diazepam.

Recommendations

(1) Declare as open the topic Heroin.

(2) Continue study on the topics Benzodiazepines and Diazepam.

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Drugs and Related Topics, except recommendation 1, and adopted by the Association. See the report of the committee, this issue.

Drugs V

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Betamethasone.—A method was developed and collaboratively studied. The collaborators' results are now being evaluated and will be reported in the near future.

Chlorpropamide.—A liquid chromatographic method was collaboratively studied and reported. Adoption as official first action is recommended.

Conjugated Estrogens, LC Methods.—A method was published (*J. Pharm. Sci.* (1985) **74,** 201–204) which appears to be quite promising. The Associate Reference hopes to conduct a collaborative study in the near future.

Prednisolone.—The study reported earlier (*J. Assoc. Off. Anal. Chem.* (1984) **67,** 674–676) showed very good agreement among collaborators. In preparing last year's report (*J. Assoc. Off. Anal. Chem.* (1985) **68,** 235), the Referee understood that all assay methods were to be accompanied by identification and purity tests and accordingly recommended non-approval of this apparently very good method. Subsequent action by the Association has shown the Referee to be in error, that identification and purity tests do not have to accompany assay methods. Thus, the Referee recommends adoption of this method as official first action and continued study on the topic.

Steroid Acetates.—The Associate Referee will report the results of the recent collaborative study at the 1985 Annual International Meeting. The preliminary results appear to be very satisfactory. The final report will be submitted soon.

Steroid Phosphates.—The Associate Referee has submitted the report of an elaborate collaborative study involving liquid chromatography to determine dexamethasone, thin layer chromatography to identify dexamethasone in elixirs, infrared identification of dexamethasone bulk, and a gas chromatographic method to determine alcohol in dexamethasone elixirs. Excellent results were obtained by the 7 participating laboratories. The Referee recommends adoption as official first action of all 4 parts of the procedure and heartily recommends Elaine Bunch for the Associate Referee of the Year Award.

Recommendations

(1) *Chlorpropamide.*—Adopt as official first action the proposed liquid chromatographic method. Continue study.

(2) *Prednisolone.*—Adopt as official first action the liquid chromatographic method for prednisolone in tablets and bulk drugs (*J. Assoc. Off. Anal. Chem.* (1984) **67,** 674–676). Appoint an Associate Referee.

(3) *Steroid Phosphates.*—Adopt as official first action the liquid chromatographic method for dexamethasone; the thin layer identification method for dexamethasone bulk and elixirs; the infrared method for identification of dexamethasone bulk; and the gas chromatographic method for determination of alcohol in dexamethasone elixirs.

(4) Continue study on all other topics.

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Drugs and Related Topics, except recommendation 3, and adopted by the Association. See the report of the committee and "Changes in Methods," this issue.

GENERAL REFEREE REPORTS: COMMITTEE ON FOODS I

Coffee and Tea

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The Associate Referee on Ash in Instant Tea, F. J. Farrell, as reported last year was waiting for results of a combined study of ash and moisture in instant tea. The results appear to be satisfactory but the report is not in its final form.

John Newton, Associate Referee on Caffeine in Coffee and Tea and on Theophylline in Tea, reported he did not have time to continue his planned work on these topics.

B. Denis Page, Referee for Solvent Residues in Coffee and Tea, had other priorities which kept him from further study of his headspace method for the determination of methylene chloride in decaffeinated tea and coffee (*J. Assoc. Off. Anal. Chem.* **67**, 757 (1984)). He reported, however, that he was able to do some work which resulted in a possible improvement in the method and hopes to proceed further during the coming year.

Mr E. de la Teja, Associate Referee on Water Extract in Tea, did not submit a report.

Recommendation

(1) *Ash in Instant Tea.*—Continue study.

(2) *Caffeine in Coffee and Tea.*—Incorporate under new topic, Methylxanthines.

(3) *Crude Fiber in Tea.*—Discontinue topic.

(4) *Moisture in Coffee and Tea.*—Continue study.

(5) *Solvent Residues in Decaffeinated Coffee and Tea.*—Continue study.

(6) *Theophylline in Tea.*—Incorporate under new topic, Methylxanthines.

(7) *Water Extract in Tea.*—Appoint Associate Referee; continue study.

(8) *Other Topic.*—Initiate new topic, Methylxanthines in Coffee and Tea.

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Foods I and adopted by the Association. See the report of the committee and "Changes in Methods," this issue.

Dairy Products

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The referee has received progress reports from several Associate Referees during the year. Based on the progress it is anticipated that several recommendations for official action will be made at next year's annual international meeting.

Based on Associate Referee reports and published literature, the Association will be asked to approve 4 methods this year. Associate Referee D. A. Biggs reported on the collaborative study of the infrared analysis of milk with the Infra-analyzer 400D at the 1983 meeting, The Committee did not recommend the new IR method at that time. The manuscript has been rewritten in accordance with instructions from the Committee and the General Referee recommends that the Association adopt as official first action the method for the determination of fat, total solids, and protein in milks by infrared analysis and include the indicated changes for use of the Infra-analyzer 400D.

The Associate Referee for Babcock tests and Babcock glassware, R. L. Bradley, has reported on a collaborative study with new glassware combining the basic Babcock test bulb with the Gerber-style calibrated neck. The name "Gercock" was coined since the new bottles are a combination of Babcock and Gerber bottles. Based on the results of the collaborative study, the Associate Referee recommends first action approval of the new 5% Gercock milkfat test bottle. The General Referee concurs with this recommendation.

The Associate Referee for reactivated phosphaste, G. K. Murthy, has presented a report on alkaline phosphatase in casein. Based on the results reported, the General Referee concurs with the recommendation that the Association adopt as official first action the rapid method of sample preparation for detecting alkaline phosphatase in casein.

A paper, "Sampling Barrel Cheese for Moisture Analysis Comparison of Methods," by Thomas M. Blattner et al. was published (*J. Assoc. Off. Anal. Chem.* **68**, 718–721 (1985)). On the basis of this published study, the General Referee recommends that the AOAC method for sampling of barrel cheese for moisture analysis, **16.015–16.016,** be revised to include the direct sampling directions described in the published paper.

Progress continues in the Joint IDF/ISO/AOAC working groups on methods of analysis for milk and milk products. Collaborative studies have been completed on several topics and manuscripts are in preparation. It is anticipated that several recommendations will be received by the Association during the forthcoming year.

Recommendations

(1) Adopt as official first action the new 5% Gercock milkfat test bottle as described by the Associate Referee.

(2) Adopt as official first action the method for the determination of fat, total solids, and protein in milk by infrared analysis and include indicated changes to provide for the use of Infra-analyzer 400D.

(3) Adopt as official first action the rapid method of sample preparation for detecting alkaline phosphatase in casein.

(4) Adopt as official first action the method for sampling barrel cheese for moisture analysis.

(5) Continue study on all other topics.

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Foods I and adopted by the Association, except adoption of the Infra-analyzer 400D for milk analysis. See the report of the committee and "Changes in Methods," this issue.

Decomposition and Filth in Foods (Chemical Methods)

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Ethanol in Seafoods.—Associate Referee H. R. Throm reported that a collaborative study on the determination of ethanol in canned salmon by gas chromatography has been completed. The procedure (*J. Food Sci.* **48**(i), 290–291 (1983)) uses headspace sampling to isolate the alcohol. The study, conducted by Throm and Hollingworth of FDA's Seafood Products Research Center, consisted of the analyses of 5 juice samples by 6 participating laboratories and covered ethanol concentrations from 0 to 100 ppm. For all samples, the coefficients of variation (repeatability) ranged from 1.4 to 10.6% and CV (reproducibility) ranged from 2.6 to 10.6%. At a level of 25.1 ppm, the collaborators recovered an average of 113.2% of added ethanol (range 109.6–120.5); at a level of 78.4 ppm, recoveries averaged 110.0% (range 106.9–113.5%). Although the ethanol and internal standard peaks eluted within 15 min, the usual time of analysis was 38 min because of late-eluting peaks. The General Referee concurs with the recommendation of the Associate Referee that the method be adopted official first action.

Gas and Liquid Chromatography.—The analyses on an authentic pack of mahimahi for histamine, cadaverine, and putrescine were completed. The samples were extracted with 75% methanol, histamine was determined by AOAC **18.067–18.071,** and diamines were determined by a gas chromatographic procedure (*J. Assoc. Off. Anal. Chem.* **64,** 584–591 (1981)) with the assistance of J. Gilchrist and C. Hamilton of the FDA Cincinnati Research Laboratory. Acceptable quality fillets contained <1 mg histamine/100 g, <5 μg cadaverine/g, and <5 μg putrescine/g. Fish decomposed at 32, 50, 70, and 90°F all contained increased amine levels. Cadaverine was the most significant compound found at lower temperatures while both histamine and cadaverine were usually found at high levels in samples spoiled at 70 and 90°F. The maximum values of the compounds found were 429 mg histamine/100 g, 469 μg cadaverine/g, and 195 μg putrescine/g. The methods performed well at all levels of decomposition. The data will be submitted for statistical analysis.

A comparison of the extraction efficiency of 100% methanol and 75% methanol for the determination of histamine, cadaverine, and putrescine was obtained for oil- and water-packed samples of skipjack and yellowfin tuna. At levels of importance for the differentiation of passable and decomposed tuna, both extraction solvents yielded the same results. Only a slight difference could be noted at high decomposition levels.

Procedures for the 3 amines were also tested on samples of commercially canned anchovies, jack mackerel, sardines, mackerel, herring, and pacific mackerel. Recovery of the amines added to the samples averaged 97% for histamine, 110% for putrescine, and 105% for cadaverine. The background values for the samples were uniformly low except for a sample of anchovies that contained 6 mg histamine/100 g, 63 μg cadaverine/g, and 20 μg putrescine/g. Two samples of a canned cheese topping intended for use in manufacturing pizza were also examined and found to contain up to 156 mg histamine/100 g, 1021 μg cadaverine/g, and 1106 μg putrescine/g.

Experiments to evaluate the frequency of occurrence of putrescine as a function of decomposition and the formation of indole in shrimp were also conducted with the assistance of the FDA Cincinnati Research Laboratory. A modified extraction solvent containing 75% methanol, 25% 0.1N HCl was used to isolate the amine, followed by GC quantitation. The procedure was applied to 14 samples of shrimp determined to be decomposed by sensory examination. In 13 samples, significant increases in putrescine were found with a range of 6–116 μg/g (average 39 μg/g). In 10 samples, indole increased with a range of 7–35 μg/100 g (av. 18 μg/100 g). Research is continuing to determine the final form of the procedure.

Because of the frequent appearance of the 3 amines (histamine, cadaverine, and putrescine) in decomposed foods, it would be useful to determine them in a single procedure. An attempt to apply a published procedure (*J. Assoc. Off. Anal. Chem.* **67,** 1040–1043 (1984)) using LC with a cation exchange column, post-column OPA reactor, and fluorescence detector was not successful. The published separation could not be reproduced and it was necessary to change the mobile phase system. Current separations currently require 15 min to complete and experiments are continuing to identify all parameters which will require control for routine analyses. The Referee recommends continued study.

Although there were no reports on the topics Ammonia in Dogfish, Coprostanol, Crabmeat, Diacetyl in Citrus Products, GC Determination of Volatile Amines—TMA and DMA, TLC Determination of Amines in Fishery Products, and Tomatoes, the General Referee recommends that they be continued.

Recommendations

(1) Adopt as official first action the GC determination of ethanol in canned salmon.

(2) Appoint an Associate Referee to the topic Shellfish; continue study.

(3) Continue study on all other topics.

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Foods I and adopted by the Association. See the report of the committee and "Changes in Methods," this issue.

Fish and Other Marine Products

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Determination of Fish Content in Coated Products (Breaded or in Batter).—Associate Referee Frederick J. King has had some communications with H. Houwing, Chairman of the West European Fisheries Technologists Association's Working Group for Analytical Methods. Three physical methods were thoroughly discussed by the Working Group during the West European Fish Technologists' Association meeting held in March 1984. The Working Group indicated last year that it would recommend the one method which has the widest applicability and the most promise. No recommendation has as yet been received. The General Referee recommends continued study.

Fish Species Identification.—Associate Referee Ronald C. Lundstrom was not able to conduct the planned collaborative study this year. Since prepared agarose-IEF gels, similar to the prepared polyacrylamide-IEF gels used in the current AOAC method, have recently become available, the Associate Referee wants to evaluate these prepared gels. If he concludes that the results are reproducible and that these commercially prepared gels work well, he will use them for this collaborative study. The General Referee concurs with this approach and recommends continued study.

Minced Fish in Fish Fillet Blocks.—Associate Referee J. Perry Lane conducted the collaborative study recommended last year using 3 levels of minced fish in fish blocks. The minced fish added was 12.50, 18.75, and 26.25%, respectively. The overall means at these 3 levels were determined to be 10.2, 17.0, and 26.0%. The standard deviations ranged from 1.51 to 1.77. The Associate Referee is preparing a manuscript which he will submit to the Association that will include appropriate recommendations. The General Referee recommends continued study.

Nitrites in Smoked Fish.—The General Referee recommends that an Associate Referee be appointed to conduct a collaborative study using the method developed by the previous Associate Referee.

Organometallics in Fish.—Associate Referee Walter Holak has tested the improved design of the interface connecting the liquid chromatograph with the atomic absorption spectrophotometer and found it to perform satisfactorily. He had planned to conduct an intralaboratory trial before initiating a collaborative study. However, his attention was temporarily diverted to a project having a higher priority. This study will be resumed shortly. The General Referee recommends continued study.

Other Topics.—Last year a recommendation was made to discontinue the topic "Drained Weight of Block Frozen, Raw, Peeled Shrimp." Recently, the General Referee has received correspondence indicating a renewed interest in this topic, particularly by the American Shrimp Processors Association. Their technical consultant has requested appointment as an Associate Referee to develop an improved method for the determination of drained weight for block frozen, raw, peeled shrimp. The General Referee recommends that this topic be reactivated and that the Associate Referee be promptly appointed.

Recommendations

(1) Reactivate the topic, Drained Weight of Block Frozen, Raw, Peeled Shrimp and appoint an Associate Referee.

(2) Continue work on all other topics.

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Foods I and adopted by the Association. See the report of the committee and "Changes in Methods," this issue.

Food Additives

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Antioxidants.—Work has continued on determining the fate of antioxidants during the process of deep fat frying of foods. A manuscript on the "Fate of Antioxidants in Deep-Fat Frying and Cookie Baking" has been published.

Denis Page has developed 2 new methods that use the basic LC determinative step of the official method for propyl gallate, trihydroxybutyrophenone, TBHQ, NDGA, BHA, Ionox-100, and BHT in fats and oils and has applied it to a number of dry food matrices. Potato and corn chips, popcorn and cheese snacks, breakfast cereals, dry beverage mixes, rice, potato flakes, French fries, and cake mixes were some of the 75 foods analyzed. Over 70% of these samples contained detectable amounts (1–2 ppm) of antioxidants, mainly BHA and BHT. More recently this same LC method was adapted to determine the aforementioned antioxidants in mayonnaise and salad dressings.

Brominated Vegetable Oils.—Two papers from the Associate Referee's laboratory have been published on the metabolism and mammary transfer of BVOs in the rat (*Lipida* **19,** 637 (1984); **19,** 704 (1984)). Methodology studies using LC and capillary GC have been completed.

LC in both normal and reverse phase modes is useful for characterizing BVOs. Seven brominated oil preparations (olive, sesame, corn, cottonseed, soybean, and 2 partially hydrogenated soybean oils) were examined by reverse phase chromatography using a C-18 column and a mobile phase of acetonitrile–water (95 + 5), and by normal phase chromatography using a silica column with a mobile phase of hexane–isopropanol (500 + 1). Both systems completely resolved the di-, tetra-, and hexabrominated methyl stearates. The composition of the brominated fatty acids in the oils compared well with previous packed-column GC, with the added advantage that the hexabromo analog could be reproducibly quantitated by the LC methods.

Capillary GC using a Durabond fused silica column also proved successful in determining the brominated fatty acid content of the 7 oils. The reproducibility of the analyses especially for the hexabromo stearate was much improved over the packed-column GC method. Analysis of an orange-flavored soft drink compared well with the packed-column method.

It was found that the partially hydrogenated soybean oils produced different brominated fatty acid patterns depending on the degree of hydrogenation, as would be expected.

Chloride Titrator.—No report was received from the Associate Referee.

Dressings.—Work on this project has been placed in abeyance until priorities change.

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Foods I and adopted by the Association, except adoption of nitrosamines in baby bottle rubber nipples. See the report of the Committee and "Changes in Methods," this issue.

EDTA in Foods.—The Associate Referee reports no progress because of higher priority commitments.

Gums.—The General Referee has been unsuccessful in finding an Associate Referee.

Indirect Additives from Food Packaging.—The Associate Referee reports the following progress during the past year.

New FDA Migration Cell: A manuscript, "New FDA Migration Cell Used to Study Migration of Styrene from Polystyrene into Various Solvents," was published in the July/August 1985 issue of *J. Assoc. Off. Anal. Chem.*

The new cell continues to be used to study migration. For example, the migration cell was used to evaluate ethanol/water solutions as an alternative fatty food simulant for impact polystyrene. The results suggest that 50% ethanol/water would be suitable as a fatty food simulant for both crystal and impact polystyrene. A different polymer/adjuvant system will be selected for future migration studies.

The results of the recent ASTM committee ballot on the proposed standard method for the use of this cell have been tabulated. The vote was in favor of adopting the method and cell with few editorial comments.

A poster session was presented at the Pittsburgh Conference in New Orleans entitled "Graphics Assisted Iterative Basic Program for Calculating Migrant/Polymer Diffusion Coefficients." This presentation described some of the computer techniques developed in our laboratory for studying polymer migration.

Vinyl Chloride (VC): The Society of Plastics Industry (SPI)-sponsored evaluation of a method to determine VC in corn oil and 50% ethanol/water at the 1–10 ppb levels was completed. A final draft of the VCM analytical method was prepared and distributed for comment. Following the comment period the draft was to be submitted to SPI for further actions.

The quality control method to determine residual VC in PVC at the 5 pbb level has been adopted by ASTM and published in their latest book of methods as Method D4443.

Butadiene (BD): FDA has developed a method suitable for the determination of BD in a number of impact-modified food packaging materials. A survey of a number of these products has indicated that most contain little or no detectable BD at a level of 1 ppb. Only one package has been found to contain a relatively high BD level; the package was an olive oil container and the migrating BD in the contained olive oil was determined to be 8 ppb. No other packaged foods were found to contain migrating BD.

A poster session was presented at the Pittsburgh Conference in New Orleans entitled "Determination of Residual 1,3-Butadiene in Rubber Modified Plastics and Its Migration from Plastic Containers into Selected Foods." This paper described the methodology developed in our laboratory for screening various plastic articles for butadiene residues.

Antioxidant Decomposition Products: The stability of the commonly used plastic antioxidants BHT and Irganox 1010 is being studied in aqueous ethanol solutions. In aqueous solution these materials degrade rapidly. The principal degradation products of BHT thus far identified are 3,5-di-*tert*-butyl-4-hydroxybenzylalcohol (BHT-CH_2OH), 2,6-di-*tert*-butyl-4-methyl-4-hydroperoxy-2,5-cyclohexadiene-1-one (BHT-OOH), 3,5-di-*tert*-butyl-4-hydroxy-benzaldehyde (BHT-CHO). An additional degradation product whose existence is suspected but lacks confirmation is 2,6-di-*tert*-butyl-4-methyl-4-hydroxy-2,5-cyclohexadiene-1-one (BHT-OH). Two minor liquid chromatographic peaks contain degradation products still to be identified. Additional samples are being prepared for FTIR studies.

Four major decomposition products have been observed in aqueous/ethanol (1 + 1) solutions of Irganox 1010® stored at 25, 50, and 90°C. Liquid chromatographic studies are under way to resolve and quantitate these components. Samples of the various Irganox decomposition products are being prepared in large enough quantities by LC to permit structure studies by NMR, MS, and FTIR.

Champion Single-Sided Extraction Cell: Champion International Corporation loaned FDA a prototype of a single-sided extraction cell that is being considered for possible marketing. This cell would be a potential replacement for the Maturi cell that is no longer commercially available. A short study of the cell demonstrated that it would be suitable for the determination of total nonvolatiles; however, it does not appear satisfactory for the determination of volatiles. No additional evaluation of the cell is planned.

Packaging-Derived Volatiles: A poster session was presented at the 1985 Pittsburgh Conference on Analytical Chemistry and Spectroscopy in New Orleans entitled, "A Headspace Capillary Gas Chromatographic Semi-Quantitative Screening Method for Volatiles in Aqueous Foods." It describes the semi-quantitative screening procedure developed for taste and odor problems due to volatiles derived from packaging. The method is based on the use of manual headspace techniques, standard addition calibration, and computer-generated linear regression curves. Since the poster submission the method has been tested on automated equipment and shown to be reliably quantitative due to greatly reduced data scatter.

Food Packaging Identity: A survey of 1600 food packages has been completed at FDA. The data base is being computerized for easier future access. Infrared spectroscopy was the principal technique used.

Nitrates (Selective Ion Electrodes).—The Associate Referee reports that the nitrate electrode is widely being used in the food and agricultural industry, especially for vegetables. A second collaborative study of an improved method using an ionic strength adjustor (ISA) is under way.

Nitrates and Nitrites.—The Associate Referee has not reported the results of the collaborative study initiated during the past year.

Nitrosamines in Foods.—During the past year Associate Referee N. P. Sen carried out research on various aspects of the nitrosamine problem. Although most of the work concentrated on the development of analytical methods, information was also obtained on the mechanism of formation of these compounds and their occurrences in foods. A summary of these investigations is presented below:

(1) *Methodology for Nonvolatile Nitrosamino Acids:* A method has been developed for the analysis of NPRO and NSAR in various foods such as malt, beer, fish, bacon, and cured meats. It is based on extraction of the sample with methanol, cleanup on commercially available extraction tubes, and preparation of methyl esters by treatment with BF_3–methanol. The final analysis is carried out by GC-TEA. The average percentage recoveries of both compounds from various spiked foods were found to be excellent (85–90%). The details have been published in *IARC Sci. Pub. No. 57,* 137 (1984).

The above method has been modified to make it amenable to the analysis of *N*-nitrosothiazolidine-4-carboxylic acid (NTCA), which is unstable in BF_3–methanol. In the modified method, the cleanup step using an extraction tube was replaced by extraction in a separatory funnel, and diazomethane (instead of BF_3–methanol) was used for esterification. The esterified food extracts were further cleaned up by using a silica gel Sep-Pak cartridge before analysis of GC-TEA. The method has been used for the analysis of a wide variety of meat and fish products (*Food Technol.* **39,** 84 (1985)).

(2) *Determination of NThZ in Foods:* A low temperature vacuum distillation from 3N KOH solution was used for the isolation and cleanup of NThZ from various foods. The method has several distinct advantages. First, the technique is applicable to a wide variety of foods (e.g., fish and seafoods, liquid smoke, both raw and fried bacon, cooked-out bacon fat, other cured meats), and second, the same extract can be used for simultaneous analysis of other volatile nitrosamines by GC-TEA or by GC-MS (after minor cleanup on basic alumina). Moreover, the method has been demonstrated to be free of

artifactual formation even in the presence of large amounts of added NThZ precursors including nitrite.

(3) *Formation of NThZ:* Our studies suggest that at least a part of the NThZ found in fried bacon originates from heat-induced decarboxylation of NTCA present in raw bacon. This was demonstrated by spiking raw bacon with NTCA and frying it under normal pan frying conditions. The yield of NThZ from NTCA, however, was very low: 1.5–3.1%. A positive correlation was also found between NTCA levels in raw bacons and NThZ levels in the fried products.

Preliminary data indicate that bacons processed by old-fashioned smoking methods contain extremely high (>1000 ppb) levels of NTCA and hence produce high (up to 654 ppb) levels of NThZ upon frying. Bacons processed with liquid smoke usually contain lower levels of both NTCA and NThZ. It is possible that higher levels of HCHO, a necessary precursor for the formation of thiazolidine ring, present in the old-fashioned smokes might be responsible for this difference.

Most fish and seafoods, including the smoked varieties, gave negative results for NThZ; only three contained traces (≈ 1 ppb). Lack of both nitrite and cysteamine were thought to be responsible for the absence of significant level of NThZ in smoked fish.

A paper has been submitted for publication in *J. Food Sci.*

(4) *Origin of NMOR in Margarine:* Previous studies from this laboratory indicated the presence of traces of NMOR in some margarines but the source of the contamination was not known. Our recent data indicate that waxed paper wrapping might be the source of NMOR. In a few samples, both the paper wrapping and margarine taken from the outer 1 cm layer of the margarine block contained NMOR, but none could be detected in margarine taken from the center of the block. This suggested migration of NMOR from wrappings to the outer layers of the margarine blocks. Samples packaged in aluminum-backed papers, specifically coated waxed papers, or plastic containers were negative.

(5) *Studies on Nitrosamine Formation in Extracts of Rubber Nipples and Pacifiers Under Simulated Gastric Conditions:* Studies with some older nipples and pacifiers indicated that significant amounts of both nitrosamines and nitrosatable amines could migrate from these products into artificial saliva. In the absence of any food, considerable amounts of additional nitrosamines were formed when such extracts (saliva and nipple) were acidified and incubated for a brief period at room temperature. However, various liquid infant foods such as orange juice, apple juice, and both milk-based and soya-based infant formulas significantly inhibited such formation. The presence of ascorbic acid, ascorbyl palmitate, and possible phenolic compounds in these foods were thought to be responsible for this inhibitory action. It was concluded that the danger of formation of nitrosamines in the babies' stomachs from ingested amines (from nipples and pacifiers) might not be as great as thought previously.

(6) *Proposed AOAC Collaborative Study on Nonvolatile Nitrosamino Acids:* At the AOAC Ad Hoc Committee meeting on nitrosamines held on June 10, 1985, Atlanta, GA, a collaborative study was proposed on the above subject. Drs Hotchkiss and Gray both supported the idea.

Since NSAR, NPRO, and NTCA have been reported to occur in cured meats it would be desirable to include all 3 compounds in the proposed study. It was agreed that smoked meats or other cured meats (e.g., fried bacon) should be chosen as the substrate.

Nitrosamines in Food Contact Items.—Associate Referee J. I. Gray has successfully completed the collaborative study, "Volatile *N*-Nitrosamines in Baby Bottle Rubber Nipples." From the statistical analysis it was concluded that the analytical method was reproducible and would support the 10 ppb *N*-nitrosamine action level for baby bottle rubber nipples. Reproducibility values were generally between 35 and 45% for *N*-nitrosamine levels ranging from 10 to 20 ppb. Lower reproducibility (higher precision) was obtained upon statistical analysis of the data from only 4 laboratories selected a priori because they were considered to be more experienced with the method. The reproducibility was consistent with that of other analytical techniques designed to measure low ppb levels of trace contaminants, for example, mycotoxins in foods (Horwitz levels).

The General Referee has recommended that the method be adopted official interim first action and published in the AOAC Journal. The study was reviewed by the AOAC Official Methods Board and accepted, and the method was resubmitted for immediate publication after some organizational changes.

Polycyclic Aromatic Hydrocarbons in Foods.—The Associate Referee reports that work on the LC method for the determination of NPAH in smoked foods has been completed. A manuscript detailing this work has been submitted to AOAC for publication.

Briefly, imported and domestic smoked foods were analyzed for basic nitrogen-containing polynuclear aromatic hydrocarbons (NPAH) content by a rapid LC technique. Nanogram quantities of NPAH standards were detected by UV and fluorescence detectors connected in series. Recoveries of 3 NPAHs (5,7-dimethylbenz*(a)*acridine, dibenz*(a,j)*acridine, and dibenz*(a,h)*acridine) each added to smoked salmon and sausage at the 5 ppb level ranged from 62 to 101% by fluorescence measurement and from 64 to 104% by UV measurement.

The imported foods chosen for study were based on investigations conducted by Grimmer in 1980 to establish gas chromatographic profiles for NPAH in high protein foods, including smoked meat, ham, fish, poultry, etc. According to his data, several NPAH were detected, including the aforementioned. Based on our studies with similar foods, no NPAHs were observed; therefore, questions must be raised regarding the identity of the peaks Grimmer reported as NPAH.

Additionally, a survey was conducted of some "fast-food" hamburgers for PAH content. The following abstract has been submitted to AOAC:

"The possible presence of PAH in hamburgers purchased from a variety of 'fast-food' restaurants has been examined. Samples from different types of restaurants were analyzed for PAH by a digestion/extraction/LC method previously developed. LC-scanning UV was used for tentative confirmation of PAH. PAHs were found in samples prepared by flame broiling, but levels varied from location to location, from no PAH detected to 5 μg/kg BaP in meat and rolls combined."

The results of this investigation are currently being compiled and will be ready for publication by the end of September.

Polysorbates.—The Associate Referee has made no progress this year because of higher priority commitments.

Quinine in Soft Drinks.—Associate Referee Leonard Valenti has not completed his current investigations and will report on his work next year.

Recommendations

(1) Appoint an Associate Referee on Sulfiting Agents in Foods as recommended to the AOAC Official Methods Board; appoint Associate Referees on Anticaking Agents; Dilaurylthiodipropionate; Gums; and Propylene Chlorohydrin.

(2) Continue study on all other topics.

(3) Adopt official first action the method for volatile *N*-nitrosamines in baby bottle rubber nipples.

Meat, Poultry, and Meat Poultry Products

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During the past year reorganization, reassignment, and retirement have taken a toll on the Associate Referees under this General Refereeship. Wherever possible, new appointments have been made, but 5 topics remain vacant: Chemical Antibiotics Methods, Bioassay Methods for Meat and Poultry Products, Steroid Analysis, Nitrosamines in Bacon, and Nitrates and Nitrites. The possible illicit and subtherapeutic uses of antibiotics in animals keep this subject in the limelight as does the use of steroids. U.S. Department of Agriculture (USDA) contracts over the past year have resulted in promising tests which could be studied collaboratively. The current methods for nitrite and nitrate need to be examined for ways to improve their repeatability and sensitivity, and newly developed procedures need to be studied. Bioassays are conspicuous for their absence in meat analysis. With reported new techniques, this topic is ripe for investigation. These Associate Referee positions need to be filled as soon as possible. Current final and first action methods for volatile nitrosamines and nitrosopyrrolidine in bacon are sufficient. It is recommended that this refereeship be filled with the emphasis on nonvolatile nitrosamine analysis.

The previously vacant referee position for Serological Identification of Animal and Poultry Species was filled as was the one for a related topic involving rapid tests for identification of beef and poultry. The topic, Protein in Meat, was terminated, as such, and is currently under 2 referees, one to study the possible use of copper catalyst in lieu of mercury for meat analysis and one investigating a peroxymonosulfuric acid digestion and nesslerization procedure as a rapid alternative to Kjeldahl determinations.

The General Referee was approached by both regulatory agencies and the industry to appoint an Associate Referee to collaboratively study an atomic absorption procedure for arsenic in animal tissues and meat and poultry to supplement the official final action spectrophotometric and molybdenum blue procedures, **41.009–41.012** and **25.050–25.055,** respectively. Because this topic is more appropriate for Metals and Other Elements at Trace Levels in Foods, the new Associate Referee, Randy Simpson, was recommended for appointment by General Referee Kenneth Boyer.

To clarify method **24.010,** Salt (Chlorine and Sodium Chloride) in Meat, the final sentence should be changed from "Det. as in **18.035.**" to "Det. as in **18.035,** beginning 'and titr. with. . . '. " The current wording leads the analyst to the beginning of **18.035** which requires the addition of 0.1N $AgNO_3$, HNO_3, boiling, etc. This is not the intent, as these analogous steps have already been completed previously in **24.010.**

Because of the ever-increasing numbers of samples requiring chemical analysis in U.S. Department of Agriculture laboratories and the requirement to look for more kinds of residues, additives, and components of meat and poultry, the Food Safety and Inspection Service (FSIS) is studying the use of many types of rapid tests for regulatory purposes. Technologies involving bioassays, radio and enzyme immunoassays, ELISA techniques, etc., are being developed within the Department and under contract. Many of these tests are developing as test kits which can be used either in a laboratory setting or in a meat packing plant or slaughter house. Some tests may even be able to be used on the farm to enable the grower to determine if animals have been withdrawn from medication long enough to eliminate residue levels in tissues or to reduce them to acceptable levels. An example of this kind of test is the Sulfa On-Site (SOS) test developed by FSIS for use as an in-plant analytical procedure, as stated in the May 20, 1985, *Federal Register* as the Proposed Rule, Sulfonamide Residues in Swine (Docket No. 84-014).

AOAC has established an ad hoc Committee on Biological and Pass/Fail Tests. This committee will be recommending new protocols for collaboratively studying these kinds of tests to obtain official action status. The Association has also appointed a General Referee for Test Kits. These two actions will enable the kinds of rapid tests needed by the industry and the regulatory agencies to be recognized. General Referees will be actively seeking Associate Referees to study these new analyses as they are developed.

It is also of interest to note that a Joint FAO/WHO Expert Consultation on Residues of Veterinary Drugs in Foods met in Rome in November 1984, and published in its report a listing identifying groups of compounds on certain specific examples which are of immediate concern as a world-wide problem. Analytical procedures will need to be developed and evaluated for antibiotics (specifically chloramphenicol), anabolics, sulfonamides (e.g., sulfamethazine), nitrofurans, nitroimidazoles, synthetic dyes used as marker compounds in therapeutic agents, tranquilizers and beta-adrenogenic blocking agents, carbadox, and cyromazine.

Automated Methods.—Associate Referee Jon Schermerhorn reports that the manuscript on the collaborative study on the use of the Technicon AA-2 AutoAnalyzer method for total protein is still being prepared. No other activity has been initiated.

Crude Protein Analysis of Meat (Peroxymonosulfuric Acid Digestion and Improved Nesslerization).—Associate Referee Dave Christians is planning a collaborative study for this technique utilizing the Hach Digesdahl® apparatus. This study will begin shortly.

Fat and Moisture Analysis, Rapid Methods.—Associate Referee Julio Pettinati is preparing a report on the collaborative study he conducted on the use of microwave ovens for rapid analysis of fat and moisture in meat. Twenty-two laboratories participated in the study, using 6 domestic ovens and 3 different laboratory models. The USDA, Agriculture Research Service has terminated this project.

Fat in Meat Products.—The Associate Referee reports that the revision to method **24.005,** Fat (Crude) or Ether Extract in Meat, regarding the drying to constant weight of the final ether extract, has apparently solved user problems. It is recommended that this Associate Refereeship be terminated and that future studies be directed toward rapid fat analysis procedures under the Associate Referee for that topic.

LC Methods for Meat Products.—Associate Referee M. Sher Ali is preparing a 3-laboratory validation study on the method previously published (*J. Assoc. Off. Anal. Chem.* **68,** 488–492 (1985)) simultaneously determining benzoic acid, sorbic acid, and 4 parabens in meat. He is also preparing a manuscript for the determination of sugars and sugar alcohols in meat and meat products by LC.

3-Methylhistidine.—Associate Referee Roger Wood is at present conducting a collaborative study for 3-methylhistidine in meat and meat products. He expects to have received collaborator reports concurrent with the 1985 annual AOAC meeting, on their analytical findings on pure solutions and model meat mixtures.

Minimum Processing Temperature.—Associate Referee James Eye reports that he is investigating the use of clinical kits that determine the presence or absence of specific enzymes, using their colored hydrozones for detection. Work which is being performed at both the USDA, Agriculture Research Service in Athens, GA, and Texas A&M University indicates

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This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Foods I and adopted by the Association, except the adoption of the method for soya protein in meat products. See the report of the committee and "Changes in Methods," this issue.

the possibility of the enzymes leucine amino-peptidase and pyruvate kinase being used as indicators of minimum processing temperatures in processed pork products.

Mr. Eye has developed a rapid test for the determination of catalase which gives a pass/fail indication at a cooking temperature of 145°F for roast beef and also detects the presence of organisms causing "flat sours" in canned meat products. The USDA Food Safety and Inspection Service has published a booklet describing this test.

Nonmeat Proteins in Meat.—Associate Referee Christopher Hitchcock has conducted 2 collaborative studies for an ELISA method for the quantitative analysis of soya protein in meat products. The results from these studies have been published (*J. Assoc. Publ. Analysts* **22,** 59–78 (1984) and *J. Sci. Food Agric.* **36,** 499–507 (1985)). Professor Hitchcock has recommended that this procedure be adopted official first action. This recommendation is under review by Committee on Foods I.

Protein in Meat and Meat Products—Mercury vs Copper Catalysts.—Associate Referee Carolyn Henry reports that she has conducted preliminary investigations into the different copper catalyst formulations that are commercially available for various forms of the Kjeldahl nitrogen determination. She has also begun a brief preliminary study to determine if it is necessary to digest meat samples for longer periods of time with copper than with mercury catalyst. Some past studies have shown that copper gives statistically low biased results for meat, but it is felt that this can be corrected. After these preliminary tests, a collaborative study is anticipated.

Serological Identification of Animal and Poultry Species.—Associate Referee Arthur Marin reports that he has been in contact with the Associate Referee for the ORBIT and PROFIT tests (see below). These tests involve serological principles and will be studied as a separate topic to expedite the process.

Species Identification by ORBIT and PROFIT.—ORBIT and PROFIT stand for Overnight Rapid Beef Identification Test and Poultry Rapid Overnight Field Identification Test, respectively. Associate Referee Mark Cutrefelli has completed the statistical design, with USDA and AOAC statisticians, for a collaborative study on these tests. He is preparing practice samples for collaborators and will be sending study samples after the practice phase. The study should be completed in a short period of time. A report with recommendations will be submitted for publication. If the study is successful, this refereeship will be terminated.

Specific Ion Electrode Applications.—Associate Referee Randy Simpson completed a collaborative study on the NOVA Biomedical Ion Analyzer for total sodium and total potassium content of meat, poultry, and meat and poultry products. He will shortly submit 2 manuscripts for publication, one containing the methodology and one covering the collaborative study. He is also preparing a paper on the analysis of total fluoride in meat by a specific ion electrode procedure. Other ion electrode procedures will be studied as time and workload permits.

Sugar and Sugar Alcohols.—It is recommended that this topic be combined with the topic LC Methods for Meat Products under the Associate Refereeship of M. Sher Ali. Dr. Ali has developed an LC procedure and is preparing a manuscript for publication.

The following topics are vacant: Chemical Antibiotic Methods, Bioassay Methods for Meat and Poultry Products, Steroid Analysis, Nitrosamines in Bacon, Nitrates and Nitrites; no report was received on Histological Identification Methods.

Recommendations

(1) Change the last sentence in **24.010** to "Det. as in **18.035,** beginning 'titr. with. . .'."

(2) Complete evaluations and submit manuscripts for collaborative studies on microwave oven procedures for moisture and fat in meat, the procedure and collaborative study for total sodium and potassium content of meat, poultry, and meat and poultry products by the NOVA biomedical ion analyzer, the method for total fluoride by specific ion electrode, the Technicon AA-2 AutoAnalyzer method and collaborative study for total protein in meat, the LC sugar and sugar alcohol method, and the 3-methylhistidine collaborative study.

(3) Adopt as official first action the ELISA method for quantitative analysis of soya protein in meat products, with all performance criteria stated, to enable the user to determine if the procedure meets his/her requirements for accuracy, repeatability, and reproducibility. Rewrite the methodology in the format for official methods.

(4) Combine the topics Sugar and Sugar Alcohols and LC Methods for Meat Products, keeping the latter title and a single Associate Referee.

(5) Discontinue the topic Fat in Meat, by placing work for this topic under Fat and Moisture Analysis, Rapid Methods.

(6) Appoint Associate Referees for Chemical Antibiotic Methods, Bioassay Methods for Meat and Poultry Products, Steroid Analysis, Nitrosamines in Bacon (with emphasis on nonvolatile nitrosamines), and Nitrates and Nitrites.

(7) Continue study on all other topics.

Mycotoxins

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In his keynote address at the 6th International IUPAC Symposium on Mycotoxins and Phycotoxins, Pretoria, South Africa, July 22–25, 1985, C. W. Hesseltine discussed the global significance of mycotoxins (1). His poll of scientists from 30 countries revealed that the most needed research on mycotoxins is on rapid and improved methods for their analysis. The mycotoxin methods proposed this year for adoption by AOAC following completion of collaborative studies meet these criteria. Study of the literature on mycotoxins in general shows that research on *Fusarium* and its toxins is on the rise (2) and the IUPAC Symposium was complemented this year by a Gordon Research Conference on Trichothecenes and Other *Fusarium* Mycotoxins, held in New London, NH, June 17–21, 1985. As background reading on *Fusarium* problems, an important new book, *Toxigenic Fusarium Species. Identity and Mycotoxicology* (1984) by W. F. O. Marasas, P. E. Nelson, and T. A. Toussoun, has recently been published (3). It is apparent that consideration will soon have to be given to establishing an Associate Refereeship on other *Fusarium* mycotoxins (with the exception of trichothecenes and zearalenone), particularly if the carcinogen(s) formed by *F. moniliforme* and the toxin(s) responsible for equine leukoencephalomalacia (LEM), also produced by *F. moniliforme*, are identified in the near future. Another important new book, of course, is the 14th edition of *Official Methods of Analysis of the Association of Official Analytical Chemists* (1984) (4). Chapter 26, "Natural Poisons" is not being published separately this time, but apart from some editorial changes, addition of the water slurry method of sample preparation in the BF method for aflatoxins in peanuts and peanut products, and inclusion of methods for determination and confirmation of aflatoxins B_1 and M_1 in liver, the 1980 edition of Chapter 26 (5) is basically unaltered in the new book of methods.

Two new Associate Refereeships are proposed. The mycotoxin emodin is widely distributed among fungal genera, including *Aspergillus* and *Penicillium*, and together with several related hydroxyanthraquinones is mutagenic in the Ames test (6). These compounds also occur in plant drugs (as glycosides). Methods for determination of emodin in feeds (7) and fungal enzyme preparations (6) have been described recently. The topic would be entitled Emodin and Related Anthraquinones. The other proposed topic is on *Penicillium islandicum* toxins. These include the carcinogenic mycotoxins luteoskyrin and cyclochlorotine for which some methodology has already been developed (8–11). Some overlap exists between the 2 topics because emodin is also a metabolite of *P. islandicum*. Persons interested in developing these topics as Associate Referees are invited to contact the General Referee.

The rapid pace of development in the field of mycotoxins is illustrated by the large number of references included in this report, which covers a time period of only 1 year (approximately), is focused primarily on method development, and covers only the more important mycotoxins that are the Associate Referee topics.

Aflatoxin M.—Associate Referee Robert D. Stubblefield (USDA, Peoria, IL) reports that data from the international collaborative study on the Foos-Warren method for determination of aflatoxins M_1 and M_2 in fluid milk (12) have been evaluated. Seven duplicate sets of artificially contaminated milk samples (0–1.31 ng M_1/mL and 0–0.13 ng M_2/mL) were prepared by each collaborator by adding solutions containing various concentrations of aflatoxins M_1 and M_2 to fresh milk. Recoveries ranged from 85.2 to 102.5% (average 93.7%) for aflatoxin M_1 (78.3 to 99.2%, average 90.1% for reverse phase LC results only) and from 99.5 to 126.7% (average 109.8%) for aflatoxin M_2 (103.0 to 166.7%, average 126% for reverse phase LC results only). Coefficients of variation averaged 21.4% (M_1) and 35.9% (M_2) for both normal and reverse phase LC results combined. The overall within-laboratory variations (S_o, repeatability) were 28.5% for M_1 and 39.9% for M_2 (27.9 and 23.9%, respectively, for reverse phase LC results only). The overall among-laboratory variations (S_x, reproducibility) were 42.1% for M_1 and 68.0% for M_2 (44.5 and 64.7%, respectively, for reverse phase LC results only). An insufficient number of collaborators (3) used normal phase LC to obtain meaningful statistical data; therefore, actual statistical comparisons with reverse phase LC were not made. Visual comparison of results did indicate that normal phase is comparable to reverse phase. For 20 aflatoxin M_1 observations for reverse phase LC, 2 false positives were reported while 11 false positives were reported for 95 aflatoxin M_2 observations. Overall, the collaborators gave positive comments about the method. One problem that occurred with 4 collaborators had been a problem in previous reverse phase LC determinations. The reaction between aflatoxin M_1 and trifluoroacetic acid (TFA) did not go to completion. The Associate Referee has studied this further and will report on an improved procedure at the fall meeting (13). Basically, he found no unreacted M_1 when equal 200 μL aliquots of hexane and TFA are mixed and the resulting mixture is heated for 10 min at 40°C. This technique has been tried successfully by the collaborators who had difficulty in the study. Therefore, this technique will be incorporated in the method. The Associate Referee is continuing research to produce and isolate sufficient quantities of the derivative formed from TFA and aflatoxin M_1 to determine its structure.

Additional methods for aflatoxin M_1 have been published that specify LC (14–16), radioimmunoassay (14), and enzyme-linked immunosorbent assay (ELISA) (17) at ppt levels. Monocolonal antibodies specific for one or more aflatoxins, including aflatoxin M_1, have been produced (18, 19). Simple fluorodensitometric HPTLC and TLC methods for aflatoxin M_1 have also been described (20–22). Aflatoxin B_1 has been chemically converted to aflatoxin M_1, an approach that could be of use for preparation of standard, but overall yields were only 0.03% (23). Aflatoxin M_1 has been found in cheese from several countries (24), underlining the widespread incidence of the toxin in milk. Two recently reported feeding studies have some bearing on levels of aflatoxin M_1 that should be regulated in milk. Newborn Holstein calves were fed concentrations of milk contaminated with aflatoxin M_1 (0–2 μg/L) for 6 weeks (25). No signs of aflatoxicosis, either physiological or biochemical, were detected. A comparative hepatocarcinogenicity study in rats indicated that aflatoxin M_1 is probably 2–10% as hepatocarcinogenic as aflatoxin B_1 (26). In a study of goats fed radioactive aflatoxin B_1 (27), 1% of the dose was found in the milk within the first 24 h, mostly in the first 6 h period. Over 74% of the radioactivity was found in the dichloromethane fraction and over 95% of this was identifiable as aflatoxin M_1. It is important to note that aflatoxin M_1 can even be present in milk of cows fed ammoniated peanut meal (28).

The Associate Referee recommends that the Foos-Warren method for determination of aflatoxins M_1 and M_2 in fluid milk, using reverse phase LC, be adopted as official first action and that the first action methods for the determination and confirmation of identity of aflatoxin M_1 in dairy products **(26.090–26.094, 26.095–26.100)** and of aflatoxins B_1 and M_1 in liver **(26.101–26.110)** be retained in that status.

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Foods I and adopted by the Association. See the report of the committee and "Changes in Methods," this issue.

Aflatoxin Methods.—Results of the collaborative study of the negative ion chemical ionization mass spectrometric (NICI/MS) confirmation procedure for aflatoxin B_1, adopted as official first action in 1984, have now been published (29). Although no adverse comments concerning the procedure have been received to date, the Associate Referee Douglas L. Park (FDA, Washington, DC) recommends that additional time be allowed for wider use of the method before consideration for official final action.

One error and an omission in aflatoxin methods in the 14th edition of the *Official Methods of Analysis* of the AOAC (4) have been noted. In section **26.002(a)**, the benzene-acetonitrile mixture should be 98 + 2 instead of 9 + 1. Sample preparation for the method for Aflatoxins in Cottonseed Products (**26.052–26.060**) was omitted in the 14th edition and in the separate Chapter 26 of the 13th edition (5) but was originally designated **26.054** in the 13th edition of *Official Methods of Analysis* (30). The section describes sample preparation procedures for whole cottonseed or kernels. Whole cottonseed has oil and lint and is not free-flowing, which causes difficulty in mixing and grinding of samples. The Associate Referee recommends that both these corrections be noted in the next "Changes in Official Methods." The Sample Preparation section can be incorporated under section **26.003.** This change has the prior recommendation of Committee on Foods I.

Data show that the water slurry sample preparation technique (**26.034**) significantly lowers analytical error associated with the determination of aflatoxins in peanut products. This technique is currently part of the BF method in the 14th edition (**26.032–26.036**). No further action is recommended.

Several laboratories have made recommendations on which methods to include in a collaborative study on an LC method for aflatoxins. Although final selection of the method has not yet been made, the list has been narrowed to 3 methods: (a) TFA derivatization of aflatoxins B_1 and G_1 and reverse phase LC (31); (b) post-column derivatization with iodine in water (32–34), which has recently been used in a survey of nut products for aflatoxins (35); and (c) normal phase LC with a silica gel-packed flow cell for fluorescence detection (36). A collaborative study is proposed on a solvent-efficient TLC method, consisting of elements of the CB and BF methods with reduced requirements for solvents (37). Samples to be included in the study are naturally contaminated peanut and corn products. A caution has been given by Zennie (38) concerning enhancement of fluorescence of aflatoxin B_1 on a TLC plate developed in chloroform–acetone (92 + 8) due to free fatty acids from corn. Such free fatty acids, revealed by iodine staining, accompany aflatoxins in the CB method.

A review article on official methods of analysis for mycotoxins has been prepared for which the Associate Referee is co-author (39). The paper presents a comprehensive listing of mycotoxin methods approved by AOAC, IUPAC, AACC, and AOCS. Chemical means for decontamination of commodities containing aflatoxins or other mycotoxins have also been reviewed (40). Meanwhile, research on methods for aflatoxin analysis continues. Aflatoxins were included in a recent multidetection procedure for 11 mycotoxins in cereals (41). Kamimura et al. (42) described a simple and rapid method for determination of aflatoxins in various foods that uses a chromatographic column containing only 0.7 g Florisil with densitometric HPTLC for quantitation; recoveries were greater than those obtained in the same laboratory by the CB method and, especially, the BF method. LC methods have been published for determination of aflatoxins in raw peanuts with disposible cartridge cleanup (43), aflatoxins B_1 and M_1 in beef liver (15), and aflatoxins in vegetable oils (44).

Alternaria Toxins.—Associate Referee Edgar E. Stinson (USDA, Philadelphia, PA) notes that a number of reviews on *Alternaria* toxins have appeared in the last year. The first review (45) discussed the toxicity of over 30 *Alternaria* metabolites, many of which have not been tested, or have been incompletely tested for toxicity. More research is needed to clarify the reasons for the relatively high toxicity of crude extracts or cultures in comparison with the mycotoxins present in greatest abundance, by testing for toxicity of individual compounds and synergistic effects and screening for thus far unidentified toxins. The second review (46) tabulated the properties of 40 metabolites of *Alternaria* and presented analytical methodology for the 3 main classes of *Alternaria* toxins (dibenzo-α-pyrones, tenuazonic acid, and the altertoxins). Analytical needs and prospects are discussed. A general review on certain *Alternaria* mycotoxins also appeared (47). The Associate Referee has published a review on the biosynthesis of *Alternaria* dibenzo-α-pyrones, tenuazonic acid, and altertoxin I; the bioregulation of these materials is also discussed (48).

The toxic potential of *Alternaria* in grains was revealed in a survey of small grains from the United States (49). Examination of 230 small grain samples (wheat, barley, and rye) for *Alternaria* revealed the presence of one or more of 9 different *Alternaria* species in 184 samples. Of the 8 species examined, all produced tenuazonic acid and 7 produced alternariol and alternariol monomethyl ether. Although extracts from the 8 species were toxic to brine shrimp, these 3 mycotoxins were not present, suggesting that additional toxic metabolites were involved. Ongoing new studies have been initiated to assess the ability of various *Alternaria* species to elaborate additional known and unknown *Alternaria* toxins. The Associate Referee heartily endorses this research.

Concerning developments in the analytical chemistry of *Alternaria* toxins, GC-MS (multiple ion detection) has been used for the determination of alternariol, alternariol methyl ether, and altenuene (as their trimethylsilyl derivatives) in fruit and vegetable products (50). The limit of detection was 20 ng/g, selectivity was high, and coextracted substances did not interfere. A reverse phase LC method for determination of tenuazonic acid and alternariol monomethyl ether in tomatoes and tomato products has been published recently (51). Mass spectrometry of the copper salt of tenuazonic acid has been studied, using both positive and negative ion techniques (52). During these investigations a homolog of tenuazonic acid was tentatively identified.

Citrinin.—Associate Referee David M. Wilson (University of Georgia, Tifton, GA) has been testing various methods for citrinin analysis. The method developed by Gimeno (53) generally gives good results if a 25 g sample is extracted and an acid-base partitioning step is included. Citrinin has a tendency to be retained by glassware and syringes and extreme care must be taken to avoid this. Some progress has been made this year and it is hoped to have a method ready for collaborative study soon. Within the last year, a multimycotoxin method that includes citrinin (41) and chemical confirmation procedures for citrinin on the TLC plate (54) have been published.

Cyclopiazonic Acids.—Associate Referee John A. Lansden (USDA, Dawson, GA) recommends continued study on a method for α-cyclopiazonic acid. He has studied some of the spectro-analytical properties of cyclopiazonic acid in relation to its determination (55). Recently, cyclopiazonic acid was isolated from a sample of Indian kodo millet seed that caused symptoms of "kodua poisoning" in humans (56). Several toxicological studies on cyclopiazonic acid have also been carried out (57–60); no teratogenic potential in rodents was indicated (59, 60).

Ergot Alkaloids.—A new method for the analysis of ergot alkaloids in cereal grains has been published, notes Associate Referee George M. Ware (FDA, New Orleans, LA). In this method (61), extraction and cleanup procedures of Scott and Lawrence (62) were used, and ergot alkaloids were separated by reverse phase LC with 20% acetone in aqueous $(NH_4)_2CO_3$ (200 mg/L) as the mobile phase. Milling experiments showed that 60–70% of alkaloid passed into the flour and the remainder was found in the bran fraction. In another analytical

method, a 3 μm particle size C_{18} column was used to separate ergot alkaloids in cereal grain extracts (63). The Associate Referee has completed work on a method for the determination of specific ergot alkaloids in wheat by liquid chromatography. This method utilizes a porous cross-linked polystyrene-divinylbenzene resin column and an isocratic LC mobile phase to resolve 8 ergot alkaloids (64). The recovery of ergot alkaloids added to wheat at levels of 16–170 ng/g averaged 85.6% with a coefficient of variation of 11.1%.

Ergot (ergopeptine) alkaloids have been determined in tall fescue forage by mass spectrometry/mass spectrometry (MS/MS) (65). The most abundant alkaloid of this class in fescue was ergovaline which appeared to be formed by *Epichloë typhina*; ergotamine standard was detectable at the 1 pg level. Subpicogram amounts of ergotamine were detected by MS/MS by other workers in a method for its quantitation in human plasma (66). Another highly sensitive determination procedure is radioimmunoassay (67).

A paper establishing experimental conditions for selective extraction of ergot alkaloids from drugs using an ion-pair extraction technique has appeared (68). Also, papers on the chromatographic separation of standard ergot alkaloids or dihydro ergot alkaloids were published last year (69–71). Information developed in these papers might be useful in developing new analytical methodology for the analysis of ergot alkaloids in cereal grains. The stability of ergot alkaloid standards in aqueous solution has been further studied (67, 72) and a patent describing substances for stabilizing different ergot alkaloid solutions was reported (73). This patent claims that ergot alkaloids in aqueous solution could be stored at room temperature for one year without decomposition.

The Associate Referee recommends that all existing methods for ergot alkaloids be evaluated and, if a method or combination of methods appears to be valid after a statistical evaluation, a collaborative study should then be conducted. Attention is drawn to the fact that analysis for ergot alkaloids is being covered by 2 other Associate Refereeships—Ergot Alkaloids (T. C. Knott) under the Committee on Drugs and Related Topics and Natural Products (G. Rottinghaus) under the Committee on Feeds, Fertilizers, and Related Areas; cooperation is encouraged.

Ochratoxin.—Associate Referee Stanley Nesheim (FDA, Washington, DC) reports that the improved method for determination of ochratoxin A in barley, corn, and pig tissues, using high performance TLC and LC, recommended for collaborative study last year (74), has been further tested in application to analysis of 5 German- and American-style pork sausages, and a sample of each of imported Westphalian ham and canned frankfurter. Recoveries of added toxin were 60–70%. The Associate Referee has not been able to conduct the collaborative study because of other duties but expects to have the study well under way by the time of the 1985 AOAC Annual International Meeting.

During 1984–85, papers were published on a rapid method for determination of ochratoxin A in pig kidneys by using LC and TLC (75), a mini-column and TLC method for screening and quantitation of ochratoxin A in corn, peanuts, beans, rice, and cassava (76), a method for determination of ochratoxins A and B in foods by LC (77), a multimycotoxin method that includes ochratoxin A (41), chemical confirmation tests for ochratoxin A on the TLC plate (54), and a very rapid micromethod for ochratoxin A in human blood, based on LC fluorescence (78). Surveys of ochratoxin A occurrence in the blood of Swedish slaughter pigs (79), in kidneys, blood, and serum samples from Polish and German slaughter pigs (80–82), and in Danish pig kidneys (83) indicate important incidence of this mycotoxin in these animals. Ochratoxin A was also detected in a human kidney, according to the German report (82). Additional surveys of commodities showed occurrence of ochratoxin A in commercial green coffee beans imported into Japan (84) and in nuts and cereals in Germany (85).

The Associate Referee recommends that the improved method for ochratoxin A (74) be collaboratively studied.

Penicillic Acid.—Associate Referee Charles W. Thorpe (FDA, Washington, DC) reports that preparations for a collaborative study of a method for the determination of penicillic acid in corn and dried beans are under way. As penicillic acid reacts with sample components over a period of time it will be necessary to supply collaborators with unknown solutions of penicillic acid which are to be added to the ground sample before analysis. It is planned to have at least one naturally contaminated sample included which should give good data on the reproducibility of the method. The method to be studied is based on procedures previously reported (86, 87) with some modifications to make it faster and more reproducible (88).

A comparatively small number of papers on penicillic acid have been published in recent months. The role of penicillic acid in the phytotoxicity of *Penicillium cyclopium* and *P. canescens* to the germination of corn seeds was reported (89). Penicillic acid was extracted from culture extracts with ethyl acetate and isolated by preparative TLC. The assay was carried out by TLC analysis using a fluorodensitometer after exposure to ammonia fumes. Analysis by glass capillary GC of the trimethylsilylether derivative of penicillic acid showed 2 closely eluting peaks which had identical electron impact mass spectra. It was postulated that these were epimeric forms of penicillic acid. A multimycotoxin method that includes penicillic acid (41) and, from the same laboratory, a confirmation test for penicillic acid performed directly on a TLC plate (54) were reported. A drop of acetic anhydride overspotted on standard and sample extract spots followed by development with toluene–ethyl acetate–90% formic acid (6 + 3 + 1) produced a new diffused gray (ammoniated) spot with a higher R_f value than a nontreated spot.

Penicillic acid was one of several mycotoxins discussed in a recent review of methods for decontamination (40). The in vitro interaction of penicillic acid with rat liver glutathione *s*-transferase (GST) has been studied (90). It was found that penicillic acid interacts with GST by direct binding and that the inhibition of the GST activity by penicillic acid in crude extracts was dose-dependent.

The Associate Referee recommends that the method presented at the 99th AOAC Annual International Meeting (88) for determination of penicillic acid in corn and beans be collaboratively studied.

Secalonic Acids.—This topic is vacant. No new method development has been published in the last year.

Sterigmatocystin.—Associate Referee Octave J. Francis, Jr (FDA, New Orleans, LA) reports that a one-dimensional TLC method has been developed in his laboratory for the determination of sterigmatocystin in cheese (91). Detection and quantitation of sterigmatocystin is possible at levels of 2 and 5 μg/kg, respectively. An intralaboratory method evaluation conducted on samples spiked from 5 to 25 μg/kg resulted in an average recovery of 92%. A collaborative study of the method has been initiated and should be completed by January 1986.

Since the last report, a TLC screening method and tests for the chemical confirmation of sterigmatocystin and several other mycotoxins have been published (41, 54). It has also been reported that sterigmatocystin was found in feed associated with acute clinical symptoms of bloody diarrhea and death in dairy cattle in the United States (92). Additional reports have been published on the inhibitory effect of sterigmatocystin on ATP synthesis in mitochondria (93) and the acute cytogenetic effect of sterigmatocystin on rat bone-marrow cells in vivo (94).

The Associate Referee recommends that surveys be continued for the natural occurrence of sterigmatocystin.

Tree Nuts.—Associate Referee Vincent P. DiProssimo (FDA, Brooklyn, NY) recommends continued study on methods for determination of aflatoxins in tree nuts. An effort

to relate the aflatoxin content of individual almond kernels to their fluorescence color has been made (95). Almonds that fluoresced violet-purple under longwave UV light were found to contain high levels of aflatoxins B_1 and B_2.

Trichothecenes.—Two collaborative studies (96, 97) for the determination of deoxynivalenol (DON) in wheat were successfully completed last year reports Associate Referee Robert M. Eppley (FDA, Washington, DC). One method uses TLC for detection and quantitation and has a determination limit of 300 ng/g. The other method is based on the use of GC for detection and quantitation with a determination limit of 350 ng/g. A thermal rearrangement product of DON has been identified (98). The rearrangement product, isoDON, was detected in samples of white and whole wheat bread to the extent of 3–13% of the DON present. Unheated samples of the wheat and flour that were positive for DON did not contain detectable quantities of isoDON. Also isoDON was detected in wheat-based breakfast cereals which had undergone heat treatment. Transformation of DON by rumen microorganisms to a de-epoxy product has been reported (99). Additional methods for preparative isolation of crystalline DON have been developed (100, 101).

Activity on new methods for the trichothecenes and improvement and adaptation of existing methods has continued. Most of the analytical methodology has been aimed at DON, nivalenol, T-2 toxin, HT-2 toxin, and diacetoxyscirpenol (102–115) and mass spectrometric techniques continue to be explored (102, 103, 108, 110–112, 115, 116). The importance of careful evaluation of extraction procedures, particularly for naturally contaminated samples, was emphasized as a result of studies by Trenholm et al. (117) with DON in grains. Gilbert et al. (102) reported on the optimization of conditions for trimethylsilylation, an area where considerable confusion has existed in the past. Incomplete derivatization of the 7-hydroxy-8-ketotrichothecenes has been a constant problem. An improved method for the simultaneous detection of DON and nivalenol has recently been published (103). Previously, procedures have been successfully applied to the determination of one or the other of these toxins, but not for both. Recoveries for both compounds ranged from a low of 83–85% for corn to 89–91% for polished rice. With this method, a high incidence of nivalenol and DON were found in Korean cereals (118). Also of note in the recent literature on occurrence of trichothecenes is a report on the presence of T-2 toxin and DON in some samples of corn imported into Taiwan from the United States (119) and co-occurrence of nivalenol and DON in samples of German wheat bran (120).

Another analytical area of considerable interest involves the use of bioassays. A bioluminescence assay (121) and a simplified protein synthesis inhibition assay (122) have been reported. The bioluminescence assay has a detection limit of 50 ng for roridin A and the protein synthesis inhibition assay ranged from 10–20 ng/mL for T-2 toxin to give 50% inhibition.

An indirect ELISA method for T-2 toxin (123) has been developed for biological fluids that can detect 0.2–1.0 ng/mL of fluid. Antibodies to diacetoxyscirpenol (124) and deoxyverrucarol (125) have been prepared, the first major step in the development of immunoassays for these mycotoxins. Also reported was the preparation and characterization of monoclonal antibodies to T-2 toxin (126). It is worthy of note that the production of antibodies to deoxynivalenol has not been achieved.

The Associate Referee recommends that the 2 collaboratively studied methods (96, 97) for determination of DON in wheat be adopted official first action at the stated determination limits. He also recommends that methods for the determination and confirmation of DON in corn and other small grains be evaluated for potential use in a collaborative study/studies to be initiated within the next year. In addition, methods for the known naturally occurring trichothecenes, nivalenol, diacetoxyscirpenol, and T-2 toxin, will be scrutinized to select the best methods for future collaborative studies. The natural occurrence of other trichothecenes will be closely monitored to evaluate the need for the development of additional methods and studies. The number of known fungal trichothecenes is increasing rapidly (e.g., ref. 127). Finally, the development of biological assays will be observed closely for future evaluation by collaborative studies.

Xanthomegnin and Related Naphthoquinones.—No additional work has been done on this topic, reports Associate Referee Allen S. Carman, Jr (FDA, New Orleans, LA). The Associate Referee recommends continued study of methodology capable of detecting both xanthomegnin and viomellein and development of screening methodology capable of determining xanthomegnin and viomellein along with ochratoxins A and B. The reason for this is some speculation that the toxic effects of the ochratoxins may interact synergistically with the toxic effects attributable to the naphthoquinone toxins and the fact that these toxins are thought to occur together (128).

Zearalenone.—Last, but certainly not least, research on the occurrence, improved methodology, metabolism, biological effects, and production of zearalenone and zearalenols continued apace during the last year, reports Associate Referee Glenn A. Bennett (USDA, Peoria, IL). A survey of mycotoxins and fungal damage in corn in North Queensland, Australia, revealed that zearalenone was found in 85% of the 293 samples examined (129). The mean concentration of zearalenone in all samples was 0.17 ± 0.225 μg/g and 4 samples contained greater than 1 μg/g. A high incidence of zearalenone (together with deoxynivalenol and nivalenol) has also been found in unpolished barley in Korea (118). Conversely, a careful examination of scab-infected hard red winter wheat samples from Nebraska showed that zearalenone was not present even in kernels severely infected with *Fusarium graminearum* (130). Field corn, which had been ear inoculated with *F. graminearum,* was examined over a growing season for the appearance of toxins in husks, axial stems, and stalks. Zearalenone was observed only in the husks (5.2 μg/g) near harvest time (131). Another study on the occurrence of zearalenols in naturally infected corn stalks showed that zearalenone, α-zearalenol, and β-zearalenol were associated with foot and stalk rot in corn from southern Italy (132); the α- and β-isomers were resolved by LC and their identity was confirmed by GC-mass spectrometry. The occurrence of zearalenone in feeds prepared in Taiwan from imported U.S. and South African corn has also been reported (119).

Improvements in methodology, especially LC, now permit low levels of zearalenone and zearalenols to be determined in both agricultural commodities and animals consuming contaminated grains or spiked feeds. The LC method tested collaboratively for the determination of zearalenone and α-zearalenol in corn (133) has been approved as official first action by AOAC (134). A rapid, sensitive reverse phase LC method for zearalenone and α- and β-zearalenols in wheat has been developed (135). This procedure employs an internal standard (zearalenone oxime) and quantitation with either fluorescence or UV detectors. Other applications of LC have been for the measurement of zearalenone by fluorescence in cereals (136), the determination of zearalenone together with deoxynivalenol and secalonic acid D in grain dust (137), the determination of mycotoxins (standards, including zearalenone, zearalenol, various aflatoxins, verrucarin A, and roridin A) by gradient LC using an alkylphenone retention index system (138), and the use of thermospray LC/mass spectrometry to analyze for zearalenone and 4 trichothecenes in porcine plasma and urine (112). The latter procedure is less sensitive than the fluorescence detection of zearalenone and α-zearalenol isolated from pig blood plasma or urine and analyzed by normal phase LC (139). The emerging technology of ELISA for mycotoxins has been applied to zearalenone with success (140) and the preparation of monoclonal antibodies offers increased specificity for the target analyte (141). Detection of zearalenone in fungal hyphae by fluorescence

microscopy has been reported (142). Among several animal studies carried out recently (and not all referred to in this report), one study concluded that ingestion of high concentrations (60–90 μg/g) of purified zearalenone by pregnant gilts interferes with embryonic development (143). These levels are much higher than those usually found in naturally contaminated feedstuffs. Intoxication of swine herds by zearalenone-contaminated corn and sorghum has been reported in Australia (144). A prepubertal gilt fed 192 μg zearalenone/kg/body weight/day for 4 days showed plasma concentrations of α-zearalenol 3–4 times higher than the parent compound (145) and another study showed that zearalenone toxicosis in rats and swine may be mediated or overcome by the fiber fraction of alfalfa, but the mechanism of this protection is unclear (146). Biotransformation of zearalenone to zearalenols (mainly β-zearalenol) by brewing microorganisms suggests that this may occur during alcoholic fermentation (147). Attempts to prevent or remove zearalenone contamination of corn and wheat have been partially successful. Density segregation of naturally contaminated wheat (22 and 17 ng/g) yielded nonbuoyant fractions that contained no detectable zearalenone (148). Also, pesticides such as carbaryl, fonofos, or maneb, were reported to cause inhibition of zearalenone production in laboratory cultures and in field corn (149).

The Associate Referee recommends that criteria be established to determine the purity of zearalenone, α-zearalenol, and β-zearalenol standards used in different laboratories and to continue development of methods to determine specific toxins and their clearing times in biological fluids.

Recommendations

(1) Adopt as official first action the methods for determination of deoxynivalenol in wheat by TLC at levels ≥300 ng/g; determination of deoxynivalenol in wheat by GC at levels ≥350 ng/g; and determination of aflatoxins M_1 and M_2 in fluid milk by reverse phase LC.

(2) Include the correction to section **26.002** and section **26.054** from the 13th edition of *Official Methods of Analysis* in the next "Changes in Official Methods."

(3) Continue study on all other topics.

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Oils and Fats

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Antioxidants.—Associate Referee B. D. Page is continuing to investigate procedures to confirm the presence of antioxidants detected by the LC method (1). Capillary GC confirmation of the relatively volatile antioxidants BHA, BHT, TBHQ, and Ionox-100 was investigated. LC peaks (in about 1 mL effluent) were collected in water and the water was passed through a C-18 Sep-Pak or similar cartridge. The antioxidant was eluted from the cartridge and 1 μL was injected onto the capillary column (on-column injector, flame ionization detector). BHA and BHT recoveries (10 ppm in sample) were about 80%, and Ionox-100 recoveries were about 70%. TBHQ recoveries were poor, no more than 10%. Additional work is planned on capillary GC confirmation of LC peaks.

Emulsifiers.—Associate Referee H. Bruschweiler has carried out a second collaborative study of a method for determination of mono- and diglycerides using capillary GC. The method involves direct conversion of the mono- and diglycerides in monoglyceride concentrates or in fats and oils into trimethylsilyl ether derivatives prior to capillary GC. The Associate Referee also conducted a collaborative study of a method for determination of lauryl sulfate in fats and oils by the methylene blue procedure. A recommendation for adoption of the methods will be made after publication of the methods and collaborative study results.

Hydrogenated Fats.—Associate Referee R. A. DePalma is planning an interlaboratory study of a capillary GC method for determination of C-18 monoene *trans* levels in hydrogenated fats and oils. The method involves conversion of the sample to methyl esters followed by analysis of the esters on a 60 m SP-2340 (75% cyanopropylmethyl silicone) fused silica capillary column.

Lower Fatty Acids.—Associate Referee G. Bigalli participated in an international collaborative study of 2 GC methods for determination of butyric acid sponsored by the International Union of Pure and Applied Chemistry (IUPAC) Commission on Oils, Fats and Derivatives. The first method, based on that published by Phillips and Sanders (2), provides for determination of free (underivatized) butyric acid after saponification of the fat sample with potassium hydroxide solution followed by acidification with phosphoric acid to liberate the fatty acids and separation of the short chain (soluble) fatty acids by filtration. The second method, based on that published by Kuzdzal-Savoie and Kuzdzal (3), requires saponification of the fat with barium hydroxide solution before acidification and filtration to separate the short chain fatty acids. Four samples (butyric acid content in the range of 0.2–3.5%) were examined by 14 collaborators. Repeatability and reproducibility coefficients of variation were 3–4% and 4–10%, respectively, for test portions with 1.8–3.5% butyric acid examined by both methods (4). For the sample containing 0.2% butyric acid, repeatability and reproducibility coefficients of variation were 4.5% (modified P-S method) and 4.9% (modified K-S method), and 12.9% (modified P-S method) and 22.6% (modified K-S method), respectively. Both methods were judged to be satisfactory, although the collaborators generally favored the P-S method in terms of simplicity and analysis time. A recommendation for adoption of a method for butyric acid will be made after IUPAC approval and publication of the collaborative study results.

Marine Oils.—Associate Referee R. G. Ackman is continuing study of methodology for analysis and identification of marine oils. The Associate Referee is planning a collaborative study on the use of flexible fused silica/bonded Carbowax columns for analysis of marine oil fatty acid methyl esters by GC.

Olive Oil Adulteration.—Methods used in Italy, supplied by the Associate Referee, are under review. Lercker and coworkers (5) proposed a method for detection of as low as 10% of esterified oil in olive oil by direct analysis of the monoglycerides (as trimethylsilyl derivatives) from enzymatic lipolysis of olive oil samples using capillary GC. Prof. V. M. Kapoulas (6) suggested validation of a standard method for detecting small amounts (<10%) of vegetable oils such as soybean oil in olive oil based on analysis of the triglycerides or specific triglyceride fractions. One of the methods suggested was based on analysis of the triglycerides by reverse phase liquid chromatography.

Oxidized Fats.—There was no Associate Referee activity on this topic during the past year.

Pork Fat in Other Fats.—There was no Associate Referee activity on this topic during the past year. Rugraff and Karleskind (7) reported a sensitive method for determining pork

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fat in beef fat, based on oxidation of the unsaturated glycerides, isolation of the saturated glycerides by silica gel column chromatography, hydrolysis of the saturated glycerides by pancreatic lipase, isolation of the 2-monoglycerides by TLC, and analysis of the 2-monoglyceride fatty acids by capillary GC. The authors stated that less than 2% of pork fat can be detected in tallow with this procedure.

Sterols and Tocopherols.—Associate Referee R. J. Reina is reviewing methodology for GC determination of sterols and tocopherols. The Associate Referee participated in a collaborative study of an LC method for determination of tocopherols, conducted by the IUPAC Commission on Oils, Fats, and Derivatives.

Other Topics.—The General Referee recommends that the Wiley method for determining the melting point of fats and fatty acids, **28.012–28.013,** be revised to allow use of 30 × 3.5–3.8 cm id test tubes. This specification revision would permit easier fabrication of the tubes, which are no longer generally available from commercial sources.

The General Referee recommends that an appropriate precision statement (see **28.068**) be added to the official first action GC method for total *trans* acid isomers in margarines (8).

The IUPAC Commission on Oils, Fats, and Derivatives completed study of a GC method for determination of triglycerides in fats and oils (9, 10). The method involves the separation of triglycerides into groups containing the same number of carbon atoms by direct GC of lipid solutions on methyl polysiloxane columns under temperature programmed conditions. Equivalent results can be obtained using a packed column or a short (≤5 m) capillary column. Statistical analysis of the collaborative results indicated that determination of the major triglyceride components of both animal and vegetable fats and oils can be carried out with an acceptable degree of precision. The General Referee recommends that the GC method for determination of triglycerides (IUPAC Method 2.323) be adopted official first action.

Commission on Oils, Fats and Derivatives, Applied Chemistry Division, IUPAC.—The Commission met on September 1–3, 1985 at the Ecole Centrale de Lyon, Lyon, France, during the 33rd IUPAC General Assembly. The Commission discussed 22 projects and topics including methods for emulsifiers, polycyclic aromatic hydrocarbons, erythrodiol in olive oil and rapeseed oil, sterols, mineral oil residues, commercial lecithin products, phospholipids in commercial lecithins, solvent residues in oils and oilseed cakes, triglyceride composition by LC, butyric acid (butterfat content), tocopherols, metals in edible oils by direct graphite furnace atomic absorption spectroscopy, fat content of foods, other vegetable oils in olive oil, antioxidants by LC, and polymers in heated oils.

The Commission discussed the results of collaborative studies of methods for emulsifiers, thiobarbituric acid value, linolenic acid and other n-3 polyenoic acids, solvent residues in oils, butyric acid, tocopherols by LC, and phospholipids in commercial lecithins. Methods were adopted for determination of phospholipids, solvent residues in oils (headspace method), and butyric acid. The texts of methods for erythrodiol, total sterols (enzymatic procedure), total hexane in oilseed cakes, and emulsifiers by GC after hydrolysis and silylation have been prepared for publication. The Commission has also prepared guidelines for conducting collaborative studies and evaluating their results. A 7th edition of the Commission's *Standard Methods* will be published in 1986.

Recommendations

(1) Revise method **28.013** to allow use of a 30 × 3.5–3.8 cm id test tube in which to carry out the Wiley melting point determination.

(2) Revise the official first action GC method for total *trans* acid isomers in margarines to include precision statement **28.068** which appears in the method for methyl esters of fatty acids in oils and fats by gas chromatography.

(3) Adopt IUPAC Method 2.323, determination of triglycerides by GC, official first action.

(4) Continue study on all other topics.

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Plant Toxins

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Intrinsic plant constituents that may exhibit toxicity continue to present problems regarding their significance and control. These questions are reflected in the debate relating to requirements for the availability of analytical procedures and for official methods for some of these analytes.

The major topics currently under development in this General Refereeship are the glucosinolates, the pyrrolizidine alkaloids, and the steroidal alkaloids. Active consideration is also being given to problems associated with hydrazine derivatives in mushrooms, estrogenic isoflavones, and 3-substituted furans.

Glucosinolate Analysis (D. Ian McGregor, Agriculture Canada, Saskatoon, Saskatchewan).—Glucosinolates or mustard-oil glucosides constitute a unique class of plant constituents that now number in excess of 90. They are characterized by a "S-C-N" moiety that has glucose attached to the sulfur and sulfate attached to the nitrogen. Individual glucosinolates are distinguished by the nature of a side-chain attached to the carbon. Glucosinolates co-exist with an enzyme called myrosinase which, when the tissue is crushed in the presence of water, catalyzes their hydrolysis to glucose, sulfate, and an unstable aglucone. The further fate of this aglucone depends, in each case, on the character of the side-chain and the conditions prevailing during the enzymatic process. Various pathways to stable end-products are known and frequently are involved simultaneously. They include intramolecular non-enzymatic rearrangements to isothiocyanates, fragmentation to nitriles, rearrangement to thiocyanates, or further hydrolysis to free thiocyanate ion.

Glucosinolates are of interest because these end-products are biologically active compounds. They impart pungency to many vegetables, such as cabbage, cauliflower, broccoli, turnip, cress, and watercress. They impart the biting taste to radish and capers and to condiments such as horseradish and mustard. But they also may be vesicants, mutagenic, goitrogenic, or otherwise toxic. When consumed by humans in small amounts they are esteemed. The hazards to humans associated with an often lifelong ingestion of diets rich in glucosinolate-containing plants is uncertain, but when consumed by animals in large amounts as part of their feed, glucosinolates or their myrosinase hydrolysis products can cause very serious growth and reproduction problems.

Glucosinolates traditionally have been analyzed indirectly, by measurement of one or another of their myrosinase hydrolysis products. Difficulty in applying this approach to determining the glucosinolate composition or content of seed, meal, or vegetable samples arises from the fact that the character of the side-chain influences the nature of the end-products of myrosinase hydrolysis. Even though most species contain only a few glucosinolates, at very low concentration levels, there can be sufficient diversity to limit methods that measure only one or other of the aglucone hydrolysis products. One solution is to measure either the glucose or sulfate which is universally produced. However, this does not provide any information on glucosinolate composition. An alternative solution would be direct measurement of the glucosinolates or their derivatives. Direct measurement has become possible in recent years with the introduction of trimethylsilyl derivatization to make the glucosinolates sufficiently volatile for separation by gas chromatography and with the application of liquid chromatography to the separation of glucosinolates or their enzymatically desulfated products. However, with the present state of the art these methods also have their limitations.

Accordingly, 3 approaches to the analysis of foods for glucosinolates are currently under consideration, one of which, it is hoped, will eventually prove suitable for consideration as an AOAC official method.

The first approach involves aqueous extraction of the glucosinolates, ion exchange purification of the extract, myrosinase release of glucose, trimethylsilyl derivatization, and quantification by gas chromatography using myoinositol as internal standard. Trimethylsilyl derivatization of glucose released from glucosinolates and the use of myoinositol as internal standard was first proposed by Theander and Aman (1), but, as with many other methods involving measurement of glucose, as a rapid and relatively simple method rather than an accurate and precise one. It is felt that coupling the approach of Theander and Aman with purification by ion exchange would afford a specific and perhaps the most sensitive means of determining glucosinolate content. It would also ensure that all of the glucosinolates present were accounted for. The disadvantages of this approach are the commercial unavailability of myrosinase and the failure of the approach to provide information on glucosinolate composition.

The second approach under consideration involves aqueous extraction, ion exchange-desulfation purification, trimethylsilyl derivatization of the desulfo glucosinolates, and separation and quantification by gas chromatography using either allyl or benzyl glucosinolate as internal standard. Gas chromatography of trimethylsilyl derivatives was first proposed by Underhill and Kirkland (2). Several improvements have been made over the years including the introduction of ion exchange and desulfation to isolate the glucosinolates and facilitate derivatization. The specific approach under consideration is that published by Daun and McGregor (3) for glucosinolate analysis of rapeseed and canola (low glucosinolate rapeseed) seed. The method has the advantage of providing information on glucosinolate composition. In particular, when applied to rapeseed it can detect contamination by common glucosinolate-containing weed seeds. It can also be applied to the analysis of mustard seed. The method has been favorably received for analysis of the seed and oil-extracted meal of rapeseed and canola, both in Canada and throughout the world. It is the approved method of the Canadian Grain Commission, used by Canadian laboratories who participate in the check sample program sponsored by the Canola Council of Canada, and the method designated by the Canola Council to define canola. It has also gained international acceptance by having been used in a number of collaborative studies, the most recent of which is a collaborative study conducted by TC 34 SC 2 of the International Standards Organization. Results of these collaborative studies have been promising but they also indicate that difficulties still exist even when application of the method is restricted to the seed of rapeseed. In addition to the fact that there is still considerable variability between laboratories in the way the analysis is performed, results indicate that calibration may be required to achieve satisfactory accuracy and precision. Also the method can not be applied to indole glucosinolates, which can constitute a significant proportion of the glucosinolate content of canola seed and meals, because of difficulties in achieving quantitative formation of the trimethylsilyl ether derivatives and/or preventing their degradation during gas chromatography.

The third approach under consideration involves aqueous extraction, ion exchange-desulfation purification, and separation and quantification of the desulfo glucosinolates by liquid chromatography with *o*-nitrophenyl-β-D-galactopyranoside as internal standard. Liquid chromatography of desulfo glucosinolates was first proposed by Minchinton et al. (4). It has the advantage that the strong basic conditions and high

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temperatures required for derivatization and separation by the previous approach may be avoided, and that labile glucosinolates, such as the indole glucosinolates found in rapeseed and other Brassica seed, are kept intact. All the glucosinolates found in Brassica seed have been detected by this method, although not all are completely resolved. The big disadvantage with the current state of the art is that response factors have been determined for only a few glucosinolates and, lacking samples of pure glucosinolates, there has been no easy way of calibrating the method.

With respect to this last point, efforts have been under way in this laboratory for a number of months to perfect techniques for isolating glucosinolates in gram quantities of suitable purity for use as internal standards and to calibrate methods of glucosinolate analysis. Of particular interest have been the alkenyl glucosinolates, which are major components of the seed of rapeseed and mustard. Efforts have concentrated on obtaining glucosinolates from sources where one glucosinolate is dominant. To this end allyl, 3-butenyl, 2-hydroxy-3-butenyl, benzyl, and 4-hydroxybenzyl glucosinolate have been isolated in crystalline form with greater than 98% purity. It is intended to use these compounds to establish the accuracy of the gas chromatography of trimethylsilyl ether derivatives of these glucosinolates. Attempts are under way to isolate 4-pentenyl and 2-hydroxy-4-pentenyl glucosinolates, which are also constituents of rapeseed. Failing this, the extrapolation of the responses for 3-butenyl and 2-hydroxy-3-butenyl to 4-pentenyl and 2-hydroxy-4-pentenyl will be considered.

The isolated glucosinolates will also be used to calibrate high pressure liquid chromatography of desulfo glucosinolates. In addition to this approach the similarity of glucose and glucosinolates to reaction with thymol recently observed by Brzezinski and Mendelewski (5) is being investigated as a possible means for calibrating the liquid chromatographic approach.

In deciding which approach—gas chromatography of glucose or desulfo glucosinolates or liquid chromatography—may be the best suited for an Association of Official Analytical Chemists official method, the scope of application must be defined. For the broadest possible application, gas chromatography of glucose would seem most suitable, but this would not yield information on composition. For analysis of the alkenyl glucosinolates of Brassica seed and meal of commercial importance, including rapeseed and both mustards (*Brassica juncea* and *B. hirta*), gas chromatography of trimethylsilyl derivatives would suffice. But this approach would not include the indole glucosinolates. It is of interest to note that since 3-methylsulfinylpropyl glucosinolate can not be quantifiably converted to the trimethylsilyl derivative, the method can not be readily extended to the analysis of Brassica vegetables that contain appreciable amounts of this glucosinolate. The only method that has potential for application to the individual analysis of all of the glucosinolates known to be present in significant amount in Brassica seed and vegetation is based on liquid chromatography of desulfo glucosinolates. However, in addition to the fact that few laboratories (particularly those which analyze Brassica seed or seed meals routinely) are equipped for liquid chromatography, this approach does not yet readily yield quantifiable results for all the glucosinolates in Brassica. Potential for wider application is unknown.

Pyrrolizidine Alkaloid Analysis (Associate Referee to be appointed).—Published TLC (6) and LC (7) methods are being evaluated for screening plant materials. MS (8) and IR (Chen, Mossoba, and Page, FDA, private communication, 1985) spectrometric confirmatory techniques are being developed.

Steroidal Alkaloid Analyses (Associate Referee to be appointed).—The initial emphasis in this topic has been on the potato glycoalkaloids. An LC screening procedure has been developed (Carman et al., FDA, private communication, 1985). A pre-collaborative study is being planned for further evaluation of this procedure.

Recommendations

(1) *Glucosinolates:* Continue study.

(2) *Pyrrolizidine Alkaloids:* Appoint Associate Referee; continue study.

(3) *Steroidal Alkaloids:* Appoint Associate Referee; initiate pre-collaborative study of LC screening method for potato glycoalkaloids; continue study.

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Processed Vegetable Products

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pH Determination.—The method for pH determination of acidified foods received official final action status in 1983. The collaborative study was reported in *J. Assoc. Off. Anal. Chem.* **64**(2), 332–336 (1981). The Associate Referee recommends that work continue on this topic.

Sodium Chloride.—The AOAC methods for determination of sodium chloride in foods are being reviewed by the Associate Referee and a paper reporting the findings is expected. The Associate Referee recommends that work continue on this topic.

Total Solids by Microwave Moisture Methods.—The method for the determination of solids in processed tomato products by microwave drying oven was adopted as official first action in 1984. Interested persons are invited to submit comments and participate in continued study of this topic.

Water Activity in Foods.—The method on water activity determination was adopted official final action in 1982. A quality assurance check sample for water activity determination by the method **(32.004–32.009)** was submitted for analysis to FDA district laboratories in 1981, 1982, and 1984. A manuscript reporting the results of these analyses has been submitted to the AOAC *Journal* and will be presented at the AOAC meeting, October 27–31, 1985. The manuscript title is "Evaluation of Water Activity Determinations Using a Fiber-Dimensional and Four Electrical Hygrometers," by William H. Stroup, James T. Peeler, and Kent Smith of the Food and Drug Administration. Other activity included a 2-day training course for FDA laboratory analysts on water activity (a_w) determinations using the AOAC method. The course included laboratory experimentation in conducting a_w analyses, construction of calibration curves from salt slushes of known a_w, and determination of the a_w of food samples. Interested persons are invited to submit comments and participate in continued study of this topic.

Soluble Solids of Tomato Products by Direct Refractometer Methods Without Filtration or Centrifugation.—No activity was reported.

Determination of Pectin Breakdown in Frozen Foods.—No comments were received.

Other Topic.—Interest has been reported in the study of a method for LC determination of sugars in processed vegetables. Initiation of this topic is recommended, and interested persons are invited to comment and participate. Appointment of an Associate Referee is to be considered.

Recommendations

(1) Discontinue the topic Determination of Pectin Breakdown in Frozen Foods.

(2) Initiate the new topic, LC Determination of Sugars in Processed Vegetables.

(3) Continue study on other topics.

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Seafood Toxins

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Since the last report, we have added 2 new associate refereeships to Seafood Toxins, namely, Diarrhetic Shellfish Poisoning and Neurotoxic Shellfish Poisoning. Since these 2 topics may not be familiar to many, they will be presented in greater detail. In addition, we have reinstituted the topic on Paralytic Shellfish Poisons—Immunoassays with a new Associate Referee.

Shellfish Poison—Chemical Methods.—Associate Referee William L. Childress (FDA, Boston, MA) has no report at this time. He is investigating the use of liquid chromatography/electrochemical detection (LCEC) to determine saxitoxin, neosaxitoxin, the gonyautoxins 1–4, and the sulfocarbamoyl analogs, B_1, B_2, C_1, and C_2, and expects to have results for the next report. Concerning other chemical methods, the LC procedure reported by Sullivan et al. (1, 2) for the fluorometric determination of the above PSP toxins is being evaluated at the FDA Center for Food Safety and Applied Nutrition.

Paralytic Shellfish Poisons—Immunoassays.—The Associate Referee on this topic is Patrick Guire of Bio-Metric Systems, Inc. (BSI), Eden Prairie, Minnesota. This R & D group has been working on the development of antibodies and immunoassay kits for saxitoxin and its analogs since 1979. Thus far they have produced a useful polyclonal antibody and a rapid, convenient radioimmunoassay (RIA) kit for saxitoxin.

Antibody production has now been reported from 3 laboratories. Guire et al. (3, 4) made polyclonal antibodies in rabbits from a stable immunogen produced from saxitoxinol by carbonyldiimidazole coupling to serum albumin. Chu and Fan (5) made polyclonal antibodies in rabbits from an unstable immunogen produced from saxitoxin by formaldehyde coupling (6) to serum albumin. Davio et al. (7) have produced both poly- and monoclonal antibodies to saxitoxin by formaldehyde coupling to keyhole limpet hemocyanin. Laboratory demonstrations of both RIA and ELISA systems have been reported for saxitoxin. BSI is producing 1000-kit lots of their 30-min RIA kit, which requires a microcentrifuge and a liquid scintillation counter and is sensitive to 15 picomoles saxitoxin/mL (1 μg/100 g shellfish). Good correlation with the mouse toxicity test has been obtained for this RIA test system by George Yang of FDA (private communication, 1985) with shellfish from Maine and Connecticut. He has found the sensitivity of the RIA test to GTX-2 to be about equal, and to GTX-3 to be about ½ that of STX. It is insensitive to neosaxitoxin and related analogs.

A related radioreceptor assay system has been reported (8) with sensitivity to saxitoxin of 0.15 ng/mL. This involves competitive binding of radiolabeled saxitoxin to crude preparation of sodium channel receptors from rat brain. In this condition, the receptor is less stable than antibodies; some difficulty remains in preparing the stable radiolabeled saxitoxin of high specific activity needed for this sensitive assay, and its convertibility to enzyme-labeled assay remains unproven (will the macromolecular enzyme-labeled saxitoxin bind to the membrane-bound receptor?). This assay system is being adapted for shellfish analysis (9). The stability of the

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receptor preparation is promising; effort is under way to improve the stability and activity of the radiolabel. The specificity of this binding protein is ideally suited for a toxicity-related assay.

Current activity is under way at Battelle Northwest (Ralph Elston, private communication, 1985) for developing additional stable immunogens and poly- and monoclonal antibodies for saxitoxin and its analogs for both potential therapeutic/prophylactic and assay applications. Improved chemical procedures for coupling PSP to proteins and improved antibodies for broad specificity and high affinity binding of PSP are expected from these efforts. BSI is also currently continuing its efforts toward development of convenient and stable EIA kit systems for PSP (for both laboratory colorimeter and non-instrumented field use) and labeled antibody preparations for fluorescent and electron microscope histochemical studies.

Diarrhetic Shellfish Poisoning.—The Associate Referee for this new topic is Takeshi Yasumoto, Department of Food Chemistry, Tokoku University, Tsutsumidori, Sendai 980, Japan.

Introduction.—The occurrence of gastroenteritis after eating mussels and scallops has been an observed health problem in the northern part of Japan since 1977. Symptoms from diagnosed patients have involved diarrhea (92%), nausea (80%), vomiting (79%), abdominal pain (53%), and chill (10%). Most patients developed symptoms within 4 h after consuming shellfish. The cause of the illness was found in the lipid fraction of the digestive gland of the shellfish, and the term "diarrhetic shellfish poisoning" (DSP) was proposed for the disease after the predominant symptom (10–12). Unlike PSP, DSP causes no human fatalities and patients usually recover within a few days without any serious after-effects. Occurrence of similar gastrointestinal disturbances has also been reported in The Netherlands (13), Spain (14), France (15), Sweden (Per-Olof Andersson, SMEF Chairman, personal communication), Chile (16), and Thailand (17). Nevertheless, with several thousand reported cases in both Europe and Japan and with its long infestation periods, DSP poses serious problems to both public health and shellfish industries. DSP may also be responsible for cases of gastroenteritis reported in the United States from eating shellfish (18).

Toxins.—Nine polyether toxins have been isolated from infected shellfish and the chemical structures for 6 of them determined (19, 20). They are classified into acidic and neutral toxins. The acidic group includes okadaic acid and dinophysistoxin-1 and -3 (DTX1 and DTX3). Okadaic acid is a potent cytotoxin agent (21) first isolated from marine sponges and later from *Prorocentrum lima* (22), a marine dinoflagellate alleged to be involved in ciguatera. The chemical structures of DTX1 and DTX3 were determined to be 35(*S*)-methylokadaic acid and its 7-O-acyl derivatives. The lethal potencies (LD_{99}, ip, mice), of okadaic acid, DTX1, and DTX3 were 200, 160, and 500 μg/kg, respectively. When orally administered to infant mice (23), they cause excessive fluid accumulation in the intestine. An oral intake of about 40 μg DTX1 is presumed to cause illness in a human adult. In European mussels, okadaic acid was the major toxin found, whereas DTX1 and DTX3 dominated the acidic fraction of Japanese shellfish. The chemical structure and the mode of action of a toxin tentatively assigned as DTX2 is under investigation. Separation of acidic toxins from neutral toxins is easily achievable on a basic alumina column, which strongly retains the acidic toxins (12). The relative ratio of the acidic toxins to the neutral toxins in Japanese shellfish varies significantly from year to year.

The neutral toxins, which are polyether lactones, were named pectenotoxins (PTX) after the family of the scallop from which the toxins were first isolated (20). The presence of 5 analogues (PTX1–5) was confirmed and the chemical structures of 3 major components (PTX1–3) were determined. So far the PTX toxins are absent in European mussel specimens tested. Unlike okadaic acid, derivatives of PTX do not cause fluid accumulation in the intestine of suckling mice (23) but cause acute liver damage. The origin and role played by PTX in cases of DSP are not presently understood.

Sources of the toxins: A species of dinoflagellate *Dinophysis fortii* was first shown to produce DTX1 and DTX3, and is considered to be responsible for toxic shellfish along the Japanese coast. Okadaic acid has been found in *Dinophysis acuminata* and presumably is produced by *Dinophysis acuta,* since shellfish consuming this species contain the toxin.

Assay methods: In Japan, DSP is currently monitored in shellfish by mouse bioassay with a maximum tolerance level of 5 mouse units/100 g meat. Since the diarrhetic symptom was only attributed to okadaic acid and its derivatives and the mouse assay cannot distinguish between the various toxins, efforts were directed to developing a rapid, sensitive, and specific LC method by labeling the toxins with 9-anthryldiazomethane (24). The fluorogenic reagent reacts quantitatively with the carboxylic acid group of the toxins at room temperature within 60 min. The resultant derivatives were separated from each other and from contaminant fatty acids on a Develosil ODS column (0.46 × 25 cm) eluted with acetonitrile–water (95 + 5), 1.0 mL/min. The fluorescent response (Ex 365 nm, Em 412 nm) for DTX1 and okadaic acid showed good linearity over a range of 25–100 ng. The minimum detection level was 5 ng for okadaic acid. To test the practical value of the method, varied amounts of okadaic acid and DTX1 were added to acetone extracts of nontoxic mussels and the extracts were treated successively on cartridge columns of basic alumina, silicic acid, and C-18 (Sep-Pak, Waters Co.). Analysis of the pretreated extracts showed good correlation between the added amounts of toxins and the peak heights of the derivatives. The method requires only 0.5 g of the digestive glands for extraction and can be completed in 3 h. However, additional experiments are necessary to improve the recovery of toxins during the cleanup procedures.

Florida Red Tide Brevetoxins/Neurotoxic Shellfish Poisons.—The Associate Referee for this new topic is Daniel G. Baden, Rosenstiel School of Marine and Atmospheric Sciences, University of Miami, Miami, FL.

Introduction: The first scientifically recorded red tide was witnessed by Walker in 1844 on the Gulf of Mexico coastline of Florida. Numerous red tide episodes have been reported since that time. Red tides occur most often along the western coast of Florida from Tarpon Springs in the north to the Dry Tortugas in the south. They are characterized by patches of discolored water, dead or dying fish and other similarly afflicted marine creatures, the presence of an upper respiratory irritant in the air, and the induction of toxicity in shellfish exposed to the red tide.

Toxicology: The planktonic marine dinoflagellate *Ptychodiscus brevis* is the causative organism in Florida's red tides (25). The organism is toxic in both the field and the laboratory (26). Both neurotoxic and hemolytic fractions have been isolated from *P. brevis* cells (27). The toxins are endotoxins and are not released into the environment until lysis of the cells. Ichthyotoxicity can remain in the water long after the motile organisms have vanished (28). In all red tides, *P. brevis* becomes the predominant organism at concentrations (about 2.5×10^5 cells/L) that begin to kill fish. The most visible environmental consequence of exposure to *P. brevis* is massive fish kills that occur as a direct result of the neurotoxins produced by the dinoflagellate (29). The widespread necrotic conditions that prevail during red tides may, in part, account for many of the observed maladies.

Human Exposure: Humans are affected by *P. brevis* toxins in primarily 2 ways. One consequence of Florida's red tides is a respiratory irritation caused by the inhalation of seaspray-associated toxic particles (28). Little research has been directed at characterization of the irritant in terms of physical state or composition. It has been hypothesized that the irritant is caused by airborne *P. brevis* particles (28), and research

groups have identified toxic fractions which cause a bronchoconstrictor response in vivo in anesthetized rats (30), and an asthmatic event in an in vivo guinea pig model (31).

The second human consequence of exposure to Florida red tide toxins is that of shellfish poisoning in humans (32). Various filter-feeding bivalves including oysters, clams, and coquina are capable of concentrating the neurotoxins without apparent harm to themselves (33). Solvent extracts of suspect oysters, collected after reports of human intoxication (34), were found to be lethal to kittens and mice by intraperitoneal injection (35). Shellfish potency correlates roughly with the concentration of *P. brevis* in surrounding water and *P. brevis* cell numbers correlate in an approximate manner with the amount of toxin extractable (34–38).

Coquina *(Donnax variabilis)* frequently becomes the most toxic bivalve, and develops toxicity at an early stage during inshore red tide blooms. Once the toxins are accumulated, they are transmitted to higher life forms by natural predation. Marine fish, shore birds, crabs, and mammals including humans consume coquina. According to Tufts (39), commercial bivalves are generally safe to eat 1–2 months after the termination of any single bloom episode, although it is known that the depuration rate is species-specific. In the laboratory, the oyster *C. virginica* accumulates toxin in less than 4 h in the presence of 5000 cells/mL, and "detoxifies" 60% in 36 h when placed in *P. brevis*-free sea water. Thus, the accumulation and depuration of *P. brevis* toxins by oysters would appear to be a function of feeding and elimination rates (40). There is, however, little quantitative data on either the rates of accumulation or depuration of Florida red tide toxins in the natural environment.

The Centers for Disease Control in Atlanta has classified shellfish poisoning in humans due to ingestion of *P. brevis* neurotoxins as being distinct from other types of shellfish poisoning. The symptoms of Neurotoxic Shellfish Poisoning (NSP) include paresthesia of lips and extremeties, a reversal of temperature sensation, vertigo, headache, G.I. tract abnormalities, and in most severe cases, paralysis (41). Debilitation is generally temporary and recovery is complete in most cases within a few days. The symptoms associated with many cases of ciguatera are virtually indistinguishable from symptoms of NSP. Ciguatera poisoning is an intoxication which follows the consumption of certain tropical marine fish that have accumulated neurotoxic substances in their flesh. The neurosensory symptoms which distinguish NSP and ciguatera from other types of marine seafood poisoning are the paresthesia and hot-cold dysesthesia described above.

The neurotoxins isolated from *P. brevis* exert their effects by altering the membrane properties of excitable cell types in a specific manner, activating voltage-dependent sodium channels in a dose-dependent manner at normal resting potential (42, 43). It has been shown recently that a specific binding site associated with the voltage-dependent sodium channel exists in rat brain, and synaptosomal receptor binding assays have been developed for characterization of the toxin-receptor association (44).

Chemistry: To date, the structures for 6 brevetoxins have been elucidated. Brevetoxins are conspicuous by their polycyclic ether nature: 4 are toxins whose structures consist of a chain of 11 *trans*-fused ether rings (brevetoxin B, or GB-2, T34; GB-3, or T17; brevetoxin-C; and GB-6); the 2 remaining toxins (brevetoxin-A and GB-1) differ from the previous 4 in that they possess more complex ring systems (45). Their functions in the dinoflagellate cell are currently unknown.

Assays: Shellfish monitoring programs in Florida currently employ a variation of the AOAC standardized mouse bioassay (46) to assess shellfish meat following a closure. Direct *P. brevis* counts in water samples are used as the primary criterion for implementing a closure. As has been discussed numerous times before, the mouse bioassay is cumbersome, is not cost effective, and as pointed out by Steidinger (47), death time versus toxin concentration is not a linear correlation. Assays that reproduce the mechanism of human intoxication would be invaluable in development of proper assessment of public health hazard, i.e., the assays in animals should ideally be oral assays. Lack of sensitivity is the obvious disadvantage of oral administration, death being the end point of the assay and most marine dinoflagellate food intoxications (with the exception of PSP) being sublethal intoxications. There are alternatives to the mouse bioassay that show promise.

Immunoassays have been developed using bovine serum albumin-linked toxin T17 (or GB-3) as antigen, and specific antibodies have been raised in a goat (48). Assay sensitivity is currently assessed at 600 pg and a linear relationship for toxin detection exists between 1.5 and 48 ng toxin. All *P. brevis* toxins examined to date are detected equally well by the assay. The assay is being refined for field use by conversion to an enzyme-linked form for general use, and by attempts to raise specific antibodies to the orally-potent component, T17, for routine public health use (49).

Molecular pharmacological analysis, using purified sodium channel binding components, may prove to be a significant advance in the specific detection of *P. brevis* toxins. With KD app (specific binding) values in the nanomolar concentration ranges (45), the technique should prove at least as sensitive as immunoassay. In addition, the binding event being measured is the initial pharmacologically significant event in the onset of toxicity. One would expect that the degree of competition for specific binding sites would directly affect potency and therefore present a public health hazard. The principal disadvantage of this assay concerns the availability of sodium channel binding component (from rat brain) and its stability once isolated and purified. Components of the sodium channel have now been cloned in bacterial vectors and thus availability may not pose a problem. However, stability may present a procedural problem for the production of a field assay kit.

It is hoped that some day specific binding assays such as the 2 mentioned will be available in field kit form for rapid on-the-spot analysis.

Ciguatoxin-Biochemical Methods.—Associate Referee Yoshitsugi Hokama (John A. Burns School of Medicine, University of Hawaii at Manoa, Honolulu, HI) reports 6 laboratories are in the process of conducting a collaborative study to evaluate the enzyme immunoassay stick test for the detection of ciguatoxin and related polyethers from fish tissues (50).

Tetrodotoxin.—Associate Referee Yuzuru Shimizu (College of Pharmacy, University of Rhode Island, Kingston, RI) reports the conjugation of tetrodotoxin (TTX) to polylysine as an alternate conjugate to TTX-BSA in obtaining antibodies for an enzyme immunoassay to determine TTX. However, the antibodies obtained from rabbits challenged with the TTX-polylysine conjugate exhibit unwanted cross reactivity with background proteins. These results are being reinvestigated.

Recommendation

Continue study on all topics.

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GENERAL REFEREE REPORTS: COMMITTEE ON FOODS II

Alcoholic Beverages

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As reported in the 1984 General Referee report, a collaborative study by Associate Referee David Chia for malic acid in wine by an enzymatic method was carried out; however, the results were disappointing and a new collaborative study will be initiated.

The LC collaborative study by Associate Referee Eric Christensen for glycerol in wine was completed, but the data were disappointing and a new collaborative study will be initiated.

Art Caputi, AOAC-ASE (American Society of Enologists) Liaison Officer and Associate Referee for carbon dioxide in wine reports that a new titrimetric method for CO_2 is undergoing collaborative testing; preliminary results may be available for the AOAC October 1985 annual meeting.

Barry Gump, Professor of Chemistry at California State University at Fresno, has been appointed Associate Referee for sulfur dioxide in wine. He reports that method evaluation is in progress and a collaborative study will be forthcoming.

John Steele, ATF, has been appointed Associate Referee for synthetic colors in alcoholic beverages. He has completed method development work, and a collaborative study using TLC is starting.

Peter Gales, AOAC-ASBC (American Society of Brewing Chemists) Liaison Officer submitted results of an ASBC collaborative study for sodium and potassium in beer with the recommendation for adoption by AOAC. The General Referee concurs.

The General Referee has been busy with the analysis of imported wine for diethylene glycol content. DEG is a poisonous chemical not natural to wine which was deliberately added to certain imported wine to simulate special harvest character. According to newspaper accounts, the suspected culprits have been identified, so the problem will not be a continuing one. There appears to be no need for an Associate Referee appointment or collaborative study; however, the methods used will be submitted for publication to the AOAC *Journal*.

Recommendations

(1) Adopt as official first action the ASBC method for determining sodium and potassium in beer.

(2) Continue study on all topics.

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee for adoption were not approved by the Committee on Foods II. See the report of the committee and "Changes in Methods," this issue.

Cereal Foods

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Phytate Method.—Associate Referee Barbara Harland completed the collaborative study on a modified ion-exchange method for phytate. The General Referee recommends that the method be adopted official first action.

Iron Determination.—Associate Referee James Martin reported (FDA Laboratory Information Bulletin 24-50) on a rapid method for iron analysis in infant formulas. The method, which uses a 3-point graph, produced results comparable to the 11-point graph required in the present method **(14.011)**. A collaborative study will be initiated this year to determine method efficacy.

Official Method Review.—The General Referee is currently reviewing the cereal and cereal products section of *Official Methods of Analysis*. He has solicited the aid of interested AOAC membership in performing this duty through a request in the *Referee* for comments on satisfaction with current methods contained in the cereal foods section.

AACC Meeting.—The General Referee attended the 70th annual meeting of the American Association of Cereal Chemists. Technical committee sessions were attended to ascertain common interests between committees of the 2 organizations.

Recommendations

(1) Adopt the ion-exchange method for phytate as official first action.

(2) Establish a collaborative study for the rapid analysis of iron in cereal-based infant formulas.

(3) Continue review of present methods in the cereal products section of *Official Methods of Analysis*.

(4) Continue as liaison between AOAC and AACC for the purpose of pursuing methods that might be developed jointly by the 2 technical committees.

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Foods II and adopted by the Association. See the report of the committee and "Changes in Methods," this issue.

Chocolate and Cacao Products

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Methods of Analysis, Comparison of AOAC and IOCC/ISCMA.—A number of methods relating to the same field of interest are published by both organizations. Since many of the old methods need revision, especially those of IOCC/ISCMA, we should study the differences and try to develop compromises acceptable to all parties.

IOCC/ISCMA has published methods specifically on analysis of cocoa and chocolate products and determination of their quality that are not listed as AOAC official methods of analysis. Some examples of these methods are:

(1) extinction/absorption values before and after washing cocoa butter with alkali (blue value);
(2) viscosity measurements in chocolate;
(3) determination of cocoa powder fineness;
(4) determination of wettability of instant cocoa powder in water;
(5) determination of SOS-type triglycerides;
(6) determination of solidification/cooling curve of cocoa butter;
(7) determination of ammoniacal nitrogen content in cocoa beans.

I will continue this survey, taking into account the relevant methods of IDF, ISO, and IUPAC pertaining to cocoa, chocolate, and cocoa butter.

New Areas of Investigation.—The determination of heavy metals in oils and fats, including cocoa butter, should be studied. A standard method was recently adopted by IUPAC on the basis of collaborative study that included cocoa butter as one of the test materials. The determination of triglyceride composition should be studied, particularly determination and detection of cocoa butter equivalents. This is a political problem within the European Community. IUPAC adopted a GC method for triglycerides in oils and fats (IUPAC standard 2.323). The General Referee for Oils and Fats will recommend this method for AOAC consideration. A draft proposal of EEC methods for testing composition and specifications of cocoa and chocolate products intended for human consumption is still under discussion. IUPAC adopted the method, solid content in fats by low resolution NMR (IUPAC standard method 2.323). Another NMR method, rapid determination of total and solid fat contents in chocolate products, was published (*J. Food Sci.* (1985) **50,** 942–945).

Recommendation

Continue study on all topics.

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Foods II and adopted by the Association. See the report of the committee and "Changes in Methods," this issue.

Color Additives

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The Associate Referee for Colors in Other Foods, N. Adamo, reports having conducted new recovery studies on colors added to both Celite and cookies. He is planning to submit a plan for a collaborative study to the methods committee statistician for approval.

The Associate Referee for Inorganic Salts, W. Brammell, is presenting 3 papers: Determination of sodium iodide in FD&C Red No. 3 by ion-selective electrode analysis; Rapid titrimetric method for pure dye in color additives; and Preparation of a more stable titanous chloride solution for determination of pure dye in color additives.

The Associate Referee for Liquid Chromatography, E. Cox, reports that a paper on the determination of synthetic colors by LC and TLC in powdered unsweetened soft drink mixes was submitted to the AOAC *Journal*. She also reports that she has begun preliminary work on a collaborative study of a reverse phase LC method for determination of intermediates, subsidiary colors, and 2 reaction products, DONS and DAADBSA, in FD&C Yellow No. 6. She would appreciate hearing from anyone interested in participating in this study.

The Associate Referee for Intermediates in Other Certifiable Colors, A. Scher, reports that he has authored a paper titled Determination of 2,4-dinitroanilin and 2-naphthol in D&C Orange No. 17 (*J. Assoc. Off. Anal. Chem.* **68,** 474–477 (1985)). He also reports that he coauthored a paper with H. Murray titled Liquid chromatographic determination of leuco base in FD&C Blue No. 1. This paper has been submitted to the AOAC *Journal* (May 1986).

Also under this topic, R. Calvey and A. Goldberg have published a paper titled Liquid chromatographic determination of intermediates in D&C Yellow No. 7 and D&C Yellow No. 8 (*J. Assoc. Off. Anal. Chem.* **68,** 471–473 (1985)).

The Associate Referee on Subsidiary Colors in Certifiable Color Additives, J. Bailey, reports that an LC procedure for the determination of the lower sulfonated subsidiary colors formed from analine and Schaeffer's Salt and sulfanilic acid and β-naphthol in FD&C Yellow No. 6 has been published in *J. Chromatogr.* The Associate Referee also reports that investigations aimed at determining the unsulfonated homolog in FD&C Yellow No. 6 are nearing completion and should be submitted for publication in the near future.

Under this topic, A. Goldberg has submitted a report on an LC procedure for determination of D&C Yellow No. 11 residues in D&C Yellow No. 10 (*J. Assoc. Off. Anal. Chem.* **68,** 477–479 (1985)).

Recommendations

Continue study on all topics.

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Foods II and adopted by the Association. See the report of the committee and "Changes in Methods," this issue.

Flavors

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In the past year, 2 collaborative studies were conducted, one by Philip Guarino and Susan Brown, and the other by Sidney Kahan. Both studies were on the same method: determination of vanillin and related flavor compounds in vanilla extract by LC.

The only difference in the 2 studies is that Kahan obtained vanilla beans directly from the importers, extracted them by the official method, and then used these extracts for the study. Guarino and Brown used commercially produced extracts of unspecified origin for their study. For this reason it is recommended that the method as studied by Kahan be adopted official first action.

Recommendations

(1) Adopt the method for determination of vanillin and related flavor compounds in vanilla extract, as studied by Kahan.

(2) Continue study on all topics.

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendation of the General Referee for adoption was not approved by the Committee on Foods II. See the report of the committee and "Changes in Methods," this issue.

Fruits and Fruit Products

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Fruit Acids.—Associate Referee E.D. Coppola has developed an LC method to determine quinic, malic, and citric acids in cranberry juice cocktail and apple juices. The method was collaboratively studied by 12 laboratories. Six samples of cranberry and 6 samples of apple juice were sent to each collaborator. Samples were treated through a disposable cartridge, filtered, and directly injected into the chromatographic apparatus. Two reverse phase columns with UV detection at 214 nm and 0.2M KH_2PO_4 mobile phase (pH 2.4) were used.

The ranges of repeatability and reproducibility CV values for quinic, malic, and citric acids are 1.2–7.6% and 2.9–14.7%, respectively, for acid content above 0.10%. The precision of the method is satisfactory. Dr. Coppola recommends that the method be adopted official first action and the General Referee concurs in this recommendation.

Orange Juice Content.—Associate Referee Carl Vandercook reports that past studies on the detection of adulteration in citrus juices have shown wide ranges in concentrations of the major constituents. For example, the concentration of malic acid in lemons decreased 10-fold during an extended storage study. A recent compositional study of navel oranges showed a 2-fold increase in both arginine and pectin between early- and late-season fruit. Furthermore, compositional variations due to growing regions have led to valid criticisms of analytical decisions based on limited data bases. The environment is known to affect the composition of fruit from the same variety. The color of California oranges is more intense than the color of the same variety grown in Florida.

This variability has plagued researchers and regulatory personnel trying to detect adulteration in fruit products since the introduction of processed products. Research at this laboratory has now shifted emphasis from a compositional approach for detecting adulteration to one of studying the environmental influences on the biochemical mechanisms controlling the composition of the fruit. Once the environmental factors are known, it should be possible to more accurately predict authenticity based on compositional data. Therefore, it is recommended that the study be continued.

Fruit Juices, Identification and Characterization.—Associate Referee R. W. Wrolstad reports that a symposium on Adulteration of Fruit Juice Beverages, organized by S. Nagy and J. A. Attaway and sponsored by the Division of Agricultural and Food Chemistry, was held May 1 and 2 at the 189th meeting of the American Chemical Society in Miami Beach, FL. Included in the symposium were 2 independent investigations, R. E. Wrolstad–V. Hong and E. D. Coppola–M. S. Starr, concerning cranberry juice. Both studies presented compositional data for authentic cranberry products and LC methodology for detecting adulteration through analysis of organic acids, sugars, and anthocyanin pigments. Both teams of investigators analyzed commercial cranberry juice drinks and concentrates and reported that several samples were adulterated. Others papers dealt with citrus and apple juice commodities and the application of statistical methods, isotopic analyses, amino acid analyses, and trace metal analyses in determining authenticity. Academic Press plans to publish the symposium as a monograph. Efforts are being continued in several laboratories to improve methodology for detecting adulteration in other fruit juices such as red raspberry, plum, and pear. The General Referee recommends continued study.

Moisture in Dried Fruits.—The General Referee recommends that work continue on this subject.

Detection of Adulteration of Apple Juice.—The General Referee recommends that work continue on this subject.

Adulteration of Orange Juice by Pulpwash and Dilution.—Associate Referee Don Petrus reports that his laboratory has been collecting background data on imports from Brazil, Mexico, Belize, and California. Four laboratories including his own have been working on a cooperative investigation to quantitate the current method. The General Referee recommends that work continue on this subject.

Sodium Benzoate in Orange Juice.—The General Referee recommends that this topic be discontinued.

Recommendations

(1) Adopt as official first action the LC method for determining quinic, and citric acids in cranberry and apple juice.

(2) Discontinue the subject of sodium benzoate in orange juice.

(3) Continue study on all other topics.

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Foods II and adopted by the Association. See the report of the committee and "Changes in Methods," this issue.

The UV/vis and fluorescence spectrophotometric method for determination of adulteration of processed Florida orange juice was not recommended for adoption by the General Referee. The method was recommended by the Official Methods Board and adopted official first action by the Association. See the report of the Official Methods Board, *J. Assoc. Off. Anal. Chem.* (1986) **69**, March issue.

Nonalcoholic Beverages

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Quinine.—Associate Referee Leonard Valenti has started a collaborative study of his proposed method and has received some results. He plans to complete the study and have a paper prepared by spring of 1986 for AOAC.

Caffeine and Methyl Xanthines in Nonalcoholic Beverages.—Associate Referee John M. Newton recommends that work continue on this subject.

Steve Miladinavich has developed a procedure that determines the caloric content of various soft drinks. The procedure is adibatic calorimetry and works well for normal soft drinks; this could be of interest to AOAC.

Recommendations

(1) Continue study of quinine.

(2) Continue study of the new procedure for determination of methyl xanthines in nonalcoholic beverages.

(3) Appoint an Associate Referee for the analysis of caloric content of nonalcoholic beverages.

(4) Appoint an Associate Referee for analysis of safrole in sassafrass roots.

This report on the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Foods II and adopted by the Association. See the report of the committee and "Changes in Methods," this issue.

Preservatives and Artificial Sweeteners

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Formaldehyde in Olives.—Associate Referee R. J. Reina has previously 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 with subsequent determination by liquid chromatography (LC) and UV detection at 254 nm. The limited study was performed by 3 analysts. The recoveries ranged from 69.1 to 83.6% at 5 ppm and from 67.6 to 95.1% at 1 ppm. Additional work was planned to improve the recoveries and reproducibilities; however, because of other commitments, no additional work was performed this year. It is recommended that study be continued.

Meats, Ground, Screening Method for Chemicals and Added Blood—Associate Referee J. J. Maxstadt previously reported on a method for determination of added blood in ground beef, which was collaboratively studied. The results of the study, however, were not satisfactory. The Associate Referee will not be able to continue work on this project and it is recommended that this topic be discontinued.

Organic Preservatives (Thin Layer Chromatography).—Associate Referee C. P. Levi previously conducted a limited within-laboratory study on 6 types of food spiked with 9 common preservatives. Because of other commitments, no additional work was performed this past year. The work will be continued by Rosella Bigornia who has been appointed Associate Referee for "Organic Preservatives, Qualitative Methods (LC)." The same objective will be pursued, that of a general qualitative method for common preservatives, but the emphasis on TLC will be dropped and LC will be investigated.

Aspartame, Benzoates, Saccharin, and Caffeine (Liquid Chromatography).—Associate Referee N. G. Webb and coworker D. D. Beckman have developed a method for the determination of aspartame in beverage mixes by liquid chromatography (*J. Assoc. Off. Anal. Chem.* **67**, 510–513 (1984)). The separation was done 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. The conditions used in this method represent a modification of the official final action method, **12.018–12.021**, for benzoates, saccharin, and caffeine. Aspartame was quantitated in the presence of these additives as well as artificial colors and flavors. The Associate Referee is planning a collaborative study of this method and will limit the study to aspartame in beverages and dry mixes. This study will involve, in part, some shelf-life studies of aspartame in beverages.

Sulfites in Foods (Polarographic Methods).—Associate Referee Walter Holak and coworker Bhailel Patel have developed a differential polarographic method capable of determining both free and combined sulfur dioxide in various food products. Preliminary testing was carried out on samples of apple sauce, soda syrup, shrimp, juice concentrate (wild cherry), baby food (strained peas), and tamarind juice. The samples were spiked at levels of 10–40 ppm and recoveries ranged from 82 to 99%. A collaborative study has been initiated. The samples being used in the study are freeze-dried shrimp, orange juice, dried apricots, strained peas, and dehydrated potatoes.

Recommendation

Continue study on all topics.

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Foods II and adopted by the Association. See the report of the committee and "Changes in Methods," this issue.

Spices and Other Condiments

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Bulk Index of Spices.—Associate Referee Tom Haney, Durkee Foods, reports that a collaborative study is completed and the report is being prepared for submission.

Piperine in Black Pepper.—Associate Referee Ted Lupina, Kalsec, reports that a collaborative study is finished, and the report has been submitted for review.

Carbon Isotopes in Vinegar.—Associate Referee Dana Krueger, Krueger Food Labs, reports that a collaborative study is in progress.

Moisture in Spices.—Associate Referee Lou Sanna, Santa Maria Chili, reports that a collaborative study has been completed, reviewed, and submitted for publication.

Ethylene Oxide Residues in Spices.—Associate Referee Lynn Theiss, R. T. French, reports that the method and protocol for a collaborative study have been submitted to collaborators, with a target date in September 1985.

Pungency of Capsicum Spices and Their Oleoresins.—Patrick Hoffman, McCormick, is chairing the investigation of this method and a collaborative study will begin shortly.

Recommendations

(1) Adopt as official first action the method for determination of moisture in spices.

(2) Continue study on all topics.

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Foods II, and adopted by the Association. See the report of the committee and "Changes in Methods," this issue.

Sugars and Sugar Products

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Chromatographic Methods.—Associate Referee Michael Gray has resigned from his position because the subject has so expanded that he feels the associate referees for this area should come from academia or industry.

Color, Turbidity, and Reflectance—Visual Appearance.—The 1984 recommendation to adopt as official first action the measurement for solution color of sugars and syrups of the International Commission for Uniform Methods of Sugar Analysis will be submitted for interim official action.

Corn Syrup and Corn Sugar.—The 1984 recommendation to adopt as interim first action the new dry substance—refractive index—composition tables for corn syrups, developed by the Augustana Research Foundation, was approved and accepted. Results have now been published (Maxwell, J. L., Kurtz, F. A., and Strelka, B. J. (1984) Specific volume (density) of saccharide solutions (corn syrups and blends) and partial specific volumes of saccharide-water mixtures, *J. Agric. Food Chem.* **32**,(5), 974–979; Wartman, A. M., Spawn, T. D., and Eliason, M. A. (1984) Relationship between density, temperature, and dry substance of commercial corn syrups, high fructose corn syrups, and blends with sucrose and invert sugar, *J. Agric. Food Chem.* **32**(5), 971–974). The General Referee recommends official final action. Associate Referee Raffael Bernetti plans to submit a paper showing how these tables can be generated by computer for any type of syrup composition. Bernetti suggests 2 types of changes in the official methods for determination of saccharides in corn syrups (**31.245–31.253** and **31.254–31.257**). The first is that "column materials" be expanded to include prepacked columns. The second is that the specifications be rewritten to more general terms for chromatographic equipment; there are now several manufacturers of appropriate equipment. It must be determined whether these changes can be incorporated as nonsubstantive changes to the current method or if new collaborative studies are required. The General Referee is of the opinion that this field of analysis has become so extensive in recent years, with a great deal of new equipment and columns available, that up-to-date collaborative studies may be desirable.

Dr. Bernetti also points out that section **31.245–31.253,** titled "Saccharides (Minor). . . ," should be titled "Saccharides (Major). . . ." Section **31.254–31.257** is correctly titled "Saccharides (Minor). . . ."

Dr. Bernetti reports that studies on sulfite analysis are continuing with emphasis on ion chromatographic methods. The Monier-Williams method has been found to overstate the SO_2 level in syrups and sugars because of interference from organic acids which distill over with sulfur dioxide. Work on headspace analysis for trace contaminants in corn syrups is progressing well.

Enzymatic Methods.—Associate Referee Marc Mason reports no new developments.

Honey.—The 1984 recommendation for official first action on the instrumental color clarification of honey was approved and accepted. The General Referee recommends official final action on this method, which uses the Lovibond 2000 Comparator.

Lactose.—Associate Referee Janice Saucerman reports no new developments and no planned studies.

Maple Saps and Syrups.—Associate Referee Mariafranca Morselli reports that she is revising the methodology for maple products in *Official Methods of Analysis.*

Stable Isotope Ratio Analysis.—The name of this subject has been changed from "Stable Carbon Isotope Ratio Analysis" at the request of the Associate Referee, Landis Doner. He reports that a study is in progress on the application of $\delta^{18}O$ values in water for detecting the addition of beet invert syrups to orange juice.

Standardization of Sugar Methods of Analysis.—Associate Referee Mary An Godshall submits the attached report on the collaborative study on the Roberts method of dextran analysis in raw cane sugars (mentioned as a planned study in the 1983 report). This analysis is an important problem in sugars, and this study is also being submitted to the International Commission for Uniform Methods of Sugar Analysis (ICUMSA) which holds no official method for this analysis, and which recommended collaborative testing for this method. The testing has been conducted in accordance with AOAC requirements. The General Referee recommends interim first action on this method, pending publication.

Godshall reports that the next ICUMSA quadrennial session will be held in May 1986, and that recommendations for updating methods for polarization will be made after that session.

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Sugars in Cereals.—Associate Referee Lucien Zygmunt reports progress in development of LC methods of analysis for sugars in cereals, using a high sensitivity refractometer to increase sensitivity and speed of analysis while maintaining resolution.

Sugars in Licorice Products.—Associate Referee Raymond Tuorto reports no new developments.

Weighing, Taring, and Sampling.—Associate Referee Melvin Lerner reports that he is not aware of any technological developments in weighing, taring, or sampling which have occurred in the last year and which indicate a need for changes in any of the current AOAC procedures for sugars or sugar products. He recommends that practices relating to weighing, taring, and sampling continue to be monitored by the Committee for Sugars and Sugar Products because of the considerable economic significance of these commodities.

Recommendations

(1) Adopt as official final action the new dry substance—refractive index—composition tables for corn syrup, developed by the Augustana Research Foundation.

(2) Adopt as official final action the instrumental color clarification of honey.

(3) Continue study on all topics.

Vitamins and Other Nutrients

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Eleven papers were presented at the poster session, 99th AOAC annual international meeting. A symposium titled Critical Analysis of Analytical Methods for Meat Foods was chaired by Robert C. Benedict. Also, a symposium was chaired by Charles W. Gehrke titled Chromatography of Amino Acids and Determination of Amino Acids Composition of Nutritionally Important Protein Sources.

As usual, a number of collaborative studies in this area are intended and collaborators are hereby solicited.

Recommendations

(1) Adopt as official final action the official first action method for dietary fiber, **43.A14–43.A20,** amended as follows:

(1) **43.A14,** *Principle,* Line 1: Change to ". . . >10% fat." The change from >5 to >10% fat before defatting is necessary is in line with results experienced routinely during the collaborative study.

(2) **43.A14,** *Principle,* Line 4: Add "When analyzing mixed diets, always ext fat prior to carrying out total dietary fiber analysis."

(3) **43.A15,** *Apparatus* **(h),** Line 1: ". . . capable of weighing to 0.1 mg."

(4) **43.A16,** *Reagents* **(b),** Line 3: Add "One vol. of DW mixed with 4 vols 95% EtOH will also give 78% EtOH final concn."

(5) **43.A16,** *Reagents* **(d),** Line 3: ". . . 6.05 g Na phosphate."

(6) **43.A16,** *Reagents* **(g),** Line 3: Add "All 3 enzymes (pretested), Cat. No. KR-185, are available. . . ."

(7) **43.A18,** *Sample Preparation,* Line 1: ". . . on dried sample." (instead of dry wt basis).

(8) **43.A20,** *Calculation,* correct formulas as shown below.

(2) Adopt as official first action the methods for chloride, phosphorus, proximate analysis, thiamine, and vitamin B_{12} studies in Phase III of the infant formula collaborative study. Amend the official first action microbiological method for vitamin B_6, **43.A27,** by adding after the fourth sentence: "Add 5 mL basal medium stock soln to all tubes for total vol. of 10 mL/tube."

(3) Continue study on all topics.

$$\text{Blank} = \text{mg blank residue} - \left(\frac{\%\text{ protein blank residue} + \%\text{ ash in blank residue}}{100}\right) \times \text{mg blank residue}$$

$$\text{TDF, \%} = \left(\frac{\left(\text{mg residue} - \left(\frac{\%\text{ protein in residue} + \%\text{ ash in residue}}{100}\right) \times \text{mg residue} - \text{av. blank}\right)}{\text{mg sample}}\right) \times 100$$

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27-31, 1985, at Washington, DC.

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GENERAL REFEREE REPORTS: COMMITTEE ON RESIDUES

Metals and Other Elements

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Arsenic in Animal Tissues and Meat Products by Atomic Absorption Analysis.—At the request of USDA, Randy Simpson was appointed Associate Referee for this topic to collaboratively study an in-house USDA dry-ash hydride generation-AAS method that the agency would like to use for regulatory purposes. The method has been validated internally by 2 USDA laboratories, but has not been published in the open literature. The Associate Referee for Hydride Generation Techniques has also been in contact with Mr. Simpson regarding this study. Mr. Simpson would like to initiate the study later in the fall.

Atomic Absorption Spectrophotometry (AAS).—Associate Referee Milan Ihnat has continued development of fundamental and verification standards for performance assessment of AAS methods. In addition, he has begun development of a multielement flame-AAS (FAAS) protocol.

Preparation of high purity standard solutions (fundamental standards) described in last year's General Referee report has been completed, and a report is in preparation for submission to the *Journal of the AOAC*. These standard solutions were used in preparation of Biological Reference Materials (BRMs) and in method development. Through the cooperative efforts of Agriculture Canada, USDA, USFDA, Health and Welfare Canada, and the National Research Council of Canada, chemical analyses and certification have been completed on 2 BRMs: dry powdered maize stalk, and maize kernel. Using different methodologies, these agencies have established recommended and information values for K, Mg, Ca, Na, Sr, Mn, Fe, Cu, Zn, Se, Al, N, F, and Cl. These materials will be issued by NBS as Reference Material 8412, Corn (Zae Mays) Stalk, and Reference Material 8413, Corn (Zae Mays) Kernel, and will be useful for FAAS method development and collaborative study. The development of several other food-agricultural BRMs is in progress.

The FAAS procedures used in developing BRMs are being integrated into a protocol for multielement analysis of foods. It is anticipated that the Associate Referee will recommend the protocol for AOAC collaborative study in 1986.

Cadmium and Lead in Earthenware.—Associate Referee Benjamin Krinitz reports no progress in this topic area. He still has an interest in investigating further leaching of lead from the lip area of teacups with decorated rims, but has not been able to obtain the needed ceramic samples for the study. The official first action method, **25.016–25.023,** for extractable Cd and Pb from ceramic and enameled cookware should be adopted as official final action.

Emission Spectrochemical Methods.—Associate Referee Fred Fricke reports that 7 laboratories have agreed to participate in the proposed collaborative study of a method for multielemental analysis of foods by inductively coupled plasma (ICP). The method, which is based on numerous publications by the Associate Referee's laboratory, uses a wet acid digestion (nitric, perchloric, sulfuric) of foods followed by determination of 12 elements (Ca, Cd, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, and Zn). The protocol for the study and the samples to be analyzed were selected and agreed to by the Statistical Consultant for the Committee on Residues. The study samples will include blind duplicates of 5 sample types representing both animal and plant matrices. Trial sample solutions will be sent to the participating laboratories before the AOAC meeting. The results of the trial will be used to screen the participating laboratories to eliminate any potential collaborators with consistently incorrect results. The full collaborative study will be issued as soon as the pre-study trial is completed.

Fluorine.—Associate Referee Robert Dabeka reports no progress on this topic for the past year.

Graphite Furnace-Atomic Absorption Spectrophotometry.—Associate Referee Robert Debeka reports that no further work has been done on the unsuccessful collaborative study for rapid-screening for Pb in canned milk and infant formula (this method was not submitted to AOAC for official first action status). However, he has developed a coprecipitation-graphite furnace-AAS method for Pb and Cd in foods at levels as low as 0.1–0.5 ppb. This method has been tested by 3 other laboratories, but has not yet been submitted for publication. Dr. Dabeka hopes to collaboratively study this method within the next year.

Hydride Generating Techniques.—Associate Referee Stephen Capar reports that his laboratory has completed development of a method for hydride generation-AAS determination of Sn in foods, but he does not plan to collaboratively study the method at this time.

Mr. Capar is actively investigating the continuous flow hydride generation apparatus of Panaro and Krull (*Anal. Lett.* (1984) **17**(A2), 157–172). Mr. Capar is also continuing his review of the literature on the analytical methodology for As and hopes to prepare a review article on the subject within the next year.

Mercury.—Associate Referee Walter Holak reports that he has had several interface units fabricated for collaborators to use in studying the LC/AAS method for methyl mercury described in *Analyst* (1982)**107,** 1457–1461. Ten collaborators have agreed to participate in a study of the method. Before initiating a collaborative study, Mr. Holak intends to conduct an interlaboratory trial of the method.

Methyl Mercury in Fish and Shellfish.—Associate Referee Susan Hight reports that she initiated a collaborative study in June 1985 of the modifications, which were presented at last year's AOAC meeting, to the official first action method, **25.146–25.152.** Results have been received from about half of the collaborators and agree well with the expected values. When all the results are received, the data will be statistically evaluated and a report will be submitted to AOAC.

Two other methyl mercury methods were submitted to the General Referee for consideration for collaborative study. The first method, published by Theron James (*J. Assoc. Off. Anal. Chem.* (1983)**66,** 128–129), was evaluated by the Associate Referee. It was considered not to offer any significant advantage over the current official first action method, **25.146–25.152,** to warrant collaborative study. A second method, which was previously collaboratively studied under the auspices of the UK Analytical Methods Committee, was submitted by Derek Abbott for consideration for adoption by AOAC. The report of the collaborative study (*Analyst* (1977) **102,** 769–776) was evaluated by the General Referee and was found not to meet the minimum requirements for an AOAC collaborative study.

Multielement Analysis of Infant Formula by ICP.—The official first action method, **43.292–43.296,** should be adopted official final action and this topic should be terminated.

Multielement Determination After Closed System Digestion.—Associate Referee Walter Holak reports no progress

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on this topic during the last year because of his heavy involvement in other topics. The official first action method **25.001–25.007** should be continued as official first action.

Polarography.—Susan Hight has been appointed Associate Referee for this topic. She has developed specific modifications to the official first action method, **25.008–25.013,** for determination of Pb and Cd in foods when high levels of Sn are present. These modifications should be published in the open literature so that they are available to workers who are using the method. She reports that the method is being used in FDA laboratories with good results for analysis of raw agricultural products, baby foods, and canned and noncanned adult foods. Recoveries are generally in the 80–120% range and the relative standard deviation of results is generally <15%. The method should be continued as official first action.

Separation Techniques for Trace Elements in Foods.—John Jones has resigned as Associate Referee and recommends that this topic be terminated. The General Referee concurs with this recommendation.

Tin.—Edgar Elkins has not conducted any additional work in this area and has resigned as Associate Referee. He recommends that the official first action method **(25.A01–25.A04)** should be granted official final action status and that official first action method **25.161–25-163** be surplused. The General Referee feels that the former method should be continued as official first action for at least one more year and that the latter method should be surplused.

Recommendations

(1) *Arsenic in Animal Tissues and Meat Products by Atomic Absorption Analysis:* Collaboratively study the USDA hydride generation-AAS method for As in animal tissues and meat products.

(2) *Atomic Absorption Spectrophotometry (AAS):* Continue development of verification standards to be used in AAS collaborative studies. Continue work on consolidating AAS methods into a general analytical scheme.

(3) *Cadmium and Lead in Earthenware:* Adopt as official final action the hot leach AAS method, **25.016–25.023,** for determining extractable Cd and Pb in ceramic and enameled cookware.

(4) *Emission Spectrochemical Methods:* Initiate a collaborative study of the ICP method for multielemental analysis of foods.

(5) *Fluorine:* Investigate the improvements necessary to recollaborate the previously collaborated method (*J. Assoc. Off. Anal. Chem.* (1981) **64,** 1021–1026).

(6) *Graphite Furnace-Atomic Absorption Spectrophotometry:* Select and collaboratively study a graphite furnace-AAS method for Pb and Cd in foods.

(7) *Hybride Generating Techniques:* Conduct an interlaboratory trial of the hybride generation-AAS method for Sn in foods developed by the Associate Referee. Conduct a collaborative study of the method if appropriate.

(8) *Mercury:* Conduct an interlaboratory study of the LC-AAS method for mercury in foods (*Analyst* (1981)**107,** 1457–1461). Conduct a collaborative study of the method if appropriate.

(9) *Methyl Mercury in Fish and Shellfish:* Continue as official first action method, **25.146–25.152.** Evaluate the results of the collaborative study of the several improvements to the method.

(10) *Multielement Analysis of Infant Formula by ICP:* Adopt as official final action the ICP method for multielement analysis of infant formula, **43.292–43.296.** Terminate the topic.

(11) *Multielement Determination After Closed System Digestion:* Continue method **25.001–25.007** as official first action.

(12) *Polarography:* Conduct an interlaboratory trial of the modifications to method **25.008–25.015** for Pb and Cd in foods developed by the Associate Referee for foods containing high levels of Sn. Determine if these modifications should become a part of the official method. Continue method **25.008–25.015** as official first action.

(13) *Separation Techniques for Trace Elements in Foods:* Terminate this topic.

(14) *Tin:* Continue as official first action method **25.A01–25.A04** for Sn in canned foods. Surplus method **25.161–25.163** for tin in food.

Multiresidue Methods (Interlaboratory Studies)

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Five Associate Referees have reported on the status of their topics, including 3 which were transferred to this General Refereeship area during the past year from the Organohalogen Pesticides General Refereeship.

Comprehensive Multiresidue Methodology.—(Co-Associate Referees Leon Sawyer, FDA, Minneapolis, MN, and Jerry Froberg, FDA, Los Angeles, CA). Leon Sawyer published the results of the collaborative study which was initiated in late 1982 (*J. Assoc. Off. Anal. Chem.* (1985) **68,** 64–71) for the method adopted last year as official first action (**29.A01–29.A04**). Based on the long history of successful use of the method, even before collaborative study, it is recommended that the method be adopted official final action for the pesticides and crops studied: acephate, α-BHC, chlorpyrifos, dieldrin, monocrotophos, and omethoate in lettuce, strawberries, and tomatoes.

It is recommended that Leon Sawyer no longer continue as Co-Associate Referee and that the topic return to single Associate Referee status with Jerry Froberg. Future efforts will focus on extending applicability of the method to additional pesticides and nonfatty foods.

Fumigants.—(James Daft, FDA, Kansas City, MO). The Associate Referee has been active in the past year and will have substantial time in the coming year to devote to this topic. The purpose of this research is to modify and expand the AOAC acetone-soak method to include more fumigant residues and more food items. Mr. Daft reports that his tentative approach can detect 11 fumigant residues in whole grain samples by gas chromatography. These are methyl bromide, methylene chloride, carbon disulfide, chloroform, ethylene dichloride, methyl chloroform, carbon tetrachloride, trichloroethylene, chloropicrin, ethylene dibromide, and tetrachloroethylene. Alternate use of electron-capture (EBD) and Hall chloride-mode (HECD-Cl) detectors provides complete detection capability for chlorinated residues. Carbon disulfide is currently determined with ECD. Attempts will be made to extend coverage to an additional 4–5 fumigants, e.g., dichloropropene, propylene dichloride, dibromochloropropane, and dichlorobenzenes.

Manuscripts covering the purification of reagents and the preparation of mixed standards for multifumigant determination have been submitted to journals for publication. Another manuscript covering all of this work to date is being prepared for submission to the *Journal of the AOAC.*

Plans have been drawn for an intralaboratory study of this whole-grain procedure in its entirety. Interlaboratory and collaborative studies are planned to follow, using 3–4 residues of interest. Sample storage experiments are also under way, i.e., the documentation of incurred residue losses when grain samples are stored at room vs freezing temperatures.

Multiresidue Methodology Miniaturization.—(D. Ronald Erney, FDA, Detroit, MI). The recommendation last year was that the miniaturized version of method **29.001–29.018** be tested again in a limited interlaboratory situation before collaborative study. In last year's report, the Associate Referee presented interlaboratory data which showed considerable variability which resulted in the General Referee recommending the additional limited interlaboratory testing with participants following a precisely described method. The Associate Referee has had no time to follow through with last year's recommendation for the additional interlaboratory testing. It is hoped that time can be found in this coming year for this recommended work.

Low Moisture–High Fat Samples (Extraction Procedure).—(Leon D. Sawyer, FDA, Minneapolis, MN). During the past year the Associate Referee has investigated use of a Unitrex cleanup procedure for the oils extracted by the method in *J. Assoc. Off. Anal. Chem.* (1982) **65,** 1122–1128. It was hoped that a "total procedure" could be developed and collaborated which would minimize the large number of solvent/solvent partitioning steps that the current procedure requires.

Investigation of Unitrex cleanup has been completed and the Associate Referee has concluded that it is *not* an acceptable procedural substitute for this substrate. The oils isolated from the various seed types possess differing physical and chemical properties; differences are great enough that uniform thermal parameters are impossible to establish. Using oils fortified with the 3 major isomers (alpha, beta, and gamma) of BHC, aldrin, chlorpyrifos, heptachlor epoxide, dieldrin, and endrin and 1 g samples of oil, the following table summarizes the results of this investigation:

Oil Type	*Range of Recoveries*	*mg Lipid Isolated/ g Oil*
Soybean	50% α-BHC—100% aldrin	1
Peanut	90% chlorpyrifos—104% β-BHC	3
Cocoa	64% endrin—97% chlorpyrifos	85
Mustard	5% aldrin—53% endrin	170
	(many EC interferences)	
Cottonseed	Too many EC interferences for any recovery information	65

[Note: The above table illustrates that the Unitrex cleanup was totally successful only with the peanut oil, which is commonly used in high temperature cooking applications because of its thermal stability.]

The Associate Referee recommends that other cleanup steps be investigated such as LC/silica column. The Associate Referee has no personal time planned to work on this particular project in the forthcoming year. However, his laboratory unit has plans to investigate these commodity types with an LC system and close monitoring of the results by the Associate Referee is anticipated.

Organophosphorus Pesticide Residues.—(Ronald Laski, FDA, Buffalo, NY). Ronald Laski will not have time to devote to this topic in the laboratory during the coming year. He will, however, remain as Associate Referee to coordinate and accumulate information being generated actively by other chemists in the 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, **29.054–29.058,** and on the extension of the coverage of this method to additional organophosphorus pesticides and crops. In the future, he will also attempt to compile and summarize available recovery data for organophosphorus pesticide residues by this method and to develop recovery data for additional organophosphorus pesticides and metabolites.

Recommendations

(1) *Comprehensive Multiresidue Methodology:* Adopt **29.A01–29.A04** as official final action for the pesticides and crops covered by the method.

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Residues and adopted by the Association. See the report of the committee and "Changes in Methods," this issue.

(2) *Fumigants:* Proceed with finalization of written method and conduct intralaboratory study of the proposed method for fumigants in whole grains and with collaborative study if the interlaboratory study is successful.

(3) *Multiresidue Methodology, Miniaturization:* Proceed with the limited interlaboratory retesting of the precisely written method before conducting a collaborative study in the longer-range future.

(4) *Low Moisture–High Fat Samples (Extraction Procedure):* Continue to evaluate alternative cleanup approaches for simplifying the published extraction method.

(5) *Organophosphorus Pesticide Residues:* Continue study to expand and extend **29.054–29.058** as outlined in this report.

Organohalogen Pesticides

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Chlordane.—(Wilbur Saxton, FDA, Seattle, WA) The Associate Referee has continued to work with the temperature-programmed capillary gas chromatographic determinative step which separates all the major components and metabolites of chlordane so that they can be individually identified and quantitated. The Referee plans to continue this study in the upcoming year by performing recovery experiments on the components and metabolites of chlordane. The method will include the Referee's determinative step and the previously recommended extraction and cleanup method.

Chlorinated Dioxins.—(David Firestone, FDA, Washington, DC) The Associate Referee prepared an excellent literature survey on the continuing development of analytical methods for the chlorinated dioxins, both the 2,3,7,8-tetrachlorodioxin and the higher chlorinated dioxins. Most of the various methods being developed still require time-consuming effort because of the very low levels of analyte that are of interest (parts-per-trillion). Several of the methods, however, now involve the use of 2-level mass spectrometry (MS/MS) for determination of the residues. It is hoped that this approach will permit the extraction and cleanup steps of methods to be simplified, since the MS/MS instruments are capable of providing greater specificity in detecting particular residues, even in the presence of some sample co-extractives which have hindered determination in the past.

The report from the Associate Referee mentions only one method's having been subjected to interlaboratory study. EPA method 613 was used by 11 laboratories to measure 2,3,7,8-TCDD in water, in a study run by F. D. Hileman and colleagues, Monsanto Research Corp., Dayton, OH, under the sponsorship of EPA. Six laboratories were successful, one did not follow the method as requested, and 4 experienced difficulties with various aspects of the method. No recoveries or statistical evaluation from this study was included in the report to the General Referee.

The Associate Referee and others have prepared a compilation of the results of FDA analyses of fish and other foods for dioxin residues. This paper was presented at the 189th National Meeting of the American Chemical Society, April 28–May 3, 1985, in Miami, FL, during the "Symposium on Chlorinated Dioxins and Dibenzofurans in the Total Environment III." The paper will appear as a chapter in the book documenting the proceedings of this symposium when it is published in 1986.

The Associate Referee recommends continued monitoring of the development of methods for dioxins and furans in food and other environmental samples. He will encourage developers to validate the methods by interlaboratory testing and eventual collaborative study.

Chlorophenoxy Alkyl Acids.—(Vacant) Chlorophenoxy alkyl acids, especially 2,4-D, are still in wide use, therefore, this topic is still of considerable interest to AOAC.

Ethylene Dibromide.—(Leon D. Sawyer, FDA, Minneapolis, MN) The Associate Referee has collaborated a method for the analysis of ethylene dibromide in whole grains, intermediate grain products (such as flour, cornmeal, etc.), and baked goods. The method involves extraction of the whole grain and intermediate products by the same acetone–water soaking procedure used in the AOAC official method for fumigants, **29.071–29.072.** The ready-to-eat baked goods are extracted with a modification of the co-distillation method of Rains and Holder (*J. Assoc. Off. Anal. Chem.* (1981) **64,** 1252–1254). In all cases, a portion of the extract is dried and analyzed by gas chromatography with electron capture detection. The Referee conducted a preliminary interlaboratory study, with 2 laboratories, to test the shipping and handling conditions he intended to use in the collaborative study. That test showed that the volatile ethylene dibromide analyte could be added to a sample and not be lost when the sample was sealed and then shipped in dry ice. The interlaboratory test also provided additional validation that the method was suitable for collaborative study.

Nine laboratories participated in the collaborative study. All 3 of the grain-type products were involved; samples with incurred residues and samples fortified with ethylene dibromide before shipping were both included. Each product type was represented by 2 different sample types, and blind duplicates were included in the study so that intralaboratory repeatability could be measured.

Ranges of mean recoveries and interlaboratory relative standard deviations for the 3 product types are as follows: 96.8–99.2% recoveries and 5.7–8.4% RSD_x for whole grains fortified at 104–1738 ppb; 92.4–101.4% recoveries and 6.2–12.8% RSD_x for intermediate products fortified at 34.8–156 ppb; and 85.2–103.4% recoveries and 13.4–20.1% RSD_x for ready-to-eat products fortified at 4.35–69.6 ppb. The precision of the quantitation of the incurred residues was similar to the RSD_xs found in the fortification studies.

The results of this study are excellent and show that the method is capable of reproducibly measuring ethylene dibromide in a variety of products. The Associate Referee has recommended that the method be adopted official first action.

Ethylene Oxide and Its Chlorohydrin.—(Vacant) A. R. Stemp, former Associate Referee, resigned his position this year. At the request of the General Referee, the vacancy was advertised in the September 1985 issue of *The Referee,* in the hope that a new Referee can be found to fill the position.

Gel Permeation Chromatography (GPC) Cleanup for Organochlorine Residues.—(Tim Spurgeon, ABC Laboratories, Columbia, MO) The Associate Referee has been unable to spend much time this year on the planned work of collaborating the GPC method, **29.037–29.043,** for polychlorinated biphenyls in meat and poultry. He hopes to be able to pursue the effort in the upcoming year. He is trying to locate a source of these products containing incurred residues of PCBs for use in the study.

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Residues and adopted by the Association, except adoption of the method for pentachlorophenol in animal and poultry tissues. See the report of the Committee and "Changes in Methods," this issue.

Methyl Bromide.—(Vacant) This refereeship was established in 1984 because there was considerable interest at the time in the expected use of methyl bromide as a fumigant to replace other banned fumigants such as ethylene dibromide. In the meantime, studies done under the auspices of the Grocery Manufacturers of America and others appear to indicate that residues are unlikely to occur with methyl bromide, because of its extreme volatility. No Referee has been appointed because of the waning concern. The topic is still of potential interest, however, if the regulatory concern over methyl bromide residues revives.

Pentachlorophenol.—(George Yip, FDA, Washington, D.C.) The Associate Referee continued work to find a suitable gas chromatographic column for free pentachlorophenol. Ten different column packings were tried, with unsatisfactory results from all. An intralaboratory study using one of the column packings, 2% diethylene glycol sebacate, for determination of pentachlorophenol in eggs, milk, fish, and gelatin produced results that convinced the Referee to reconsider methylation of pentachlorophenol to its anisole for improved gas chromatography. None of the methylation techniques used thus far is completely satisfactory. The Referee is now attempting to include a methylation step at an early stage of the procedure. The extract could then be cleaned up after the methylation step to remove interfering background peaks. The Referee will continue development and will collaborate a method in the upcoming year if a suitable method is found.

Pentachlorophenol in Animal and Poultry Tissue.—(Douglas Gillard, U.S. Environmental Protection Agency, Washington, D.C.) The Associate Referee has submitted a manuscript describing the collaborative study previously run on a method for pentachlorophenol in chicken, pork, and beef liver. The method tested was that used by the U.S. Department of Agriculture, Food Safety and Inspection Service, and published in their Chemistry Division Guidebook as Section 5.015. The method consists of digesting liver with 12M sulfuric acid and extracting pentachlorophenol from the digestate with cyclohexane. The extract is further cleaned up by additional treatment with concentrated sulfuric acid, and the cyclohexane layer is then examined by gas chromatography for pentachlorophenol. An internal standard of pentabromoethylbenzene is added to the sample after the digestion but before the digestate is extracted with cyclohexane. Gas chromatography is performed on a column of 1% SP-1240DA with detection by a ^{63}Ni electron capture detector. Residues are quantitated by comparison to a standard curve which plots the ratio of peak heights of equal amounts of pentachlorophenol and pentabromoethylbenzene vs ppb pentachlorophenol. Known amounts of pentachlorophenol and pentabromoethylbenzene are mixed with blank sample matrix (extract) for preparation of the standard curve. This is done because the presence of sample matrix enhances the chromatography, and this step ensures that the analyte chromatographs as well in the standard solution as in the actual sample extract.

Five laboratories participated in the study. Blind duplicate samples of each liver, each fortified at 3 different concentrations, were analyzed by the participants. Liver samples containing incurred residues of pentachlorophenol were also included. Pentachlorophenol fortifications ranged from 50 to 500 ppb, and incurred residues were determined to be 36–129 ppb. Mean recoveries and interlaboratory relative standard deviations ranged from 87.1 to 94.4% recovery and 9.8 to 16.5% RSD_x for chicken liver fortified at 50–250 ppb; 79.4–92.9% recovery and 11.8–14.0% RSD_x for swine liver fortified at 100–500 ppb; and 71.2–80.4% recovery and 9.7–14.6% RSD_x for bovine liver fortified at 100–500 ppb. Measurements of interlaboratory precision for the samples with incurred residues were within the same ranges.

The Associate Referee recommends that the method be adopted official first action for the determination of pentachlorophenol in liver.

The Associate Referee has changed jobs and will be resigning from this refereeship. If the method is adopted, there may be no more need for this topic to continue. The advice of the Committee on this decision is sought.

Polychlorinated Biphenyls.—(Leon D. Sawyer, FDA, Minneapolis, MN) The Associate Referee is closely following the new developments in PCB analysis, especially those involving analysis for individual congeners. It has been shown that the toxicological effects vary considerably among the congeners. Currently, efforts are being made to develop analytical techniques which will provide a report of levels of the more toxic congeners in samples. Individual congener analysis is being developed at EPA's Large Lakes Research Station, Grosse Ille, MI, where the analysis of Great Lakes fish containing PCBs is a high priority. Researchers from this laboratory and their colleagues from the University of Guelph, Ontario, Canada, and Texas A&M University, College Station, TX, have published literature on the development of reference materials of the individual congeners (Mullin et al., *Environ. Sci. Technol.* (1984) **18,** 468–476), and on the application of the technique to human milk samples (Safe et al., *J. Agric. Food Chem.* (1985) **33,** 24–29). Capillary gas chromatography is used in this analysis.

The Associate Referee will attempt to duplicate this work in his laboratory, and will make comparisons between the results produced by this determinative technique and those of the existing AOAC official method.

Polychlorinated Biphenyls (PCBs) in Blood.—(Virlyn Burse, Centers for Disease Control, Atlanta, GA) The Associate Referee has begun a collaborative study of the method for PCBs in blood, published as CDC Laboratory Update 81-108, *Polychlorinated Biphenyl Determination at Parts-Per-Billion Level in Serum.* Because the method involves the use of silica gel for cleanup, and adsorbents are often known to be the source of non-reproducible results, the first stage of the collaborative study was a check on the lots of silica gel purchased by the collaborators.

Thus far, results have been received from 6 of the 10 collaborators on the silica gel phase of the study. Organic extracts of serum, fortified with Aroclor 1254, were eluted from silica gel and measured by the collaborators. Not all of the collaborators were able to quantitate all peaks identified for analysis by AOAC method **29.018** (Table 29:02) because of poor resolution or interferences from other compounds. Results of 3 silica gel checks, by each of the 6 laboratories who reported, range from 63.4 to 125.0% recovery of the Aroclor 1254. Average recovery was 87.1% and interlaboratory relative standard deviation was 14.80%, according to the statistical analysis performed by the Associate Referee.

The second stage involves analysis of serum fortified with PCBs, and also serum containing in vivo PCBs and further fortified with pesticides. Phase 2 of the study is currently under way. The Associate Referee is presenting a report on the results he has received thus far during the 1985 fall meeting of the AOAC. Once he has received all the results from collaborators, he will submit his report for interim approval.

Tetradifon, Endosulfan, and Tetrasul.—(Lawrence R. Mitchell, FDA, Atlanta, GA) No report was received from the Associate Referee this year.

Toxaphene.—(Larry Lane, Mississippi State Chemical Laboratories, Mississippi State, MS) No report was received from the Associate Referee this year. The Referee has been unable for the past 2 years to make progress on this topic because of a change in job status. In the meantime, most uses of toxaphene have been cancelled. Residues of toxaphene are expected to remain in the environment for some time because of the extensive use of it in the past. However, it can be expected that residues will decline with time, so that by the time research and testing on this topic could be completed, there may no longer be a need for a quantitation technique for the residues of toxaphene. The more important current interest in toxaphene involves the identity of possible

residues of toxaphene in Great Lakes fish. This research area is not one of method development at this time, however, so does not seem to come under the direct interest of this committee.

Recommendations on Associate Refereeships

(1) Perform recovery experiments, as planned, on the components and metabolites of chlordane through the recommended method. If results are satisfactory, plan an interlaboratory study, preliminary to a future collaborative study, to test the interlaboratory application of the method to the analysis of residual chlordane. The method to be tested consists of extraction, **29.011–29.012;** acetonitrile partitioning cleanup, **29.014;** Florisil column chromatographic cleanup and residue separation, **29.046–29.048;** and the capillary column GLC determinative step developed by the Associate Referee. This study should include analysis of residues of *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor, 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-tetrachloro-dioxin, and for hexachloro-, heptachloro-, and octachloro-substituted dibenzo-*p*-dioxins and dibenzofurans in foods. Continue to evaluate methods toward the ultimate goal of establishing one as an official method through AOAC collaborative procedures.

(3) Appoint a new Associate Referee for Chlorophenoxy Alkyl Acids.

(4) Adopt as official first action the method collaborated by the Associate Referee on Ethylene Dibromide. Certain changes in the manuscript have been recommended to the Referee, most involving changing the statistical values in the tables to those developed by the Statistical Consultant; this will also require changes in a few paragraphs of the text. When these changes are made, the manuscript of the study should be published.

Since all food uses of ethylene dibromide have been banned as of September 1985, it is unlikely that methods will be needed in the future for this fumigant in other product types. It is recommended that the Associate Referee continue in this position for at least one more year, to provide consultation as needed. After that, it may be determined that this topic is no longer necessary.

(5) Find and appoint a new Associate Referee for Ethylene Oxide and Its Chlorohydrin. The new Referee should study the GC method of Scudamore and Heuser (*Pestic. Sci.* (1971) **2,** 80–91) for determining ethylene oxide, ethylene chlorohydrin, and ethylene bromohydrin in foods.

(6) Collaboratively study the use of method **29.037–29.043** for analyzing poultry, beef, and swine fat for PCBs.

(7) Maintain the refereeship on methyl bromide; advertise to fill the position so that a focal point on methodology for this compound will be available.

(8) Continue work on developing a method for analysis of pentachlorophenol in eggs, fish, milk, and gelatin; plan and execute a limited interlaboratory study of the method before planning a collaborative study.

(9) Adopt as official first action the method collaborated by the Associate Referee for Pentachlorophenol in Animal and Poultry Tissue. Certain changes in the manuscript have been recommended to the Referee, and when these are finalized, the manuscript should be published.

(10) Study the application of the individual congener analysis of PCB residues and report on the comparison of results produced by that technique and by the official AOAC technique.

(11) Complete the collaborative study of the method for PCBs in blood serum as planned. Report the results for consideration for interim official action.

(12) Complete the intralaboratory trials on **29.044–29.049,** the method for tetradifon, endosulfan, and tetrasul, to validate the use of this method on all the nonfatty foods for which **29.001–29.018** is official.

(13) Discontinue the topic on toxaphene.

Other Recommendations

(14) Adopt as official final action the official first action status of the GPC method, **29.037–29.043,** for 15 organochlorine pesticides in beef and swine fat. The method already has official final action status on poultry fat.

(15) Continue the first action status of the method for pentachlorophenol in gelatin, **29.A14–29.A18.** Questions still exist on the gas chromatography step of this method, and the Referee is continuing to work on this area.

(16) Based on the inadequacy of the number of samples involved in the studies submitted by Derek Abbot to AOAC (*Analyst* (1979) **104,** 425–433, and *Analyst* (1976) **101,** 386–390), do not adopt these methods as official first action. These methods, for organochlorine pesticides in animal fats and eggs, and for inorganic bromide in grain, respectively, had been studied by committees of the United Kingdom Ministry of Agriculture, Fisheries and Food. Interlaboratory protocols for the studies did not conform to those required of AOAC collaborative studies, in terms of number of samples, multiple levels of analyte, and inclusion of blind duplicates for measuring intralaboratory repeatability. These studies represent useful preliminary work but do not justify adopting the methods as official.

Organonitrogen Pesticides

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Associate Referee reports on 6 topics were received. An additional 11 topics are vacant and require Associate Referees to conduct collaborative work.

Anilazine.—An Associate Referee is required to select and study a method for anilazine residues in food crops.

Benzimidazole-Type Fungicides.—Associate Referee Mikio Chiba reports that conditions necessary to degrade benomyl in crops to BBU (1-(2-benzimidazolyl)-3-*n*-butyl urea) quantitatively also degrades carbendazim to 2-aminobenzimidazole. An LC system consisting of a C-18 reverse phase column and an aqueous acetonitrile mobile phase was found to produce the best separation. Further work is required to improve the cleanup of 2-aminobenzimidazole, since problems have been experienced with interferences.

Captan and Related Fungicides.—Associate Referee Dalia Gilvydis reports that the collaborative study protocol for captan, captafol, and folpet has been changed to reflect current tolerances for these compounds. Three crops, tomato, cucumber, and cauliflower, have been chosen for spiking at concentrations ranging from 0.2 to 25 ppm. Application of the method to tomatoes fortified with 25 ppm of the 3 fungicides yielded satisfactory recoveries. The collaborative study will proceed upon approval of the statistical design and completion of instructions to collaborators.

Carbamate Herbicides.—An Associate Referee is required to select and test an analytical method for carbamate herbicides.

Carbamate Insecticides, Liquid Chromatographic Methods.—Associate Referee Richard Krause reports that since no adverse reports of the official first action method, **25.A05–25.A13,** have been received, he recommends it be approved for official final action.

Carbofuran.—An Associate Referee is required to develop and test collaboratively an analytical method for carbofuran and its phenolic and carbamate metabolites in foods.

Chlorothalonil.—An Associate Referee is required to select an analytical method for chlorothalonil and subject it to collaborative study.

Daminozide.—Associate Referee Hafez Abdel-Kader reports that intralaboratory trials of the GC method for daminozide are still in progress. Mean recoveries from apples and apple juice fortified with 0.05–5 ppm were 90–95%. An experimental protocol is being prepared.

Dinitro Compounds.—Associate Referee Richard Krause reports that 33 organonitro compounds including nitrophenol, nitroaniline, and nitrobiphenyl ether pesticides have been studied for separation by LC and for their electrochemical reduction characteristics. All but 5 pairs were separated adequately using a gradient mobile phase. Study of the LC separation with electrochemical detection at the nanogram level is continuing.

Diquat and Paraquat.—Associate Referee Brian Worobey reports that development of a method for diquat and paraquat has been completed with recoveries of 80–98% being obtained over a spiking range from 0.05 to 1.0 ppm of both diquat and paraquat. Coefficients of variation were 8.3% or less. Instructions to collaborators are being prepared and a collaborative study will be initiated when the statistical design is approved.

Dithiocarbamates, General Residue Method.—A collaborative study of a headspace method for dithiocarbamate residues on lettuce was carried out by the Panel on Determination of Dithiocarbamate Residues, Ministry of Agriculture Fisheries and Food (*Analyst* (1981) **106,** 782) and submitted for review.

Maleic Hydrazide.—An Associate Referee is required to study a method for the determination of maleic hydrazide in crops.

Organo-Tin Fungicides.—An Associate Referee is required to study a method for fenbutatin, triphenyltin, and cyhexatin residues.

Sodium o-Phenylphenate.—An Associate Referee is required to test a specific method for *o*-phenylphenol in foods.

Substituted Ureas.—Associate Referee Ronald Luchtefeld reports that he has developed a method for phenylurea herbicides which involves extraction with methanol, partition into methylene chloride, Florisil column cleanup, and determination by LC with post-column photodegradation and spectrofluorometry. Recovery studies are presently being conducted.

Thiolcarbamate Herbicides.—An Associate Referee is required for this topic.

s-Triazines.—An Associate Referee is needed to conduct a collaborative study of a method for atrazine and cyanazine residues.

Trifluralin.—An Associate Referee is required to conduct a collaborative study of trifluralin analysis by existing multiresidue methodology.

Recommendations

(1) *Anilazine.*—Appoint an Associate Referee to select an appropriate method for anilazine in food crops and subject it to collaborative study.

(2) *Benzimidazole-Type Fungicides.*—Continue study of a method for benomyl and carbendazim by alkaline degradation to BBU and 2-aminobenzimidazole, respectively. Establish a cleanup procedure for the resulting 2-aminobenzimidazole which permits accurate and reproducible determination of carbendazim.

(3) *Captan and Related Fungicides.*—Complete instructions to collaborators and initiate a collaborative study.

(4) *Carbamate Herbicides.*—Appoint an Associate Referee to carry out an interlaboratory study.

(5) *Carbamate Insecticides, Liquid Chromatographic Methods.*—Adopt the method for *N*-methylcarbamates, **25.A05–25.A13,** as official final action.

(6) *Carbofuran.*—Appoint an Associate Referee to study a method for carbofuran and its carbamate and phenolic metabolites.

(7) *Chlorothalonil.*—Appoint an Associate Referee to evaluate methods for chlorothalonil and conduct a collaborative study of the procedure selected.

(8) *Daminozide.*—Submit a description of the analytical procedure and precision data to the General Referee for review and comment. Prepare instructions for collaborators and obtain approval of statistical design.

(9) *Dinitro Compounds.*—Develop data on recovery, precision, and detection limits of nitroaniline pesticides when added to foods at various levels.

(10) *Diquat and Paraquat.*—Initiate a collaborative study of the Associate Referee's method for diquat and paraquat in potatoes.

(11) *Dithiocarbamates, General Residue Method.*—Continue study of a method capable of determining dialkyl and bisdithiocarbamates as distinct entities. Do not adopt headspace method as submitted because of failure to meet minimum requirements of AOAC, such as use of blind duplicates.

(12) *Maleic Hydrazide.*—Appoint an Associate Referee to study a GC or LC method.

(13) *Organo-Tin Fungicides.*—Appoint an Associate Referee to study methods for fenbutatin, triphenyltin, and cyhexatin.

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The recommendations of the General Referee were approved by the Committee on Residues and adopted by the Association. See the report of the committee and "Changes in Methods," this issue.

(14) *Sodium o-Phenylphenate.*—Appoint an Associate Referee. Continue study.

(15) *Substituted Ureas.*—Prepare and submit an experimental protocol and recovery data to the General Referee for review and comment. Prepare instructions to collaborators and obtain approval of the statistical design before initiation of the collaborative study.

(16) *Thiolcarbamate Herbicides.*—Appoint an Associate Referee to study methods for thiolcarbamate herbicides.

(17) *s-Triazines.*—Appoint an Associate Referee. Examine application of existing methods to a collaborative study of atrazine and cyanazine in foods.

(18) *Trifluralin.*—Appoint an Associate Referee to determine trifluralin by existing multiresidue procedures and subject the method to interlaboratory trials.

Organophosphorus Pesticides

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The General Referee has monitored the published literature for analytical methods for organophosphorus pesticide residues. Acephate (1) residues were determined in the rind and pulp of oranges by gas chromatography (GC) with nitrogen phosphorus detector (NPD). Residues were extracted with ethyl acetate and cleaned up on Florisil with elution of acephate and methamidophos by methanol–acetone (1 + 4). Frank et al. (2) reported the determination of acephate and methamidophos residues in beans, carrots, celery, lettuce, peppers, potatoes, strawberries, and tomatoes. Residues were extracted with acetone; the extract was partitioned with hexane and the residues were partitioned from the aqueous phase with ethyl acetate. No further cleanup was required before GC determination with flame photometric detector (FPD). Acephate and methamidophos (3) residues were extracted from mouse liver with acetonitrile–methanol (1 + 1) before cleanup on a Nuchar carbon–Whatman cellulose column. Following elution with methanol, residues were determined by GC-FPD. Acephate and methamidophos residues were determined in biological samples (blood, brain) (4) and snow peas (5) by GC–mass spectrometry (MS).

James et al. (6) reported a method for chlorpyrifos and its oxygen analog in greenhouse vegetables by GC with electron capture detection (ECD). Residues were extracted with acetone and partitioned into hexane. Extracts from dark green vegetables were cleaned up with carbon and silica gel while light green vegetables were cleaned up on disposable cartridges. Sherma and Slobodien (7) published a quantitative thin layer chromatographic (TLC) method for chlorpyrifos and its metabolite 3,5,6-trichloro-2-pyridinol in tap water and bananas. Dimethoate and dimethoxon residues were determined in asparagus (8) by GC-NPD following ethyl acetate extraction and cleanup on a Nuchar carbon-cellulose column. Residues were eluted with 20% methanol in ethyl acetate. Goodwin et al. (9) reported the determination of dimethoate residues in strawberries following extraction with acetonitrile, partitioning into chloroform, replacement of the chloroform with hexane, and estimation by GC with specific thermionic detector.

Disulfoton and its oxidation products were extracted from fish (10) with aqueous acetonitrile, partitioned into ethyl acetate, and, following removal of ethyl acetate, partitioned between hexane and acetonitrile. After removal of the acetonitrile, residues were dissolved in acetone for determination of disulfoton and its sulfone by GC-FPD. For the measurement of disulfoton sulfoxide and its oxygen analog, the acetone solution was oxidized with potassium permanganate before GC determination. Holmes et al. (11) determined fenitrothion residues in fish by GC with alkali flame ionization detector (AFID). Residues were extracted with ethyl acetate, partitioned between acetonitrile and hexane, and further cleaned up on an activated charcoal–celite column with elution of residues with benzene–ethyl acetate (4 + 1). Krause (12) reported peculiarities in the GC analysis of fenamiphos sulfone when determining total fenamiphos residues by oxidation of fenamiphos and the sulfoxide to the sulfone. He found that Carbowax and Reoplex columns appeared to reduce the sulfone partly to the sulfoxide and recommended a DC-200 column for the analysis of fenamiphos sulfone. Fenthion (13) residues were determined in samples from the estuarine environment by GC-NPD. Water was extracted with petroleum ether. Tissues and sediments were extracted with acetonitrile and residues partitioned into hexane. Cleanup was done on a deactivated silica gel column, and residues were eluted with 5% ethyl ether in hexane.

Newton et al. (14) reported procedures for the determination of glyphosate residues in forest brush field ecosystems. Samples were extracted with deionized water and residues were eluted through anion-exchange resins, Duolite A101D for soil, stream sediment, streamwater, and mammals and AG1-X8 for foliar substrates and fish. Further cleanup and separation of glyphosate, (aminomethyl)phosphonic acid, and *N*-nitrosoglyphosate was done on a cation-exchange column, Bio-Rad AG50W-X8. Residues of glyphosate and (aminomethyl)phosphonic acid were determined by GC-FPD in fish and by liquid chromatography (LC) with postcolumn ninhydrin detection in all other substrates. *N*-Nitrosoglyphosate residues were determined in all substrates by LC with a Griess reaction postcolumn detection system. A differential pulse polarography method for glyphosate in crops and soils was described by Friestad and Bronstad (15). Crops were extracted with aqueous methylene chloride and the aqueous phase mixed with a cation-exchange resin, Dowex 50-X8. Soils were extracted with 0.5N KOH. Residues from crops or soil were cleaned up on an anion exchange resin, Dowex 1-X8, and eluted with 1N HCl.

Cairns et al. (16) used GC with chemical ionization MS to characterize isofenphos and 3 of its metabolites. This technique was used to confirm residues of isofenphos in alfalfa pellets.

Monocrotophos and quinalphos (17) residues were extracted from bittergourd fruits with chloroform and cleaned up on charcoal–Celite 545–neutral aluminum (2 + 2 + 1). Residues were eluted with chloroform and determined colorimetrically with 4-(*p*-nitrobenzyl)pyridine reagent. Methyl paraoxon was determined in plasma by LC with UV detector (18). Residues were extracted with methylene chloride, evaporated to dryness, and dissolved in acetonitrile for chromatography.

Total phorate residues were determined in soil and plant samples colorimetrically with 4(*p*-nitrobenzyl)pyridine reagent (19, 20). Soils were mixed with ammonia solution and further mixed with sodium sulfate, Florisil, and activated charcoal (1 + 1 + 1). The mixture was placed in a column that had already been packed with an adsorbent mixture of Hyflo-super Cel, activated charcoal, sodium sulfate, and magnesium oxide (1 + 1 + 1 + 1). Plant samples were extracted

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with dichloromethane and extracts passed through a column of sodium sulfate, charcoal, and Hyflo-super Cel (2 + 1 + 1). Residues were eluted from the cleanup columns with dichloromethane. Pirimiphos-methyl residues (21) were determined in citrus fruit by GC-NPD. Residues were extracted with acetone–hexane (1 + 4) and cleaned up on Florisil. Pak-column, and eluted with hexane–diethyl ether (5 + 1). A method (22) for pirimiphos-methyl and some of its degradation products in dates was reported. Residues were extracted with acetone–hexane (1 + 4) and cleaned up on Florisil, Pirimiphos-methyl was eluted with diethyl ether–hexane (3 + 7) and the oxygen analog, 2-ethylamino-6-methylpyrimidin-4-yl-dimethylphosphorothioate, and 2-ethylamino-4-hydroxy-6-methylpyrimidine were eluted with acetone–diethyl ether (1 + 9) with determination by GC with specific nitrogen detection.

Saeed and Abu-Tabanja (23) compared the purge-and-trap method of Dumas (24) and the sulfuric acid treatment method of Nowicki (25) for the determination of phosphine residues. They found that the rate of desorption of phosphine by the purge-and-trap method to be much slower and also reported that the 30 min purge suggested by Dumas to be inadequate for complete recovery of phosphine. Phosphine fumigated samples of apricots, figs, beans, walnuts, apricot pulp, and dates were analyzed by the 2 methods and much higher residues were reported by the sulfuric acid treatment method. Al-Omar and Al-Bassomy (26) reported the use of Nowicki's method for the determination of phosphine residues in dates.

Temephos residues were determined in rat plasma by LC following extraction with ethyl acetate (27).

Stijve (28) reported a multiresidue method for organophosphorus pesticides in milk and milk products. Milk was extracted with chloroform, the chloroform was evaporated, and the residue was dissolved in light petroleum. A portion was taken for cleanup of dicrotophos and dimethoate residues on a Celite 545 column. Dimethoate was eluted with light petroleum–methylene chloride (4 + 1) and dicrotophos with light petroleum–methylene chloride (2 + 1). Butter and cheese fat were dissolved in light petroleum. Residues were partitioned into acetonitrile and then into light petroleum–methylene chloride (4 + 1) and determined by GC with thermionic or FPD. Recoveries were given for 18 organophosphorus pesticides.

A report (29) was published on results of a collaborative exercise on the determination of some organophosphorus pesticide residues in grain. The method that was developed and tested was based on extraction of residues with acetone–water (8 + 1), partitioning into dichloromethane, and cleanup on a chromatographic column of silica gel–activated charcoal (15 + 1). Residues were eluted with acetone–toluene–dichloromethane (1 + 1 + 5) and determined by GC with a phosphorus specific detector. The method was considered to provide an effective cleanup procedure and the mean overall recovery of 8 pesticides (etrimfos, chlorpyrifos-methyl, malathion, bromophos, methacrifos, dimethoate, pirimiphos-methyl, fenitrothion) analyzed by 14 laboratories was 74%. Sawyer (30) reported a successful collaborative study of the Luke et al. method for multipesticide residues. Four organophosphorus pesticides (chlorpyrifos, acephate, omethoate, monocrotophos) were studied in lettuce, tomatoes, and strawberries.

Matsumoto (31) reported the use of gradient silica gel dry column chromatography for the recovery of 40 organophosphorus pesticides. The elution patterns using 2 kinds of solvent systems, hexane, benzene, and acetone and benzene and acetone, were given. A multiresidue method (32) for 25 organophosphorus pesticides in honey bees by capillary column GC with ECD was described. Residues were extracted with acetone and cleaned up on a silica gel–charcoal (15 + 1) column with elution by dichloromethane–acetone–toluene (5 + 1 + 1). Residues of 9 organophosphorus compounds were determined in soils and sediments by GC-NPD (33). Samples were acidified with HCl and Soxhlet extracted with acetone–hexane (4 + 1). Following partitioning into methylene chloride, cleanup was done on a column of activated carbon–magnesium oxide–Celite 545 (1 + 4 + 8) with residues eluted by ethyl acetate–acetone–toluene (1 + 1 + 2). A droplet counter-current chromatography method (34) for the separation of contaminants from lipids in fish samples was developed for food contamination monitoring by GC-MS. Diazinon, EPN and fenitrothion were included in the pesticide contaminants studied.

Priebe and Howell (35) developed a post-column reaction detection system for the LC determination of organophosphorus compounds, based on their photodegradation to orthophosphate followed by the formation of reduced heteropolymolybdate. The determination of dylox, dimethoate, and dichlorvos in tomatoes demonstrated the potential of the methodology.

Stan and Goebel (36) evaluated automated splitless and manual on-column injection techniques using capillary GC for pesticide residue analysis. Fourteen organophosphorus pesticides were included in the study. Daft (37) reported relative retention time data for organochlorine and organophosphorus pesticides on a mixed-bed column of 3% OV-225/5% OV-101 (1 + 1).

Permethrin residues were determined in lake water and sediments by GC-ECD (38). Residues were extracted from water with hexane and from sediment with 20% acetone–hexane in a Soxhlet extractor. Extracts were cleaned up on a Bio-Sil A column and residues were eluted with 2.5% diethyl ether in hexane. Belanger et al. (39) reported a method for the determination of cypermethrin, deltamethrin, fenvalerate, and permethrin in apples. Residues were extracted with acetone, partitioned into hexane, cleaned up on a Florisil column with elution by 10% diethyl ether in hexane, and determined by GC-ECD.

A brief summary of each Associate Referee topic follows:

Confirmation Procedures.—(Bill Lee, Environment Canada, Burlington). The Associate Referee reports no progress on the topic this year. There appears to be little interest by residue analysts in chemical derivatization for the confirmation of residues. It is doubtful if sufficient collaborators could be found to carry out the recommended collaborative study. The Associate Referee recommends that the GC-MS approach be investigated for the confirmation of residues.

Disulfoton.—(S. Szeto, Agriculture Canada, Vancouver). The Associate Referee has been newly appointed to study analytical methods for disulfoton and its metabolites in foods.

High Fat Samples.—(R. Scharfe, Agriculture Canada, Ottawa). The Associate Referee did not report this year.

Phorate.—(Jim Devine, American Cyanamid Co., Princeton). The Associate Referee has been recently appointed to study analytical methods for determining phorate and its metabolites in foods.

Phosphine.—(T. Dumas, Agriculture Canada, London). The Associate Referee has resigned.

Sweep Codistillation.—(B. Luke, State Chemistry Laboratory, East Melbourne, Australia). The Associate Referee reported the results of interlaboratory studies carried out in Australia utilizing the Unitrex cleanup system. In one study meat fat samples were fortified with bromophos-ethyl at the 0.6 and 0.8 ppm levels. These samples were analyzed by 5 laboratories. Mean recoveries were 90 and 85% with coefficient of variation (CV) of 11.6 and 14.4%, respectively, for 0.6 and 0.8 ppm levels.

In another trial, 2 meat fat samples were each fortified with lindane (0.15 and 0.18 ppm) and heptachlor epoxide (0.18 and 0.21 ppm) and analyzed by 7 laboratories. Mean recoveries ranged from 78 to 86% with CV ranging from 15.5 to 23.3%. The results from one laboratory were very low and may have been due to inexperience with their newly acquired Unitrex unit. When the data from this laboratory were deleted, the mean recoveries were 83–90% with CV of 11.6–15.1%. The

Associate Referee recommends a collaborative study of the Unitrex cleanup system for chlorpyrifos, bromophos ethyl, and ethion residues in beef fat.

Fenvalerate.—(T. D. Spittler, New York State Agriculture Experiment Station, Geneva). The Associate Referee has continued work on the analysis of various food commodities field treated with fenvalerate. EPA no longer requires residue data for the photo-metabolite SD-54597. The Associate Referee considers the current manufacturers method to be adequate for the determination of fenvalerate residues in treated crops.

Resmethrin.—(Calvin Corley, Environmental Protection Agency, Beltsville). The Associate Referee did not submit a report this year.

Other Topics.—The topics Azinphos-methyl, Extraction Procedures, General Method for Organochlorine and Organophosphorus Pesticides, Methamidophos, Monocrotophos, Soils, and Permethrin are vacant.

Recommendations

(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.*—Discontinue topic.

(3) *Disulfoton.*—Initiate study to evaluate analytical methods for determining disulfoton and its metabolites in foods.

(4) *Extraction Procedures.*—Appoint an Associate Referee to study the efficiency of extraction procedures for residues of organophosphorus pesticides in crops, i.e., to extend extraction efficiency studies of Watts (*J. Assoc. Off. Anal. Chem.* (1971) **54,** 953–958) to additional pesticides and crops.

(5) *General Method for Organochlorine and Organophosphorus Pesticides.*—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 determination step of this method.

(6) *High Fat Samples.*—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 photometric detector for determining organophosphorus pesticide residues by gas chromatography.

(7) *Methamidophos.*—Appoint an Associate Referee to evaluate and collaboratively study analytical methods for determining residues of methamidophos and its residues in foods.

(8) *Monocrotophos.*—Appoint an Associate Referee to evaluate and collaboratively study analytical methods for determining residues of monocrotophos in foods.

(9) *Phorate.*—Initiate study to evaluate analytical methods for determining phorate and its metabolites in foods.

(10) *Phosphine.*—Appoint an Associate Referee to evaluate and collaboratively study analytical methods for determining the total residue of intact phosphine and phosphine derived from residual aluminum phosphide in grains.

(11) *Soils.*—Appoint an Associate Referee to develop multiresidue extraction and cleanup methods for determining residues of organophosphorus pesticides and their metabolites in soils.

(12) *Sweep Codistillation.*—Prepare protocol for approval and collaboratively study the Unitrex cleanup system (*J. Assoc. Off. Anal. Chem.* (1984) **67,** 902–904) for chlorpyrifos, bromophos-ethyl, and ethion residues in beef fat.

(13) *Fenvalerate.*—Continue study of methods for determining fenvalerate residues in foods with the goal of selecting a method(s) suitable for collaborative study.

(14) *Permethrin.*—Appoint an Associate Referee to evaluate and collaboratively study analytical methods for determining residues of permethrin in foods.

(15) *Resmethrin.*—Continue study of analytical methods for determining resmethrin residues in foods.

(16) The following reports of studies on methods for organophosphorus pesticides were submitted for review/consideration.

(A) The Determination of Malathion and Dichlorvos Residues in Grain (*Analyst* (1973) **98,** 19–24): The method recommended was based on methanol extraction and determination by GC-FPD or phosphorus sensitive thermionic detection without cleanup. The study was based on only one level of dichlorvos in commercially treated wheat. Duplicate analyses were reported on 5 sub-samples of the sample of wheat by 9 laboratories. Statistical analysis was not performed on the data. No data were presented for malathion. The study did not provide data that meet the AOAC requirements for collaborative studies.

(B) Determination of Residues of Organophosphorus Pesticides in Fruits and Vegetables: The method of Abbott et al. (*Pestic. Sci* (1970) **1,** 10–13) was modified and recommended for the determination of malathion, dichlorvos, dimethoate, omethoate, and parathion in carrots, beans, peas, apples, tomatoes, and lettuces. In most trials reported, only 3, 4, or 5 laboratories participated and only one level of each pesticide was used. Data were reported from 3 different collaborative exercises. Means and standard deviations were the statistical data reported. The study was not designed to provide sufficient data to meet AOAC requirements for collaborative studies.

In the same report, the method of Watts et al. (*J. Assoc. Off. Anal. Chem.* (1969) **52,** 522–526) was modified and recommended for the determination of malathion, dichlorvos, dimethoate, omethoate, azinphosmethyl, and parathion in lettuces, carrots, peas, apples, tomatoes, and plums. Varying numbers of laboratories (4–8) participated in the different trials (4). Only one level of pesticide was studied in each trial and means and standard deviations were the statistical data reported. The study was not designed to provide sufficient data to meet AOAC requirements for collaborative studies.

(C) Determination of a Range of Organophosphorus Pesticide Residues in Grain (*Analyst* (1980) **104,** 515–517): The purpose of the study was to extend the scope of the method studies in (A) above. Results from 7 laboratories were presented for dimethoate, fenitrothion, phorate, pirimiphos-methyl, malathion, bromophos, iodofenphos, diazinon, and disulfoton at 2 levels in wheat. Excluding outliers, the mean recovery was 80% and the arithmetic mean of the 18 coefficients of variation was 15%. It was considered that further work was required because of the occasional difficulty encountered with the quantitative aspects of the GC analysis.

(D) Determination of a Range of Organophosphorus Pesticide Residues in Grain (*Analyst* (1985) **110,** 765–768): The method was based on the extraction of residues with acetone–water (8 + 1), partitioning into dichloromethane, and cleanup on a chromatographic column of silica gel–activated carbon (15 + 1). Residues were eluted with acetone–toluene–dichloromethane (1 + 1 + 5) and determined by GC with phosphorus specific detector. The method was considered to provide an effective cleanup procedure. Spike grain samples at one level of each pesticide (etrimfos, chlorpyrifos-methyl, malathion, bromophos, methacrifos, dimethoate, pirimiphos-methyl, fenitrothion) were analyzed by 14 laboratories. The mean overall recovery of all pesticides was 74%. The study did not provide data that meet AOAC requirements for collaborative studies.

In general, the 4 studies on the determination of organophosphorus pesticides in various substrates were not designed for nor do they meet the minimum requirements for AOAC collaborative studies.

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Radioactivity

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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 report has been revised according to the recommendations of the Committee on Residues and has been resubmitted. The results of the collaborative study show that the method should be considered for official first action adoption. Appointment of a new Associate Referee will be considered. A study for the coming year and evaluation of methods for determining cesium-137 in milk, foods, and biological materials at lower levels will be considered as the next project.

Iodine-131.—The method for determination of iodine-131 in milk has also been evaluated and collaboratively tested for iodine-131 in foods. This method is an extension of **48.025–48.029.** The report of the study shows that the method should be considered for official first action adoption.

Ruggedness testing has been completed on the more sensitive method as outlined by the General Referee (*J. Assoc. Off. Anal. Chem.* (1979) **63,** 387–389) and recommended by the Nuclear Regulatory Commission for determining iodine-131 in milk. A collaborative study protocol will be prepared for review by the General Referee and a study will be initiated in late 1985 or early 1986.

Neutron Activation Analysis.—Results have been received from 5 collaborators. A sixth collaborator has been found and is in the process of analyzing the sample. When all results are available, the Associate Referee will perform a statistical analysis of the data. The method involves the determination of sodium in neutron-irradiated biological materials. The study, if successful, will be presented at the 1986 annual AOAC meeting. Also, the Associate Referee plans to perform a study on multielements (metals) using neutron activation and, if feasible, design a collaborative study for review and comment by the General Referee.

Plutonium.—Richard E. Needham, FDA, has been appointed Associate Referee. He is in the process of testing the method of the Department of Energy for determining plutonium in food, biological materials, and water (HASL-300-Ed 25, EML Procedures Manual (1982) pp. E-Pu-01-01). He is continuing his work on a comprehensive bibliography of plutonium chemistry and analytical procedures.

Radium-228.—A ruggedness test is being conducted on a collaborative test of the method using gamma-ray spectrometry. Ten laboratories are participating, using samples supplied by EPA. Two sets are in triplicate. The final results have been received from all collaborators. The results will be evaluated and if the study was successful, the method will be submitted for interim first action adoption.

Strontium-89 and -90.—Joseph A. Hutchinson of the New York State Division of Laboratories and Research has been appointed as Associate Referee for this topic. He is in the process of initiating a collaborative study of the method described by Baratta and Reavey (*J. Agric. Food Chem.*

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Residues and adopted by the Association. See the report of the committee and "Changes in Methods," this issue.

(1969) **17,** 1337–1339) for determining strontium-89 and -90 in foods.

Tritium.—The General Referee reviewed this topic and decided that it is still needed. The first order of business is still to appoint an Associate Referee. The next step will be for the Associate Referee to study the literature for a possible method to be tested for ruggedness and to be collaboratively studied. A candidate is being considered.

Recommendations

(1) Adopt as official first action the method for cesium-137 in foods proposed by the Associate Referee; appoint a new Associate Referee; study methods for determining lower levels of cesium-137 in milk, foods, and biological materials.

(2) Adopt as official first action the method for iodine-131 in foods proposed by the Associate Referee.

(3) Analyze results of 2 collaborative studies, neutron activation and radium-228, before recommending official action.

(4) Appoint an Associate Referee for Tritium.

(5) Continue study on all other topics.

GENERAL REFEREE REPORTS: COMMITTEE ON MICROBIOLOGY

Analytical Mycology of Foods and Drugs

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A Howard mold count sample preparation method for whole capsicums was developed. Whole capsicum pods may be macroscopically examined for external or internal mold decomposition, but it is not feasible to macroscopically examine chopped or crushed pods. Whole or chopped pods prepared by the official method for ground spices, **44.213,** yield uncountable preparations with heavy background plant material that obscures mold hyphae and results in low counts. The new method incorporates *(1)* chopping, *(2)* alcohol extraction, *(3)* rehydration, *(4)* pulping, *(5)* centrifugation, and *(6)* dilution to standard volume with stabilizing solution. Although this is an involved, 6 step method, it yields an eminently countable preparation. Samples with 7 and 12% by weight of visibly moldy pods resulted in respective mold counts of 23 and 27%. This method will undergo further study and will be used to examine additional lots of whole or chopped capsicums containing known percentages of moldy pods.

The official Howard mold count method, **44.195,** scores positive or negative microscopic fields based on whether the aggregate lengths of up to 3 mold hyphal fragments exceeded ⅙ of the microscopic field. The analyst who has periodically examined tomato products over the years will have noticed the increased use of comminution by the industry, which has led to an overall reduction in the size of mold filaments being counted. Because of the size criteria used in the official method, milling has tended to increase mold counts, and high pressure homogenization has reached the point of decreasing mold counts. In an attempt to overcome the decreasing mold filament size, 2 modifications of the Howard method are being investigated. Paired tomato catsup samples are being compared (before and after milling or homogenization). All visible mold fragments are being counted by using the standard eyepiece grid, and are being placed into 9 size categories ranging from less than 1/36 of a field to greater than 1 field diameter. The second modification uses an alternative eyepiece grid divided into fiftieths of a field and scores the aggregate lengths of all visible mold filaments in 15 size categories ranging from less than 1/50 of a field to greater than 1 field diameter. A report on these methods will be given at the 99th Annual International Meeting in October 1985.

The introduction to method **44.213,** Mold in Ground Spices, which reads "applicable to garlic powder, paprika . . . and other grand capsicums," should be changed to read "Applicable to garlic powder, paprika . . . and other ground capsicums."

Recommendations

(1) Editorially revise the Howard mold count sample preparation, **44.213,** as described.

(2) Discontinue the following topics: Microscopic Appearance of Mold Hyphae, Effect of Freezing; Refractive Index of Tomato Porducts; and Tomato Rot Fragment Count.

(3) Continue study on all other topics.

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Microbiology and adopted by the Association. See the report of the Committee and "Changes in Methods," this issue.

Section numbers refer to *Offical Methods of Analysis* (1984) 14th edition.

Cosmetics

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Essential Oils and Fragrance Materials, Composition.—Associate Referee Harris H. Wisneski is studying the composition of peru balsam in an effort to identify the ingredient responsible for sensitization. When identified, he intends to develop an analytical method for its determination.

Nitrosamines.—Associate Referee Hardy J. Chou is presently studying the nitrosation potential of the cosmetic preservatives 2-hydroxymethyl-2-nitro-1,3-propanediol and 5-bromo-5-nitro-1,3-dioxane.

Preservatives.—Associate Referee Ann R. Stack is planning a collaborative study on the liquid chromatographic determination of methyl, propyl, and butyl parabens in creams and lotions.

Recommendations

(1) Continue official first action status of methods **35.001–35.006** and **35.020–35.024.**

(2) Continue study on all other topics.

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Microbiology and adopted by the Association. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1984) 14th edition.

Drug and Device Related Microbiology

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Limulus Amebocyte Lysate Tests for the Presence of Endotoxin.—(Associate Referee, Christine W. Twohy, Food and Drug Administration, Minneapolis, MN).

The variation of 9 drug product inhibition and enhancement end points with 2 commercially available chromogenic Limulus Amebocyte Lysate (LAL) reagents was determined with 3 lots of LAL from each manufacturer. Because only 1 lot of chromogenic LAL was available from 1 of the manufacturers, 2 lots of gel LAL reagents from that manufacturer were adapted to the chromogenic procedure. EC-5 was used as the endotoxin standard. At 0.5 EU/mL absorbance ±25%, the end points for 3 of the 9 products were within a 2-fold dilution among LAL reagents, 2 products were inhibitory for both LAL reagents, and the end points for 1 product varied more than one 2-fold dilution among LAL reagents. One product was compatible with only 1 LAL reagent, and 2 products were enhanced by 1 LAL reagent but inhibited by the other. When standard curves were used, the end points of 3 of the 9 products did not vary more than a 2-fold dilution among LAL reagents, 1 product was inhibitory for both LAL reagents, and the end points of 1 product varied by more than a 2-fold dilution. Four products were compatible with only 1 of the LAL reagents. No lot-to-lot variation was observed between LAL reagents from the same manufacturer. The LAL reagents varied in their ability to change the pH of drug product/LAL combinations.

Packaging Integrity for Medical Devices.—(Associate Referee, Ana M. Placencia, Food and Drug Administration, Sterility Research Center, Minneapolis, MN).

Two methods were developed and tested to determine the biobarrier capabilities of medical grade packaging materials and the presence of microholes. The membrane agar plate strike-through method was evaluated for: *(1)* transfer of bacteria from membrane onto packaging material, and *(2)* challenge sensitivity studies, using packaging materials with 1 microhole. The FDA exposure chamber method was evaluated to determine: *(1)* the amount of bacteria being drawn into each filtration cup in the exposure chamber; *(2)* pore sensitivity, using membrane filters (Nucleapore Corp., Pleasanton, CA 94566); *(3)* challenge sensitivity, using coated Tyvek®; and *(4)* the effect of low flow rates of 1 mL/min/cm and 281 mL/min/cm. The developed method was used to test 5 materials.

The Associate Referee prepared and presented the developed methods at the 1985 West Conference of the Medical Device and Diagnostic Industry (MD&DI) and prepared and submitted a paper to be presented and published at the Technical Association of Pulp and Paper Industry (TAPPI). Letters have been sent to potential collaborators, requesting participation in the studies. Preliminary statistical plans for the study are being evaluated. Blueprints for construction of the chamber are being prepared.

Three presentations of these methods were scheduled for September and October, 1985 (TAPPI, International Conference on Packaging, and AOAC). Two papers were scheduled for publication in October, 1985 (MD&DI).

The Associate Referee plans to submit patent information on the exposure chamber by October, 1985. A research project scheduled for FY 1986 involves collaborative study of both methods; study of the correlation between porosity and microbial penetration, using both the ARO test method and the test methods developed for package integrity; and study of the detection of microholes in packaging materials, using both the microbiological and physical methods.

Biological Indicator Testing and Standardization.—(Associate Referees Robert R. Berube, 3M Company, St. Paul, MN, and Gordon S. Oxborrow, Food and Drug Administration, Minneapolis, MN). The Associate Referees are jointly working on this topic to provide broader input to the complicated testing protocols.

Biological indicators used to determine the minimum time required for growth of sterilant-injured spores are being evaluated in preliminary studies of self-contained units obtained from 2 manufacturers.

Variability studies will be conducted on biological indicator evaluation resistometers used for ethylene oxide testing. Work will begin on a chemically defined medium that can be used for outgrowth testing and D-value testing.

Sterility Testing.—(Associate Referee, Michael Palmierei, Food and Drug Administration, New York Regional Laboratory, New York, NY).

A collaborative study of a sterility test vessel is being prepared. The study will include sterility test procedures for 3 types of medical devices. Methodology will be developed for various drugs and devices to be incorporated into standard methods.

Recommendation

Continue study on all topics.

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The recommendations of the General Referee were approved by the Committee on Microbiology and adopted by the Association. See the report of the committee, this issue.

Extraneous Materials in Foods and Drugs

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Collaborative studies were successfully completed on *(1)* a method for extraction of light filth from papaya, lemon balm, alfalfa, and spearmint; *(2)* an improved uric acid thin layer chromatographic (TLC) visualization reagent; and *(3)* a colorimetric method for determination of alkaline phosphatase as an indicator of mammalian feces in corn meal.

Collaborative studies are in progress on *(1)* a TLC method for determination of coprostanol as an indicator of mammalian feces; and *(2)* a method for light filth in spirulina.

A collaborative study design was approved for a modification of the method for light filth in chocolate.

A collaborative study design of an AutoAnalyzer method for determination of uric acid as an indicator of insect excreta in flour is being organized.

Joseph K. Nagy, FDA-Phildelphia District, was appointed Associate Referee to test and study analytical methods for light filth in baked goods with fruit and nut tissue. James D. Barnett, FDA-New Orleans District, was appointed Associate Referee to develop brine extraction methods for light filth in canned crabmeat, shrimp, and tuna. Bernice Beavin, FDA-Baltimore District, was appointed Associate Referee to develop brine extraction techniques for determining filth in tomatoes and mushrooms. Wilfred A. Sumner, FDA-San Francisco District, was appointed Associate Referee to develop enzyme methods for light filth in canned fish and fish products.

The topic Brine Extraction Techniques is being discontinued because of the retirement of Clarence C. Freeman, Associate Referee.

The topic on Particulates in Large Volume Parenterals is being discontinued at the request of the Associate Referee.

Methods development research will continue on all other topics.

Recommendations

(1) Adopt official first action the colorimetric method for determination of alkaline phosphatase as an indicator of mammalian feces in corn meal.

(2) Adopt official first action the improved uric acid TLC visualization reagent and technique to replace the detection spray, **44.186(c)**, and color development, **44.188(c)**. Editorial changes for correction and clarification are also included.

(3) Continue study on all other topics.

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The recommendations of the General Referee were approved by the Committee on Microbiology and adopted by the Association. See the report of the committee and "Changes in Methods," this issue.

Section numbers refer to *Official Methods of Analysis* (1984) 14th edition.

Food Microbiology

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The history and development of various microbiological topics covering a 50-year period, as well as the current status of these topics, was recently reviewed (*J. Assoc. Off. Anal. Chem.* (1984) **67**, 661–673). This report marks the 51st year of coverage of microbiological methods by AOAC. Preparation of that review provided the General Referee with an invaluable insight on the direction in which the development and validation of microbiological methods by AOAC is proceeding. Methods developed for the determination of microorganisms such as *Salmonella, Escherichia coli,* and *Bacillus cereus* have relatively high degrees of sensitivity, but now the emphasis, which may be considered 2-fold, has shifted from the continued development of even more highly sensitive methods for these and other microorganisms to (i) the development of rapid methods without compromising the sensitivity of existing methods, and (ii) the elucidation of the mechanisms of pathogenicity. Of the 9 methods adopted official first action by the AOAC at the 1982–1984 meetings, 4 were concerned with the rapid detection of microorganisms or their metabolites, while 2 were concerned with demonstrating pathogenicity mechanisms of microbial isolates. Based on collaborative studies currently under way or soon to be performed, there is every indication that this trend will continue. Moreover, the development of microbiological methods appears to be at the very threshold of a rapid proliferation that can be expected to result in validation of a number of these methods.

Collaborative Studies

Genetic Methods for Bacterial Pathogens.—Any strain of *E. coli* that is capable of causing diarrhea in humans and animals may be considered enteropathogenic. Four categories have been recognized (*J. Food Prot.* (1982) **45**, 1051–1067): classical enteropathogenic *E. coli,* facultatively enteropathogenic *E. coli,* enterotoxigenic *E. coli,* and enteroinvasive *E. coli.* Enterotoxigenic *E. coli* include those strains that produce a heat-labile (LT) enterotoxin only, a heat-stable (ST) enterotoxin only, or both LT and ST enterotoxins. In addition to their difference in tolerance of a temperature of 60°C for 30 min, the 2 enterotoxins may be distinguished by their dialyzability, susceptibility to an alteration in pH, and biochemical and physiological behavior in humans and animals.

Toxigenicity of *E. coli* strains is plasmid-mediated. Plasmid DNA may be cleaved into specific fragments that encode for these enterotoxins. In a previous collaborative study of a DNA colony hybridization method (*J. Assoc. Off. Anal. Chem.* (1984) **67**, 801–807), specific DNA fragments were isolated, purified, and radioactively labeled to detect LT enterotoxin-producing strains of *E. coli* that had been lysed and spotted on nitrocellulose filters overlaid on MacConkey agar, secs **46.035–46.048.** Colonies that contained the same gene as the radioactively labeled DNA fragment bound this DNA, and radioactive colonies were detected by radiography. Although this method was able to correctly identify 96.9% of the LT-producing strains used in the collaborative study, it is necessary to isolate and purify cloned gene fragments for the LT gene. Another disadvantage is that lysed colonies are spotted on nitrocellulose filters that are difficult to manipulate and are expensive.

Once the nucleotide sequence of genes that encode for these enterotoxins has been determined, it is possible to synthesize specific sequences of oligonucleotides that are identical to part of the gene encoding for these enterotoxins, making the isolation and purification of cloned DNA frag-

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Section numbers refer to *Official Methods of Analysis* (1984) 14th edition, and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* (1985) **68**, 369–411.

ments unnecessary. In this current collaborative study, synthetic radioactively labeled deoxyribonucleotides were used to detect 2 *E. coli* ST enterotoxins: STH, originally isolated from humans, and STP, first isolated from pigs. Because it is not necessary to distinguish STH from STP when this method is used for screening purposes, the synthetic oligonucleotide probes for each of these 2 enterotoxins were combined into a single ST probe. Moreover, colonies were lysed and hybridization was allowed to occur on cellulose (rather than nitrocellulose) filters as used in the previous collaborative study. Associate Referee Walter Hill furnished each of 23 collaborators with 3 known control and 20 (10 ST-positive and 10 ST-negative) bacterial strains. Of 460 cultures examined, 440 (95.7%) were correctly classified by the collaborators. Based on the results of this study, the Associate Referee recommends that the DNA colony hybridization method using synthetic oligodeoxyribonucleotides and cellulose filters for the detection of ST-producing strains of *E. coli* be adopted official first action, and the General Referee concurs.

Hydrophobic Grid Membrane Filter Methods.—Associate Referee Phyllis Entis performed a collaborative study comparing the hydrophobic grid membrane filter (HGMF) method and the conventional pour plate method for determining aerobic plate count values of foods. The conventional pour plate procedure of the American Public Health Association (APHA) (*Standard Methods for the Examination of Dairy Products* (1978) 14th Ed.) was used for samples of raw milk, secs **46.007–46.008** for whole egg powder, and secs **46.013–46.015** for raw ground poultry meat, flours, and spices. Twenty-one laboratories analyzed 1 or more of the 5 food types by the HGMF and conventional methods.

In the HGMF method, the sample is blended or agitated with diluent at a 1:9 sample/diluent ratio, and serial 10-fold dilutions are made. The appropriate enzyme, if needed, is added to the sample dilutions and the enzyme-treated dilution is incubated for 20–30 min at 35–37°C. The HGMF is filtered, aseptically removed from the filtration unit, and placed on the surface of a plate of pre-dried tryptic soy agar with added fast green FCF dye (0.25 g dye/L agar). These plates are incubated under conditions of time and temperature specific for the food being analyzed, and the number of squares containing 1 or more colonies is enumerated. An MPN value is obtained from this count of occupied squares by using a conversion formula.

The data for each food were subjected to a 3-way analysis of variance and precision estimates of repeatability and reproducibility for each sample pair were determined. With raw milk, raw poultry, flours, and spices, the HGMF and conventional pour plate methods gave statistically equivalent counts. With whole egg powder, the HGMF method gave significantly higher counts than the conventional method. Precision estimates were generally similar for both methods. The Associate Referee recommends that the HGMF method for determining the aerobic plate count values of foods be adopted official first action and the General Referee concurs.

Petrifilm Methods.—The Petrifilm system, a "sample ready" system requiring no media preparation, has been proposed as an alternative to conventional media for the enumeration of specific microorganisms or group of microorganisms. Each Petrifilm plate consists of 2 films, one containing appropriate nutrients and the other containing a cold water-soluble gelling agent and a tetrazolium indicator dye. Contact between the 2 contiguous films is temporarily broken by lifting the top film, and 1.0 mL of the appropriate sample dilution is pipetted onto the surface of the bottom film. Gentle pressure is applied to assure thorough contact of the 2 films and the sample dilution. Petrifilm plates are subsequently incubated at time and temperature conditions prescribed for the organisms to be enumerated.

Co-Associate Referees Roy Ginn and Vernal Packard conducted a collaborative study of the Petrifilm system for the enumeration of total aerobic microflora and of total coliforms. Eleven laboratories compared the Petrifilm SM and the standard plate count (SPC) methods of the APHA (*Standard Methods for the Examination of Dairy Products* (1978) 14th Ed.) for the enumeration of total aerobic microflora. These same laboratories compared the Petrifilm VRB and the violet red bile agar (VRBA) standard methods of the APHA for the enumeration of total coliforms. Each laboratory analyzed 16 samples (8 different samples in blind duplicate) by the Petrifilm VRB and VRBA standard methods. The repeatability standard deviation values (square root of the between-replicates variance) were 0.05104 (SPC), 0.0444 (Petrifilm SM), 0.14606 (VRMA), and 0.13806 (Petrifilm VRB). The reproducibility standard deviation values were 0.07197 (SPC), 0.06380 (Petrifilm SM), 0.15326 (VRBA), and 0.13806 (Petrifilm VRB). The difference between mean $\log_{10}$ values were 0.027 (SPC and Petrifilm SM methods) and 0.013 (VRBA anmd Petrifilm VRB methods). These results indicate comparability between the Petrifilm systems and the methods using conventional culture media. In March 1984, the co-Associate Referees recommended adoption of the Petrifilm SM and Petrifilm VRB method for the enumeration of total aerobic microflora and total coliforms, respectively, in raw and pasteurized milk as interim official first action, and the General Referee concurred. In April, the Committee on Microbiology approved this recommendation, and the method is in interim official first action status. The General Referee now recommends that the method be adopted official first action.

Salmonella.—Because of the threat to human health and the adverse economic impact of detecting *Salmonella* organisms in foods, interest in developing alternative rapid methods to the lengthy conventional culture method, secs **46.115–46.128,** continues unabated. One of these rapid methods, marketed as the *Salmonella* Bio-EnzaBead Immunoassay Method (Organon Teknika Corp., Charleston, SC), is an enzyme immunosorbent assay (EIA) that provides a definitive negative result for *Salmonella* in 2–3 days compared to 4–5 days required by the conventional culture method.

The EIA method has been detailed by Mattingly et al. (*Food Technol.* (1985) **39**(3), 90–94) but essentially this method uses monoclonal antibodies, i.e., specific molecules able to bind with antigens. With the Bio-EnzaBead system, 2 different monoclonal antibodies attached to a metal bead are needed to detect all *Salmonella* serotypes. When this bead is placed into a broth containing *Salmonella,* the antigens of the organism are bound by these antibodies which, in turn, are bound by the bead.

To detect the bound *Salmonella* antigens, another antibody, separate from the monoclonal antibodies yet reactive against *Salmonella,* is coupled with the enzyme horseradish peroxidase. When the bead is exposed to this antibody, a sandwich is created among the monoclonal antibodies, the enzyme-linked antibody, and the intervening *Salmonella* antigen. Exposing this complex to a substrate for the horseradish peroxidase results in a color reaction that presumptively indicates the presence of *Salmonella.* Absence of a color reaction is considered a definitive negative result.

Russell Flowers of Silliker Laboratories, Chicago Heights, IL, conducted a comparative collaborative study of the EIA and conventional culture method, secs **46.115–46.128.** A total of 25 laboratories participated in the study, but not all 6 foods (black pepper, soy isolate, dry whole egg, nonfat dry milk, milk chocolate, and raw ground turkey) were examined by each of these laboratories. Data from this study indicated no significant difference between the 2 methods for the determination of *Salmonella.* Accordingly, it was recommended that the EIA method be adopted official first action for the determination of *Salmonella* in foods, and the General Referee concurs.

Associate Referee Reports

Bacillus cereus, Isolation and Enumeration—Co-Associate Referee Stanley Harmon reports that the official final

action methods, secs **46.106–46.114,** have given acceptable results in several laboratories that have used the methods. Only one adverse comment was received during the previous year, and this comment was concerned with the occasional failure of *B. cereus* isolates to reduce nitrate to nitrite, one of several reactions used to identify this microorganism. Since this reaction is not an essential one for the identification of this organism, the Associate Referee suggests that it is sufficient to include a footnote in the method to acknowledge this exception.

Canned Foods.—Associate Referee Cleve Denny reports that it would be useful to conduct a collaborative study of a method to diagnose the cause of spoilage in a wide variety of canned foods. Although the method in the APHA *Compendium of Methods for the Microbiological Examination of Foods* is widely used, it could not be readily collaboratively studied because of the wide diversity of tests required to analyze various foods.

Clostridium perfringens.—There are currently 2 official final action methods for the enumeration of *C. perfringens* in foods. One of these methods, secs **46.092–46.097,** is a plating method and is applicable only when relatively large numbers of vegetative cells are expected to be present. This method uses tryptose–sulfite–cycloserine agar, which suppresses the growth of practically all facultative anaerobes, but other anaerobic organisms may grow on this medium. The presence of lecithinase, significant for identifying *C. perfringens,* is evidenced by the presence of a halo or a zone of white precipitation around suspect *C. perfringens* colonies. The analyst should be aware, however, that some strains will not produce this characteristic.

The second method, secs **49.098–46.105,** is concerned with the determination of the level of *alpha*-toxin produced by *C. perfringens*. This toxin increases in proportion to an increase in the cell population of this organism and is detectable when the level of *C. perfringens* is at least 10^6 cells per g of food. In many instances, however, the number of organisms surviving sample shipment has been substantially lowered, and it becomes necessary to estimate the original level of this organism by determining the *alpha*-toxin level.

Because of shortcomings of both of these methods, Associate Referee Stanley Harmon reports that studies have been initiated at the Food and Drug Administration (FDA) Seafood Laboratory, Seattle, WA, and the FDA New York Regional Laboratory to develop a sensitive most probable number method for the enumeration and confirmation of relatively low numbers of *C. perfringens* in foods. Upon development of a suitable method, it will be collaboratively studied.

Genetic Methods for Bacterial Pathogens.—Associate Referee Walter Hill reports that the development of a gene probe specific for *Salmonella* species is underway. Radioactive DNA hybridization will be conducted on lysates of postenrichments, and results will be determined by scintillation counting. Additionally, the feasibility of undertaking a study to detect and/or enumerate certain bacterial species in food directly, without any enrichment whatsoever, is being considered.

Hydrophobic Grid Membrane Filter Methods.—The HGMF method for the detection of *Salmonella* in nonfat dry milk, powdered egg, cheese powders, pepper, chocolate, and raw poultry was adopted official first action at the 1984 Annual International Meeting. Since that time, Associate Referee Phyllis Entis reports that several laboratories have conducted their own comparative evaluation of the HGMF method, secs **46.A06–46.A11,** and the conventional culture method, secs **46.115–46.128.** The 2 methods gave equivalent results for detecting *Salmonella* in artificially and naturally contaminated samples of nonfat dry milk, whey powder, soy products, "instant shake" mixes, and poultry meat. Moreover, several laboratories have reported that the use of a water bath for the 6–8 h incubation of the tetrathionate broth was essential and that any deviation from this condition resulted in reduced method sensitivity. One laboratory compared several *Salmonella* selective plating agars used in conjunction with the HGMF method and found selective lysine iron agar to be the most productive, followed by Hektoen enteric agar, these 2 agars being used in the HGMF method for *Salmonella*.

The Associate Referee reports that, as a result of favorable experience with the HGMF method for *Salmonella*, several laboratories have begun to use this method routinely in the analysis of foods for the purpose of quality control. Moreover, the Microbiology Committee of the American Association of Cereal Chemists voted to accept the HGMF *Salmonella* method as an approved method. Based on the foregoing favorable reports of the HGMF method and on the absence of adverse reports, the Associate Referee recommends that the official first action HGMF method for the determination of *Salmonella* in the 6 foods mentioned above, secs **46.A06–46.A11,** be adopted official final action, and the General Referee concurs.

The Associate Referee reports that development of a color-based, nonradioisotopic DNA probe for detecting *Salmonella* has begun. It is anticipated that an enzyme color probe used in colony hybridization on the HGMF will be developed. This color-based detection system will subsequently be used to screen the *Salmonella* genome for *Salmonella*-specific DNA fragments. After the choice of fragments for the probe has been decided and conditions for hybridization have been optimized, an in-house evaluation of this method will be initiated.

Listeria monocytogenes.—Associate Referee Joseph Lovett reports that at least 4 methods have recently been developed for the detection of *L. monocytogenes* in milk and foods: (i) Donnelly and Baigent (Annual Meeting of the American Society for Microbiology, 1985) used labeling with species-specific immunofluorescent antibodies and flow cytometry for discriminating *L. monocytogenes* from cross-reacting *Staphylococcus aureus* and *S. epidermidis* in enriched foods. (ii) Hayes et al. (Annual Meeting of the American Society for Microbiology, 1985) reported that the optimal procedure in their laboratory for isolating *L. monocytogenes* in samples of raw milk consisted of cold-enriching diluted milk in a nonselective broth at 4°C for 1 month followed by selective enrichment. (iii) Michael Doyle and associates at the Food Research Institute of the University of Wisconsin have isolated *L. monocytogenes* from milk by enriching the sample under microaerobic conditions (85% N_2, 10% CO_2, and 5% O_2) in tryptose broth supplemented with 5% defibrinated sheep blood, 0.5% glucose, 0.15% dipotassium phosphate, polymyxin B (16 IU/mL), acriflavine HCl (10 μg/mL), and nalidixic acid (40 μg/mL) followed by plating on McBride *Listeria* agar. Plans for evaluating this method for the isolation of *L. monocytogenes* from soft cheese are under way. (iv) Lovett et al. (Annual Meeting of the American Society for Microbiology, 1985) developed a method that consisted of enriching samples of raw and pasteurized milk in trypticase soy broth with 0.6% yeast extract supplemented with acriflavine HCl (15 mg/L), nalidixic acid (50 mg/L), and cycloheximide (50 mg/L) After 24 and 48 h incubation at 30°C, enrichment cultures are streaked to McBride *Listeria* agar.

During the coming year, the Associate Referee intends to conduct a comparison of methods in preparation for a collaborative study. Immediate research needs, according to the Associate Referee, should be directed towards reducing the analytical time required by current methods, improving method sensitivity, constructing gene probes for *Listeria,* and developing methods applicable to the analysis of foods other than milk and milk products.

Salmonella.—Co-Associate Referee Paul Poelma reports that the initiation of 2 collaborative studies is being considered by other laboratories. One of these studies involves the evaluation of Gene-Trak (Integrated Genetics, Framingham, MA), a system using radioisotopic DNA hybridization for the rapid detection of *Salmonella* in foods. Results are available

2 days after analyses begin, compared to 4–5 days with the conventional AOAC culture method, **46.115–46.128.** In an in-house comparison, the DNA hybridization and conventional culture methods gave essentially equivalent results for the detection of *Salmonella* in a variety of high- and low-moisture foods.

Another proposed collaborative study will evaluate the option of refrigerating sample preenrichments and selective enrichments. Because at present the conventional culture method cannot be interrupted until after incubation of selective agar plates, analyses that include sample preenrichment cannot be begun later than Monday if weekend work is to be avoided. If sample preenrichments and selective enrichments can be refrigerated for up to 3 days without a significant reduction of *Salmonella* organisms, the number of days for beginning analyses can be increased.

In an in-house evaluation (*J. Food Prot.* (1980) **43,** 343–345), and in a collaborative study sponsored by the International Commission on Microbiological Specifications for Foods (*J. Food Prot.* (1983) **46,** 391–399), D'Aoust et al. demonstrated that there was no significant decrease in the recovery of *Salmonella* when the analysis of selected low-moisture foods was interrupted by refrigerating the sample preenrichments and selective enrichments for up to 72 h at 4°C. However, the sensitivity of the refrigeration techniques was significantly greater with the low-moisture foods than with the high-moisture foods.

Section **46.117(f)** is concerned with the sample preparation of nonfat dry milk by the soak method. After this method was adopted official final action, the co-Associate Referee demonstrated that non-instant nonfat dry milk sample composites of 100 g and 375 g were incompletely wetted when analyzed by the soak method. Incomplete wetting of the sample is likely to result in decreased recovery of *Salmonella*, particularly when the level of contamination is low. Analysis of sample composites of instant nonfat dry milk by the soak method is not a problem. Accordingly, the co-Associate Referee recommends specifying that the food type in sec. **46.117(f)** is instant nonfat dry milk, and the General Referee concurs.

Co-Associate Referee Dean Wagner presented a paper, "*Salmonella* Surveillance by the FDA: A Review, 1974–1984," at the Midwest Regional AOAC Meeting, June 17–19, 1985, in Hillside, IL. The paper summarized the numbers, types, and sources of *Salmonella* isolates reported by the FDA during this period.

Staphylococcal Enterotoxin.—Associate Referee Reginald Bennett reports that a collaborative study of the single radial immunodiffusion method for screening staphylococcal isolates for the production of enterotoxin is planned for the coming year.

Staphylococcus.—Associate Referee Gayle Lancette conducted a collaborative study in which trypticase soy broth with 10% NaCl, the recovery medium used in the current official final action method, sec. **46.062,** was compared with the same medium containing 1% sodium pyruvate. Fifteen collaborators analyzed samples of milk, tuna salad, and ground turkey inoculated with 3 levels of *S. aureus*. Recovery of this organism was significantly higher with the medium containing pyruvate except for the tuna salad inoculated with the low level of *S. aureus*.

Virology.—A method for the enumeration of poliovirus 1 plaque-forming units in oysters, secs **46.A12–46.A22,** was adopted official first action at the 1984 Annual International Meeting. Associate Referee Edward Larkin reports that when this method was compared to the method of choice at the University of North Carolina for the viral analysis of oysters, the AOAC method gave equivalent recoveries of Hepatitis A virus and slightly lower recoveries of enteroviruses. When compared to the method of choice at the National Marine Fisheries Laboratory, Charleston, SC, for the analysis of oysters, the AOAC method gave slightly lower recoveries of polyvirus 1. Before raising the official first action method to final action, the Associate Referee recommends that the method be studied for at least another year, and the General Referee concurs.

Yeasts, Molds, and Actinomycetes.—Associate Referee Philip Mislivec has concluded a study on the use of NaCl (final concentration 7.5%) in potato dextrose agar (PDA) for restricting the growth of "spreader" molds, common to many foods. Viable mold counts of several foods were determined using PDA with and without NaCl. "Spreader" growth was effectively inhibited by the addition of NaCl to the PDA, and mold counts were very similar on the 2 types of PDA. Results of this study will be presented at the 1985 Annual International Meeting.

The Associate Referee reports that an in-house study comparing FDA's 5-day *Bacteriological Analytical Manual* procedure and the 48 h HGMF procedure for determining viable yeast and mold counts in 10 foods was conducted at Anheuser-Busch, St. Louis, MO. The data indicate that there were no significant differences in counts by the 2 methods. Thus a comparative collaborative study of these 2 methods is planned for the coming year.

Yersinia enterocolitica.—During the past year co-Associate Referees James Cholensky and Sallie McLaughlin continued their comparative evaluation of 2 methods for the recovery of *Y. enterocolitica* from foods. With artificially contaminated raw oysters, one method was superior to the other with respect to sensitivity, detection time, and numbers of strains recovered, including the problematic serotype 0:8. When the in-house data are complete, a collaborative study is planned.

Recommendations

(1) Adopt official first action the DNA colony hybridization method using synthetic oligodeoxyribonucleotides and cellulose filters for the detection of ST-producing strains of *E. coli*.

(2) Adopt official final action the official first action HGMF method for the detection of *Salmonella* in nonfat dry milk, powdered egg, cheese powders, pepper, chocolate, and raw poultry, **46.A06–46.A11.**

(3) Adopt official first action the HGMF method for determining aerobic plate count values of foods.

(4) Adopt official first action the dry rehydratable film interim official first action method for determining aerobic plate count and total coliform values in raw and pasteurized milk.

(5) Adopt official first action the EIA method for the determination of *Salmonella* in foods.

(6) Change "nonfat dry milk" to "instant nonfat dry milk" in sec. **46.117(f).**

(7) Delete the topics, Helium Leaks in Canned Foods, Low Acid Canned Foods—Spore-formers and Non-spore-formers, Parasitology, and *Vibrio parahaemolyticus*.

(8) Continue study on all other topics.

GENERAL REFEREE REPORTS: COMMITTEE ON FEEDS, FERTILIZERS, AND RELATED MATERIALS

Drug Residues in Animal Tissues

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The 1975 General Referee report on Drug Residues in Animal Tissues expressed great concern that a number of analytical methods that were being used had not been adequately validated, or were not supported by appropriate metabolic and toxicological data. There are reasons now in 1985 to be more optimistic about the progress that is being made in developing analytical methodology for trace drug residues in animal tissues.

Significant attempts are being made by (*1*) the U.S. Department of Agriculture, Agricultural Research Service, Food Safety and Inspection Service, (*2*) U.S. Food and Drug Administration, (*3*) drug sponsors, (*4*) commercial laboratories, and (*5*) university research centers to improve the quality and efficiency of the available analytical methodology. Both FSIS and FDA have more than one laboratory involved in development and validation of analytical methods for drugs in animal tissues. Both are funding either methods development or validations by extramural contracts or by cooperative agreements.

Several firms have developed and made commercially available, in simple kit form, assays that are based on competitive binding techniques. Generally, these are being proposed for screening large numbers of samples for drug residues. Positive samples are then assayed by officially approved analytical methods. Many of these competitive binding assays have the potential for substantial specificity and accuracy, and merit consideration as official determinative or confirmatory procedures.

Zone inhibition-type procedures are being developed for use on the farm and in stock yards for screening blood, urine, or tissue extracts for antibiotics. Zone inhibition procedures are also being made available in very simple formats. Some are as simple and short as placing a few drops of blood, urine, or meat extract on a card or in a vessel either before or after application of other reagents. Appropriate standardization and usage are issues that need attention.

Liquid chromatography with either electrochemical, UV-vis, or fluorescence detection, coupled with post-column derivatization, is increasingly replacing some of the older zone inhibition techniques for assay of antibiotic compounds that are produced by microorganisms. However, zone inhibition techniques still have an important role in tissue residue assays.

Comments and recommendations on the animal tissue drug assay topics are as follows:

Benzimidazoles.—The Associate Referee reported that 5 of the participating laboratories have completed the analyses of their assigned tissue samples for the collaborative study of a multiresidue determinative assay for thiabendazole, hydroxythiabendazole, oxfendazole, mebendazole, and fenbendazole in cattle tissues. Sample analyses are expected to be completed and the study data analyzed by the end of October 1985.

Dimetridazole.—The Associate Referee reports that she was unable to work on this project this year. She expects to be provided time to work on the project during the coming year.

Levamisole.—Laboratory work on a collaborative study of a gas chromatographic determinative assay for levamisole in swine, bovine, and ovine tissues has been completed. Recommendations from the Associate and General Referees are expected to be completed by the end of October 1985.

Recommendations

(1) Appoint Associate Referees on the topics Diethylstilbestrol, Tiamulin, and Tiamulin (Screening Method).

(2) Discontinue the topics Steroids, Sulfa Drugs, and Sulfonamide Drugs.

(3) Continue study on all other topics.

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Feeds, Fertilizers, and Related Materials and adopted by the Association. See the report of the committee, this issue.

Drugs in Feeds

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A collaborative study is in process on carbadox and pyrantel tartrate. Progress has been made in the area of precollaborative interlaboratory studies, particularly on carbadox, lasalocid, and pyrantel tartrate. A continued effort will be made to use the AOAC spring training workshop as a forum for investigators to discuss problems concerning specific analytes and general procedures.

Carbadox.—(Virginia Thorpe). A collaborative study including pyrantel tartrate is currently in progress.

Ethylenediamine Dihydroiodide.—(Gary Ross). Continue study on a colorimetric procedure.

Furazolidone.—(Robert Smallidge). Collaborative study has been completed and approved as official first action.

Ethopabate.—(Joseph Hillebrandt). Ruggedness testing of a method is currently being done. A collaborative study is planned for 1986.

Pyrantel Tartrate.—(James Braswell). Plans depend on outcome of the current collaborative study.

Roxarsone.—(Glenn George). A collaborative study has been completed.

Sulfa Drug Residues.—(Robert Munns). A collaborative study has been completed.

Sulfamethazine and Sulfathiazole.—(Dwight Lowie). Continue study.

Recommendations

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

(2) Appoint Associate Referees on the topics Amprolium and Arsanilic Acid.

(3) Continue study on all other topics.

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Feeds, Fertilizers, and Related Materials and adopted by the Association. See the report of the committee and "Changes in Methods," this issue.

Feeds

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The Associate Referees were active in the animal feed area this year. The Laboratory Methods Committee of the American Association of Feed Control Officials has requested that Associate Referees be appointed for analysis specific to animal feeds, e.g., vitamins. Their suggestion meets with the General Referee's approval and only awaits volunteers experienced in these areas.

Amino Acids in Animal Feeds.—Associate Referee Wayne Stockland submitted samples for collaborative study to some 30 laboratories, with instructions on a hydrolysis procedure, but not limiting the analytical device. He hopes that the study will result in an amino acid analysis method that is acceptable for animal feeds.

Fiber, Crude.—Associate Referee David O. Holst reports that he collaborated with Leon Prosky in a collaborative study on total dietary fiber. He also reports that he receives 4 or 5 questions a month concerning fiber analysis.

Fiber, Crude, in Milk Replacers.—Associate Referee Jim Pierce recommends that the topic be discontinued because there appears to be little concern at present.

Infrared Reflectance Techniques for Mixed Animal Feeds.—Associate Referee Franklin E. Barton II initiated a collaborative study which included 94 samples of hay.

Karl Fischer/Moisture in Corn Products.—Associate Referee Raffaele Bernetti plans to initiate a collaborative study this year using the new crop grains.

Microbial Additives and Enzymes.—Associate Referee William Y. Cobb was appointed this year. This is a new topic and persons interested in this field are urged to contact the Associate Referee.

Minerals.—Joel M. Padmore has been appointed Associate Referee and is working on an inductively coupled argon plasma method to allow a single digest analysis for Ca, Mg, Cu, Co, Zn, Mn, P, and S.

Protein, Crude.—Associate Referee Peter Kane has been investigating a new catalyst mixture.

Sampling.—Associate Referee Darrel Sharpe and the Missouri Department of Agriculture have been gathering data on dissimilarity of trier and hand sampling of textured feeds. There is a plan to study present molasses sampling procedures for use on liquid feeds.

The remaining Associate Referees on Feeds did not report any activity this year.

Recommendations

(1) Discontinue the topic Fiber, Crude, in Milk Replacers.

(2) Appoint Associate Referees for topics recommended and nominated by AAFCO.

(3) Continue study on all other topics.

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Feeds, Fertilizers, and Related Materials and adopted by the Association. See the report of the committee, this issue.

Fertilizers and Agricultural Liming Materials

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Biuret in Urea and Mixed Fertilizers.—Associate Referee Luis Corominas conducted a precollaborative study on a method utilizing a copper ion selective electrode. The results were somewhat disappointing, but further investigation is being done to strengthen the method.

Boron.—After some modification of the report on the collaborative study, Associate Referee James Melton has recommended adoption of the inductively coupled plasma method as official first action. The General Referee concurs.

Dicyanodiamide.—Associate Referee Michael Rohrl has distributed the liquid chromatographic method, practice samples, and standards to 8 collaborators. The study samples will be distributed in October.

Iron.—Associate Referee James Silkey continues his investigation of method **2.157–2.160.** He recommends that Fe *N,N*-bis(2-hydroxy-5-sulfobenzyl)glycine (DPS) be dropped from the list of chelates to which the method is applicable. The General Referee concurs.

Potash.—Associate Referee Peter Kane recommends that methods **2.108–2.113** and **2.114–2.118** be declared surplus. These methods are seldom used, depend on obsolete equipment, and have been superseded by newer methods. The General Referee concurs with this recommendation.

Sampling and Preparation of Sample.—Associate Referee Douglas Caine reports that the bag sample study noted here the last 2 years has been completed; the data are being evaluated by the statistician. The Associate Referee has also been requested to evaluate the specifications of the D-tube listed in method **2.001.** The specifications noted in Table **2:01** may be in variance with the D-tubes manufactured and in use.

Slow-Release Mixed Fertilizers.—Continue official first action status of method **2.073–2.074.** Associate Referee Stanley Katz is making laboratory evaluations of the slow release characteristics of a specialty fertilizer at the request of the manufacturer.

Sodium.—Associate Referee Luis Corominas conducted a collaborative study on a modified flame emission method. He recommends adoption of the modified method as official first action, and the General Referee concurs. Continue official first action status for method **2.177–2.181.**

Soil and Plant Amendment Ingredients.—Continue official first action status of method **2.194–2.197.**

No reports were received from Associate Referees on Free and Total Water, Nitrogen, Phosphorus, Sulfur, Water-Soluble Methylene Ureas, and Zinc.

Recommendations

(1) Adopt as official first action the inductively coupled plasma method for boron.

(2) Declare methods **2.108–2.113** and **2.114–2.118** for potash as surplus.

(3) Delete from method **2.157–2.160** applicability to chelate Fe *N,N*-bis(2-hydroxy-5-sulfobenzyl)glycine (DPS).

(4) Adopt as official first action the modified flame emission method for sodium.

(5) Continue official first action status of the following: water elution method for slow-release mixed fertilizers, **2.073–2.074;** atomic absorption spectrophotometric method for sodium, **2.177–2.181;** atomic absorption spectrophotometric method for aluminum, **2.194–2.197.**

(6) Continue study on all topics.

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Feeds, Fertilizers, and Related Materials and adopted by the Association, except recommended methods for boron and sodium. See the report of the committee and "Changes in Methods," this issue.

Section numbers refer to *Official Methods of Analysis* (1984) 14th edition.

Tobacco

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Tar and Nicotine in Cigarette Smoke.—(Associate Referee Harold C. Pillsbury). No further collaborative studies have been conducted on this method.

Recommendation

Continue study on the tar and nicotine method.

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendation of the General Referee was approved by the Committee on Feeds, Fertilizers, and Related Materials and adopted by the Association. See the report of the committee, this issue.

Veterinary Analytical Toxicology

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Two methods, nitrate in forage by the nitrate electrode (1) and arsenic in animal tissues (2), received interim approval during the past year. These approvals were based on collaborative studies reported here 1 year ago. Both methods will be considered for official first action at the 1985 Annual International Meeting.

Three more collaborative studies are close to completion and are discussed in the Associate Referee reports. Results from 2 studies of lead in blood and 1 study of selenium in blood, tissue, and feed will be evaluated and considered for action after all data have been tabulated and analyzed. Reports of several interlaboratory studies and methods surveys that have been completed are also discussed. Of special note is the study on cholinesterase determination, where important information was gained on 2 different methods.

The Second Annual Workshop on Veterinary Analytical Toxicology was held at the Midwest Regional AOAC Meeting on June 18, 1985, in Hillside, IL. The program included demonstrations on the topics zinc phosphide, anticoagulants, cyanide, cholinesterase, molybdenum, aflatoxins in liver and rumen contents, bioautography of antibiotics, data base management, remote computer accessing of data bases, cyclopiazonic acid, slaframine, selenium, and ergot alkaloids. A summary of the methods and data presented is available on request from this author. Other technical sessions were also held at the American Association of Veterinary Laboratory Diagnosticians (AAVLD) Meeting, October 21, 1984, in Fort Worth, TX, and at the AOAC Annual International Meeting, November 1, 1984, in Washington, DC. The AAVLD/American Academy of Veterinary and Comparative Toxicology/AOAC Committee on Veterinary Analytical Toxicology met and their recommendations have been reported (3).

The Symposium on the Diagnosis of Mycotoxicoses of Importance in the United States and Japan, October 11–13, 1984, at the National Animal Disease Center in Ames, IA, featured comprehensive coverage of the entire subject of mycotoxicoses. Technical presentations ranged from clinical syndromes to chemical analyses. Proceedings of the symposium, sponsored by the United States-Japan Cooperative Program on Development and Utilization of Natural Resources Joint Panel on Toxic Microorganisms and the U.S. Department of Agriculture, will be published in the near future.

Several assignment changes occurred during the past year. The topic formerly known as Monensin has been expanded and renamed Antibiotic Screening Methods, and an Associate Referee Committee has been assigned. This topic has considerable breadth and could be subdivided once problems are better defined. Three new topics, Pentachlorophenol in Animal Tissues, Atomic Absorption Methods, and Animal Serum Thyroxine, have been added.

Reports were received from all Associate Referees, whose efforts are greatly appreciated. Their commitment to improving quality is reflected in the following summaries of their reports:

Animal Serum Thyroxine.—Daniel J. Sullivan (Vet-A-Mix, Shenandoah, IA), the newly assigned Associate Referee, reports on plans to study thyroxine (T-4) in equine serum. A study involving 5 laboratories, conducted by the Associate Referee, has provided support for further studies of a radioimmunoassay (RIA) technique. In that study, interlaboratory variation (CV, %) ranged from 16 to 25% for concentrations of 2.1–2.9 μg/100 mL serum, respectively. Each of the laboratories used a RIA technique with different commercially prepared T-4 diagnostic kits. The data indicate systematic bias by some of the kits. The Associate Referee plans to evaluate the different kits available on the market and determine which kits might be most suitable for a collaborative study, as well as conduct a second interlaboratory study. The second study would involve a wider range of sample concentrations, incorporate the use of the same calibration standards by all participants, including an external control and a known spiked sample.

Antibiotic Screening Methods.—Wynne Landgraf (National Veterinary Services Laboratories, Ames, IA) and Stephen Ross (Animal Disease Laboratory, Centralia, IL) are the newly appointed Associate Referee Committee. They report on plans to study screening methods for those antibiotics of diagnostic interest. A charcoal column thin layer chromatographic (TLC) colorimetric screening method for monensin in feeds has been reported (4). A more recent study has led to the development of a lead acetate cleanup in place of the charcoal column. Both techniques are suitable for detecting monensin as low as 1 ppm in feeds, although recovery problems exist with the charcoal column at these levels (G. E. Rottinghaus, 1985, private communication). The lead acetate cleanup is also suitable for determining lasalocid, narasin, and salinomycin.

The Associate Referees also report they have completed an interlaboratory survey to decide which antibiotics are considered problems, as well as to determine what types of methods are currently being used. While many diagnostic laboratories are interested in antibiotics, only a few conduct routine testing. The survey did indicate that a variety of methodologies exist: bioautography, TLC, liquid chromatography (LC), plate cylinder assay, and spot tests are but a few of the techniques used. Clearly, more specific methods are needed for several of the antibiotics.

The Associate Referees recommend continued study and that an interlaboratory study for determining monensin be conducted.

Arsenic in Animal Tissues.—R. Tracy Hunter (Division of Consolidated Laboratories, Richmond, VA) reports that the modification of the method for determining As residues (**41.009–41.012**) reported here last year has been adopted interim first action. The collaborative study of that method will be published in the May 1986 issue of this journal. The Associate Referee recommends that the method be adopted official first action.

The Associate Referee has resigned from this topic due to a change in position. He recommends continued study and that a new Associate Referee be appointed.

Atomic Absorption Methods.—Steve Kasten (Animal Disease Laboratory, Centralia, IL) is the newly assigned Associate Referee. He will initially study Mo methods. Based on the experience of the former Associate Referee on Molybdenum, whose interlaboratory study showed difficulty with Mo determination in blood and serum, the new study will be conducted with only one matrix, serum. Once all the problems with serum Mo determinations are solved, other matrices, such as feed, blood, and liver, will be studied.

The new Associate Referee reports on his experience with Mo analysis in serum. A simple dilution and determination by graphite furnace atomic absorption (AA) has been shown to be reproducible and accurate (5), based on intralaboratory and interlaboratory determinations. Recovery was assessed by the method of known addition. Experience with blood and liver has not been as successful; significant matrix effects are encountered with both types of samples. More work is needed.

The new Associate Referee recommends that a collaborative study be conducted on the determination of Mo in animal serum.

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Feeds, Fertilizers, and Related Materials and adopted by the Association. See the report of the committee and "Changes in Methods," this issue.

Section numbers refer to *Official Methods of Analysis* (1984) 14th edition, and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* (1985) **68**, 369–411.

Table 1. Preliminary data for collaborative study on fluoride in bone ash samples (11 laboratories reporting)

	Sample No.				
Statistic	02	33	64	79	84
Mean, ppm F	681	1115	647	1096	1103
CV, %	9.0	6.9	10.0	5.8	6.5

Chlorinated Phenols in Animal Tissues.—Paul Marsden (Lockheed, Las Vegas, NV) is the newly appointed Associate Referee. He plans to initially study the determination of pentachlorophenol in bovine tissues (6).

Cholinesterase.—Paula Martin (Iowa State University, Ames, IA) reports that she has completed an interlaboratory study of cholinesterase (CHE) determination by 2 different methods. Seventeen laboratories were provided 2 human serum samples (one with depressed CHE and one with normal activity) for CHE determination by method of choice. Nine laboratories used the colorimetric method, or a modification, described by Ellman (7); the other 8 laboratories used the ΔpH method described by Michel (8). Almost all of the laboratories routinely do CHE determinations and were familiar with their technique.

The results of the survey show good reproducibility by the Michel technique. All participants clearly demonstrated the different CHE activities of the samples. The average result for the normal sample was 1.02 ΔpH units/mL/h with a CV of 19%. All of the results for the normal sample were within 2 standard deviations of the mean and could not have been misinterpreted. The average for the depressed sample could not be calculated because some laboratories reported "zero." For the depressed sample, none reported greater than 0.09 ΔpH unit/mL/h, clearly indicating that the sample was depressed.

The results from those laboratories doing the Ellman method were not as clear. There were some differences in the reporting units, making recalculation necessary so that some comparisons could be made. All of the results from the depressed sample clearly showed it was depressed. Some laboratories again reported the acceptable result of "zero." In the case of the normal sample, there was a wide variety of results. The average result was 3000 IU/min/L with a CV of 40%. The range was from 1700 to 4600, making interlaboratory comparison difficult. Differences in reaction temperature, substrate, reagents, and/or operator are likely reasons. The results indicate that extreme care should be taken when comparing interlaboratory results from the Ellman method. Laboratories must establish their own "normal method ranges."

Based on the results of the study, the Associate Referee recommends that a collaborative study be conducted, using the Michel method on animal blood, and that study continue on the Ellman technique to determine where the interlaboratory variability originates.

Copper in Animal Tissues.—David Osheim (National Veterinary Services Laboratories, Ames, IA) reports continued success with the official first action methods for serum copper (**49.003–49.007**) and liver copper (**49.A01–49.A04**). He recommends that the serum copper method be considered for official final action and that the liver copper method remain in official first action status.

The Associate Referee recommends that consideration be given to studying copper in feeds at concentrations of concern in veterinary toxicology.

Fluoride in Animal Tissues.—David L. Osheim reports progress during the past year on the analysis of fluoride in bone and bone ash by the fluoride electrode (9, 10). Several lots of bone meal and bone ash were evaluated for use in a collaborative study. In-house analyses of these samples have produced data with CVs of less than 5% for bone meal and less than 3% for bone ash. Accuracy has been determined by the method of addition, with recovery consistently greater than 90%. Attempts to prepare a spiked bone meal or spiked bone ash have met with limited success. Some success was obtained from spiking bone ash with CaF_2 where in-house analysis showed recovery of greater than 90%; however, when this sample was sent to other laboratories, the recovery was low and the variability was extremely high (CV greater than 20%). The Associate Referee believes that the CaF_2 adhered to the walls of the container during shipment. All suggestions and comments addressing this problem are welcome.

The Associate Referee conducted a modified collaborative study on bone ash samples (bone samples ashed by the Associate Referee). Results obtained from most of the 13 participating laboratories have been promising. Table 1 summarizes the results to date. No estimate of accuracy can be made from the data; however, the levels are in good agreement with results obtained from in-house values by the method of known addition.

The Associate Referee recommends continued study.

Lead in Animal Tissues.—Robert J. Everson (Purdue University, West Lafayette, IN) reports that 2 collaborative studies on the determination of Pb in animal blood are currently in progress. Based on results from an extensive interlaboratory study completed 1 year ago, 2 different methods for determining Pb in blood were determined to be good candidates for study. The 2 methods are extraction/AA flame (11) and extraction/AA furnace (12). Due to the differences in equipment necessary to run the methods (flame or furnace), the decision was made to study both techniques. The collaborative studies involve the analysis of animal blood with varying amounts of naturally occuring and spiked Pb levels. Results will be analyzed and action considered after data from these studies have been collected.

The Associate Referee recommends continued study.

Lead in Bone.—The Associate Referee has resigned.

Molybdenum.—R. Tracy Hunter (Division of Consolidated Labs, Richmond, VA) reports on an interlaboratory study on the determination of Mo in blood and serum. Seven blood and serum samples were provided to 10 laboratories for analysis. The method used in the study is a dry ashing/AA graphite furnace procedure (13) which is quite labor intensive. Only a few results have been received and no statistics are available, but apparently several problems exist. NBS Standard Reference Material (SRM) 1577 Bovine Liver was included as an external control. It is not an exact matrix match and contains Mo at a much higher concentration than normally encountered in blood or serum; therefore, it may not have been a suitable control. Problems were encountered with ashing of blood; re-ashing was necessary for several of the samples. Quantitation was done by the method of addition, which is an involved process and may cause problems. Considerable work is needed on blood methodology.

The Associate Referee has resigned due to a job change, but recommends that study on this topic continue.

Multielement Analysis by ICP.—W. Emmett Brasleton (Michigan State University, East Lansing, MI) reports on continued work with the development of frequency distribution of element concentrations in samples of serum, liver, and kidney of various species using inductively coupled plasma (ICP) characterization of the mean (arithmetic and geometric). Histograms of the frequency distribution of Ca, Cu, Fe, Mg, Na, P, and Zn in 607 bovine serum samples; Ca, Cu, Fe, K, Mg, Mn, Mo, Na, P, and Zn in 299 bovine liver samples; and Ca, Cd, Cu, Fe, K, Mg, Mn, Mo, Na, P, and Zn in 90 bovine kidney samples have been recently reported (14). Similar studies in the canine have also been completed (15). Work is currently under way on equine and porcine samples.

The Associate Referee recommends continued study on the use of ICP techniques as diagnostic tools.

Multiple Anticoagulant Screening.—Jim Stedelin (Animal Disease Laboratory, Centralia, IL) is the newly appointed Associate Referee and the plans to continue study of the

Table 2. Conversion factors for units of nitrate and nitrite concentrations[a]

From / To	NO_3^- – N (14.01)	NO_3^- (62.01)	$NaNO_3$ (85.01)	KNO_3 (101.11)	NO_2^- – N (14.01)	NO_2^- (46.01)	$NaNO_2$ (69.01)	KNO_2 (85.11)
NO_3^- – N (14.01)	1.000	0.2259	0.1648	0.1386	1.000	0.3045	0.2030	0.1646
NO_3^- (62.01)	4.426	1.000	0.7294	0.6133	4.426	1.348	0.8986	0.7286
$NaNO_3$ (85.01)	6.068	1.371	1.000	0.8408	6.068	1.848	1.232	0.9988
KNO_3 (101.11)	7.217	1.631	1.189	1.000	7.217	2.198	1.465	1.188
NO_2^- – N (14.01)	1.000	0.2259	0.1648	0.1386	1.000	0.3045	0.2030	0.1646
NO_2^- (46.01)	3.284	0.7420	0.5412	0.4550	3.284	1.000	0.6667	0.5406
$NaNO_2$ (69.01)	4.926	1.113	0.8118	0.6825	4.926	1.500	1.000	0.8108
KNO_2 (85.11)	6.075	1.373	1.001	0.8418	6.075	1.850	1.233	1.000

[a]Find current unit of concentration on top row. Find desired unit of concentration in left column. Obtain conversion factor at intersection of row and column. Desired concentration = current concentration × conversion factor. Numbers in parentheses below units of concentration are formula masses. Atomic masses: N: 14.01; O: 16.00; Na: 23.00; K: 39.10. 1% = 10 000 ppm.

previously reported liquid chromatographic (LC) screening method (16). Recent study has shown that fluorescence detection in series with UV detection greatly increases sensitivity and specificity for several of the anticoagulants.

Warfarin, difenacoum, bromodiolone, brodifacoum, coumachlor, racumin, and fumarin can be detected at levels below 1 ppm by fluorescence with essentially no background problems. Diphacinone, chlordiphacinone, valone, and pival are still detected by UV since they fluoresce weakly. The Associate Referee has collected sufficient quantities of most of the anticoagulants to conduct interlaboratory studies.

A previous survey (17) indicated reluctance by some laboratories to participate in study of the screening method due to the use of an LC solvent with a pH of greater than 9.0. The Associate Referee has found that the Brownlee PRP C-18 polymer column is unaffected by high pH and recommends this column for the portion of the method involving high pH.

Two recent reports (18, 19) have provided important data toward understanding the levels of concern in tissues of anticoagulant poisoned animals. In dogs poisoned with diphacinone, levels in postmortem samples of blood ranged from 6 to 22 ppm, while liver levels were 2–5 ppm. In the case of dogs dosed with brodifacoum, the levels were less than 100 ppb. More studies of this type are necessary to ultimately determine how sensitive analytical methods need to be.

The Associate Referee recommends that an interlaboratory study using bait samples containing 1 or 2 anticoagulants each be conducted to further evaluate the method.

Natural Products.—George Rottinghaus (University of Missouri, Columbia, MO) reports on development of a method for detection of ergot alkaloids in fescue seed and other plant material. Specifically, ergovaline and ergotamine are detected by reverse phase LC/fluorescence after extraction with methanol and cleanup with a small exclusion column. The method is more sensitive than conventional techniques, resulting in detection of the low levels of ergotamine and ergovaline which occur in tall fescue infected by the fungus *Epichloe typhina*. This infection is associated with fescue toxicity in cattle (20). The minimum detection limits are 10 ppb for ergovaline and 5 ppb for ergotamine. Recoveries of 100% were obtained from samples spiked with 500 ppb ergovaline and 300 ppb ergotamine.

The method is currently being used to analyze samples of fescue seed and other plant material to determine if a correlation exists between percent fungus infection and ergot alkaloid concentration. Preliminary data indicate some correlation exists for ergovaline, but not for ergotamine.

The Associate Referee recommends continued study.

Nitrates/Nitrites.—Michael P. Carlson and Norman R. Schneider (University of Nebraska, Lincoln, NE) report significant progress during the past year. The method for determination of nitrate in forage by the nitrate electrode (1) has been adopted interim first action. The collaborative study evaluating that method will be published in the March 1986 issue of this journal. The Associate Referees recommend that the method be adopted official first action.

Several inquiries have been received concerning the collaborative forage nitrate method. Laboratories have been supplied with the method and have reported success. Forage nitrate control samples were used as part of the collaborative study and proved very useful; however, a continuing source of forage nitrate control samples is not available. The Associate Referees plan to create several pools of forage with known nitrate concentrations that would be distributed to several laboratories for confirmation analysis. These results would then be analyzed and made available with packets of the pooled samples. The control samples would be made available at a nominal fee. This plan will be implemented as soon as possible.

The Associate Referees plan to concentrate on the analysis of biological fluids for nitrate/nitrite during the next year. A survey of several laboratories has been completed and valuable information was obtained. Indications are as follows: (*1*) There is a need for analysis of biological specimens (other than plants) for nitrate/nitrite; (*2*) a variety of methods are used to analyze these specimens; (*3*) ocular fluids, serum, and water are most often analyzed; and (*4*) the analyses are most often performed for diagnostic purposes. Effort to conduct a collaborative study on the analysis of biological fluids for nitrate is now under way. A recent report on nitrate determination in serum by HPLC (21) offers an alternative to the commonly used cadmium reduction technique (22), which is labor intensive and difficult to reproduce. A thorough literature search, identification of laboratories willing to collaborate, and a statistically designed interlaboratory study are planned.

The Associate Referees addressed another problem that exists in nitrate/nitrite analysis. Several units of concentration for nitrate and nitrite are used in the scientific literature. Certain disciplines prefer %KNO_3, others ppm NO_3^-. Although interconversion of units is trivial, confusion can occur. The Associate Referees recommend that a table of conversion factors be included with the section of *Official Methods of Analysis* that deals with nitrate to help avoid problems that may ensue. The suggested conversions are shown in Table 2.

Pesticides in Toxicological Samples.—H. M. Stahr (Iowa State University, Ames, IA) reports his work on a cleanup technique for determination of organophosphate, carbamate, and organochlorine pesticides. Using a C-18 minicolumn, the

Associate Referee has successfully recovered added quantities of all 3 classes of compounds and has applied this cleanup procedure to field cases where tissue levels of 10–100 ppb organophosphates have been detected. He also reports some success in detecting pesticides with the Microtox® bioluminescence screening system.

The Associate Referee recommends continued study.

Selenium in Animal Tissue.—James Roof (Bureau of Animal Industry, Harrisburg, PA) reports that he has completed a collaborative study on a method for determination of Se. The method, a combination of 2 procedures (23, 24) uses dry ashing, complex formation, and gas chromatography (GC)/electron capture detection. Feed, liver, and blood samples were included in the study. Two of the 7 samples in the study were NBS Standard Reference Materials (SRM); a third SRM was used as an external control. Statistical analysis of the results is presently being conducted.

Preliminary analysis of the data indicates some interesting, but possibly troublesome trends. The results of a duplicate pair of blood samples showed a high bias for one of the samples from every reporting laboratory, but no bias was observed in the results from a duplicate pair of liver samples. This bias may indicate a problem with preparation of the test samples. Another interesting result is the significantly lower interlaboratory variation (9.5%) for a spiked blood sample containing 0.34 ppm Se, contrasted with the variation (20%) for its unspiked mate which contained 0.24 ppm. For the entire study, recovery ranged from 80% (feed sample containing 0.75 ppm Se) to 100% (blood sample containing 0.34 ppm Se).

The Associate Referee recommends that the method be considered for action after the statistical analysis has been completed.

Sodium Monofluoroacetate.—H. M. Stahr (Iowa State University, Ames, IA) reports on further work with the indirect determination of sodium monofluoroacetate (1080) in tissues by alkaline defluorination/fluoride electrode detection. Levels of fluoroacetate obtained from tissues of dogs poisoned with 1080 agree with data obtained from poisoned coyotes (25).

The Associate Referee also reports on the determination of 1080 and its metabolically related products fluorocitrate and citrate by GC using Porapak Q and SE30/QF1. In the case of 1080-poisoned animals, the tissue levels of citrate are elevated to 25–35 ppm compared with the normal level of 5 ppm. Fluorocitrate has not been detected at high levels, indicating that secondary poisonings result from 1080 and not from fluorocitrate, as some have hypothesized.

The Associate Referee recommends continued study.

Zinc in Animal Tissues.—Dana Perry (Veterinary Diagnostic Laboratory, Tucson, AZ) reports that he is conducting a survey of diagnostic laboratories to determine (*1*) the frequency of Zn analyses; (*2*) the methods used for those analyses; and (*3*) the number of laboratories interested in participating in interlaboratory studies of Zn methodology.

The Associate Referee recommends continued study.

Recommendations:

(1) Adopt as official first action the interim method for determination of arsenic in animal tissues.

(2) Adopt as official first action the interim method for determination of nitrate in forage by the nitrate electrode. Include as a portion of the official methods for nitrate/nitrite, the conversion table described by the Associate Referees.

(3) Adopt as official final action the first action method for determination of copper in serum (**49.003–49.007**). Continue in official first action status the liver copper method (**49.A01–49.A04**).

(4) Discontinue the topic of Molybdenum. Transfer the study of molybdenum to the Associate Referee on Atomic Absorption Methods.

(5) Discontinue the topic of Lead in Bone. Transfer any future studies of lead in bone to the Associate Referee on Lead in Animal Tissues.

(6) Continue study on all other topics.

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GENERAL REFEREE REPORTS: COMMITTEE ON HAZARDOUS SUBSTANCES IN WATER AND THE ENVIRONMENT

Chemical/Waste Interactions in Soil

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Two Associate Referees have reported on the status of their topics. It is recommended that both of these topics be continued.

Soils—Distribution Coefficients—Nonvolatile Organics.—Danny R. Jackson, Battelle-Columbus Laboratories, was appointed to test and develop a standard test method for determining distribution coefficients in soil of nonvolatile organic chemicals. This method will be tested in his laboratory and then undergo collaborative study.

Volatile Organic Chemicals in Soil and Sediments—Adsorption Isotherm Test.—Leverett R. Smith was appointed to test and develop a standard adsorption isotherm test for volatile organic chemicals. Dr. Smith has a revised draft method. He will subject this method to ruggedness testing; based on those results, the draft method will be revised and used in an interlaboratory collaborative study.

Recommendations

(1) Continue developing standard method for volatile organic chemicals in soil and sediments.

(2) Continue developing standard method for distribution coefficients of nonvolatile organics in soils.

(3) Continue to search for Associate Referees and collaborators who can study and develop methods discussed in the 1984 Referee report.

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Hazardous Substances in Water and the Environment and adopted by the Association. See the report of the committee and "Changes in Methods," this issue.

Drinking, Ground, and Surface Waters

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(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) Ethylene Dibromide (EDB) in Water.—Finalize the evaluation of a steam distillation procedure in the Associate Referee's laboratory, as well as the investigation of the stability of EDB in water. Initiate ruggedness testing. Submit protocol for collaborative study of the gas chromatographic determination of EDB in water to the General Referee and the Statistical Consultant for review and comment. If the protocol is approved, initiate a collaborative study.

(4) Herbicides in Water and Sediment.—Delay collaborative study until study for phenols in water has been initiated.

(5) 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.

(6) Organophosphorus Pesticides in Water.—Appoint an Associate Referee to evaluate and collaboratively test the method proposed by the Environmental Protection Agency or other methods for determining organophosphorus pesticides in water and wastewater.

(7) Phenols in Water.—Finalize study of methods for identification and quantitation of pollutant phenols in water, including the GC pentafluorobenzyl ether derivatization method reported previously (*J. Assoc. Off. Anal. Chem.* (1983) **66,** 1029–1038); select method for collaborative study and submit protocol.

(8) Salt.—Appoint an Associate Referee to review methods **33.138–33.150** (all of which have remained in official first action status since their adoption more than 40 years ago) to evaluate the need for these or other methods for determining moisture, insoluble matter, sulfate, calcium, magnesium, and iodine in salt. Defer consideration of the recommendation by the General Referee and previous Associate Referee to change the temperature in the method for determining moisture in salt, **33.139,** from ca 250°C to ca 105–120°C to make the method applicable for determining moisture in sea salt, pending submission of collaborative data supporting the change.

(9) Triazine Herbicides in Water.—Appoint an Associate Referee to evaluate and collaboratively test methods for determining triazine herbicides in water and wastewater.

(10) Munitions in Wastewater.—Evaluate the submitted report on the interlaboratory evaluation of a method, "Reverse Phase Liquid Chromatographic Determination of Nitro-Organics in Munitions Wastewater." This report meets AOAC criteria for collaborative study and is recommended for adoption by the General Referee.

Recommendations

(1) Adopt as official first action the method for determining nitro-organics in munitions wastewater.

(2) Continue study on all other topics.

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Hazardous Substances in Water and the Environment and adopted by the Association. See the report of the committee and "Changes in Methods," this issue.

Industrial Process Waste

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This area has 5 associate refereeships: Inorganic Analytes, Gerald McKee; Organic Analytes, Paul Friedman; Physical Chemical Properties, Florence Richardson; Bioassays, Llewellyn Williams; Sampling, Associate Referee needed.

Progress has been slower than expected. This has resulted from a combination of technical problems encountered by researchers conducting the method evaluations and workload changes for the Associate Referees. However, progress has been made, which will be discussed below.

Physical Chemical Properties.—(1) Cyanide/Sulfide Evolution Method: §RCRA 261.23 identifies as hazardous wastes those cyanide or sulfide-bearing wastes which, when exposed to pH conditions of 2–12.5, generate toxic gases or fumes in a quantity sufficient to present a danger to human health. The test method being evaluated is designed to identify those waste materials which meet the above definition. The test measures the amount of hydrogen cyanide or sulfide generated under a defined set of conditions.

The method had been the subject of a collaborative study during 1983. The results of that study indicated that, while the method might be suitable for identifying hazardous wastes, it suffered from a number of problems. During 1984–85, additional laboratory work was conducted, which resulted in a new draft method. Further work to calibrate the draft procedure will be conducted early in 1986 and a second collaborative study will be performed. Collaborators are needed for this study.

(2) Explosivity: §RCRA 261.23 identifies as hazardous wastes those materials which are capable of detonation. The U.S. Card Gap Test and the U.S. Internal Ignition Test have been under evaluation as a means of determining if a given waste material can undergo detonation under reasonable mismanagement conditions. The methods were the subject of a single laboratory evaluation last year.

Because of problems with the single laboratory study, additional work will have to be performed before method submission.

(3) Ignitability: The accumulation of waste at landfill sites may pose a number of hazards, e.g., the possibility of ignition and uncontrolled burning. To identify wastes subject to this hazard, a study was conducted to develop methodology suitable for determining ignitability of solid waste materials. Methods addressed the following properties of a solid material: ease of ignition, rapidity of burning once ignited, and difficulty of extinguishing the fire.

Single laboratory evaluation of these protocols were conducted using simulated actual waste sample. The data were encouraging and a collaborative study, with several laboratories, was performed. The study identified some deficiencies in the apparatus which were corrected. A second study is now being organized.

Inorganic and Organic Analyte Methods.—During the past year, several methods were evaluated. However, workloads have prevented the effort needed for a first action recommendation.

Toxicity Characteristic Leaching Procedure.—A new procedure is being developed by EPA for use in simulating the leaching of a waste disposed in a sanitary landfill. The toxicity characteristic leaching procedure (TCLP) is suitable for determining the mobility of both organic and inorganic compounds present in liquid, solid, and multiphasic wastes.

A meeting was held with the AOAC meeting in Dallas to present results of the development program and to solicit collaborators for the study to begin in November 1985.

Miscellaneous.—EPA's Office of Solid Waste is preparing a guidance manual on how to submit an equivalent methods petition for the hazardous waste program. AOAC is assisting EPA in developing the statistical methods that the agency will use in evaluating such petitions. Persons interested in assisting in peer review of the draft manual are advised to contact Florence Richardson (202/382-4778).

This referee area is suffering from a lack of participation from AOAC's general membership. We have advertised for collaborators on 3 method evaluations this year and no one has volunteered. The hazardous waste problem is a major one and development of valid testing protocols deserves the support of all chemists and laboratories. Let's make a viable contribution to this critical national program. Volunteer!

Recommendations

AOAC management should mount a coordinated effort to broaden member interest and participation in this committee's activities.

This report of the General Referee was presented at the AOAC 99th Annual International Meeting, Oct. 27–31, 1985, at Washington, DC.

The recommendations of the General Referee were approved by the Committee on Hazardous Substances in Water and the Environment and adopted by the Association. See the report of the committee and "Changes in Methods," this issue.

TRANSACTIONS: ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS

The ninety-ninth annual international meeting of the Association of Official Analytical Chemists was held at the Shoreham Hotel, Washington, DC, on October 27, 28, 29, 30, and 31, 1985. The following reports, along with the actions of the Association, were given at the business meeting, held Thursday, October 31, 1985, Richard J. Ronk, presiding.

Committee on Pesticide Formulations and Disinfectants: 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);
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JAMES J. KARR (Pennwalt Technical Center, 900 First Ave, Box C, King of Prussia, PA 19406);
JAMES P. MINYARD, JR (Mississippi State Chemical Laboratory, PO Box CR, Mississippi State, MS 39762);
RICHARD S. WAYNE (American Cyanamid Co., Agricultural Division, Box 400, Princeton, NJ 08540);
RICHARD H. COLLIER (Purdue University, Department of Entomology, West Lafayette, IN 47907), *Secretary*;
JAMES HANSEN (Union Carbide, Box 8361, South Charleston, WV 25303), *Statistical Consultant*;
BERTRAM D. LITT (Environmental Protection Agency, Office of Pesticide Programs, 1921 Jefferson Davis Hwy, Arlington, VA 22202), *Statistical Consultant*

The Committee on Pesticide Formulations and Disinfectants met on October 27 and 28, 1985, in Washington, DC. The meeting was attended by the General Referees whose topics are assigned to the Committee.

The Committee approved the renaming of a number of pesticide formulations topics to consistently use common names of chemicals as topic titles. Chemical names are to be used as topic names when no common name has been approved by the International Standards Organization (ISO), the American National Standards Institute (ANSI), or other generally recognized standard setting organization. Frequently used trade names may be included in a topic title for the sake of clarity. When trade names are included, they will be listed in parentheses. These guidelines have been applied to the current topics in this report. The Committee plans to apply these guidelines to future topics and similar guidelines to method titles.

The Committee discussed the status of the hydrolyzable chlorine method for dicofol in relation to the LC method, which is recommended for adoption as official first action in this report. The Committee considered recommending deletion of the hydrolyzable chlorine method, but decided to delay such a recommendation until the need for the method outside the United States can be assessed.

The Committee discussed the topic of methods of analysis of seed treatment chemicals (pesticides and "safeners") and treated seed. The Committee determined that methods of analysis of seed treatment chemicals are within the scope of this Committee. The need for methods in this area will be investigated. The Committee feels that the analysis of treated seed is more closely associated with pesticide residue analysis than with pesticide formulations analysis, and suggests that the Committee on Residues assess the need for methodology in the area of treated seed.

The Committee also discussed the rapid growth of test kits for the qualitative analysis of pesticides in groundwater, on treated properties, and the like. The Committee refers this topic to the Committee on Hazardous Substances in Water and the Environment for investigation of the desirability of official methods.

At the request of the Associate Referee for alachlor, the Committee reviewed a proposed revision of the official first action GLC method to include a new type of formulation, an encapsulated product called "Micro-Tech." The Committee recommended to the Associate Referee that a mini-collaborative study be designed to test the applicability of the method for this new formulation.

The Committee discussed several issues in the disinfectants area. There are current controversies relative to both the use-dilution test and the tuberculocidal test. In regard to the use-dilution test, the co-Associate Referee is evaluating results of a collaborative study aimed at determining the problem areas of the method. The General Referee has indicated that a fully modified method will be ready for collaborative study in May or June 1986. The Committee will request an opportunity to review the protocol of the study before it is initiated. That protocol should provide means to determine and control the bacterial load and the quality of the reagents and apparatus, such as the letheen broth and the stainless steel cylinders.

In regard to the tuberculocidal test, an independent panel, appointed by the U.S. Environmental Protection Agency, has been selected to review the AOAC method and a method proposed by a disinfectant manufacturer. The Committee decided to delay making recommendations regarding the AOAC tuberculocidal test until the EPA panel has made its report. That report is expected in November 1985.

The Committee discussed terms of office for its General Referees. The Committee Chairman will make recommendations through the appropriate channels.

The Committee recommends discontinuing the General Referee areas of Pesticide Formulations: General Methods and Pesticide Formulations: Inorganic Pesticides. Current

*An asterisk before a recommendation indicates that official action on that recommendation resulted in its appearance in "Changes in Methods."

Section numbers refer to *Official Methods of Analysis* (1984) 14th edition, and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* (1985) **68**, 369–411.

The recommendations submitted by the Committee on Pesticide Formulations and Disinfectants were adopted by the Association.

Associate Referee topics in these areas should be transferred to other appropriate areas as follows:

Associate Referee Topic	*General Referee Area*
Dioxins in Pentachlorophenol	Fungicides and Disinfectants
Sampling	Rodenticides and Miscellaneous Pesticides
Water-Soluble Copper in Water-Insoluble Fungicides	Fungicides and Disinfectants
Aluminum Phosphide	Other Insecticides, Synergists, and Insect Repellants
Sodium Chlorate	Herbicides III

Four first action methods have been recommended to the Committee for final approval. Two of these are recommended to the Association for adoption. Regarding the rejected recommendations, (*1*) the Committee will request that the Associate Referee for allethrin and the General Referee in that area provide comparative information on the GLC and the titrimetric methods before taking action on the recommendation for the final action on the titrimetric method; and (*2*) the Committee recommends that the GLC method for alachlor remain in first action until the method has been in broad use for at least one year.

Eleven methods were submitted to the Committee for first action approval. Nine of these are recommended to the Association for adoption, 3 of which have previously achieved interim first action status. The 2 methods not recommended for adoption will be returned to the authors with suggestions for improvement. It is expected that these methods will be eligible for interim first action approval later in the year.

DISINFECTANTS

(1) *Antimicrobial Agents in Laundry Products:* Continue study.
(2) *Textile Antibacterial Preservatives:* Continue study.
(3) *Sporicidal Tests:* Continue study.
(4) *Tuberculocidal Tests:* Continue study.
(5) *Use-Dilution Tests, Variation and Amendments:* Continue study.
(6) *Virucidal Tests:* Continue study.

PESTICIDE FORMULATIONS: GENERAL METHODS

(1) *Dioxins (2,3,7,8-Tetrachloro-p-dibenzo-p-dioxin in 2,4,5,-T):* Discontinue topic.
(2) *Dioxins in Pentachlorophenol:* (a) Transfer topic to Fungicides and Disinfectants General Referee area (b) Continue study.
(3) *Physical Properties of Pesticides:* Discontinue topic.
(4) *Sampling:* (a) Continue official first action status of the sampling procedures for fertilizers (**2.001–2.002**) as applied to Pesticide-Fertilizer Mixtures. (b) Transfer topic to Rodenticides and Miscellaneous Pesticides General Referee area. (c) Continue study.
(5) *Water-Soluble Copper in Water-Insoluble Copper Fungicides:* (a) Continue official first action status of the CIPAC-AOAC atomic absorption and bathocuproine methods (**6.066–6.072**). (b) Transfer topic to Fungicides and Disinfectants General Referee area.

PESTICIDE FORMULATIONS: INORGANIC PESTICIDES

(1) *Aluminum Phosphide:* (a) Transfer topic to Other Insecticides, Synergists, and Insect Repellants General Referee area. (b) Appoint an Associate Referee. (c) Continue study.
(2) *Sodium Chlorate:* (a) Transfer topic to Herbicides III General Referee area. (b) Appoint an Associate Referee. (c) Continue study.

PESTICIDE FORMULATIONS: FUNGICIDES AND DISINFECTANTS

(1) *Anilazine (Dyrene):* Continue study.
(2) *Benomyl:* (a) Continue official first action status of the liquid chromatographic method (**6.522–6.527**). (b) Continue study.
(3) *Carboxin and Oxycarboxin:* Continue study.
(4) *Chlorothalonil:* Continue study.
(5) *Copper Naphthenate:* Continue study.
(6) *Dinocap:* Continue study.
(7) *Dithiocarbamate Fungicides:* Continue study.
*(8) *Oxythioquinox (Morestan):* (a) Adopt as official first action the liquid chromatographic method described by the Associate Referee. (b) Continue study.
(9) *Pentachloronitrobenzene:* Discontinue topic.
(10) *o-Phenylphenol:* Continue study.
(11) *Quaternary Ammonium Compounds:* Continue study.
(12) *Thiram:* Continue study.
(13) *Triadimefon (Bayleton):* (a) Continue official first action status of the liquid chromatographic method (**6.A31–6.A36**). (b) Continue study.
(14) *Triphenyltin (Fentin):* (a) Continue official first action status of the gas chromatographic method (**6.079–6.084**). (b) Continue study.

PESTICIDE FORMULATIONS: HERBICIDES I

(1) *Chlorophenoxy Herbicides:* (a) Continue official first action status of the following liquid chromatographic methods: Combinations of 2,4-D, dicamba, and MCPP amine salts (**6.321–6.327**); 2-methyl-4-chlorophenoxyacetic acid (**6.372–6.377**); 2,4,5-trichlorophenoxyacetic acid (**6.391–6.395**); 2,4-dichlorophenoxyacetic acid esters and amine salts (**6.288–6.292**). (b) Continue study.
(2) *Dicamba:* Continue study.
(3) *Pentachlorophenol:* (a) Appoint an Associate Referee. (b) Continue study.
(4) *Plant Growth Regulators:* (a) Appoint an Associate Referee. (b) Continue study.
(5) *2,3,6-Trichlorobenzoic Acid:* Discontinue topic.

PESTICIDE FORMULATIONS: HERBICIDES II

(1) *Barban (Carbyne):* Continue study.
(2) *Benefin, Trifluralin, Pendimethalin, and Ethafluralin:* Continue study.
(3) *Bensulide (Betasan):* Continue study.
(4) *Benzoylprop-ethyl:* Continue study.
(5) *Bromacil and Lenacil:* Continue study.
(6) *Chlorsulfuron (Glean):* Continue study.
(7) *Dimethyl Tetrachloroterephthalate:* Continue study.
(8) *Dinoseb:* (a) Appoint an Associate Referee. (b) Continue study.
(9) *Diuron:* Combine into new topic "Substituted Urea Herbicides."
(10) *Fluometuron:* Continue study.
(11) *Linuron:* Combine into new topic "Substituted Urea Herbicides."
(12) *Metsulfuron-methyl (Ally):* Continue study.
(13) *Methazole:* Continue study.

(14) *Monuron:* Combine into new topic "Substituted Urea Herbicides."
(15) *Naptalam (Alanap):* Continue study.
(16) *Oryzalin (Surflan):* (a) Appoint an Associate Referee. (b) Continue study.
(17) *Siduron:* Combine into new topic "Substituted Urea Herbicides."
(18) *Substituted Urea Herbicides:* (a) Appoint an Associate Referee. (b) Continue study.
(19) *Sulfuron-methyl (Oust):* Continue study.
(20) *Thiocarbamate Herbicides:* (a) Continue official first action status of the gas chromatographic method for thiocarbamate herbicides (**6.581–6.585**). (b) Continue study.

PESTICIDE FORMULATIONS: HERBICIDES III

*(1) *Alachlor, Butachlor, and Propachlor:* (a) Continue official first action status of the gas chromatographic method for alachlor (**6.A05–6.A08**). (b) Adopt as official first action the gas chromatographic methods for butachlor and propachlor as described by the Associate Referee, with statistical and editorial changes. (c) Continue study.
(2) *Amitrole:* (a) Appoint an Associate Referee. (b) Continue study.
(3) *Bentazon (Basagran):* Continue study.
(4) *Bromoxynil:* Continue study.
(5) *Cacodylic Acid, MSMA, DSMA:* Continue study.
(6) *Cyanazine (Bladex):* Continue study.
(7) *Dalapon:* (a) Continue official first action status of the liquid chromatographic method (**6.296–6.301**). (b) Continue study.
(8) *Dichlobenil:* Continue study.
(9) *Fluazifop-butyl (Fusilade):* Continue study.
(10) *Glyphosate:* Continue study.
(11) *Metolachlor:* (a) Continue official first action status of the gas chromatographic method (**6.A14–6.A18**). (b) Continue study.
*(12) *Metribuzin (Lexone or Sencor):* (a) Adopt as official final action the gas chromatographic method (**6.553–6.559**). (b) Continue study.
(13) *Pesticides in Fertilizers:* Continue study.
(14) *Propanil:* Continue study.
(15) *s-Triazine Herbicides:* Continue study.

PESTICIDE FORMULATIONS: CARBAMATE INSECTICIDES AND SUBSTITUTED UREA INSECTICIDES

(1) *Aldicarb:* Continue study.
(2) *Aminocarb:* (a) Continue official first action status of the liquid chromatographic method (**6.A25–6.A30**). (b) Continue study.
*(3) *Bendiocarb:* (a) Adopt as official first action the interim first action liquid chromatographic method described by the Associate Referee. (b) Continue study.
(4) *Carbaryl:* Continue study.
*(5) *Carbofuran and Carbosulfan:* (a) Adopt as official first action the interim first action liquid chromatographic method for Carbofuran described by the Associate Referee. (b) Continue study.
(6) *Methiocarb:* (a) Continue official first action status of the liquid chromatographic method (**6.546–6.552**). (b) Continue study.
(7) *Methomyl:* Continue study.
(8) *Mexacarbate (Zectran):* Continue study.
(9) *Oxamyl:* Continue study.
(10) *Pirimicarb:* (a) Continue official first action status of the gas chromatographic method (**6.560–6.564**). (b) Continue study.
(11) *3,4,5- and 2,3,5-Trimethylphenyl Carbamate Isomers:* Continue study.
(12) *Propoxur (Baygon):* (a) Continue official first action status of the liquid chromatographic method (**6.565–6.571**). (b) Continue study.

PESTICIDE FORMULATIONS: HALOGENATED INSECTICIDES

*(1) *Benzene Hexachloride and Lindane:* (a) Adopt as official final action the AOAC-CIPAC gas chromatographic method for BHC, gamma isomer, and lindane (**6.221–6.226**). (b) Continue study.
(2) *Chlordane:* Continue study.
(3) *Chlordimeform:* (a) Continue official first action status of the gas chromatographic method (**6.A09–6.A13**). (b) Continue study.
*(4) *Dicofol (Kelthane):* (a) Adopt as official first action the interim first action liquid chromatographic method described by the Associate Referee. (b) Continue official first action status of the hydrolyzable chloride method (**6.332–6.337**). (c) Continue study.
(5) *Ethylan (Perthane):* Continue study.
(6) *Fenvalerate:* Continue study.
(7) *Heptachlor:* Continue study.
(8) *Methoxychlor:* Continue study.
(9) *Methyl Bromide:* Continue study.
(10) *Toxaphene:* Continue study.
(11) *Trichlorfon (Dylox):* Continue study.

PESTICIDE FORMULATIONS: ORGANOTHIOPHOSPHORUS PESTICIDES

(1) *Acephate (Orthene):* Continue study.
(2) *Azinphos-methyl (Guthion):* Continue study.
(3) *Coumaphos:* Continue study.
(4) *Demeton (Systox):* Continue study.
(5) *Demeton-S-methyl (Metasystox-I):* Continue study.
(6) *Diazinon:* (a) Continue official first action status of the gas chromatographic method for diazinon (**6.425**). (b) Continue study.
(7) *Dimethoate:* Continue study.
(8) *Dioxathion:* Continue study.
(9) *Encapsulated Organophosphorus Pesticides:* (a) Continue official first action status of the gas chromatographic method for encapsulated diazinon (**6.426–6.429**). (b) Continue study.
(10) *EPN:* Continue study.
(11) *Ethoprop:* Continue study.
(12) *Fenitrothion:* (a) Continue official first action status of the gas chromatographic method (**6.A19–6.A24**). (b) Continue study.
*(13) *Fensulfothion:* (a) Adopt as official first action the gas chromatographic method described by the Associate Referee. (b) Continue study.
(14) *Fenthion:* Continue study.
(15) *Fonophos:* Continue study.
(16) *Isofenphos:* Continue study.
(17) *Malathion:* Continue study.
(18) *Methamidophos (Monitor):* Continue study.
(19) *Methidathion (Supracide):* Continue study.
(20) *Oxydemeton-methyl (Metasystox-R):* Continue study.
(21) *Parathion and Methyl Parathion:* (a) Continue official first action status of the volumetric (**6.472–6.478**), colorimetric (**6.479–6.483**), gas chromatographic (**6.463–**

6.467), and liquid chromatographic (**6.468–6.471**) methods for parathion, and the gas chromatographic (**6.484–6.488**) and liquid chromatographic (**6.489–6.492**) methods for methyl parathion. (b) Continue study.

(22) *Phorate:* Continue study.

(23) *Pirimiphos-methyl:* Continue study.

(24) *Temephos:* (a) Continue official first action status of the CIPAC-AOAC liquid chromatographic method (**6.509–6.515**). (b) Continue study.

(25) *Tributyl Phosphorotrithioate:* Continue study.

PESTICIDE FORMULATIONS: OTHER ORGANOPHOSPHORUS INSECTICIDES

(1) *Crotoxyphos:* Continue study.

(2) *Cruformate (Ruelene):* Discontinue topic.

(3) *Dichlorvos (DDVP):* (a) Continue official first action status of the infrared methods (**6.417–6.420** and **6.421–6.424**). (b) Continue study.

(4) *Fenamiphos (Nemacur):* Continue study.

(5) *Mevinphos:* Continue study.

(6) *Monocrotophos:* Continue study.

(7) *Naled:* Continue study.

(8) *Tetrachlorvinphos (Gardona, Rabon):* Continue study.

PESTICIDE FORMULATIONS: OTHER INSECTICIDES, SYNERGISTS, AND INSECT REPELLANTS

(1) *Allethrin:* (a) Continue official first action status of the titrimetric method (**6.165–6.170**). (b) Continue study.

(2) *2,3,4,5-Bis(2-butylene)tetrahydro-2-furfural (MGK Repellant 11):* Continue study.

(3) *Cyhexatin (Plictran):* Continue study.

*(4) *Cypermethrin:* (a) Adopt as official first action the CIPAC gas chromatographic method described by the General Referee, with editorial changes. (b) Continue official first action status of the gas chromatographic method (**6.A01–6.A04**). (c) Continue study.

(5) *Cyromazine (Larvadex):* Continue study.

(6) *Dipropyl Isocinchomeronate (MGK Repellant 326):* Continue study.

(7) *Fumigants:* Continue study.

(8) *Nicotine:* Continue study.

*(9) *Permethrin:* (a) Adopt as official first action the CIPAC gas chromatographic method described by the General Referee, with editorial changes. (b) Continue study.

(10) *Piperonyl Butoxide and Pyrethrins:* Continue study, including low levels and mixed formulations.

(11) *Resmethrin:* Continue study.

(12) *Rotenone and Other Rotenoids:* (a) Continue official first action status of the liquid chromatographic method (**6.182–6.186**) and the infrared method (**6.179–6.180**). (b) Continue study.

PESTICIDE FORMULATIONS: RODENTICIDES AND MISCELLANEOUS PESTICIDES

(1) *Brodifacoum (Talon):* (a) Continue official first action status of the liquid chromatographic method (**6.597–6.601**). (b) Continue study.

(2) *Chlorophacinone:* Continue study.

(3) *Diphacinone:* Continue study.

(4) *Alpha-Naphthylthiourea (Antu):* (a) Continue official first action status of the surplus method (**6.157**). (b) Continue study.

(5) *N-3-Pyridyl-N'-p-nitrophenyl Urea (Vacor):* Discontinue topic.

(6) *Strychnine:* Continue study.

(7) *Warfarin:* (a) Appoint an Associate Referee. (b) Continue study.

Committee on Drugs and Related Topics: Recommendations for Official Methods

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BIOCHEMICAL METHODS

(1) *Aminoglycosides in Animal Tissue:* Discontinue topic.
(2) *17β-Estradiol and Diethylstilbestrol in Tissues (Immunochemical Methods):* Discontinue topic.
(3) *Hormones in Tissues (Immunospecific Affinity Chromatography):* Discontinue topic.
(4) *Human Chorionicgonadotropin by Non-RIA Procedures (Performance Evaluation Method):* Change the title of this topic to Heparin Quantitation by Non-RIA Procedures. Continue study.
(5) *Hybridoma-Monoclonal Antibodies:* Continue study.
(6) *Immunochemical Species Identification of Meat:* Continue study.
(7) *Performance Evaluation Protocols for Clinical Chemical and Immunochemical Diagnostic Products:* Discontinue topic.
(8) *Steroid Quantitation (Enzymatic Methods):* Discontinue topic.
(9) *Sulfa Drugs in Animal Tissues (Immunoassay):* Discontinue topic.

DIAGNOSTICS AND TEST KITS

(1) *Oxalates-Enzymatic Procedures:* Continue study.

DRUGS I

(1) *Acetaminophen in Drug Mixtures:* Continue study.
(2) *Acetaminophen with Codeine Phosphate:* Continue study.
(3) *Allopurinol:* Continue in official first action status the liquid chromatographic method for the determination of allopurinol in tablets, **37.A01–37.A06.** Continue study.
*(4) *Amitriptyline HCl in Dosage Forms (LC Method):* Adopt as official final action the first action liquid chromatographic method for the determination of amitriptyline in tablets and injectables, **36.207–36.211.** Discontinue topic.
(5) *Aspirin and Caffeine with Other Drugs:* Continue study.
(6) *Barbiturates:* Discontinue topic.
(7) *Benzothiazine Derivatives:* Merge with the topic Benzthiazides by LC.
(8) *Benzthiazides by LC:* Continue study.
(9) *Chlordiazepoxide by LC:* Continue study.
(10) *Chlorpromazine:* Discontinue topic.
(11) *Methyldopa:* Continue in official first action status the liquid chromatographic method for the determination of methyldopa and methyldopa-thiazide combinations in tablets, **37.A11–37.A16.** Continue study.
(12) *Phenothiazines and Tricyclic Antidepressants in Formulations by LC:* Continue study.
(13) *Phenothiazine-Type Drugs by TLC:* Continue study.
*(14) *Primidone:* Adopt as official first action the interim first action liquid chromatographic method for the determination of primidone in tablets, **37.A17–37.A22.** Continue study.
(15) *Probenecid:* Discontinue topic.
(16) *Sulfamethoxazole:* Continue in official first action status the liquid chromatographic method for the determination of sulfamethoxazole in tablets, **37.A07–37A.10.** Continue study.
*(17) *Sulfisoxazole:* Adopt as official final action the first action liquid chromatographic method for the determination of sulfisoxazole in tablets, solutions, and ointments, **37.166–37.172.** Discontinue topic.
(18) *Sulfonamides by LC:* Discontinue topic.
(19) *Thiazide Diuretics, Semiautomated Individual Unit Analysis:* Discontinue topic.

DRUGS II

(1) *Aminacrine:* Continue study.
(2) *Atropine in Morphine and Atropine Tablets and Injections:* Continue study.
(3) *Belladonna Alkaloids:* Appoint an Associate Referee. Continue study.
(4) *Colchicine in Tablets:* Continue in official first action status the liquid chromatographic method for the determination of colchicine in tablets, **38.A01–38.A06.** Continue study.
(5) Curare Alkaloids: Continue study.
(6) *Dicyclomine Capsules:* Appoint an Associate Referee. Continue study.
(7) *Epinephrine-Lidocaine Combinations:* Appoint an Associate Referee. Continue study.
(8) *Epinephrine and Related Compounds by LC-Electrochemical Detectors:* Continue study.
(9) *Ergot Alkaloids:* Continue study.
(10) *Homatropine Methyl Bromide in Tablets:* Appoint an Associate Referee. Continue study.
(11) *Morphine Sulfate in Morphine Injection:* 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 the Committee on Drugs and Related Topics were adopted by the Association.

Section numbers refer to *Official Methods of Analysis* (1984) 14th edition, and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* (1985) **68**, 369–411.

(12) *Neostigmine:* Appoint an Associate Referee. Continue study.
(13) *Phenethylamine Drugs, Semiautomated Individual Unit Analysis:* Continue study.
(14) *Pheniramine with Pyrilamine, Phenylpropanolamine, and Phenylephrine:* Continue study.
(15) *Physostigmine and Its Salts:* Continue in official first action status the liquid chromatographic method for the determination of physostigmine salicylate in solution and physostigmine sulfate in ointment, **38.074–38.080.** Continue study.
(16) *Pilocarpine:* Continue in official first action status the liquid chromatographic method for the simultaneous determination of pilocarpine, isopilocarpine, and pilocarpic acid, **38.144–38.150.** Continue study.
(17) *Rauwolfia Alkaloids:* Appoint an Associate Referee. Continue study.
(18) *Rauwolfia serpentina:* Continue study.

DRUGS III

(1) *Ampicillin and Amoxicillin:* Continue study.
(2) *Bisacodyl:* Continue study.
(3) *Coumarin Anticoagulants:* Continue study.
(4) *Flucytosine:* Continue study.
*(5) *Fluoride:* Adopt as official final action the first action fluoride-selective electrode method for determination of fluoride in sodium fluoride preparations, **36.063–36.069.** Continue study.
(6) *Halogenated Hydroxyquinoline Drugs:* Continue study.
(7) *Hydralazine:* Continue study.
(8) *Insulin—Human, Porcine, and Bovine—by LC:* Continue study.
(9) *Levodopa:* Continue study.
(10) *Medicinal Gases:* Continue study.
(11) *Meprobamate Tablets:* Continue study.
(12) *Mercury-Containing Drugs:* Continue study.
(13) *Metals in Bulk Drug Powders:* Continue study.
(14) *Miconazole:* Continue study.
(15) *Microchemical Tests:* Continue in official first action status the microchemical identification test for promethazine, trifluopromazine, perphenazine, and thiethylperazine in pure drug substances, **36.A07–36.A10.** Continue study.
(16) *Penicillins:* Continue study.
*(17) *Protein Nitrogen Units in Allergenic Extracts:* Amend official final action method **38.205–38.207** to specify use of the Kjeldahl method for determination of nitrogen as shown in *J. Assoc. Off. Anal. Chem.* (1981) **64,** 1436–1437.
(18) *Salts of Organic Nitrogenous Bases:* Continue study.
(19) *Thyroid and Thyroxine Related Compounds:* Discontinue topic.
(20) *Trimethobenzamide:* Continue in official first action status the ion-pair chromatographic method for the determination of trimethobenzamide hydrochloride in capsules, **36.A01–36.A06.** Continue study.

DRUGS IV

(1) D- *and* L-*Amphetamines—LC Separations:* An Associate Referee has been appointed. Continue study.
(2) *Amphetamines in Mixtures:* Discontinue topic.
(3) *Benzodiazepines:* Continue study.
(4) *Chemical Microscopy:* Discontinue topic.
(5) *Diazepam:* Continue in official first action status the liquid chromatographic method for determination of diazepam in tablets, **40.A01–40.A06.** Continue study.
(6) *Dimethyltryptamine (DMT), Diethyltryptamine (DET), and Dipropyltryptamine (DPT):* Discontinue topic.
(7) *Heroin:* An Associate Referee has been appointed. Continue in official first action status the method for determination of diacetylmorphine (heroin) in tablets, **40.007.** Continue study.
(8) *Lysergic Acid Diethylamide (LSD):* Discontinue topic.
(9) *Marihuana and Synthetic Tetrahydrocannabinol (THC):* Discontinue topic.
(10) *Methadone:* Discontinue topic.
(11) *Methamphetamine:* Discontinue topic.
(12) *Methylphenidate Phenidine Hydrochloride:* Discontinue topic.

DRUGS V

(1) *Betamethasone:* Continue study.
*(2) *Chlorpropamide:* Adopt as official first action the interim liquid chromatographic method described by the Associate Referee for the determination of chlorpropamide in tablet dosage forms. Continue study.
(3) *Digitoxin, Automated Individual Tablet Analysis:* Continue study.
(4) *Estrogens (Conjugated):* Continue study.
(5) *Hydrocortisone:* An Associate Referee has been appointed. Continue in official first action status the liquid chromatographic method for the determination of hydrocortisone in drug substances and tablets, **39.047–39.055.** Continue study.
(6) *Methocarbamol:* Continue in official first action status the liquid chromatographic method for the determination of methocarbamol in pharmaceutical dosage forms, **36.212–36.218.** Continue study.
(7) *Pentaerythritol:* Initiate topic. Appoint an Associate Referee.
*(8) *Prednisolone in Tablets and Bulk:* Establish an Associate Refereeship. Adopt as official first action the liquid chromatographic method described by the Associate Referee for determination of prednisolone in tablets and bulk drugs (*J. Assoc. Off. Anal. Chem.* (1984) **67,** 674–676). Continue Study.
(9) *Progestins in Tablets—Automated Methods:* Continue study.
(10) *Steroid Acetates:* Continue study.
(11) *Steroid Phosphates:* Continue in official first action status the spectrophotometric method for determination of steroid phosphates, **39.056–39.060.** Continue study.

FORENSIC SCIENCES

(1) *ABO Blood Typing:* Continue study.
(2) *Biological Fluids (Immunoelectrophoresis):* Continue study.
(3) *Blood:* Continue study.
(4) *Bloodstains, ABH Typing:* Discontinue topic.
(5) *Bloodstains, Species Determination:* Discontinue topic.
(6) *Bomb Residues:* Continue study.
(7) *Documents:* Discontinue topic.
(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:* Discontinue topic.
(17) *Safe Insulation:* Discontinue topic.
(18) *Serial Number Restoration (Chemical Etching Techniques):* Discontinue topic.
(19) *Soil Analysis:* Continue study.
(20) *Voice Print Identification:* Discontinue topic.

Committee on Foods I: Recommendations for Official Methods

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COFFEE AND TEA

(1) *Ash in Instant Tea:* Continue study.
(2) *Caffeine:* Incorporate under new topic, Methyl Xanthines in Coffee and Tea.
(3) *Crude Fiber in Tea:* Discontinue topic.
(4) *Moisture in Coffee and Tea:* Continue study.
(5) *Solvent Residues in Decaffeinated Coffee and Tea:* Continue study.
(6) *Theophylline in Tea:* Incorporate under new topic, Methyl Xanthines in Coffee and Tea.
(7) *Water Extract in Tea:* Appoint Associate Referee; continue study.
(8) *Other Topic:* Initiate new topic, Methyl Xanthines in Coffee and Tea.

DAIRY PRODUCTS

(1) *Adulteration of Dairy Products with Vegetable Fat:* Continue study.
*(2) *Babcock Test and Babcock Glassware:* Adopt as official first action the provision for the use of the 5% Gercock milkfat test bottle as described by Associate Referee R. L. Bradley; continue study.
(3) *Casein and Caseinates:* Continue study.
(4) *Chocolate Milk (Fat Test):* Continue study.
(5) *Cryoscopy of Milk:* Continue study.
(6) *Fat (Automated Methods):* Continue study.
(7) *Fat in Milk (AutoAnalyzer):* Continue study.
(8) *Infrared Milk Analyzer (IRMA):* Continue study.
(9) *Iodine in Milk and Dairy Products:* Continue study.
(10) *Lactose in Dairy Products (Chromatographic Determination):* Continue study.
(11) *Lactose in Dairy Products (Enzymatic Determination):* Continue study.
*(12) *Moisture in Cheese:* Adopt as official first action the method for sampling barrel cheese for moisture analysis as described by Blattner, Olson, and Wichern (*J. Assoc. Off. Anal. Chem.* **68,** 718–721 (1985)); continue study.
(13) *Nitrates in Cheese:* Continue study.
(14) *Phosphatase (Rapid Method):* Continue study.
*(15) *Phosphatase (Reactivated):* Adopt as official first action the rapid method of sample preparation for detecting alkaline phosphatase in casein as presented by G. K. Murthy and J. T. Peeler; continue study.
(16) *Phosphorus:* Continue study.
(17) *Protein Constituents in Processed Dairy Products:* Continue study.
(18) *Protein in Milk (Rapid Tests):* Continue study.
(19) *Protein Reducing Substance Tests:* Continue study.
(20) *Solids-Not-Fat:* Continue study.
(21) *Total Solids and Moisture (Microwave Drying):* Continue study.
(22) *Vapor Pressure Osmometry:* Continue study.
(23) *Whey Proteins in Non-Fat Dry Milk:* Continue study.

DECOMPOSITION AND FILTH IN FOODS (CHEMICAL METHODS)

(1) *Ammonia in Seafood:* Continue study.
(2) *Coprostanol:* Continue study.
(3) *Crabmeat:* Continue study.
(4) *Diacetyl in Citrus Products:* Continue study.
*(5) *Ethanol in Seafoods:* Adopt as official first action the headspace GC method for determination of ethanol in canned salmon; 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 Determination of Amines in Fishery Products:* Continue study.
(10) *Tomatoes:* Continue study.

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Section numbers refer to *Official Methods of Analysis* (1984) 14th edition.
The recommendations submitted by the Committee on Foods I were adopted by the Association.

FISH AND OTHER MARINE PRODUCTS

(1) *Determination of Fish Content in Coated Products (Breaded or in Batter):* Continue study.
(2) *Drained Weight of Block Frozen, Raw, Peeled Shrimp:* Re-initiate topic; appoint Associate Referee; continue study.
(3) *Fish Species Identification (Thin Layer Isoelectric Focusing):* Continue study.
(4) *Minced Fish in Fish Fillet Blocks:* Continue study.
(5) *Nitrites in Smoked Fish:* Appoint Associate Referee; continue study.
(6) *Organometallics in Fish:* Continue study.

FOOD ADDITIVES

(1) *Anticaking Agents:* Appoint Associate Referee; continue study.
(2) *Antioxidants:* Continue study.
(3) *Brominated Vegetable Oils:* Continue study.
(4) *Chloride Titrator:* Continue study.
(5) *Dilauryl Thiodipropionate:* Continue study.
(6) *Dressings:* Continue study.
(7) *EDTA in Food Products:* Continue study.
(8) *Gums:* Appoint Associate Referee; continue study.
(9) *Indirect Additives from Food Packages:* Continue study.
(10) *Nitrates (Selective-Ion Electrode):* Continue study.
(11) *Nitrates and Nitrites:* Continue study.
(12) *Nitrosamines:* Continue study.
(13) *Polycyclic Aromatic Hydrocarbons in Foods:* Continue study.
(14) *Polysorbates:* Continue study.
(15) *Propylene Chlorohydrin:* Appoint Associate Referee; continue study.
(16) *Quinine in Soft Drinks:* Continue study.
(17) *Sodium Lauryl Sulfate:* Appoint Associate Referee; continue study.
(18) *Other Topic:* Initiate new topic Sulfiting Agents in Foods; appoint Associate Referee.

MEAT, POULTRY, AND MEAT AND POULTRY PRODUCTS

(1) *Automated Methods:* Continue study.
(2) *Bioassay Methods for Meat and Poultry Products:* Appoint Associate Referee; continue study.
(3) *Chemical Antibiotic Methods:* Appoint Associate Referee; continue study.
(4) *Crude Protein Analysis of Meat (Peroxymonosulfuric Acid Digestion):* 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:* Continue study.
(9) *3-Methyl Histidine:* Continue study.
(10) *Nitrates and Nitrites:* Appoint Associate Referee; continue study.
(11) *Nitrosamines in Bacon:* Appoint Associate Referee; continue study.
(12) *Nonmeat Proteins in Meat:* Continue study.
(13) *Proteins in Meat and Meat Products:* Continue study.
(14) *Serological Identification of Animal and Poultry Species:* Continue study.
(15) *Species Identification by ORBIT and PROFIT:* Continue study.
(16) *Specific Ion Electrode Applications:* Continue study.
(17) *Steroid Analysis:* Appoint Associate Referee; continue study.
(18) *Sugars and Sugar Alcohols:* Combine subject under LC Methods for Meat and Poultry Products.
(19) *Temperature, Minimum Processing:* Continue study.

MYCOTOXINS

*(1) *Aflatoxin M:* Adopt as official first action the Foos and Warren method for determination of aflatoxins M_1 and M_2 in fluid milk by reverse phase LC as described by Stubblefield; continue first action status of the TLC methods for determination and confirmation of aflatoxin M_1 in dairy products (**26.090–26.094** and **26.095–26.100**), and aflatoxins B_1 and M_1 in liver (**26.101–26.110**); continue study.
(2) *Aflatoxin Methods:* Continue study.
(3) *Alternaria Toxins:* Continue study.
(4) *Citrinin:* Continue study.
(5) *Cyclopiazonic Acids:* Continue study.
(6) *Ergot Alkaloids:* Continue study.
(7) *Ochratoxins:* Continue study.
(8) *Penicillic Acid:* Continue study.
(9) *Secalonic Acid:* Continue study.
(10) *Sterigmatocystin:* Continue study.
(11) *Tree Nuts:* Continue study.
*(12) *Tricothecenes:* Adopt as official first action the interim first action TLC method for determination of deoxynivalenol in wheat at levels ≥300 ng/g (Eppley method); adopt as official first action the GC method for the determination of deoxynivalenol in wheat at levels ≥350 ng/g (Ware method); continue study.
(13) *Xanthomegnin and Related Naphthoquinones:* Continue study.
(14) *Zearalenone:* Continue study.
(15) *Other Topics:* Initiate new topics Emodin and Related Anthraquinones, and *Penicillium islandicum* Toxins; correct section **26.002** and insert missing sections **26.052–26.060** as indicated in the General Referee report.

OILS AND FATS

(1) *Antioxidants:* Continue study.
(2) *Emulsifiers:* Continue study.
(3) *Hydrogenated Fats:* Continue study.
(4) *Lower Fatty Acids:* Continue study.
(5) *Marine Oils:* Continue study.
(6) *Olive Oil Adulteration:* Continue study.
(7) *Oxidized Fats:* Continue study.
(8) *Pork Fat in Other Fats:* Continue study.
(9) *Sterols and Tocopherols:* Continue study.
*(10) *Other Topics:* Revise **28.013** to provide for the use of 30 × 3.5 to 30 × 3.8 cm id test tube to carry out the Wiley melting point determination; revise the official first action GC method for total *trans* acid isomers in margarine, **28.A06,** to include precision statement, as it appears in **28.068**; adopt as official first action, IUPAC method 2.323, determination of triglycerides by GC.

PLANT TOXINS

(1) *Glucosinolates:* Continue study.
(2) *Pyrrolizidine Alkaloids:* Appoint Associate Referee; continue study.
(3) *Solanaceous Alkaloids:* Redesignate topic as Steroidal Alkaloids; appoint Associate Referee; initiate pre-collaborative study of LC screening method for potato glycoalkaloids; continue study.

SEAFOOD TOXINS

(1) *Ciguatoxins (Biochemical Methods):* Continue study.
(2) *Diarrhetic Shellfish Poisoning:* Continue study.
(3) *Neurotoxic Shellfish Poisoning (P. brevis):* Continue study.
(4) *Paralytic Shellfish Poisoning (Immunoassay Method):* Continue study.
(5) *Shellfish Poisons (Chemical Methods):* Continue study.
(6) *Tetrodotoxins:* Continue study.

Committee on Foods II: Recommendations for Official Methods

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ALCOHOLIC BEVERAGES

(1) *Alcohol Content by Oscillating U-Tube Density Meter:* Continue study.
(2) *Alcohol Content of High Solids Distilled Spirits:* Continue study.
(3) *Carbon Dioxide in Wine:* Continue study.
(4) *Citric Acid in Wine:* Continue study.
(5) *Color Intensity for Distilled Alcoholic Beverage Products:* Continue study.
(6) *Ethanol in Wine by GC:* Continue study.
(7) *Flavor Compounds in Malt Beverages:* Continue study.
(8) *Glycerol in Wine:* Continue study.
(9) *Hydrogen Cyanide:* Continue study.
(10) *Malic Acid in Wine:* Continue study.
(11) *Malt Beverages and Brewing Materials:* Continue study.
(12) *Sorbic Acid in Wine:* Continue study.
(13) *Synthetic Colors:* Continue study.
(14) *Sugars in Wine:* Continue study.
(15) *Sulfur Dioxide in Wine (Ripper Method):* Continue study.
(16) *Tartrates in Wine:* Continue study.
(17) *Thujone in Alcoholic Beverages:* Continue study.
(18) *Vanillin and Ethyl Vanillin:* Continue study.
(19) *Other Topics:* Continue official first action status of the following methods: **9.013,** artificial colors; **9.113–9.115,** cyanide; **9.135,** total acidity; **9.139,** total malic acid; **9.145,** thujone; **10.182,** aphids; **10.215–10.218,** yeast; **11.052,** citric and malic acids; **11.072,** cyanide; **11.062–11.066** and **11.067–11.069,** carbon dioxide, manometric and volumetric methods, respectively.

CEREAL FOODS

(1) *Iron:* Continue study.
*(2) *Phytates:* Adopt as official first action the modified ion-exchange method for phytate as described by the Associate Referee; continue study.
(3) *Other Topics:* Continue official first action status of the following methods: **14.048–14.052,** α-amylase; **14.108–14.110,** lactose; **14.112–14.115,** mineral oil; **14.144–14.147,** sterols.

CHOCOLATE AND CACAO PRODUCTS

(1) *Caffeine and Theobromine:* Discontinue 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; **13.040,** unsaponifiable matter in cacao butter; **13.045,** lecithin; **13.055,** glucose. Establish the following new topics and appoint Associate Referees: Nonfat Dry Cocoa Solids, Triglycerides Composition in Cocoa Butter and Fat from Chocolate, Total and Solid Fat Content in Chocolate Products by NMR.

*An asterisk before a recommendation indicates that official action on that recommendation resulted in its appearance in "Changes in Methods."

Section numbers refer to *Official Methods of Analysis* (1984) 14th edition, and "Changes in Methods," J. Assoc. Off. Anal. Chem. (1985) **68**, 369–411.

The recommendations submitted by the Committee on Foods II were adopted by the Association.

The UV/vis and fluorescence spectrophotometric method for determination of adulteration of processed Florida orange juice was not recommended for adoption by the Committee on Foods II. The method was recommended by the Official Methods Board and adopted official first action by the Association. See the report of the Official Methods Board, *J. Assoc. Off. Anal. Chem.* (1986) **69**, March issue.

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 Nonfrozen Dairy Desserts:* Continue study.
(7) *Color in Other Foods:* Continue study.
(8) *FD&C Red No. 4 in Maraschino Cherries:* Continue study.
(9) *Inorganic Salts:* Continue study.
(10) *Intermediates, Uncombined in Certifiable Water-Soluble Azo Colors:* Continue study.
(11) *Intermediates, Uncombined in Other Certifiable Colors:* Continue study.
(12) *Liquid Chromatography:* Continue study.
(13) *Subsidiary Colors in Certifiable Color Additives:* Continue study.
(14) *X-Ray Fluorescence Spectroscopy:* Continue study.

FLAVORS

(1) *Additives in Vanilla Flavorings:* Continue study.
(2) *Citral:* Continue study.
(3) *Essential Oils:* Continue study.
(4) *Glycyrrhizic Acid and Glycyrrhizic Acid Salts:* Continue study.
(5) *Imitation Maple Flavors, Identification and Characterization:* Continue study.
(6) *Organic Solvent Residues in Flavorings:* Continue study.
(7) *Vanillin and Ethyl Vanillin in Foods:* Continue study.
(8) *Other Topics:* Continue official first action status of the following methods: **12.022,** essential oils; **12.023–12.027,** 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; **19.122** and **19.123,** anise and nutmeg extracts; **19.125, 19.126,** and **19.127–19.128,** other extracts and toilet preparations.

FRUITS AND FRUIT PRODUCTS

(1) *Adulteration of Apple Juice:* Continue study.
(2) *Adulteration of Orange Juice by Pulpwash and Dilution:* Continue study.
*(3) *Fruit Acids:* Adopt as official first action the LC method for determining quinic, malic, and citric acids in apple juice and cranberry juice cocktail as described by the Associate Referee; continue study.
(4) *Fruit Juices, Identification and Characterization:* Continue Study.
(5) *Moisture in Dried Fruits:* Continue study.
(6) *Orange Juice Content:* Continue study.
(7) *Sodium Benzoate in Orange Juice:* Discontinue study.

NONALCOHOLIC BEVERAGES

(1) *Caffeine and Methyl Xanthines in Nonalcoholic Beverages:* Continue study.
(2) *Glycyrrhizic Acid Salts in Licorice-Derived Products:* Continue study.
(3) *Lasiocarpine and Pyrrolizidines in Herbal Beverages:* Continue study.
(4) *Quinine:* Continue study.
(5) *Safrole in Sassafrass:* Continue study.
(6) *Other Topics:* Establish the new topic Caloric Content of Nonalcoholic Beverages and appoint an Associate Referee.

PRESERVATIVES AND ARTIFICIAL SWEETENERS

(1) *Aspartame, Benzoates, Saccharin, and Caffeine by LC:* Continue study.
(2) *Formaldehyde:* Continue study.
(3) *Meats, Ground, Screening Methods for Chemical Preservatives and Added Blood:* Discontinue study.
(4) *Organic Preservatives (LC):* Continue study.
(5) *Sulfites (Polarographic Methods):* Continue study.
(6) *Other Topics:* Continue official first action status of the following methods: **20.043–20.048,** preservatives in ground beef; **20.029–20.033,** benzoic acid by TLC; **20.034–20.042,** benzoic and sorbic acid by GC; **20.062–20.067,** boric acid by atomic absorption spectrophotometry; **20.073–20.074,** soluble fluorides by fluorescence quenching of aluminum 8-hydroxyquinolate; **20.079–20.081,** formaldehyde; **20.090–20.092,** nitrites; **20.094–20.095,** qualitative tests for quaternary ammonium compounds (QAC); **20.107–20.109,** eosin yellowish method for QAC; **20.115–20.118,** sorbic acid oxidation method; **20.138–20.139,** thiourea in frozen peaches; **20.174–10.178,** identification of nonnutritive sweeteners; **20.179,** qualitative method for cyclamates; **20.181–20.184,** cyclamates in canned fruit; **20.185–20.189,** cyclohexylamine in cyclamates; **20.190–20.193,** dulcin; **20.194,** P-4000; **20.210,** saccharin by sublimation.

PROCESSED VEGETABLE PRODUCTS

(1) *pH Determination:* Continue study.
(2) *Pectin Breakdown in Frozen Foods:* Discontinue study.
(3) *Sodium Chloride:* Continue study.
(4) *Soluble Solids of Tomato Products (Direct Refractometer Methods):* Continue study.
(5) *Total Solids by Microwave Moisture Analyzer:* Continue study.
(6) *Water Activity in Foods:* Continue study.
(7) *Other Topics:* Establish the new topic LC Determination of Sugars in Processed Vegetables and appoint an Associate Referee.

SPICES AND OTHER CONDIMENTS

(1) *Ash and Pungent Principles in Mustard:* Continue study.
(2) *Bulk Index of Spices:* Continue study.
(3) *Ethylene Oxide and Ethylene Chlorohydrin Residues in Dried Spices:* Continue study.
(4) *Extractable Color in Capsicum Spices and Oleoresins:* Continue study.
*(5) *Moisture in Dried Spices:* Adopt as official first action the interim action distillation method for moisture in spices as described by the Associate Referee; continue study.
(6) *Monosodium Glutamate in Foods:* Continue study.
(7) *Piperine in Black Pepper:* Continue study.
(8) *Pungency of Capsicums and Oleoresins:* Continue study.
(9) *Vinegar:* Continue study.

SUGARS AND SUGAR PRODUCTS

(1) *Chromatograph Methods:* Continue study.
(2) *Color, Turbidity, and Reflectance—Visual Appearance:* Continue study.
*(3) *Corn Syrup and Corn Sugar:* Adopt as final action the official first action tables for dry substance in corn

syrups and high fructose corn syrups, **31:A1–31:A2;** adopt as official final action the official first action refractive index table for dry substance in corn syrups **31:A3;** make editorial change in title of **31.245** to read, "Saccharides (Major) in Corn Syrup"; continue study.

(4) *Enzymatic Methods:* Continue study.

*(5) *Honey:* Adopt as official final action the official first action method for color classification, **31.A01–31.A03;** continue study.

(6) *Lactose Purity Testing:* Continue study.

(7) *Maple Sap and Syrups:* Continue study.

(8) *Stable Carbon Isotope Ratio Analysis:* Continue study.

(9) *Standardization of Sugar Methods of Analysis:* Continue study.

(10) *Sugars in Cereal:* Continue study.

(11) *Sugar in Licorice Products:* Continue study.

(12) *Weighing, Taring, and Sampling:* Continue study.

VITAMINS AND OTHER NUTRIENTS

*(1) *Amino Acids:* Adopt as official final action the official first action ion-exchange chromatographic method for sulfur amino acids in food and feed ingredients, **43.A08–43.A13;** continue study.

(2) *Automated Nutrient Analysis:* Continue study.

(3) *Biotin:* Continue study.

(4) *Carotenoids:* Continue study.

*(5) *Dietary Fiber:* Adopt as official final action the official first action method for total dietary fiber in food, **43.A14–43.A20,** as amended by the General Referee; continue study.

(6) *Fat in Food by Chloroform—Methanol Extraction:* Continue study.

(7) *Folic Acid:* Continue study.

(8) *LC Assay for Total Vitamins A, B, and E Content:* Continue study.

(9) *Iodine:* Continue study.

*(10) *Nutrient Assay of Infant Formula:* Adopt as official first action the methods for chloride, phosphorus, proximate analysis, thiamine, and vitamin B_{12} as described by the Associate Referee; amend official first action microbiological method for vitamin B_6, **43.A27,** by adding sentence, "Add 5 mL basal medium stock soln to all tubes for total vol. of 10 mL/tube." after fourth sentence ending ". . . to 5 mL/tube."

(11) *Pantothenic Acid, Total Acidity:* Continue study.

(12) *Protein Quality, Evaluation:* Continue study.

(13) *Sodium:* Continue study.

(14) *Thiamine Assay, Enzyme and Column Packing Reagents:* Continue study.

(15) *Vitamin A:* Continue study.

(16) *Vitamin D:* Continue study.

(17) *Vitamin E:* Continue study.

(18) *Vitamin E in Pharmaceuticals (Gas Chromatography):* Continue study.

(19) *Vitamin K:* Continue study.

Committee on Residues: Recommendations for Official Methods

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METALS AND OTHER ELEMENTS

(1) *Arsenic in Animal Tissues and Meat Products by Atomic Absorption Analysis:* Initiate topic for collaborative study of the USDA dry-ash hydride-generation AAS method for determining arsenic in animal tissues and meat products; develop protocol for collaborative study of this method and initiate collaborative study if the General Referee and Statistical Consultant to the Committee on Residues approve the protocol.

(2) *Atomic Absorption Spectrophotometry (AAS):* Continue study to develop biological reference materials with well established concentrations of selected elements for use in assessing performance of AAS methods; continue effort to integrate present AOAC official AAS methods for individual elements into unified AAS protocol for multielement analysis of foods and other biological substrates.

*(3) *Cadmium and Lead in Earthenware:* Adopt as official final action the official first action hot leach AAS method, **25.016–25.023,** for determining extractable cadmium and lead in ceramic and enameled cookware; continue study to evaluate methods for determining leachable lead in decorated teacup rims.

(4) *Emission Spectrochemical Methods:* Initiate collaborative study of method combining wet acid digestion of plant or animal commodities with inductively coupled plasma (ICP) emission spectroscopy for multielement analysis of foods, using collaborators who have dem-

*An asterisk before a recommendation indicates that official action on that recommendation resulted in its appearance in "Changes in Methods."

Section numbers refer to *Official Methods of Analysis* (1984) 14th edition.

The recommendations submitted by the Committee on Residues were adopted by the Association.

onstrated proficiency with ICP determinative step in pre-study test with trial sample solutions.

(5) *Fluorine:* Continue study of microdiffusion and fluoride-specific electrode method for fluoride in foods (*J. Assoc. Off. Anal. Chem.* (1979) **62,** 1065–1069) to incorporate needed improvements identified in Associate Referee report on the collaborative study for determination of fluoride in infant foods (*J. Assoc. Off. Anal. Chem.* (1981) **64,** 1021–1026).

(6) *Graphite Furnace-Atomic Absorption Spectrophotometry (GF-AAS):* Evaluate results of interlaboratory trial of Associate Referee's coprecipitation GF-AAS method for determining sub-ng/g levels of lead and cadmium in foods; if warranted by the results of trial, prepare protocol for collaborative study of method for review and comment by General Referee and Statistical Consultant to Committee on Residues.

(7) *Hydride Generating Techniques:* Conduct interlaboratory trial of hydride generation-AAS method using the Associate Referee's simple hydride generator (designed to be constructed from common laboratory items) for determining tin in foods and, if warranted by results of trial, conduct collaborative study of method; continue study to evaluate other hydride generators, such as continuous flow hydride generation apparatus of Panaro and Kroll (*Anal. Lett.* (1984) **17,** 157–172), for use in AAS methods for determining arsenic and selenium in foods.

(8) *Mercury:* Conduct interlaboratory trial of Associate Referee's method combining liquid chromatography (LC) with AAS for determination of methyl mercury in fish (*Analyst* (1982) **107,** 1457–1461), using improved LC-AAS interface developed since publication of method; if trial results are satisfactory, prepare protocol for collaborative study of method for review and comment by General Referee and Statistical Consultant to Committee on Residues.

(9) *Methyl Mercury in Fish and Shellfish:* Continue official first action status of electron capture gas chromatographic method, **25.146–25.152,** for determining methyl mercury in fish and shellfish; evaluate results of collaborative study of modified version of this method developed by Associate Referee to eliminate use of toxic extractant benzene and to simplify analytical procedure; prepare report recommending adoption of modified method if warranted by evaluation of collaborative study results.

*(10) *Multielement Analysis of Infant Formula:* Adopt as official final action the official first action inductively coupled plasma (ICP) emission spectroscopy method, **43.292–43.296,** for determining calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc in infant formula; discontinue topic.

(11) *Multielement Determination After Closed System Digestion:* Continue official first action status of multielement method, **25.001–25.007,** for determining arsenic, cadmium, lead, selenium, and zinc in foods; continue study to extend applicability of this method as described by Associate Referee (*J. Assoc. Off. Anal. Chem.* (1983) **66,** 620–624) to include determination of copper by anodic stripping voltammetry and determination of chromium and nickel by differential pulse polarography.

(12) *Polarography:* Continue as official first action the dry ash, anodic stripping voltammetric method, **25.008–25.015,** for determining cadmium and lead in foods other than fats and oils; conduct interlaboratory trial of modified version of **25.008–25.015** developed by Associate Referee to eliminate interference from canned foods containing high levels of tin in determination of cadmium by this method.

(13) *Separation Techniques for Trace Elements in Foods:* Discontinue topic.

*(14) *Tin:* Continue official first action status of the nitrous oxide-acetylene flame atomic absorption spectrophotometric method for determining tin in canned foods, **25.A01–25.A04** (*J. Assoc. Off. Anal. Chem.* (1985) **68,** 209–213); surplus official first action atomic absorption method for determining tin in canned fruits, vegetables, and juices, **25.161–25.163.**

(15) *Other Topic:* The Committee on Residues concurs with the General Referee's recommendation against adoption of the method proposed for the determination of mercury and methylmercury in fish (*Analyst* (1977) **102,** 769–776).

MULTIRESIDUE METHODS (INTERLABORATORY STUDIES)

*(1) *Comprehensive Multiresidue Methodology:* Adopt as official final action the official first action GC method, **29.A01–29.A04** (*J. Assoc. Off. Anal. Chem.* (1985) **68,** 64–71), for determination of acephate, α-BHC, chlorpyrifos, dieldrin, monocrotophos, and omethoate in lettuce, strawberries and tomatoes; continue study to extend applicability of this broad-spectrum pesticide multiresidue method to additional pesticides and additional fruits and vegetables.

(2) *Fumigants:* Continue study to extend applicability of official final action GC method for volatile fumigants in grain, **29.071–29.072,** to provide coverage for additional fumigants (e.g., methyl bromide, methylene chloride, carbon disulfide) and additional foods (e.g., citrus fruits and milled grain products); prepare complete description of modified version of **29.071–29.072** developed by Associate Referee for determining broad range of fumigant residues in whole grain and conduct interlaboratory and intralaboratory trials of modified method; if results of trials are satisfactory, develop protocol for collaborative study of method for review and comment by General Referee and Statistical Consultant to Committee on Residues.

(3) *Low Moisture–High Fat Samples, Extraction Procedure:* Continue study of Associate Referee's sample preparation/extraction procedure (*J. Assoc. Off. Anal. Chem.* (1982) **65,** 1122–1128) to evaluate cleanup techniques as alternatives to acetonitrile partitioning, **29.014,** for removing fats and oils from extracts of oil seeds and other low moisture–high fat foods before determination of pesticide residues by electron capture GC, **29.018.**

(4) *Multiresidue Methodology, Miniaturization:* Continue study of Associate Referee's miniaturized version of multiresidue method, **29.001–29.018,** consisting of the following parts for analysis of nonfatty foods: acetonitrile extraction of 100 g sample (as in **29.011**(a)), transfer of residues to petroleum ether (as in FDA Laboratory Information Bulletin 2592), Florisil cleanup (as in miniaturized method for fish, **29.029–29.036**), and electron capture GC analysis (as in **29.018**); conduct second interlaboratory trial of this method, requiring participants to follow instructions exactly as in complete description of analytical procedure written in format of *Official Methods of Analysis;* if results of interlaboratory trial are

satisfactory, prepare collaborative study protocol for approval by General Referee and Statistical Consultant to Committee on Residues.

(5) *Organophosphorus Pesticide Residues:* Continue study of official final action carbon column cleanup method for residues of parathion, paraoxon, EPN, carbophenothion, and its oxygen analog in apples and green beans, **29.054–29.058,** to extend 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.

ORGANOHALOGEN PESTICIDES

(1) *Chlordane:* Continue study to determine recovery levels for all major components and metabolites of chlordane in the method combining multiresidue extraction, **29.011–29.012,** acetonitrile partitioning cleanup, **29.014,** Florisil column chromatographic cleanup and residue separation, **29.046–29.048,** and electron capture (EC) capillary column GC determinative step developed by Associate Referee for separation and individual quantitation of terminal residues of chlordane; if recoveries are satisfactory, conduct interlaboratory trial of method and prepare plan for approval of General Referee for collaborative study to assess method's performance for determining residues of *cis-* and *trans*-chlordane, *cis-* and *trans*-nonachlor, octachlor epoxide (oxychlordane), and heptachlor epoxide in butter, eggs, fish, and poultry fat.

(2) *Chlorinated Dioxins:* Continue study to monitor development of methods for determining 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and hexachloro-, heptachloro-, and octachloro-substituted dibenzo-*p*-dioxins and dibenzofurans in foods and environmental samples, with ultimate goal of establishing through AOAC collaborative procedures an official method or methods for determining residues of these compounds at parts-per-trillion levels in fish, milk, and other foods.

(3) *Chlorophenoxy Alkyl Acids:* Appoint an Associate Referee to develop, evaluate, and collaboratively study methods for analyzing foods to determine free and bound residues of chlorophenoxyalkanoic acid herbicides, with primary emphasis on residues of 2,4-D.

*(4) *Ethylene Dibromide:* Adopt as official first action the electron capture GC method collaboratively studied and reported by the Associate Referee in 1985, for determination of ethylene dibromide in whole grain (rice, wheat), in intermediate milled-grain products derived from corn or wheat (flour, bread mix), and in grain-derived ready-to-eat foods (white bread, oat-based baby cereal).

(5) *Ethylene Oxide and Its Chlorohydrin:* Appoint an Associate Referee to evaluate GC method of Scudamore and Heuser (*Pestic. Sci* (1971) **2,** 80–91) and alternative methods for determining ethylene oxide, ethylene chlorohydrin, and ethylene bromohydrin in foods.

*(6) *Gel Permeation Chromatography (GPC) Cleanup for Organochlorine Residues:* (a) Adopt as official final action the official first action method for determining 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 beef fat and pork fat, as described in official final action GPC method for determining the same 15 organochlorine residues in poultry fat, **29.037–29.043;** (b) revise the final action method, **29.037–29.043,** as follows: (i) change title to read: "Organochlorine Pesticide Residues in Animal Fats," (ii) add the statement: "(Applicable to beef, poultry, and swine fat)," and (iii) change "poultry fat" to "fat" in first line of **29.038** and the first line of **29.041;** (c) prepare protocol for collaborative study to extend official status of this method to determination of polychlorinated biphenyls (PCBs) in beef, poultry, and swine fat, and if protocol is approved by General Referee, initiate collaborative study.

(7) *Methyl Bromide:* Appoint an Associate Referee to evaluate and collaboratively study methods for determining residual methyl bromide in foods.

(8) *Pentachlorophenol:* Continue as official first action the GC method for pentachlorophenol in gelatin, **29.A14–29.A18;** continue study to improve GC determinative step of this method or to introduce methylation step for converting pentachlorophenol to its anisole before GC determination.

(9) *Pentachlorophenol in Animal and Poultry Tissue:* (a) Defer adoption of method collaboratively studied by Associate Referee in 1981 for electron capture GC determination of pentachlorophenol in animal livers until the General Referee and the Committee on Residues approve collaborative study report after it has been revised to provide (i) an explanation and supporting data to justify inclusion in the method of 2 requirements that are not usually acceptable in a regulatory analytical method for pesticide residues in foods (specifically, the requirement for use of a reference compound as an internal standard, and the requirement for use of blank sample matrix), and (ii) a description of the analytical procedure that is written in the style and format of *Official Methods of Analysis* and that does not indicate applicability of the method to the determination of pentachlorophenol concentrations beyond the range of concentrations tested in the collaborative study; (b) if the information required to address part (a)(i) of this recommendation is available now or is developed later (including data on the method's variability for pentachlorophenol concentrations found in "blank" livers from different sources and in spiked samples where livers used as blanks are from different animals than those fortified with pentachlorophenol, and data on the behavior of pentachlorophenol and the internal standard, pentabromoethylbenzene, in the parts of the procedure to which both compounds are subjected, i.e., the cleanup extractions with H_2SO_4 and the GC determinative step), rewrite the report, correcting the statements, errors, and omissions indicated by the Committee on Residues on a copy of the present manuscript and submit the revised report for consideration under the interim first action approval procedure; (c) continue study to develop and collaboratively test a GC method that does not require use of an internal standard or blank sample matrix for determination of pentachlorophenol in animal tissues.

(10) *Polychlorinated Biphenyls (PCBs):* Continue study of methods for separating PCBs from organochlorine pesticide residues that remain with them in extraction/cleanup steps of the multiresidue method, **29.001–29.018;** continue study to compare results produced by GC quantitation techniques based on analysis for individual congeners of PCBs and those produced by the quantitation techniques described in the official method, **29.018** and Table **29:02.**

(11) *Polychlorinated Biphenyls (PCBs) in Blood:* Complete collaborative study of method reported in the Centers for Disease Control Laboratory Update 80-108 (Polychlorinated Biphenyl Determination at Parts-Per-Billion Levels in Serum) and report results for consideration under the interim first action approval procedure.

(12) *Tetradifon, Endosulfan, and Tetrasul:* Complete intralaboratory study of 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 method recovery data for these pesticides in the few nonfatty foods that remain to be tested to determine if method is applicable to all Group I and Group II nonfatty foods (Table **29:01**).

(13) *Toxaphene:* Discontinue topic.

(14) *Other Topic:* The Committee on Residues concurs with the General Referee's recommendations against adoption of the methods for determining residues of inorganic bromide in grain (*Analyst* (1976) **101,** 386–390) and organochlorine pesticides in animal fats and eggs (*Analyst* (1979) **104,** 425–433).

ORGANONITROGEN PESTICIDES

(1) *Anilazine:* Appoint an Associate Referee to evaluate LC method of Lawrence and Panopio (*J. Assoc. Off. Anal. Chem.* (1980) **63,** 1300–1303) and other LC or GC methods for determining anilazine residues in food crops and to design and conduct collaborative study of method selected.

(2) *Benzimidazole-Type Fungicides:* Continue study to improve cleanup of 2-aminobenzimidazole (2-AB) in LC method for simultaneous determination of benomyl and its hydrolysis product methyl 2-benzimidazolecarbamate (MBC, also known as the fungicide carbendazim) in apples and cucumbers, in which alkaline degradation procedure developed by Associate Referee is used to convert benomyl and MBC to 1-(2-benzimidazolyl)-3-*n*-butyl urea (BBU) and 2-AB, respectively, and resulting BBU and 2-AB are separated by reverse phase LC for individual quantitation by UV absorbance.

(3) *Captan and Related Fungicides:* Complete preparation of protocol for collaborative study on determination of captan, captafol, and folpet in tomato, cucumber, and cauliflower by method that combines acetone extraction and Florisil cleanup procedures in secs 232.4 and 212.2, respectively, of *FDA Pesticide Analytical Manual* with the electron capture GC determinative step developed by Associate Referee; upon approval of protocol by General Referee and Statistical Consultant to Committee on Residues, begin collaborative study.

(4) *Carbamate Herbicides:* Appoint an Associate Referee to select and collaboratively study method for determining residues of carbamate herbicides in crops.

*(5) *Carbamate Insecticides, Liquid Chromatographic Methods:* Adopt as official final action the official first action LC method, **25.A05–25.A13** (*J. Assoc. Off. Anal. Chem.* (1985) **68,** 726–733), for determination of aldicarb, aldicarb sulfone, bufencarb, carbaryl, carbofuran, 3-hydroxycarbofuran, methiocarb, methomyl, and oxamyl in grapes and potatoes; continue study to extend applicability of this method to other crops and to residues of additional *N*-methylcarbamate insecticides and metabolites.

(6) *Carbofuran:* Appoint an Associate Referee to evaluate analytical methods for determining carbofuran and its carbamate and phenolic metabolites in milk and meat and to collaboratively test method(s) selected.

(7) *Chlorothalonil:* Appoint an Associate Referee to evaluate existing GC multiresidue methods, such as **29.044–29.049,** as well as methods specifically designed for determination of chlorothalonil residues in foods and to design and conduct collaborative study of method selected.

(8) *Daminozide:* Prepare summary of recovery and precision data from intralaboratory study of GC method proposed by Associate Referee for determining daminozide residues in foods and description of analytical procedure in format of *Official Methods of Analysis* for review and comment by General Referee; develop plan for collaborative study of method and proceed with study upon approval of plan and instructions to collaborators by General Referee and Statistical Consultant to Committee on Residues.

(9) *Dinitro Compounds:* Continue study of Associate Referee's procedure for LC separation and electrochemical detection of nitro- and dinitro-substituted pesticides at nanogram levels to develop this procedure into electrochemical detection-LC method for determining multiple residues of these compounds in foods.

(10) *Diquat and Paraquat:* Prepare protocol for collaborative study of paired ion LC method developed by Associate Referee for determination of diquat and paraquat in potatoes; if protocol is approved by General Referee and Statistical Consultant to Committee on Residues, begin collaborative study.

(11) *Dithiocarbamates, General Residue Methods:* Appoint an Associate Referee to develop residue methods for determining dimethyldithiocarbamate and ethylenebisdithiocarbamate fungicides as separate entities in foods.

(12) *Maleic Hydrazide:* Appoint an Associate Referee to develop and collaboratively study GC or LC method for determining maleic hydrazide in crops.

(13) *Organotin Fungicides:* Appoint an Associate Referee to evaluate and collaboratively study GC methods for determining residues of cyhexatin, triphenyltin hydroxide, and fenbutatin oxide in foods.

(14) *Sodium o-Phenylphenate:* Appoint an Associate Referee to develop and collaboratively study GC or LC method for determining *o*-phenylphenol in foods.

(15) *Substituted Ureas:* Continue study to obtain recovery and precision data for LC method developed by Associate Referee for determining phenylurea herbicides in food crops; submit data and description of method in format of *Official Methods of Analysis* for review and comment by General Referee.

(16) *Thiolcarbamate Herbicides:* Appoint Associate Referee to evaluate and collaboratively study methods for determining residues of thiolcarbamate herbicides in crops.

(17) *s-Triazines:* Appoint an Associate Referee to evaluate methods for determining residues of atrazine and cyanazine in food crops and to collaboratively study method selected.

(18) *Trifluralin:* Appoint an Associate Referee to evaluate existing pesticide multiresidue methods, such as **29.001–29.018,** for use in determining trifluralin residues in crops, and to conduct collaborative study of selected method.

(19) *Other Topic:* The Committee on Residues concurs with the General Referee's recommendation against adoption of the dithiocarbamate pesticide residue method reported in *Analyst* (1981) **106,** 782–787.

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:* Discontinue topic.

(3) *Disulfoton:* Continue study to select and collaboratively test a method for determining disulfoton and its metabolites in foods.

(4) *Extraction Procedures:* Appoint an Associate Referee to study efficiency of procedures for extracting field-incurred residues of organophosphorus pesticides from crops and to develop improved extraction procedures for incorporation into existing multiresidue methods.

(5) *Fenvalerate:* Submit intralaboratory recovery and precision data for method selected by Associate Referee for determining fenvalerate in food crops and description of analytical procedure in format of *Official Methods of Analysis* to General Referee; conduct interlaboratory trial of method and develop collaborative study protocol for review and comment by General Referee and Statistical Consultant to Committee on Residues.

(6) *General Method for Organochlorine and Organophosphorus Pesticides:* Appoint an Associate Referee to design and conduct collaborative studies to extend applicability of AOAC multiresidue method, **29.001–29.018,** to determination of additional organophosphorus pesticide residues in fatty and nonfatty foods and to evaluate nitrogen/phosphorus (N/P) detector as alternative to KCl thermionic detector in GC determinative step of this method.

(7) *High Fat Samples:* Continue study to evaluate gel permeation chromatography (GPC) as cleanup technique for organophosphorus pesticides and their metabolites in extracts of high fat samples; continue study to evaluate N/P detector for GC determinations of organophosphorus residues after GPC cleanup of fatty foods.

(8) *Methamidophos:* Appoint an Associate Referee to evaluate methods for determining methamidophos and its metabolites in foods and to collaboratively test selected method.

(9) *Monocrotophos:* Appoint an Associate Referee to select and collaboratively study method for determining residues of monocrotophos in foods.

(10) *Permethrin:* Appoint an Associate Referee to evaluate and collaboratively study analytical methods for determining residues of permethrin in foods.

(11) *Phorate:* Continue study to evaluate and collaboratively test analytical methods for determining phorate and its metabolites in foods.

(12) *Phosphine:* Appoint an Associate Referee to evaluate methods for determining residual phosphine in grains (including GC methods reported in *J. Assoc. Off. Anal. Chem.* (1978) **61,** 5–7 and 829–836) and to design and conduct collaborative study of selected method.

(13) *Resmethrin:* Terminate appointment of Associate Referee and appoint a new Associate Referee to begin evaluation, selection, and collaborative study of methods for determining resmethrin residues in foods.

(14) *Soils:* Transfer topic to the Committee on Hazardous Substances in Water and the Environment.

(15) *Sweep Codistillation:* Prepare protocol for collaborative study of Associate Referee's sweep codistillation cleanup method for GC determination of organophosphorus pesticide residues in fats (*J. Assoc. Off. Anal. Chem.* (1984) **67,** 902–904), as applied to residues of chlorpyrifos, bromophos-ethyl, and ethion in beef fat; upon approval of protocol by the General Referee and Statistical Consultant to Committee on Residues, initiate collaborative study.

(16) *Other Topic:* The Committee on Residues concurs with the recommendations of the General Referee against adoption of the methods proposed for determining malathion and dichlorvos residues in grain (*Analyst* (1973) **98,** 19–24), organophosphorus pesticide residues in fruits and vegetables (*Analyst* (1977) **102,** 858–868), organophosphorus pesticides in grain (*Analyst* (1980) **105,** 515–517), and organophosphorus pesticides in grain (*Analyst* (1985) **110,** 765–768).

RADIOACTIVITY

*(1) *Cesium-137:* Adopt as official first action the extension of the official final action gamma-ray spectroscopic method for Ba-140, Cs-137, and I-131 in milk, **48.025–48.029,** to include determination of Cs-137 in other foods, *provided* that the General Referee approves the report on the collaborative studies conducted jointly by the Associate Referees for Cs-137 and I-131 after report has been revised by Editor of *Official Methods of Analysis* to incorporate the instructions for sample preparation into present method; appoint an Associate Referee to evaluate and collaboratively study radiochemical methods for determining Cs-137 in foods and biological materials at lower levels than determinable with the official method, **48.025–48.029.**

*(2) *Iodine-131:* Adopt as official first action the extension of the official final action gamma-ray spectroscopic method for Ba-140, Cs-137, and I-131 in milk, **48.025–48.029,** to include the determination of I-131 in other foods, *provided* that the General Referee approves the collaborative study report after it has been revised by Editor of *Official Methods of Analysis* (*see* rec. 1); prepare protocol for collaborative study of more sensitive method recommended by Nuclear Regulatory Commission for determining I-131 in milk, as outlined in *J. Assoc. Off. Anal. Chem.* (1979) **62,** 387–389, and if protocol is approved by General Referee and Statistical Consultant to Committee on Residues, initiate the collaborative study.

(3) *Neutron Activation Analysis:* Evaluate results from collaborative study of the Associate Referee's method for determining sodium in neutron-irradiated biological materials and prepare report on study, including recommendation for adoption of method if warranted by statistical evaluation of its performance; continue study to assess feasibility of conducting collaborative studies of methods for simultaneous multimetal determinations in neutron-irradiated foods and, if feasible, develop collaborative study protocol for review and comment by General Referee.

(4) *Plutonium:* Appoint an Associate Referee to evaluate methods for determination of plutonium in foods, including Department of Energy method for determining plutonium in foods, biological materials, and water (HASL-300-Ed 25, *Energy Monitoring Laboratory Procedures Manual* (1982) pp. E-Pu-01-01), and to conduct collaborative study of selected method.

(5) *Radium-228:* Evaluate results of interlaboratory trial of method for determining Ra-228 in water by gamma-ray spectrometry; if results of trial are satisfactory, develop protocol for collaborative study of method for review

and comment by General Referee and Statistical Consultant to Committee on Residues.

(6) *Strontium-89 and -90:* Continue study of method of Baratta and Reavey (*J. Agric. Food Chem.* (1969) **17**, 1337–1339) for determining strontium-89 and -90 in foods; subject method to ruggedness testing and develop intralaboratory and interlaboratory precision data in preparation for collaborative study.

(7) *Tritium:* Appoint an Associate Referee to evaluate and collaboratively study methods for determining tritium in foods and biological materials.

Committee on Microbiology: Recommendations for Official Methods

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ANALYTICAL MYCOLOGY OF FOODS AND DRUGS

(1) *Baseline Mold Counts by Blending:* Continue study.
(2) *Chemical Methods for Detecting Mold:* Continue study.
(3) *Direct Count of Molds, Yeasts, and Spores by Fluorescence Microscopy:* New topic; continue study.
(4) *Geotrichum candidum Morphology:* Continue study.
(5) *Geotrichum Mold in Canned Fruits, Vegetables, and Fruit Juices:* Continue study.
(6) *Geotrichum Mold in Frozen Fruits and Vegetables:* Continue study.
(7) *Howard Mold Counting, Use of Widefield Eyepiece:* Continue study.
(8) *Howard to Viable Mold Counts of Frozen Fruits and Vegetables, Comparison:* Continue study.
(9) *Microscopic Appearance of Mold Hyphae, Effect of Freezing:* Discontinue topic.
(10) *Microscopic Mold Count Methods, Use of Compound Microscope:* Continue study.
(11) *Microscopic Mold Counts, Effects of Interfering Plant Material:* Continue study.
(12) *Mold in Spices:* Continue study.
(13) *Molds and Yeasts in Beverages:* Continue study.
(14) *Refractive Index of Tomato Products:* Discontinue topic.
(15) *Standardization of Plant Tissue Concentration for Mold Counting:* Continue study.
(16) *Tomato Rot Fragment Count:* Discontinue topic.

COSMETICS

(1) *Essential Oils and Fragrance Materials, Composition:* Continue study.
(2) *Nitrosamines:* Continue study.
(3) *Preservatives:* Continue study.
(4) *Other Topics:* Continue official first action status of (a) the gas chromatographic method for water and alcohol, **35.001–35.006,** and (b) the method for soluble zirconium in antiperspirant aerosols, **35.020–35.024.**

EXTRANEOUS MATERIALS IN FOODS AND DRUGS

(1) *Botanical Drugs, Adulteration by Foreign Plant Materials:* Continue study.
(2) *Botanicals:* Continue study.
(3) *Brine Extraction Techniques:* Discontinue topic.
(4) *Chocolate Products:* Continue study.
(5) *Cocoa Powder and Press Cake:* Continue study.
(6) *Fecal Sterols:* Complete collaborative study; continue study.
(7) *Filth in Canned and Dried Soups:* Continue study.
(8) *Food Supplement Tablets:* Continue study.
(9) *Grains, Whole, Cracking Flotation Methods:* Continue study.
(10) *Insect Excreta in Flour:* Continue study.
(11) *Soluble Insect and Other Animal Filth:* Continue study.
(12) *Meats, Processed:* Continue study.
(13) *Mite Contamination Profiles and Characterization of Damage to Foods:* Continue study.
(14) *Mites in Stored Foods:* Continue study.
(15) *Mushroom Products, Dried:* Continue study.
(16) *Mushrooms, Canned:* Continue study.
(17) *Particulates in Large Volume Parenterals:* Discontinue topic.
(18) *Performance Evaluation of Methods for Filth:* Continue study.
(19) *Rye Bread:* Continue study.
(20) *Shrimp:* Continue study.
(21) *Spices:* Continue study.
(22) *Spirulina:* Complete collaborative study; continue study.

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*An asterisk before a recommendation indicates that official action on that recommendation resulted in its appearance in "Changes in Methods."

The recommendations submitted by the Committee on Microbiology were adopted by the Association.

Section numbers refer to *Official Methods of Analysis* (1984) 14th edition, and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* (1985) **68**, 369—411.

*(23) *Urine, Methods for Detection:* Adopt as official first action the improved TLC visualization reagent and color development technique for uric acid to replace **44.186(c)** and **44.188(c).**

(24) *Vegetable Products, Dehydrated, Isolation of Filth:* Continue study.

(25) *Vertebrate Excreta, Chemical Identification Tests:* Adopt as official first action the colorimetric method for determination of alkaline phosphatase as an indicator of mammalian feces in corn meal.

(26) *Other Topics:* Initiate the new topics, Baked Goods with Fruit and Nut Tissues, Light Filth in; Brine Extraction Techniques for Determining Filth and Tomatoes in Mushrooms; Canned Crabmeat, Shrimp, and Tuna—Brine Extraction Technique; Canned Fish and Fish Products, Light Filth in.

MICROBIOLOGY—DRUG AND DEVICE RELATED

(1) *Biological Indicator Testing and Standardization:* Continue study.

(2) *Biological Sterility Indicators:* Continue study.

(3) *Chemical Indicators:* Continue study.

(4) *Endotoxins by Limulus Amebocyte Lysate:* Continue study.

(5) *Medical Devices—Packaging Integrity:* Continue study.

(6) *Medical Devices—Sterility Testing:* Continue study.

MICROBIOLOGY—FOODS

(1) *Automated Methods for Foods:* Continue study.

(2) *Automated Methods for Milk—Bactoscan Method:* Continue study.

(3) *Bacillus cereus—Enterotoxin:* Continue study.

(4) *Bacillus cereus—Isolation and Enumeration:* Continue study.

*(5) *Bacterial Pathogens—Genetic Methods:* Adopt as official first action the DNA colony hybridization method using synthetic oligodeoxyribonucleotides and cellulose filters for the detection of ST-producing strains of *E. coli.*

(6) *Campylobacter Species:* Continue study.

(7) *Canned Foods:* Continue study.

(8) *Clostridium botulinum:* Continue study.

(9) *Clostridium perfringens:* Continue study.

(10) *Escherichia coli and Other Coliforms:* Continue study.

(11) *Enzymatic Method for E. coli:* Continue study.

*(12) *Hydrophobic Grid Membrane Filter Methods:* (a) Adopt as official final action the first action method, **46.A06–46.A11,** for detection of *Salmonella* in nonfat dry milk, powdered eggs, cheese powders, pepper, chocolate, and raw poultry. (b) Adopt as official first action the HGMF method for determination of aerobic plate count values of foods.

(13) *Helium Leaks in Canned Foods:* Discontinue topic.

(14) *Listeria monocytogenes:* Continue study.

(15) *Low Acid Canned Foods—Sporeformers and Non-Sporeformers:* Discontinue topic.

(16) *Microorganisms—Identification by Biochemical Kits:* Continue study.

(17) *Parasitology:* Discontinue topic.

*(18) *Petrifilm Methods:* Adopt as official first action the interim first action dry rehydratable film method for determination of aerobic plate count and total coliform values in raw and pasteurized milk.

*(19) *Salmonella:* (a) Adopt as official first action the enzyme immunoassay method for detection of *Salmonella* in foods. (b) In **46.117(f),** change "Nonfat dry milk" to "Instant nonfat dry milk."

(20) *Somatic Cell—Automated Optical Methods:* Continue study.

(21) *Somatic Cell—Fossomatic Method:* Continue study.

(22) *Staphylococcal Enterotoxin:* Continue study.

(23) *Staphylococcus:* Continue study.

(24) *Sugars:* Continue study.

(25) *Vibrio cholerae:* Continue study.

(26) *Vibrio parahaemolyticus:* Discontinue topic.

(27) *Virology and Animal Oncology:* Continue study.

(28) *Yeast, Molds, Actinomycetes:* Continue study.

(29) *Yersinia enterocolitica:* Continue study.

Committee on Feeds, Fertilizers, and Related Materials: Recommendations for Official Methods

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The committee voted to recruit a Co-General Referee for Feeds.

Of special interest during the meetings with the General Referees was the 2-page listing of "Method Criteria" for drugs in feeds prepared and presented by Mary Lee Hasselberger.

ANTIBIOTICS

(1) *Affinity Quantitative Determination of Penicillin in Milk:* Continue study.
(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) *Cup Plate System for Antibiotic Analysis:* Continue study.
(8) *Design and Computerization of Microbiological Assays:* Continue study.
(9) *Erythromycins:* Discontinue topic.
(10) *Lasalocid in Feeds (LC Method):* Continue study.
(11) *Lasalocid in Feeds (Microbiological Assay):* Continue study.
(12) *Lincomycin in Feeds:* Continue study.
(13) *Monensin:* Continue study.
(14) *Neomycin:* Appoint Associate Referee; continue study.
(15) *Oxytetracycline:* Continue study.
(16) *Qualitative Delvo-test for β-Lactam Residues in Milk:* Continue study.
(17) *Qualitative Determination of β-Lactam Antibiotic Residues in Milk:* Continue study.
(18) *Quantitative Determination of β-Lactam Antibiotic Residues in Milk:* Continue study.
(19) *Screening Procedures for Antibiotics in Feeds:* Continue study.
(20) *Statistics of Microbiological Assay:* Continue study.
(21) *Tetracyclines in Tissues (Chromatographic Assay):* Continue study.
(22) *Tetracyclines in Tissues (Microbiological Assay):* Continue study.
(23) *Turbidimetric Virginiamycin Assay:* Continue study.
(24) *Tylosin:* Continue study.

DRUG RESIDUES IN ANIMAL TISSUES

(1) *Benzimidazoles:* Continue study on multiresidue screening procedure.
(2) *Esterogenic Compounds:* Appoint Associate Referee; continue study (formerly Diethylstilbestrol).
(3) *Dimetridazole:* Continue study.
(4) *Ipronidazole in Turkey and Swine Muscle:* Appoint Associate Referee; initiate study.
(5) *Levamisole:* Continue study.
(6) *Screening Methods:* Continue study.
(7) *Steroids:* Discontinue topic.
(8) *Sulfa Drugs:* Discontinue topic.
(9) *Sulfonamide Drugs:* Discontinue topic.
(10) *Tiamulin:* Discontinue topic.
(11) *Tiamulin (Screening Method):* Appoint Associate Referee; continue study.

DRUGS IN FEEDS

(1) *Amprolium:* Appoint Associate Referee; continue study.
(2) *Arsanilic Acid:* Appoint Associate Referee; continue study.
(3) *Carbadox:* Continue study.
(4) *Ethopabate:* Continue study.
(5) *Ethylenediamine Dihydroiodide:* Continue study.
(6) *Furazolidone and Nitrofurazone:* Continue as official first action the LC method for determination of furazolidone in premixes and complete feeds **(42.A01–42.A06).**
(7) *Melengestrol Acetate:* Continue study.
(8) *Morantel Tartrate:* Continue study.
(9) *Phenothiazine:* Continue study.
(10) *Pyrantel Tartrate:* Continue study.
*(11) *Roxarsone:* Adopt as official first action with editorial revisions the graphite furnace atomic absorption method described by the Associate Referee.
(12) *Sulfa Drug Residues:* Continue study.
(13) *Sulfamethazine and Sulfathiazole:* Continue study.
(14) *Discontinued Topics:* Discontinue the topics, Arprinocid, Dibutyltin Diluarate, Ipronidazole, Microscopy, Nifursol, Ormetoprin, and Sulfaquinoxaline.

FEEDS

(1) *Amino Acids in Mixed Feeds:* Continue study.
(2) *Fiber, Crude:* Continue study.

*Asterisk before a recommendation indicates that official action on that recommendation resulted in its appearance in "Changes in Methods."

The recommendations submitted by the Committee on Feeds, Fertilizers, and Related Materials were adopted by the Association.

Section numbers refer to *Official Methods of Analysis* (1984) 14th edition.

(3) *Fiber, Crude, in Milk Replacers:* Discontinue topic.
(4) *Infrared Reflectance Techniques for Mixed Animal Feeds:* Continue study.
(5) *Iodine in Feeds:* Continue study.
(6) *Microbial Additives and Enzymes:* Continue study.
(7) *Minerals:* Continue study.
(8) *Non-Nutritive Residues:* Continue study.
(9) *Protein, Crude:* Continue official first action status of the copper catalyst method **(7.033–7.037)**; continue study.
(10) *Sampling:* Continue study.
(11) *Vitamins:* Appoint Associate Referees; initiate studies.
(12) *Water (Karl Fischer Method):* Continue study.

FERTILIZERS AND AGRICULTURAL LIMING MATERIALS

(1) *Biuret in Urea and Mixed Fertilizers:* Continue study.
(2) *Boron:* Deny official first action status for inductively coupled plasma method described by the Associate Referee; continue study.
(3) *Dicyanodiamide:* Continue study.
(4) *Free and Total Water:* Continue study.
*(5) *Iron:* Delete from method **2.157–2.160** the applicable chelate Fe *N,N*-bis(2-hydroxy-5-sulfobenzyl)glycine (DPS); continue study.
(6) *Melamine:* New topic; initiate study.
(7) *Nitrogen:* Continue study.
(8) *Phosphorus:* Continue study.
*(9) *Potash:* Declare methods **2.108–2.113** and **2.114–2.118** surplus; continue study.
(10) *Sampling and Preparation of Sample:* Continue study.
(11) *Slow-Release Mixed Fertilizers:* Continue official first action status of the water elution method **(2.073–2.074)**; continue study.
(12) *Sodium:* Continue official first action status of the atomic absorption method **(2.177–2.181)** and the flame photometric method **(2.173–2.176)**; deny official first action status for potassium interferences in flame emission method for determination of sodium in fertilizers described by the Associate Referee; continue study.
(13) *Soil and Plant Amendment Ingredients:* Continue official first action status of the atomic absorption method for aluminum in aluminum sulfate soil acidifiers **(2.194–2.197)**; continue study.
(14) *Sulfur:* Continue study.
(15) *Water-Soluble Methylene Ureas:* Continue study.
(16) *Zinc:* Continue study.

PLANTS

(1) *Ashing Methods:* Continue study.
(2) *Atomic Absorption Methods:* Continue official first action status of the atomic absorption method for calcium, copper, iron, magnesium, manganese, potassium, and zinc **(3.013–3.016)**; continue study.
(3) *Boron:* Continue study.
(4) *Chromium:* Continue study.
(5) *Emission Spectroscopy:* Continue official first action status for plasma emission spectroscopy (ICP) method **(3.A01–3.A04)**; continue study.
(6) *Fluoride:* Continue official first action status of the potentiometric method **(3.075–3.080)** and the semi-automated method **(3.082–3.094)** for fluoride; continue study.
(7) *Nitrogen, Non-Protein:* Continue study.
(8) *Selenium:* Continue study.
(9) *Starch:* Continue study.
(10) *Sulfur:* Continue study.

TOBACCO

(1) *Alkaloid Analysis:* New topic; initiate study.
(2) *LC of Polyphenols:* New topic; initiate study.
(3) *Nicotine, Gas Chromatography:* Discontinue topic.
(4) *Tar and Nicotine in Cigarette Smoke:* Continue study.

VETERINARY ANALYTICAL TOXICOLOGY

(1) *Animal Serum Throxine:* New topic; initiate study.
(2) *Antibiotic Screening:* New topic; initiate study.
*(3) *Arsenic in Animal Tissue:* Adopt with editoral changes as official first action the determination of diagnostic levels of arsenic in animal tissue described by Associate Referee; it is a modification of **41.009.**
(4) *Atomic Absorption:* New topic; initiate study.
(5) *Chlorinated Phenols in Animal Tissues:* New topic; initiate study.
(6) *Cholinesterase:* Continue study.
*(7) *Copper in Animal Tissues:* Adopt as official final action the official first action method for determining copper in serum **(49.003–49.007)**; continue as official first action the liver copper method **(49.A01).**
(8) *Fluoride in Animal Tissues:* Continue study.
(9) *Lead in Animal Tissues:* Continue study.
(10) *Lead in Bone:* Discontinue topic.
(11) *Molybdenum:* Discontinue topic.
(12) *Monensin:* Combine with Antibiotic Screening topic.
(13) *Multielement Analysis by ICP:* Continue study.
(14) *Multiple Anticoagulant Screening:* Continue study.
(15) *Natural Products:* Continue study.
*(16) *Nitrates and Nitrites:* Adopt as official first action the determination of nitrates in forages using a selective ion electrode described by the Association Reference.
(17) *Pesticides in Toxicological Samples:* Continue study.
(18) *Selenium in Animal Tissues:* Continue study.
(19) *Sodium Monofluoroacetate:* Continue study.
(20) *Zinc in Animal Tissues:* Continue study.

Committee on Hazardous Substances in Water and the Environment: Recommendations for Official Methods

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NILE FRAWLEY (Dow Chemical Co., 574 Bldg, Midland, MI 48640);
ROBERT GRAVES (Environmental Protection Agency, Environmental Monitoring and Support Laboratory, 26 W St. Clair, Cincinnati, OH 45268);
ERIKA E. HARGESHEIMER (Glenmore Waterworks, Laboratory (35), City of Calgary, PO Box 2100, Calgary, Alberta, Canada T2P 2M5);
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LASZLO TORMA (State Department of Agriculture, Montana State University, Bozeman, MT 59717);
MARK F. MARCUS (Chemical Waste Management, Inc., 150 W 137th St, Riverdale, IL 60627), *Secretary*

AIR

No General Referee. Continue study.

BIOMONITORING

No General Referee. Continue study.

BIOTA

No General Referee. Continue study.

DRINKING, GROUND, AND SURFACE WATERS

(1) *Chemical Pollutants in Water and Wastewater.*—Continue study.
(2) *Chlorinated Solvents in Water.*—Continue study.
(3) *Ethylene Dibromide (EDB) in Water.*—Continue study.
(4) *Herbicides in Water and Sediment.*—Continue study.
(5) *Major Ions and Nutrients in Water.*—Appoint Associate Referee; continue study.
(6) *Organophosphorus Pesticides in Water.*—Appoint Associate Referee; continue study.
(7) *Phenols in Water.*—Continue study.
(8) *Salt.*—Appoint Associate Referee; continue study.
(9) *Triazine Herbicides in Water.*—Appoint Associate Referee; continue study.
*(10) *Munitions in Wastewater.*—Adopt as official first action the reverse phase liquid chromatographic determination of nitro-organic compounds in munitions wastewater.

EFFLUENTS

No General Referee. Continue study.

HAZARDOUS SUBSTANCES

(1) *Benzene in Consumer Products.*—Continue study.
*(2) *Nitrosamines in Infant Pacifiers.*—Adopt as official first action the gas chromatographic method for determining volatile *N*-nitrosamines in latex infant pacifiers.
(3) *Pentachlorophenol in Toy Paints.*—Continue study.
(4) *Toxic Metals in Paints.*—Continue study.

CHEMICAL/WASTE INTERACTIONS IN SOIL

(1) *Soils—Distribution Coefficients—Nonvolatile Organics.*—Continue study.
(2) *Soils—Distribution Coefficients—Volatile Organics.*—Appoint Associate Referee; continue study.
(3) *Soil Column Leaching.*—Appoint Associate Referee; continue study.
(4) *Volatile Organic Chemicals in Soil and Sediments—Adsorption Isotherm Test.*—Continue study.

INDUSTRIAL PROCESS WASTE

(1) *Bioassays.*—Continue study.
(2) *Inorganic Analytes.*—Continue study.
(3) *Organic Analytes.*—Continue study.
(4) *Physical Chemical Properties.*—Continue study.
(5) *Sampling.*—Continue study.

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*An asterisk before a recommendation indicates that official action on that recommendation resulted in its appearance in "Changes in Methods."

The recommendations submitted by the Committee on Hazardous Substances in Water and the Environment were adopted by the Association.

Section numbers refer to *Official Methods of Analysis* (1984) 14th edition.

Executive Director's Report

DAVID B. MacLEAN
AOAC, 1111 N 19th St, Suite 210, Arlington, VA 22209

This year we began the second century of AOAC attending to our basic mission: the approval and publication of methods. But we also paid considerable attention to building the organization: the development of membership and a firm financial base through the sale of publications and other activities. The numbers of laboratories and numbers of scientists indicate the broad participation in AOAC activities and point to successful endeavors on both these fronts.

Methods Approval

In 1985, AOAC received 68 methods, and approved 40 official first action methods which will be published in the 1986 "Changes in Official Methods" supplement to the 14th edition. More than 300 laboratories from all over the world participated in these interlaboratory collaborative studies. These 40 methods are applicable to the following areas: Food Analysis 28, Pharmaceutical Analysis 3, and Pesticide Formulations 9. Industry and the U.S. Food and Drug Administration (FDA) each sponsored 15 interlaboratory collaborative studies. The infant formula industry and FDA continue working together on a long term project to obtain AOAC approved methods for analysis of nutrients in infant formula. This year, AOAC approved methods for 6 nutrients, including one each for thiamine and vitamin B_{12}.

AOAC Methods Committees held a number of submitted methods for consideration for interim official first action in 1986.

During the year, AOAC published 47 interlaboratory collaborative studies; 631 persons in 18 countries participated in these studies.

AOAC approved 186 methods official first action, beginning in 1981; since 1971, AOAC has approved 600 methods.

Needs and Resources

We recognize the need to be responsive to industry and government needs for methods, and to convince laboratory managers to allow their scientists to participate in the collaborative studies. For example, government and industry's concern about sulfites in food prompted AOAC to conduct two special meetings for presenting and comparing methods and to organize collaborative studies. The Nordic Committee for Food Analysis (NMKL) has organized a collaborative study for a spectrophotometric method for sulfites in food. AOAC members are among the participants.

Industry and government have stated they need faster, simpler methods to analyze larger numbers of samples at the site of collection, with lesser-trained analysts. The development of immunochemical and enzyme test kits partially fulfills this need. AOAC approved two immunochemical methods in food microbiology, using genetically engineered antibodies.

New Methods Committee on Hazardous Substances

Following establishment of the Methods Committee on Hazardous Substances in Water and the Environment, AOAC approved methods for nitrosodibutylamine in infant pacifiers, and for TNT, etc., in munitions wastewater. The Environmental Protection Agency (EPA) has and is sponsoring many past, present, and projected collaborative studies of methods for environmental analysis, including methods for determining leaching of toxic organic compounds from waste. Future AOAC efforts in environmental analysis will include obtaining active Associate Referees, and bringing previously collaboratively studied methods for AOAC consideration. Methods related to hazardous waste and organic contaminants in groundwater will be of interest in 1986 and 1987.

Future Methods Issues

Future needs include evaluation of labor-saving methods in food microbiology and simple screening methods for use in production facilities and ports of entry. The rapid development of genetically engineered antibodies for use in immunoassay test kits will provide many of these needed low cost methods for food and environmental analysis. The impact of "engineered foods" on future demand for new AOAC official methods is not yet clear.

International Activities

AOAC continues cooperation with international and national organizations including the International Organization for Standardization, International Dairy Federation, Nordic Committee for Food Analysis, Office International du Cacao et du Chocolat, Collaborative International Pesticides Analytical Council (CIPAC), and American Association of Cereal Chemists. Each has carried out collaborative studies of methods for a variety of analytes. AOAC adopted, as official first action, 4 CIPAC methods under the AOAC-CIPAC cooperative agreement. AOAC approved 2 veterinary toxicology methods in cooperation with 2 veterinary toxicology groups.

AOAC and the Nordic Committee for Food Analysis signed a cooperative agreement. AOAC will continue seeking existing collaborative studies from other organizations to support approval of methods.

Statistical Issues

Associate and General Referees still desire guidance on the statistical evaluation and presentation of collaborative study data, including protocols for calculating measures for precision and identifying outliers. AOAC committees are developing protocols for the design, conduct, and evaluation of collaborative studies for biological pass/fail methods. AOAC will present its approach to interlaboratory collaborative studies at the Third International Conference on Harmonization of Collaborative Studies in Geneva, Switzerland, in May 1987.

Candidate Methods and Instrumental Methods

The Official Methods Board presented its proposal for a new class of "candidate methods", as outlined in its report. The object of the proposal is separate publication, through AOAC, of methods urgently needed, or in wide use and subjected to less than a full size interlaboratory collaborative study. This new class is *not* intended to be equivalent to AOAC official first and final action methods.

The AOAC approval of methods containing proprietary equipment and reagents suggests a need for guidelines to describe proprietary items in methods in generic terms, with performance specifications and tests. The Instrumental Methods and Data Handling Committee has recommended specification for specific laboratory equipment and chromatographic columns. The Subcommittee on Laboratory Computers and Microprocessors has begun consideration of draft criteria for approval of methods that use laboratory robots. They appear to be the fourth generation of devices for laboratory automation. Use of these will expand rapidly in the future.

Methods Approval Process

The Official Methods Board will change the methods approval process to assure proper design of collaborative

studies before they are conducted, and resolution of all scientific questions before a method is approved. In 1986, all methods will be considered for interim official first action before receiving official first action.

The Official Methods Board conducted the first training session for Associate and General Referees and Methods Committee members in AOAC structure, duties, and responsibilities, collaborative study guidelines, manuscript preparation, and approval procedures. The purpose of this training is to help participants carry out successful collaborative studies, leading to approval of methods.

Membership

Discussion continues about who is a member of AOAC, who should serve on AOAC decision-making committees and the Board of Directors, and who should vote on approval of methods. The Membership Committee has recommended that there be one class of individual members of AOAC, and that voting on final approval of methods be vested in the Official Methods Board. The Board of Directors has asked the Committee to present an option paper with recommendations on classes and privileges of membership and voting.

Sentiment for equal participation by government and industry is growing among AOAC members. We anticipate extensive discussion of equal participation among these two groups in AOAC in 1986. Subjects of discussion will include balance of representation among these groups on decision-making committees and requirements for openness of meetings. Retaining the official status of AOAC methods and the credibility of the methods approval process is the goal of all AOAC participants.

Publications

Our publications continue to be the major source of income and recognition of AOAC.

The first supplement to the 14th Edition was published in March 1985, and the second is to be published in March 1986.

The *Journal* published 243 technical papers on methods, collaborative studies, special reviews, and reports of chemical contaminants monitoring. The Editorial Board has authorized an Ad Hoc Journal Review Committee to begin work in 1986.

Other new publications included *Principles of Food Analysis for Filth, Decomposition and Foreign Matter; Key for Identification of Mandibles of Stored Food Insects; Use of Statistics to Develop and Evaluate Analytical Methods;* and *Production, Regulation, and Analysis of Infant Formula, a Topical Conference.*

Short Courses

AOAC presented the short course "Quality Assurance for Analytical Laboratories" in Dallas, TX, Arlington, VA, and Washington, DC; and a new short course, "Quality Assurance for Microbiological Laboratories," in Washington, DC. Presentations in 1986 include "Quality Assurance for Analytical Laboratories," April 26–27, Seattle, WA; July, 8–9 and August 12–13, Arlington, VA; August 29–30, Antwerp, Belgium; and September 13–14, Scottsdale, AZ; "Quality Assurance for Microbiological Laboratories;" April 30–May 1, Seattle, WA. AOAC will conduct a short course on "Sampling" for Agriculture Canada on March 25–26, 1986, in Ottawa, Ontario.

Annual Meeting and Other Meeting Activities

The Annual International Meeting held at the Shoreham Hotel, Washington, DC, October 27–31, 1985, included presentations by the Harvey W. Wiley award winner Daniel Schwartz, from USDA, Agricultural Research Service, in Philadelphia, PA: AOAC President, Richard Ronk, Food and Drug Administration, Washington, DC; and Paul Hile, Food and Drug Administration, Rockville, MD. The meeting included the scientific equipment exhibition, poster presentations, and a regulatory round table on pesticide formulations, registration, regulation, and residue methodology. The following symposia were conducted: Immunotoxicity of Pesticides and Toxic Compounds, Safety Training of Laboratory Analysts and Managers, Critical Analysis of Analytical Methods for Meat Foods, Chromatography of Amino Acids, and Practical Applications of Quality Assurance for Analytical Laboratories. Registration was 1038 and banquet attendance was 180.

For the first time in more than 70 years, the Annual International Meeting will be held outside Washington, DC, as follows: September 15–18, 1986, Scottsdale, AZ; September 14–17, 1987, San Francisco, CA; August 29–September 1, 1988, Palm Beach, FL; September 1989, St. Louis, MO.

AOAC held the Spring Training Workshop, April 8–11, in Dallas, TX, with an attendance of 450. The 1986 Spring Training Workshop will be held April 27–30 in Seattle, WA.

AOAC sponsored the first Topical Conference: Production, Regulation, and Analysis of Infant Formula, May 13–16, in Virginia Beach, VA.

AOAC Europe met March 25, 1985, in Antwerp, Belgium, and will meet again May 12, 1986, in Milan, Italy.

Five regional sections met in 1985: Northeast, June 24–26, Amherst, MA; New York-New Jersey, May 16 and December 5, New York, NY; Eastern Ontario-Quebec, June 11, Ottawa, Ontario, and November 14, Montreal, Quebec; Midwest, June 17–19, Hillside, IL; Pacific Northwest, June 20–21, Olympia, WA.

Financial

AOAC recognizes the need to develop additional income in the period 1986–1989, including new publications, more cost-effective meetings, and other new products and services to maintain the quantity and quality of services. The Membership Committee has recommended to the Board that *1987* individual dues be increased from $25 to $45, and private sustaining member dues from $500 to $750. AOAC will make the decision on 1987 dues at the 1986 Annual International Meeting.

Board of Directors

AOAC chose the following Officers and Board Members for 1985–1986: President Earle Coffin, Health Protection Branch Canada; President Elect Frank Johnson, Tennessee Valley Authority; Secretary-Treasurer, Prince Harrill, Food and Drug Administration; Robert Rund, Office of the Indiana State Chemist; Odette Shotwell, Agricultural Research Service; Thomas Layloff, Food and Drug Administration; and immediate Past President, Richard Ronk, Food and Drug Administration.

We are grateful to the 11 national, 44 state, and 5 provincial government and 125 industry organizations listed in this Journal who provided financial support to AOAC in 1985. AOAC now has 2771 members in 66 countries including 50 states (United States), and 9 provinces (Canada).

The Board of Directors met January 23–24, 1985, in Arlington, VA, and took the following actions:

(1) Endorsed the Process for Appeals of Recommendations on Methods put in effect by the Official Methods Board.
(2) Reaffirmed mail ballot of November 15, 1985, approving as an operating guideline, "Guidelines for Interlabora-

tory Collaborative Study Procedure to Validate Characteristics of a Method of Analysis."

(3) Approved a recommendation of the Intercommittee Task Force to withdraw all of the statistical parameters for the "A" set of Changes in Official Methods, 14th edition, published in 1985.

(4) Directed that all methods approved in 1984 be directed back to the Official Methods Board for the purpose of conducting a review.

(5) Amended and approved Terms of Reference for the Safety Committee.

(6) Approved hiring Kenneth Helrich as a consultant to be Scientific Editor of *Official Methods of Analysis*, 15th edition.

(7) Declined to hire a full-time fund raiser recommended by the Ways and Means Committee.

(8) Approved revised Terms of Reference for the Committee on the Constitution.

(9) Approved reconsideration of the total awards program at AOAC.

(10) Decided to continue the AOAC scholarship program while the total awards program is under consideration.

(11) Thanked the Centennial Committee for the outstanding AOAC Centennial Meeting.

(12) Approved the cooperative agreement between the Nordic Committee for Food Analysis (NMKL) and AOAC.

(13) Directed that cooperative agreements with other organizations do not change the requirement that methods and collaborative study data from outside AOAC must go through AOAC review as any method and data from within AOAC.

(14) Approved the terms and responsibilities part of the Terms of Reference for the International Coordination Committee.

(15) Approved a no-interest loan of $5000 U.S. to the Chemical Institute of Canada, effective January 1986, for the IUPAC Congress on Pesticides.

(16) Approved supplemental funds of $4000 for Derek Abbott for FY 1985 as AOAC United Kingdom representative.

(17) Approved expansion of publication of *The Referee* from 10 issues to 12 issues annually.

(18) Amended and approved Terms of Reference for the Long Range Planning Committee.

The Board of Directors met April 11–12 in Dallas, TX, during the Spring Training Workshop and took the following actions:

(19) Approved expenditures of a maximum of $287,000 to purchase a VAX 750 computer with peripherals, software, design, site preparation, and training. Subsequently approved by telephone ballot an additional $70,500 for this purpose.

(20) Approved discontinuance of a prepublication list of forthcoming papers in the Journal for Government and Private Sustaining Members.

(21) Commended the Joint Committee on Mycotoxins for its valuable work in obtaining uniform, accepted, collaboratively studied methods for mycotoxins.

(22) Terminated the Ad Hoc Committee on Nitrates, Nitrites, and Nitrosamines, effective after the 1985 Annual International Meeting.

(23) Approved placing the 1987 Annual International Meeting in a city other than Washington, DC. Dates and places subsequently are September 14–18, 1986, Scottsdale, AZ; September 14–18, 1987, San Francisco, CA; August 29–September 3, 1988, Palm Beach, FL.

(24) Reaffirmed openness of all AOAC meetings except as otherwise provided by the Board.

(25) Approved a provisional charter for the Eastern Ontario Quebec Regional Section with a provision that the Section bylaws be amended to avoid conflict with AOAC bylaws or standard regional bylaws (in preparation).

(26) Established 1986 *Journal* subscription rate at $89.75, nonmember, U.S.

(27) Approved a bank resolution at the First American Bank of Virginia.

(28) Approved a maximum of $12,000 for symposia speaker expenses at the 1985 Annual International Meeting. This action shall not be considered setting a precedent for future funding of symposia speaker expenses.

(29) Discussed membership classes and rights in AOAC, roles of President and Executive Director under the present bylaws and possible changes therein, and a performance agreement with the Executive Director.

The Board of Directors met September 18–19, 1985, in Arlington, VA, and took the following actions:

(30) Directed President Richard Ronk to present by September 1986, proposed actions necessary to establish the position of Executive Vice President of AOAC.

(31) Directed the Executive Director to present a report on the monetary value of volunteer time to design, conduct, and evaluate interlaboratory collaborative studies.

(32) By consensus approved the FY 1986 budget as amended transferring $4900 travel funds from the Official Methods Board to the Editorial Board. Directed the AOAC auditors to provide a management letter with the annual audit.

(33) Directed the AOAC Executive Director to present several alternatives for AOAC paid travel for committees by January 1986.

(34) Directed the Executive Director to present to the Board of Directors an outline of the history and policy issues related to statistical performance parameters for AOAC methods in October 1985.

(35) Deferred inclusion of statistical performance parameters in the "B" set of Changes in Official Methods, 14th edition.

(36) Approved guidelines to define terms of service as a committee member or chairman, effective 1985. Committee terms of service will be: members a maximum of 2 consecutive 3-year terms, chairman one 3-year term, with immediate past chairman serving one additional year.

(37) Approved submission of a proposal for "Candidate Methods" to the membership for review and comment.

(38) Approved 3-year terms with possibility of reappointment, depending on performance, for General Referees.

(39) Approved waiving 1985 membership dues for 97 persons.

(40) Authorized the Executive Director to sign and execute contracts for hotels for AOAC meetings with approval of the President or, if unavailable, the President-Elect.

(41) Approved 1986 Annual International Meeting registration fees ($U.S.) as follows:

Full Meeting	*Member*	*Nonmember*
Advance	$80	$105
On Site	$95	$120
One Day	*Member*	*Nonmember*
Advance	$55	$65
On Site	$70	$80

The Board of Directors met October 27, 1985, and took the following actions:

(42) Directed a study on the impact of all AOAC cooperation agreements (national and international).
(43) Directed that a task force be assembled of individuals from the International Coordination Committee (ICC) to study the proposed AOAC/APHA cooperative agreement and how it affects existing agreements.
(44) Directed that a task force be assembled to look at AOAC involvement in dairy microbiology.
(45) Decided that AOAC needs to develop guidelines for the following: (*1*) General guidelines for writing agreements between AOAC and other organizations. (*2*) Specific guidelines for writing national and/or international organizations.
(46) Requested that the Long Range Planning Committee develop a plan that will provide for thorough consideration of structural changes within the Association on an orderly basis and permit reasoned decisions over the next 10 years regarding the future structure of the Association. The plan should provide for membership review and decisions to be made by the Association on the issues of: (*1*) Membership classification. (*2*) Voting rights. (*3*) Roles and relationships of the Board of Directors, Official Methods Board, Editorial Board, and staff. (*4*) The role of regional sections and the structural changes necessary to fulfill the role. (*5*) The international role of the Association and the structural changes necessary to fulfill the role.
(47) Approved authorization for the Executive Director to act with full authority of the Board of Directors, according to existing policies of the Board, in matters of discipline of the staff. This is to remain in effect until a new personnel policy is developed and adopted by the Board of Directors.
(48) Requested that the Executive Director develop a revision of the personnel policy manual of the AOAC to present to the Board of Directors.
(49) Requested that the Statistics Committee provide the Board of Directors with recommendations for the best means of calculating statistical performance parameters for methods by April 1986.

Sources of financial support up to December 1985 were:

Government:
Agriculture Canada
Association of Public Analysts
Environmental Protection Agency
(Office of Pesticide Programs)
Food and Drug Administration
Health and Welfare Canada
(Health Protection Branch)
Irish State Laboratory
Laboratory of the Government Chemist
Ministry of Agriculture, Fisheries & Food
National Marine Fisheries Service
State Administration for the Inspection of Import/Export Commodities of The People's Republic of China
U.S. Department of Agriculture
(Agricultural Research Service)

Alabama Dept of Agriculture and Industries
Alberta Agriculture
Alberta Dairymen's Assn. Research Unit (ADARU)
Arkansas State Plant Board
Assiut University
Arizona State Agric. Lab.
California Dept of Food and Agriculture
Colorado Dept of Agriculture
Delaware Dept of Agriculture
Florida Dept of Agriculture and Consumer Services
Georgia Dept of Agriculture
Hawaii Dept of Agriculture
Hawaii Dept of Health
Illinois Dept of Agriculture
Indiana Office of State Chem.
Instituto di Tecnica e Sperimentazione Lattiero-Caseari Di Thiene
Indiana State Board of Health
Iowa Dept of Agriculture
Iowa State Veterinary Diagnostic Laboratory
Kansas State Board of Agric.
Kentucky Agricultural Experiment Station Div. of Regulatory Services
Kentucky Dept of Agriculture
Maryland Dept of Agriculture
Michigan Dept of Agriculture
Minnesota Dept of Agriculture
Mississippi State Chem. Lab.
Missouri Exp. Station Chem. Lab.
Montana Dept of Agriculture
Nebraska State Dept of Agriculture
New Jersey Dept of Agriculture
New Mexico Dept of Agriculture
New York Dept of Agriculture and Markets
North Carolina Dept of Agriculture
North Dakota State Labs Dept
Oklahoma State Dept of Agriculture
Ontario Ministry of Agriculture and Food
Oregon Dept of Agriculture
Pennsylvania Dept of Agriculture
Pennsylvania Liquor Control Board
Quebec Dept of Agriculture
South Carolina Dept of Agriculture
South Dakota State University
Tennessee Dept of Agriculture
Texas Agric. Exp. Station
Utah State Dept of Agriculture
Vermont Agric Exp. Station
Virginia Division of Consolidated Lab Svcs.
Wisconsin Dept of Agric., Trade/Consumer Affairs
Wyoming Dept of Agriculture

Industry:
3M Medical Products Div.
Agrico Chemical Co.
Agway, Inc.
Alcon Laboratories, Inc.
American Council of Independent Labs, Inc.
American Cyanamid Co.
Andersons, The
Aqualab, Inc.
Archer-Daniels-Midland
A/S N. Foss Electric
Avon Products, Inc.
BASF Wyandotte Corp.
Beatrice/Hunt-Wesson Foods, Inc.
Beckman Instruments, Inc.
Beech-Nut Nutrition Corp.
Biochem Laboratorium b.v.
Bionetics Lab Products
Boehringer Mannheim Biochem.

Borden, Inc.
Bristol-Myers Co.
Burroughs-Wellcome Co.
Campbell Inst. Res. & Techn.
Campbell Taggart
Cargill, Inc.
Carnation
Castleton Beverage Corp.
Chef Reddy Foods Corp.
Chemical Waste Mgmnt, Inc.
Chevron Chemical Co.
Ciba-Geigy Corp.
Coca-Cola Co., The
Comibassal International
Compu-chem Labs, Inc.
ConAgra Consumer Frozen Foods
Corn Refiners Assn, Inc.
CPC North America, CPC Int'l, Inc.
DFA of California
Dickey-john Canada, Inc.
Dionex Corp.
Dow Chemical Co.
Duphar BV
Dupont Co.
Dupont Pharmaceuticals
Dynatech Labs, Inc.
E & J Gallo Winery
Eastman Chemical Prods. Inc.
Eli Lilly and Co.
Express Foods, Inc.
FBC Ltd
FMC Corp.
Fertilizer Institute, The
Foss Food Techn. Corp.
GAF Corp.
Galbraith Laboratories, Inc.
General Foods Corp.
General Mills, Inc.
Gerber Products Co.
Gist Brocades USA
Golden State Foods Corp.
Hazleton Labs America, Inc.
Heinz U.S.A.
Hershey Foods Corp.
Heublein Wines
Hoechst-Roussel Pharm., Inc.
Hoffmann-La Roche, Inc.
ICI Americas, Inc.
IMC Corp.
ITT Continental Baking Co.
Jack Daniel Distillery, Motlow, Prop.
Kellogg Co., The
Kraft, Inc.
Kroger Co., The
Laboratory Specialists, Inc.
Lancaster Laboratories, Inc.
Lehn & Fink Products Co.
Lipton, Thomas J.
Loma Linda Foods, Inc.
McCormick & Co., Inc.
McKee Baking Co.
McLaughlin Gormley King Co.
McNeil Consumer Prods. Co.
Marion Laboratories, Inc.
Mead Johnson & Co.
Merck Sharpe & Dohme Res. Labs
Miles Laboratories, Inc.
Mississippi Chemical Corp.
Mobay Chemical Corp.
Monsanto Agric. Prods, Co.
Moorman Manufacturing Co.
Nabisco Brands, Inc.
National Food Processors Assn.
National Starch & Chem. Corp.
Nestec Ltd
Norwich Eaton Pharmaceuticals
Ocean Spray Cranberries, Inc.
O. M. Scott & Sons Co.
Orion Research, Inc.
Ortho Pharmaceutical Corp.
Pennwalt Corp.
Perdue, Inc.
Pfizer, Inc.
Philip Morris USA
Pillsbury Co., The
Procter & Gamble Co., The
Ralston Purina Co.
Rhone-Poulenc Chemical Co.
R. J. Reynolds Industries, Inc.
R. J. Reynolds Tobacco Co.
Ross Laboratories
Royal Iceland Corp.
Royster Company
Seagram (Joseph E.) & Sons, Inc.
Shaklee Corp.
ShriRam Institute for Industrial Research
Silliker Laboratories, Inc.
SmithKline Animal Health Prods.
Stauffer Chemical Co.
Sunkist Growers, Inc.
Tastybird Foods
Technicon Industrial Systems
Union Carbide Agricultural Products Co.
Upjohn Company, The
Velsicol Chemical Corp.
Waters Chromatography Division of Millipore
Welch Foods, Inc.
Zoecon Corp.

Secretary/Treasurer and the Finance Committee

PRINCE G. HARRILL, *Secretary/Treasurer, Chairman of Finance Committee*
Food and Drug Administration, Division of Food Technology, Washington, DC 20204

Other Members: T. G. Alexander; E. R. Elkins; J. E. McNeal

The Secretary/Treasurer and the Finance Committee have confirmed in Fiscal Year 1985, (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 financial reports. The Committee reviewed and judged the June 30, 1985, financial reports to be satisfactory. The Association had assets in the amount of $1 979 619 including an investment portfolio of $1 099 443 and a fund balance of $1 639 974 and liabilities of $339 645. As of September 30, 1985, we have sold 9062 copies of the 14th edition of *Official Methods of Analysis*.

The Finance Committee has had the task of monitoring the conversion of the Association's computer system. All hardware and software has been purchased and installed, and the conversion is on schedule.

The Committee recommended to the Board of Directors that the Association's present accounting firm be retained for Fiscal Year 1986.

STATEMENT OF FINANCIAL CONDITION—SEPTEMBER 30, 1985

Assets

Current Assets:		
Cash, Bank of Virginia*		$ 64,238
Cash, 1st American Bank*		177,951
Cash, 1st American Bank, payroll*		1,312
Cash, office fund		500
Cash, Mid-West fund		6,797
Cash, Northeast fund		1,270
Cash, Northwest fund		1,403
Cash, New Brunswick Savings Bank		302
Cash, Eastern Ontario-Quebec		198
Cash, Texas Commerce Bank		3,613
Accounts receivable		
Books and publications		49,198
Contracts and grants		72,527
Private Sustaining Members		14,500
Membership		602
Short Course		6,445
Spring Workshop		315
Annual Meeting		200
Infant Formula		750
Accrued interest receivable		2,146
Inventory, books and publications—at cost		295,052
Prepaid expenses		6,899
Advances		7,391
Total Current Assets		$713,609
Investments:		
Securities	$ 74,087	
Certificates of Deposit	1,117,679	1,191,766
Fixed Assets:		
Office furniture, fixtures, and equipment	$149,840	
Computer and computer software	218,844	
	$368,684	
Less: accumulated depreciation	176,639	192,045
Deferred Costs:		
Sampling Manual Text	$ 150	
Statistical Manual Text	13,496	
Short Courses	7,964	
Spring Workshop—1986	4,251	
Annual Meeting—1985	59,846	
Annual Meeting—1986	3,986	$ 89,693
Total Assets		$2,187,113

*Interest bearing account

Liabilities and Fund Balances

Current Liabilities:		
Accounts payable		$ 63,696
Accrued and withheld payroll taxes		3,090
Total Current Liabilities		$ 66,786
Deferred Income:		
Journal subscriptions	$86,181	
Private Sustaining Members	33,125	
Membership	10,979	
Spring Workshop—1986	300	
Short Course—QA Program	35,859	
Annual Meeting—1985	39,912	206,356
Reserve for Publications		200,000
Restricted Reserve for FAAM		5,189
Restricted Reserve for MAM		817
Restricted Fund—Harvey Wiley		48,807
Restricted Fund—15th Edition		618,139
Fund Balance:		
Balance, October 1, 1984	$ 996,462	
Add: excess of income over expenses for the twelve months ended September 30, 1985	662,696	
	$1,659,158	
Less: Restricted fund—15th Edition	618,139	
Balance, September 30, 1985		1,041,019
Total Liabilities and Fund Balances		$2,187,113

Editorial Board

The Editorial Board met on two occasions during the past year. The first was March 4–5, 1985, at the Westpark Hotel in Rosslyn, VA, and the second on October 27, 1985, at the Shoreham Hotel, Washington, DC. The Journal Editors met for consultation with the Board Chairman and Publications Manager on October 28, 1985.

The Board functioned throughout the year with a membership one less than authorized because Kenneth Helrich, originally appointed to the Board, was nominated for and assumed the position of Scientific Editor of *Official Methods of Analysis,* 15th edition. Mr. Helrich continues to serve the Editorial Board in an ex officio status.

We continue to be served well by our six Journal Editors, Rodney J. Noel, James F. Lawrence, Malcolm C. Bowman, Joseph Sherma, Helen L. Reynolds, and Joseph Westheimer. The meritorious service of the Association editorial staff is acknowledged at the outset of this report and grateful appreciation is intended herein.

The Board wishes to pay tribute by notation in this report, and by other means, to Kenneth R. Hill (USDA) and Milan Ihnat (Agriculture Canada) for their long service and many contributions. Drs. Hill and Ihnat leave the Board after serving terms of 9 and 11 years, respectively.

In 1981, the Board's first job description, "Terms of Reference," was adopted. That document contained 9 short term goals the Board assigned to itself. A recent assessment indicates that 7 of those 9 goals have been accomplished in whole or in part. At our most recent meeting, the Board has proposed 16 new short term goals. The actions of the past and those proposed show the vitality of the Editorial Board and publication staff.

With slight variations, the total number of papers submitted to the *Journal* for publication has remained fairly constant during the past 5 years. Those originating from organizations within the United States provide the bulk of these and accounted for 64% of all submitted in 1985. Within this group, the Food and Drug Administration submitted 67 papers, 43% of the U.S. contribution and 28% of the worldwide effort. Of those submitted by organizations outside the United States, Canada provided 19 papers, 22% of the group and 8% of the worldwide contribution. Other major sources of papers outside the United States include India, Japan, and Egypt.

Page costs of the *Journal* continue to rise. To maintain control of the cost within budget constraints, we will restrict the total pages of volume 69 (1986) to 1200. This will be 78 pages less than the 1985 volume and about the same as the 1984 volume.

As of September 30, 1985, the *Journal* had 3964 subscribers. Fifty-nine percent of these were from beyond the United States. The total is 121 (3%) less than the total number of subscribers a year earlier. Better than half the reduction in subscriptions occurred within the United States, which is contrary to what might be expected in view of the fact that the U.S. support is but 41% of the total number.

The 14th Edition of *Official Methods of Analysis* is doing well in sales. As of September 30, 1985, total sales amounted to $1,223,480.00. This is equivalent to 9062 copies. 10,991 copies remain in inventory.

The long awaited new publication on statistical analysis of analytical data by Wernimont, begun in 1981, is now in press and will be available in December 1985. The title is, *Use of Statistics to Develop and Evaluate Analytical Methods.*

In addition, AOAC has published in the past year 1000 copies of the manual, *Principles of Food Analysis for Filth, Decomposition, and Foreign Matter;* 500 copies of *Key for Identification of Mandibles of Stored Food Insects;* 500 copies of the *Animal Drug Analytical Manual;* and 300 copies of *Production, Regulation, and Analysis of Infant Formula* (conference proceedings).

A task force composed of individuals outside the Editorial Board has been appointed and funded to review and evaluate the *Journal* for content, focus, reader perception, and needs. The task force will initiate its activities in the spring of 1986 and file a final report at the 1987 annual meeting of the Association.

Another task force will be raised to study alternative ways for publication of the 15th edition of *Official Methods of Analysis*. Such alternative ways will include electronic transmission of requested methods as well as other sophisticated procedures, and division of the single bound volume into smaller groupings or individual chapters.

Other actions taken by the Board include:

(a) Reviewed and accepted the scope of duties for the Scientific Editor of *Official Methods of Analysis.*

(b) Approved guidelines for reviewers of papers proposed for the newest *Journal* section, "Chemical Contaminants Monitoring."

(c) Revised the policy on advertising in the *Journal.*

(d) Revised "Terms of Reference" for submission to the Board of Directors for approval.

(e) Revised guidelines for "Technical Communications" section of the *Journal.*

(f) Approved the 1986 *Journal* subscription rate of $89.75 for U.S. nonmembers.

Official Methods Board

H. MICHAEL WEHR, *Chairman*
Oregon Department of Agriculture, Laboratory Services Division, Salem, OR 97310

Other Members: P. M. Brickey; H. B. S. Conacher; D. J. Dube; E. George; A. R. Hanks; T. P. Layloff; G. R. Myrdal; and R. J. Noel

The Official Methods Board has the responsibility for administering the methods approval process within the Association and, in conjunction with the Board of Directors, determining policy in the methods validation area. This past year has continued to be an interesting and challenging one. The year has seen the conclusion of most parts of a complete review of the methods approval process. As described below, major elements of this review include a change in the mechanics of the collaborative study review process and a training program for Associate Referees, General Referees, Method Committee members, and Method Committee statisticians. The Board has also considered certain other important areas in response to interests within the Association; chief among these areas has been the development of a proposal for candidate methods as described below. Appendix A lists the major accomplishments for the 1983–1985 period. In addition, the Board has handled the responsibilities traditionally assigned to it.

During the past year, the Board met 3 times: December 17–19, 1984, in Chicago, IL; April 25–26, 1985, in Baltimore, MD, and July 18–19, 1985, in Chicago, IL.

We are pleased that 62 collaborative study reports were received by the Method Committees during this past year. Of these, 5 were given interim official first action approval.

As in previous years, the Board coordinated the selection of the individual receiving the AOAC Associate Referee of the Year Award. This award represents excellence in designing, conducting, and reporting a collaborative study. Nominees for this year's award included: Committee on Pesticide Formulations and Disinfectants—Stephen C. Slahck, Mobay Chemical Corp.; Committee on Drugs and Related Topics—no nominee; Committee on Foods I—Robert M. Eppley, Food and Drug Administration; Committee on Foods II—no nominee; Committee on Residues—Leon D. Sawyer, Food and Drug Administration; Committee on Microbiology—Walter Hill, Food and Drug Administration; Committee on Feeds, Fertilizers, and Related Areas—Michael Carlson, Nebraska Department of Veterinary Science; Committee on Hazardous Substances in Water and the Environment—no nominee.

Leon D. Sawyer, Food and Drug Administration, was selected as the 1985 Associate Referee of the Year.

This year, the Board instituted an Annual General Referee Award. Recognizing the key role the General Referees play in the method development and validation carried out by AOAC, this award recognizes and honors an individual who has successfully carried out all duties of a General Referee. Nominees for this year's award included: Committee on Pesticide Formulations and Disinfectants—James Launer, Oregon Department of Agriculture; Committee on Drugs and Related Topics—James Fitzgerald, Food and Drug Administration; Committee on Foods I—David Firestone, Food and Drug Administration; Committee on Foods II—Mike Deutsch, Food and Drug Administration; Committee on Residues—Bernadette McMahon, Food and Drug Administration; Committee on Microbiology—Wallace Andrews, Food and Drug Administration; Committee on Feeds, Fertilizers, and Related Areas—Frank Ross, U.S. Department of Agriculture; Committee on Hazardous Substances in Water and the Environment—no nominee.

Bernadette McMahon was selected as the first recipient of the General Referee Award.

The Board also sent the appropriate annual information and instructional letters to all General Referees and Associate Referees in January 1985 and June 1985.

As noted above, the Board considered a number of areas of importance to the methods approval activities of the Association. These areas included:

Proposal for Change in Review Process for Collaborative Studies

The AOAC collaborative study process has been exceedingly successful as a means to validate methods. Many organizations worldwide have modeled their analytical methods validation approaches after AOAC. Nevertheless, the Board has recognized that the collaborative study review process as currently carried out presents some deficiencies. Problems specifically noted include: (A) A difference in the extent of review between collaborative studies submitted as interim official first action and those submitted for approval at the annual international meeting; (B) Frequent lack of advance review by General Referees and Method Committee statisticians; (C) Frequent lack of inclusion of required statistical and safety information; (D) Frequent lack of incorporation of reviewer's comments until after method committee approval has been given; (E) Lack of adequate review of completed collaborative studies by General Referees and Method Committee members; (F) Inadequate meeting time for methods committees at the annual international meeting to carry out the necessary collaborative study review and to conduct all other necessary business.

As a result of these problems, the Board will institute a change in the procedure by which all collaborative studies will be reviewed and approved. These changes will involve: (A) Using the current interim official first action approach to review all collaborative studies; (B) Strengthening the intitial (front end) and final (back end) approval process to ensure initial and final review by the General Referee and Method Committee statistician, and to ensure inclusion of statistical information and safety information; (C) Instituting a written peer review by Method Committee members with a requirement for response from the Associate Referee on all adverse comments. Details of these procedures are outlined in Appendix B.

Training of Individuals Involved in Method Approval Process; Revision of Duty Guidelines

As an outgrowth of concerns described above relating to collaborative study approval, and recognizing the frequency of new individuals becoming a part of the AOAC method validation and approval process, it was determined that training of individuals involved in designing, implementing, reporting, and approving collaborative studies would be helpful. Also, the Board agreed that updating and revising duty guidelines for Associate Referees, General Referees, Method Committee members, and Method Committee statisticians to incorporate the changes in the collaborative study process recommended by the Board would be helpful. The Board has therefore prepared a series of revised duty guidelines for volunteers involved in the collaborative study process. Further, elements of the proposed training program will include a review of the following: (A) AOAC organization and struc-

ture; (B) Duties and responsibilities of Associate Referees, General Referees, Method Committee members, and Method Committee statisticians; (C) Guidelines for design and conduct of a collaborative study, including statistical analysis; (D) Manuscript preparation; (E) Mechanics of collaborative study review and approval.

While initial training was instituted at the 1985 annual international meeting, final development and full implementation will be accomplished during 1986.

Statistical Parameters for Official Methods of Analysis

In initiating efforts on the 15th edition of *Official Methods of Analysis*, the Board recognized the need to confirm which statistical parameters will be incorporated in the book and, therefore, which parameters should be included in the design and reporting of collaborative studies. In agreement with the "Guidelines for Interlaboratory Collaborative Study Procedure to Validate Characteristics of a Method of Analysis," the Board will recommend the use of the following statistical parameters: percent recovery; reproducibility coefficient of variation (CV_x) at the appropriate concentration level; minimum detectable level for the analyte (method sensitivity); applicable concentration range; repeatability (within laboratory) standard deviation (S_o); among laboratory standard deviation (S_l); reproducibility (including repeatability) standard deviation (S_x).

Candidate Methods

Recognizing (a) the need for the Association to respond to concerns that the current collaborative study procedure is unresponsive to the rate of method change and speed requested by some regulatory agencies; (b) that certain regulatory agencies currently use less rigorous means to validate methods; and (c) the need felt by some members for AOAC to recognize lesser validated methods to continue the viability of the Association, the Board established a subcommittee to consider and develop a proposal for the review and dissemination of methods which had not undergone a full collaborative study. Following extensive review, discussion, and input from interested individuals within the Association, the Board recommended to the Board of Directors for review by the membership a proposal for a lesser level of validated method termed "candidate" methods. Appendix C fully describes the proposal for candidate methods. As proposed, a candidate method is one which has been validated in a minimum of 3 laboratories on a minimum of 5 samples, including one set of blind duplicates. The method should include evidence of analyte identification, resolution of the analyte from interferring substances, adequate sensitivity, determination of the minimum detectable level, and specific limits on acceptable coefficient of variation values. Candidate methods would not be official methods of analysis, would be printed in looseleaf form, and would lose status after 4 years unless continued by the appropriate Method Committee.

Pass/Fail, Biological Methods Task Force

In response to concerns expressed by various members that the current collaborative study process does not lend itself adequately to the validation of many biological methods, including microbiological procedures and certain pass/fail tests, the Board established a task force to review this area and recommend approaches to resolving the problem. Two meetings took place in 1985. Recommendations from the task force are anticipated within a year. The Board will work with the Interlaboratory Studies Committee to provide a final set of recommendations to the Association to resolve this area of concern.

Descriptive Names for Method Committees

The use of letters to designate the various Method Committees provides no clear indication of what these committees do or for what analytical areas each has responsibility. Indeed, to many members of the Association, such letter designations are confusing and may hinder understanding of and involvement in the Association. To help resolve this problem, the Board recommended the use of descriptive terms related to the analytical or commodity area of responsibility of each committee. These new terms, along with the old letter designations for each method committee are as follows: A: Pesticide Formulations & Disinfectants; B: Drugs & Related Topics; C: Foods I; D: Foods II; E: Residues; F: Microbiology; G: Feeds, Fertilizers, & Related Areas; H: Hazardous Substances in Water & the Environment.

Model Purpose, Activities, and Objective Statement for Method Committees

To provide for consistency among Method Committees and continuity for operation, the Board developed a model statement of purpose, activities, and objectives for Method Committees.

Terms of Service for Method Committees and Official Methods Board

To provide for consistency with the Board of Directors policy on length of service and rotation of committee members, the Board agreed to the following terms of service for Method Committees and the Official Methods Board: (A) Method Committee members shall be appointed annually, not to exceed 6 years; (B) Method Committee secretaries shall be appointed annually, not to exceed 6 years; (C) Method Committee chairmen shall be appointed annually, not to exceed 3 years; (D) A Method Committee chairman may remain on the committee for one year following the last year as chairman; (E) The Official Methods Board chairman shall be appointed annually, not to exceed 3 years.

In other areas, the Board: (1) Assisted with establishing an agreement between AOAC and the American Public Health Association regarding the collaborative study of methods published in "Standard Methods for the Examination of Dairy Products," (2) Reviewed General Referee performance; (3) Implemented the new Method Committee on Hazardous Substances in Water and the Environment; (4) Reviewed procedures for improving feedback on the adoption of official first action methods; (5) Reviewed use of endorsement of AOAC method approval in advertising, and recommended the phrase "This product conforms with the requirements of an official method of AOAC" by vendors who wish to incorporate a statement on AOAC method approval for a product or instrument produced by them. (6) Handled 2 appeals regarding the approval of collaboratively studied methods.

In conclusion, we express our thanks to the General Referees, Associate Referees, Method Committee members, Method Committee statisticians, and collaborators for their work and dedicated service over the past year. AOAC would not function without their efforts and services. The Official Methods Board also expresses sincere thanks to members of the Board who are completing their terms of service including Elmer George, Chairman of the Method Committee on Foods II; and Paris Brickey, Chairman of the Method Committee on Microbiology. We have appreciated their input and dedi-

cated service. We express our thanks to AOAC staff, particularly Rita Bahner, AOAC Assistant Executive Director; and Claire McGee, Assistant to Rita Bahner. Their particular contributions helped immeasurably to carry out the great amount of work needed to make the collaborative study system operate effectively. We also thank the Association Officers and Board of Directors for their guidance and assistance. It has been a pleasure to work with all involved in AOAC.

The Board looks forward to an interesting and challenging year.

Addendum

The Official Methods Board recommends for adoption as official first action the interim official first action method, spectral characteristics of Florida orange juice and orange pulpwash, with continued study. The Board acted on this change in status of this method based on its review of an appeal of an earlier Association action.

APPENDIX A

Major Accomplishments, 1983–1985

(1) Development and establishment of an appeals process to resolve differences regarding approval of collaborative studies.
(2) Development and implementation of a strengthened review process, an all-interim approval procedure for collaborative studies.
(3) Establishment of a Method Committee on Hazardous Substances in Water and the Environment.
(4) Implementation of a General Referee award.
(5) Development of revised duty guidelines for General Referees, Associate Referees, Method Committee members, Method Committee statisticians, and collaborators.
(6) Development of a training program for General Referees, Associate Referees, and Method Committee members.
(7) Development of a proposal for candidate methods.
(8) Development of a model goals and objectives statement for Method Committees.
(9) Initiation of performance reviews for General Referees.
(10) Review and revision of terms of service for the Official Methods Board and Method Committees.
(11) Recommendation to the Board of Directors on a memorandum of agreement between the Association of Official Analytical Chemists and the American Public Health Association regarding dairy product method approval.
(12) Development of statistical parameter requirements.

APPENDIX B

Proposed Review Process for Collaborative Studies

Traditionally, AOAC Method Committees review all collaborative studies conducted during the immediate preceding year for recommendation for official first action adoption by the membership. Studies received after some deadline before the meeting (approximately August 15) and otherwise between meetings (prior to approximately May 15) are considered for interim official first action subject to adoption as official first action at the next annual meeting.

The Official Methods Board has observed the following problems with the current collaborative study review process.

(1) There is a difference in the extent of review between papers submitted for consideration as interim official first action and those submitted directly for consideration at the annual meeting, with interims receiving a more indepth review. This is primarily due to greater available time for review of interim papers and the unavoidable time constraints placed on Method Committees at the annual meeting.

(2) Reviewers' comments may not be responded to by Associate Referees. Recommendations for changes in manuscripts may not be incorporated until after approval has been given, with responsibility for making changes often left to the scientific editor. These problems have been noted with respect to both interim studies and studies submitted for consideration at the annual meeting.

(3) Completed studies frequently do not contain the required statistical parameters necessary for inclusion in *Official Methods of Analysis*.

(4) Collaborative study proposals may not have been reviewed by the General Referee or Method Committee statistician, leading to disagreements as to acceptable experimental design and statistical analysis *after* the study has been completed.

(5) Completed collaborative studies may not have been reviewed by the General Referee or Method Committee statistician.

(6) Much of the business of Method Committees is usually conducted in a very short period of time. This includes review of new and old policies, resolutions of disputes or problems, and examination of developing areas in addition to consideration of collaborative studies. All too frequently, questions concerning a study must be addressed to General and/or Associate Referees with minimal time to receive responses which must be evaluated before decisions on approval can be made by a Method Committee. Indeed, some concerns may not even be addressed due to the lack of time for discovery caused by the magnitude of the task faced at the annual meeting. The interim review process provides a better opportunity for a more indepth consideration of collaborative studies.

Because of these problems, the Board is proposing a change in the procedure by which collaborative studies are reviewed and approved. These changes involve: (1) Using the interim official first action approach to review *all* collaborative studies; (2) Strengthening the initial (front end) and final (back end) approval process to ensure initial and final review by the General Referee and Method Committee statistician and to ensure inclusion of statistical parameters and safety information; (3) Instituting a written peer review by Method Committee members with a requirement for response from the Associate Referee on all adverse comments.

Specifically, this proposal would use the following approach for review of collaborative study proposals and acceptance of completed collaborative studies:

(1) All methods considered for collaborative studies would be submitted by the Associate Referee in proposal form to the appropriate General Referee and Method Committee statistician in advance of initiation of the study. The collaborative study proposal would outline the protocol to be used for the study, statistical parameters to be determined, and other appropriate information. The General Referee and Method Committee statistician would: (a) recommend; (b) recommend with modification; or (c) not recommend the study as appropriate. A request for modification or a recommendation against determination by the General Referee or Method Committee statistician would not prohibit the Associate Referee from conducting the study; however, studies conducted with such a comment are carried out at the risk that such studies may not be accepted by the Association.

General Referees and Method Committee statisticians must prepare a written review of the proposal, using a standard form prior to initiation of the study.

(2) The AOAC office would continue to use a checklist to track the study from proposal stage to final determination by the Association. This checklist would help to assure that all reviews of the proposal and completed study have been done, and that the *Official Methods of Analysis* statistical parameters and safety information are included.

(3) All completed collaborative studies would be submitted for approval as interims. Studies would be reviewed, approved by the appropriate Method Committee, and granted interim official first action. Method Committees would ratify for official first action all interim methods at the annual international meeting. A final date for acceptance of interims for any given year would be selected (suggest 30 days prior to annual meeting). All studies for ratification as official first action would have to have been fully reviewed as outlined. *No* unreviewed or partially reviewed methods would be considered at the annual international meeting.

(4) The review process for acceptance of a completed collaborative study manuscript would consist of: (A) *Written* reviews by General Referee, Method Committee statistician, and Methods Committee members; (B) Acceptance, revise and review, or rejection of the manuscript; (C) For revise and review, the method and reviews would be returned first to the Committee Chairman, and then to the Associate Referee for response and/or revision. The revised collaborative study manuscript would be rereviewed. The cycle would be repeated until acceptance or final rejection; (D) For rejection, the Associate Referee could respond to the review comments with reconsideration by the Method Committee. The appeal process would be used to resolve differences; (E) Requirements for acceptance would include: manuscript written in proper AOAC format/style, including all changes or modifications as a result of the collaborative study and the review process; inclusion of *Official Methods of Analysis* statistical parameters; and inclusion of appropriate safety information; Manuscripts and method write-up *will not* be reviewed by Method Committee members to the extent that all appropriate AOAC abbreviations, etc., have been incorporated. Rather, their reviews will emphasize that needed information is present and understandable, that studies were correctly conducted, that results support recommendations, and, above all else, that there are no apparent technical flaws.

(5) Method Committee meetings at the annual international meeting would involve: (A) Incorporation of all interim collaborative studies (i.e., *all* collaborative studies conducted during the past year up to cutoff date) in the Method Committee secretary's report; (B) Resolution of concerns with any collaborative study carried out during the year; (C) Review of old and/or new policies, resolution of disputes or problems; (D) Examination of developing analytical areas and consideration of needed and/or proposed collaborative studies.

(6) Collaborative studies from other organizations can be accepted on the following basis: (A) Each collaborative study ought to meet all technical requirements as outlined in the *Handbook for AOAC Members;* (B) Statistical analysis must be adequate to judge the performance of the method; (C) The study must be reviewed through the normal procedure, that is, the method must be reviewed and approved by the appropriate General Referee, statistician, and Method Committee; (D) Preferably, the method will meet the AOAC format and writing style. This is not a requirement for acceptance, however; (E) The study, if previously published elsewhere, need not be republished by AOAC. Publication of a summary of the study may be helpful, however, and is recommended; (F) The scientific editor will undertake what editorial revisions are necessary.

APPENDIX C
Candidate Methods

Background and History

The Official Methods Board established a subcommittee consisting of H. B. S. Conacher, A. R. Hanks, and T. P. Layloff to consider and develop a proposal for review and dissemination of methods which had not been submitted to the full rigors of the AOAC collaborative study process. Since these methods would not be fully collaborated, they *would not* have the legal status of the *Official Methods of Analysis,* which is specifically cited in legislation, e.g., U.S. CFR 21, Article 2.19. The reasons for the development of the proposal is based on the following observations and statements:

(1) Warren Bontoyan, in his President's Address, stated, "With a few exceptions, the present collaborative regime is at times too slow for regulatory agencies. . . . I am saying we must respond by developing scientific methods that will stand cross-examination in courts of law, even though the methods have not been subjected to a full collaborative study. . . ."

(2) The Food and Drug Administration (FDA) Center for Veterinary Medicine uses in their law enforcement program methods which have been ". . . evaluated in at least 2 FDA laboratories; in addition, tissue residue methods are tested in a minimum of one USDA laboratory. . . ." (Quoted from the presentation, "NADA Methods Validation: Tissue and Residue Methods, Field Analytical Methods, Dosage Form Methods" by Robert C. Livingston, given at the FDA Division of Field Sciences Drug Analytical Workshop, September 10, 1984, Bethesda, MD.)

(3) The Canadian Health Protection Branch currently uses a 2 or 3 laboratory validation procedure to "Extend the Scope of a Method," "Justification of Modification in Method," and "Handle Crises Situations." (Quote from letter Conacher/Layloff dated July 9, 1984.)

(4) AOAC methods either have no legal status or are a fourth-tier choice (human drugs) for many regulatory/law enforcement groups, e.g., all of the topical areas of human drugs, veterinary methods, forensics, illicit drugs, and clinical methods. It is unlikely that AOAC methods will receive legal status in these areas. A new class of methods requiring a 2 or 3 laboratory validation procedure would have good market potential for these areas.

Proposal for Candidate Methods

A candidate method is one which has been reviewed by the appropriate General Referee, statistician, and Method Committee and has been approved by them for publication as a candidate method. Candidate methods should not be confused with the AOAC official methods of analysis which are formally adopted by the Association.

A candidate method must be validated in a minimum of 3 laboratories on a minimum of 5 samples including one set of blind duplicates. The method should include evidence of analyte identification, resolution of analyte from interfering substances, sensitivity of the principle of measurement to the analyte concentration, and determination of the lower limits of detection of the analyte. The coefficient of variation obtained for the determination of the analyte should not exceed the appropriate guidelines. (Horwitz, W., Kamps, L., & Boyer, K. (1980) *J. Assoc. Off. Anal. Chem.* **63**, 1344).

Methods which fulfill the above mentioned criteria may be submitted for consideration by the appropriate General Referee and statistician for review and comments. It is assumed that the GR will discuss the method with Associate Referee(s) who have the same or overlapping topic assignments. If the GR and statistician recommend the method for consideration, it will be sent through the collaborative study peer review process. After the reviews have been successfully completed, the Method Committee can approve the method as a candidate method.

Candidate methods are kept in active status for 4 years from the first date of publication and are then dropped from the current cumulative listings unless the appropriate Method Committee votes to continue the method for an additional 4 years. (Alternative statement without sunset provision: delete the previous sentence.) Candidate methods must be submitted to a full collaborative study to be considered for elevation to official method status. Comments on or problems reported with a method will be referred to the Method Committee which approved the method for their review and investigation. If a Method Committee deems that a method is flawed, the approval of the candidate method will be rescinded.

Newly approved methods and notices of rescinded methods could be published quarterly on looseleaf paper. The date of publication of the method should be noted on each page. A cumulative index of all current candidate methods should be included with each quarterly publication.

The candidate methods would not go to the membership for approval.

Committee on Collaborative Interlaboratory Studies

WILLIAM HORWITZ, *Chairman*
Food and Drug Administration, Center for Food Safety and Applied Nutrition, Washington, DC 20204

Other Members: R. Albert; P. W. Britton; P. R. Caudill; C. J. Dahl; T. Dols; R. Ellis; D. W. Fink; J. Gallagher; M. Gallo; E. M. Glocker; R. Grappin; M. Ihnat; P. Kahn; S. E. Katz; C. J. Kirchmer; M. Margosis; K. McCully; A. Munson; J. O'Rangers; H. S. Ragheb; S. Sherken; E. Smith; W. Steller; L. Stoloff; B. K. Thompson; L. Williams; E. S. Windham; J. Winter

The Committee discussed at its 2 meetings an algorithm FLOWCHART which is intended to calculate the same measures of precision that are provided by the directions in the "Youden Manual" and the Guidelines on a material-by-material basis (one-way analysis of variance). The Committee recommends that this document and the explanatory matter, supplemented by the quick method for the calculation of the measures of precision of a collaborative study, balanced or unbalanced design, using a scientific calculator with only a square root key, be circulated to all Associate Referees, General Referees, and Method Committee members. The recipients should be asked to try the procedure for the statistical analysis of their collaborative studies and to report their reactions to the Committee.

In this connection the Committee discussed the importance of screening the original raw collaborative data to remove invalid data. *Invalid data* are values produced by collaborators who did not follow the method, who had instrumental problems, whose results on practice materials were not satisfactory, or similar stiuations. The inclusion of such invalid data may result in incorrect statistical analyses when performed by personnel who do not appreciate the significance of the technical footnotes. The data are being incorporated by Associate Referees under the admonition to "report all data," a statement meant to imply "report all 'valid' data." Invalid data should be relegated to the text, footnotes, or separate tables and should not be included in the primary tables used to calculate the performance parameters. Invalid data should not be confused with "outliers" for which there is no explanation of their abberation.

The Committee recommends that a document defining various types of interlaboratory studies also be circulated to the same group for additional comment. This document is intended to eliminate the confusion which has arisen in the definitions and procedures of several organizations because of failure to recognize that collaborative studies test *method* performance (the same method must be used by all participants) while proficiency studies test *laboratory* performance (any method may be used by the participants).

The Committee reviewed a document containing guidelines for collaborative studies of biological assay systems. It recommends that this document also be circulated to Associate Referees. General Referees, and Method Committee members for review and application to the design of protocols that incorporate biological reagents. It contains a number of suggestions for including ruggedness into biological assay procedures in an attempt to reduce their variability.

The Committee desires to review the comments on these documents in order to make any necessary revisions before recommending that the documents be considered by the Association for adoption.

Twenty-Ninth Annual Meeting of the Collaborative International Pesticides Analytical Council (CIPAC)

WARREN R. BONTOYAN, *Representative*
Environmental Protection Agency, Office of Pesticide Programs, Beltsville, MD 20705

The 29th meeting of CIPAC was held in Vaerloese, Denmark, September 3–6, 1985. The annual informal meeting of the Food and Agriculture Organization (FAO) of the United Nations was held September 9–11, 1985.

Jean Henriet of Belgium resigned as CIPAC Chairman after serving for 7 years. CIPAC Secretary, A. Martijn of The Netherlands, expressed on behalf of all members their gratitude for Jean Henriet's dedicated and stimulating leadership which resulted in the publication of CIPAC Handbooks 1A, 1B, and 1C and the adoption of numerous pesticide methods of analysis used by government regulatory agencies throughout the world.

The CIPAC Management Committee elected H. P. Bosshardt of Switzerland as Chairman. Dr Bosshardt is a respected scientist known for his expertise in analysis for pesticides and related materials. He is internationally known for his significant contributions to the development of analytical methods for analysis for dioxins and benzofurans in the environment.

W. Dobrat was elected as the West Germany government member to CIPAC. Dr Dobrat replaces W. Weinmann who retired in 1984.

At the FAO informal meeting, the FAO Group of Experts on Pesticide Specification reviewed and critiqued proposed guidelines for the preparation of FAO Specifications for Plant Protection Products. These guidelines are similar in many respects to the U.S. Pesticide Regulation Guidelines for Product Chemistry.

Summary of the CIPAC decisions made at the 29th meeting (4 AOAC methods adopted)

Code No.	*Name*	*Status of Method*
24.	thiram	The LC method for thiram technical and powder formulations, CIPAC/3242, was adopted as *full* CIPAC method.
143.	chlormequat	The argentometric method for determination of chlormequat in aqueous solutions, CIPAC/3263, was adopted as *provisional* CIPAC method.
204.	alachlor	The GC method for alachlor formulations, **6.A05–6.A08** (JAOAC (1985) **68,** 370–371), was adopted as *provisional* AOAC-CIPAC method.
239.b	primiphos-ethyl	The GC method for pirimiphos technical and emulsifiable concentrates, CIPAC/3254, was adopted as *tentative* CIPAC method.
242.	ethirimol	The GC method for ethirimol technical, CIPAC/3251, was adopted as *provisional* CIPAC method.
277.	chlordimeform	The GC method for determination of chlordimeform in formulations, **6.A09–6.A13** (JAOAC (1985) **68,** 371), was adopted as *provisional* AOAC-CIPAC method.
289.	cyhexatin	The LC method for cyhexatin technical and formulations, CIPAC/3265, was adopted as *full* CIPAC method.
292.	aminocarb	The LC method for aminocarb technical and formulations, **6.A25–6.A30** (JAOAC (1985) **68,** 372–373), was accepted as *provisional* AOAC-CIPAC method.
333.	deltamethrin	The LC method for deltamethrin technical and formulations, CIPAC/3219, was adopted as *full* CIPAC method.
364.	phoxim	The provisional LC method for phoxim technical and formulations, CIPAC Handbook 1C, p. 2187–2189, was accepted as *full* CIPAC method.
366.	bentazone	The provisional LC method for bentazone technical and water-soluble concentrates, CIPAC Handbook 1C, p. 1973–1976, was accepted as *full* CIPAC method.
381.	metamitron	The LC method for metamitron technical and wettable powders, CIPAC/3259, was adopted as *provisional* CIPAC method.
387.	cartap	The spectrophotometric method for cartap technical and water-soluble powder formulations, CIPAC/3280, was adopted as *full* CIPAC method.
388.	thiobencarb	The GC method for thiobencarb technical and emulsifiable concentrates, CIPAC/3282 was adopted as *provisional* CIPAC method.
390.	fenobucarb	The TLC-spectrophotometric method for fenobucarb technical and emulsifiable concentrates, CIPAC/3286, was adopted as *provisional* CIPAC method.
396.	isoprocarb	The LC method for isoprocarb technical and hot fogging concentrates, CIPAC/3261, was adopted as *provisional* CIPAC method.
400.	metolachlor	The GC method for metolachlor formulations, **6.A14–6.A18** (JAOAC (1985) **68,** 371), was accepted as provisional AOAC-CIPAC method.
MT130	identity tests for dithiocarbamates	The revised method, CIPAC/3244, was accepted as *full* CIPAC method.
MT151	TCDD	The provisional GC-MS method for determination of TCDD in technical 2,4,5-T and technical 2,4,5-T esters, CIPAC Handbook 1C, p. 2284–2289, was adopted as *full* CIPAC method.
MT163	identity tests for pyrethroids	The methods for identification of permethrin, cypermethrin, and fenvalerate, CIPAC/3248, were adopted as *full* CIPAC methods.

MT164	identity tests for pirimidines	The methods for identification of pyrimidines, CIPAC/3257, were adopted as *full* CIPAC methods.
MT165	UV absorption test for ethylenebisdithiocarbamates	The UV absorption test for evaluation of ethylenebisdithiocarbamates, CIPAC/3245, was accepted as *full* CIPAC method.
PP231	pirimicarb	The method for preparation of pure pirimicarb, CIPAC/3256, was accepted.
PP239.b	pirimiphos-ethyl	The method for the preparation of pure pirimiphos-ethyl, CIPAC/3255, was accepted.
PP242	ethirimol	The method for the preparation of pure ethirimol, CIPAC/3252, was accepted.

Committee on the Constitution

JAMES B. KOTTEMANN, *Chairman*
Food and Drug Administration, 200 C St, SW, Washington, DC 20204

Other Members: W. R. Bontoyan; R. Bowers; D. E. Coffin; A. Conetta; A. Gardner; F. J. Johnson; R. Luskin; J. P. Minyard; M. Rhodes; A. W. Tiedemann

F. J. Johnson served as acting chairman at the Fall 1985 meeting.

The activities of the Constitution Committee during the year were minimal. One action that was finalized was adoption and recommendation to the Board of the bylaws for regional sections. We thank those people who have served and look forward to much more activity in the immediate future.

Committee on Instrumental Methods and Data Handling

KENNETH R. HILL, *Chairman*
U.S. Department of Agriculture, Agricultural Research Service, Beltsville, MD 20705

Other Members: L. Bailey; S. Barnett; R. Beine; W. R. Bontoyan; J. V. Bruno; P. Caudill; A. Conetta; L. Dusold; W. Furman; R. J. Gajan; T. Gale; C. Gehrke; L. Gelber; L. Hambleton; W. J. Hurst; W. Hyde; R. Issac; W. Y. Ja; P. D. Jung; P. F. Kane; G. MacEachern; M. Margosis; H. M. McNair; W. J. Morris; R. Sweeney; W. Trujillo; M. Walters; R. S. Wayne; P. Whittier

The Committee met on October 30, 1985, to receive the reports and review the activities of the subcommittees for the preceding year. Also attending as invited observers and guests were Noreen Lally, Food Materials Corp.; Robert Briggs, Fisher Scientific; Sam Chappell, NBS (OIML); Joseph Gensic, Central Soya Co.; Fred Rabel, Whatman, Inc.; Edward Smith, FDA; and Fred Baur, AOAC.

W. Furman, Chairman of the Subcommittee on Instrumental Specifications, reported that the final draft performance specifications for UV/visible spectrophotometers had been forwarded to AOAC for transmission to SAMA (Scientific Apparatus Manufacturers Assoc.) for comment. No response from SAMA had been received at this time. The second draft of the performance specifications for voltammetric instruments and the second draft of the specifications for instrument analog voltage output were under review by the full Committee, and, if no substantial comments were received, would be forwarded for SAMA review. A first draft specification for single-pan analytical balances had been prepared, edited for style, and would be ready soon for full Committee review. First draft specifications for infrared spectrophotometers and mass spectrometers are in preparation and should be ready for Committee review in mid-1986. Efforts are under way to begin new projects on atomic absorption/emission spectrometers, fluorometers, and possibly antoinjectors. After the preceding specification papers have been reviewed by SAMA and revised if necessary, they will be submitted to the *Journal of the AOAC* for publication, along with the rationale and comments received as appropriate and example data to substantiate the proposed requirements. Comments from *Journal* readers will be solicited. After a final round of revisions, if needed, these specifications will be submitted to AOAC for adoption as "official," with the intent of proposing their inclusion as a separate reference chapter in future editions of *Official Methods of Analysis*.

P. Kane, Co-Chairman of the Subcommittee on Automated Methods and Equipment, reported that the paper on automated phosphorus pentoxide analysis by Rose Sweeney, which is intended to serve as an example of how to write a method description based on performance criteria, had been published in *J. Assoc. Off. Anal. Chem.* (1985) **68**, 466–470. A draft document, "Guidelines for Writing AOAC Instrument Methods," which had been previously approved by the Subcommittee, was distributed to the Committee for review. Following review and revisions, this document will be submitted to the Official Methods Board (OMB) with the suggestion that its provisions be used as a guide for revising future editions of the *Handbook for AOAC Members*.

R. Wayne, Chairman of the Subcommittee on Gas and Liquid Chromatographic Column Specifications, reported progress on 4 projects. The Committee approved a paper on "Preparation of Performance Criteria for C-18 Columns to Establish Equivalency" by Milda Walters and agreed that it should be reformatted and submitted to the *Journal* for publication. A draft paper on "Criteria of Performance of Integration Algorithms" by Loren Gelber is in revision, and draft papers on "Establishing the Purity of a Reference Standard" by Art Hofberg and Tom Gale and "Simple Methods and Useful Criteria for Column Peformance Measurements for AOAC Collaborative Studies to Establish Column Equiva-

lency" by Michel Margosis are in preparation. The Subcommittee plans to review, update, combine, and republish its previous reports on gas chromatographic (1976) and liquid chromatographic (1983) column recommendations. Potential future projects on capillary columns and supercritical fluid chromatography were discussed.

R. Beine, Chairman of the Subcommittee on Laboratory Computers and Microprocessors, reported that his recommendations on a new approach for specifying weights and volumes had been accepted by OMB for weights but not for volumes. The Committee agreed that the recommendation on volumes should be revised, clarified, and resubmitted to OMB for reconsideration. This Subcommittee has now expanded to include laboratory robotics in recognition of the impact that such systems will have on AOAC official methods. Draft recommendations were submitted to the Committee to establish criteria under which methods, which include robot sample preparation, might become official. Briefly summarized, these criteria are: *(1)* via normal collaborative study procedures for new methods and for certain existing methods where required, and *(2)* by establishing through comparative experiments that the substitution of robotic sample manipulation for human procedures could be considered as a minor modification to an existing method. K. Hill reported briefly on the one-day symposium on Laboratory Robotics held in connection with the AOAC 10th Annual Spring Training Workshop, April 8–11, 1985, in Dallas, TX.

The Committee received a report from S. Chappell, National Bureau of Standards and International Organization for Legal Metrology (OIML), on the current status of the OIML's Committee on "Measurement of Pollution" second draft recommendations on gas chromatographs and gas chromatograph-mass spectrometers, which had been reviewed by the Committee during the year. These recommendations are now being voted on by representatives of participating member nations. New projects on atomic absorption spectrophotometers and liquid chromatographs have been initiated by the U.S. National Working Group.

The Committee accepted the resignation of W. Bontoyan as OIML Liaison for IMDH with appreciation for his services. The Chairman of the Committee will reassume this function.

The Committee accepted the resignations of 6 other members and received suggestions for 6 new members to assist with the new projects. Additional members with specific expertise are still needed and will be sought.

International Coordination Committee

BEN BORSJE, *Chairman*
P. H. Van Rynstraat 44, 3904 HJ Veendndaal, The Netherlands

Other Members: L. Appelqvist; W. Bontoyan; C. Cheng; R. Coleman; E. Elkins; E. Hopkin; W. Horwitz; J. Iturbe; P. Martin; K. Naguib (Vice Chairman); D. Park; H. Poulsen; M. Rogers; R. Rund; I. Santich; B. Smith; R. Weik

The Committee at the 99th AOAC Annual International Meeting was pleased to welcome as its guests Dr. Packham, Laboratory of the Government Chemist, UK; R. Tarleton and E. Christiansen, American Association of Cereal Chemists; Marie Siewierski, Rutgers University; L. Chen, Food and Drug Bureau, Department of Health, Executive Yuan, Republic of China; and Roger Wood, Ministry of Agriculture, Foods, and Fisheries, UK.

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 (APA), Analytical Division of the Royal Society of Chemistry (AMC), Collaborative International Pesticides Analytical Council (CIPAC), International Union of Pure and Applied Chemistry (IUPAC), Food and Agriculture Organization (FAO), International Dairy Federation (IDF), Nordic Committee on Food Analysis (NMKL), the Pan American Health Organization (PAHO), European Economic Community (EEC), American Association of Cereal Chemists (AACC), and the American Society of Brewing Chemists (ASBC).

Effective October 1, 1985, AOAC no longer has P status for ISO/TC 134—Fertilizers and Soil Conditioners.

Effective January 1, 1985, the ISO Central Secretariat (Geneva, Switzerland) confirmed the Category "A" liaison status for AOAC in all 14 subcommittees of ISO/TC 34—Agricultural Products.

New Category "A" liaisons with 2 ISO Technical Committees will be established with TC 69 (Statistics) and TC 93 (Starch).

The Committee expressed concern about the procedures for evaluation and adoption of collaborative studies published in journals other than the *Journal of the AOAC*. The Committee requests that the Board of Directors instruct the appropriate methods committees and the Official Methods Board to evaluate such studies regardless of the existing format. Also, the differences in statistical nomenclature should be explained to the appropriate methods committee by its appointed statistician.

The AOAC/AMC and AOAC/APA agreements are still under review and in this respect the following motion was developed and carried: The Board of Directors is requested to review any new proposed agreement between AOAC and any other organization to ensure that such agreements are consistent with existing agreements and are consistent with AOAC policies and bylaws.

Concern was expressed by the African member that AOAC should study the possibilities of setting up training courses in developing countries. The Committee will study the possibilities of future projects as well as appropriate representation of AOAC in various countries together with priorities for arranging such representation. A decision on further AOAC activities in Japan was tabled.

Pending future activities to develop substructures of AOAC, if was stated that AOAC Europe should study the possibility of a 2–3 day meeting which is not in conjunction with another meeting.

A report by the Asian member revealed a substantial interest in AOAC. It was reported that AOAC methods are becoming considered as government standards in Taiwan.

Intersociety Committee (ISC) on Methods of Air Sampling and Analysis

BERNARD E. SALTZMAN, *Representative*
University of Cincinnati, Kettering Laboratory, Cincinnati, OH 45267-0056

During the past year, the committee held one meeting on June 18, 1985, in Detroit, MI. The Secretary reported that 1447 copies of the 2nd edition of the manual remain in inventory. The available funds for the committee are now very low, and may barely suffice to cover the costs of travel for subcommittee meetings this year. The Chairman reported that he had been unsuccessful in obtaining support from EPA and NSF. Prospects for additional funding efforts were discussed.

The subcommittee chairmen reported good progress on updating old methods and writing new methods for the 3rd edition of the manual. Some ion chromatography methods have been added. A state-of-the-art report on trace metal analyses by neutron activation has been prepared. Some methods for radioactivity in air have been deleted, and methods recently adopted for water will be adapted for air. A state-of-the-art paper on stationary-source sampling will be updated. It was agreed to delete the designation of "Tentative" for methods in the next edition. The editor, Dr. Lodge, set a target date of September 15th for receipt of all outstanding material. A new chairman will be elected next year.

Joint AOAC-AOCS-AACC-IUPAC Mycotoxin Committee

PETER M. SCOTT, *Chairman*
Health and Welfare Canada, Food Research Division, Health Protection Branch, Ottawa, Ontario, Canada K1A 0L2

Other Members: J. W. Dickens; D. L. Park (AOAC); A. E. Pohland (IUPAC); R. Bernetti; O. L. Shotwell; A. H. Bowers (AACC); A. E. Waltking; L. A. Goldblatt; R. D. Stubblefield *(Secretary)* (AOCS)

The annual meeting of the Joint Mycotoxin Committee was held on October 29, 1985, in Washington, DC, and was chaired by P. M. Scott. The following topics were discussed:

(1) The minutes of the previous meeting (October 30, 1984) were approved, with minor corrections.

(2) Joint Committee membership appointments were reviewed. Members are appointed by the 4 organizations represented; the AOCS, AACC, and IUPAC representatives have been appointed for indefinite terms. AOAC is recommending 3 year terms on committees, in general. Only AOAC representatives are affected. O. L. Shotwell reported that R. Bernetti has replaced J. Routh for AACC.

(3) The AOAC report was given by General Referee P. M. Scott. Three methods were submitted to the Committee on Foods I for adoption as official first action methods: (1) determination of deoxynivalenol in wheat by TLC (≥ 300 ng/g), (2) determination of deoxynivalenol in wheat by GC (≥ 350 ng/g), and (3) determination of aflatoxins M_1 and M_2 in fluid milk by reverse phase LC. It was also recommended to include the solvent proportion changes in **26.002** and the cottonseed sample preparation in **26.054** from the 13th edition of *Official Methods of Analysis* in the next "Changes in Official Methods" (March 1986). P. M. Scott reported that he needed to appoint new Associate Referees for secalonic acids, emodin and related anthraquinones, and *Penicillium islandicum* toxins. Anyone interested in developing any of these refereeships should contact the General Referee. In response to a request concerning constituency issues and list of projects planned for the next 5 years, it was moved, seconded, and passed to (1) submit a proposal to hold a mycotoxin symposium at the 1987 AOAC annual meeting and (2) request an oral presentation session at the 1986 meeting with the General Referee selecting the papers to be presented in the allotted time. Additional papers will be given at a poster session.

It was recommended by O. L. Shotwell, and concurred by the committee, that we express our appreciation to AOAC for providing a room for our committee to meet each year.

Chairman Scott read a letter from the AOAC Board of Directors commending the committee for their excellent work on mycotoxin methods development and testing.

(4) A. E. Waltking summarized the minutes of the AOCS Mycotoxin Committee. He reported that he is now the chairman since the resignation of L. A. Goldblatt. Results from the Smalley Check Sample Program indicate that the proficiency of analysts is improving over the years as evidenced by the within-laboratory coefficients of variation (SD = 0.8, initial year/SD = 0.2–0.3, 1985). Four AOAC methods will be written in AOCS style and submitted for approval for the AOCS Book of Methods: (1) minicolumn method for corn and raw peanuts, (2) B_1 and G_1 derivative confirmation on TLC, (3) aflatoxin M_1 in dairy products (and confirmation on TLC), and (4) zearalenone in corn by LC.

(5) O. L. Shotwell presented the report of the Mycotoxin Committee Meeting of AACC. The committee is recommending adoption of the method for determining α-zearalenol and zearalenone in corn by LC. A decision will be made after a poll of the complete committee. The committee agreed that the "black light" BGY presumptive test for aflatoxin should be published in the AACC Book of Methods. The committee discussed the decision by the U.S. Court of Appeals on March 26, 1985, which declared the FDA aflatoxin guidelines illegal. This decision greatly affects aflatoxin analytical methodology. The court decision has been appealed. S. Watson wrote a rebuttal to a controversial syndicated news article concerning this court decision. Copies of the rebuttal can be obtained from O. L. Shotwell.

(6) A. E. Pohland summarized the minutes of the IUPAC Commission on Food Chemistry meeting. This commission deals with all aspects of food chemistry, of which mycotoxins are only a part. Of new projects approved by the Commission, 3 deal with mycotoxins: (1) collaborative study of patulin in juices, (2) spectroanalytical parameters for *Fusarium* toxins, and (3) compendium of limits and regulations for mycotoxins. The Commission sponsored a successful symposium on mycotoxins and phycotoxins in Pretoria, South Africa (July 22–25, 1985). The next one will be in Japan in 1988.

(7) P. M. Scott presented a brief report on the South African Symposium. The keynote address was given by C. W. Hesseltine. He reported on an international poll of mycotoxin scientists which indicated that their most needed research is

for rapid and improved methods. The proceedings of the meeting are to be published late in 1985.

(8) A report of the International Dairy Federation—Group E 33 (Mycotoxins) meeting was given by P. M. Scott.

(9) A discussion of the status of immunochemical methodology was led by P. L. McMahon (AgriTech Systems). He pointed out the problems one encounters when developing ELISA screening kits: (1) ease of comprehending directions, (2) stability of kit components, (3) sensitivity and specificity, (4) quantitation equipment, and (5) preparation of enzyme conjugate and antisera. Development of screening kits is continuing, and further research is encouraged by the committee.

(10) L. Pennington, FDA, Atlanta, GA, reported her experience with HPTLC determination of aflatoxins. Under the conditions she uses in her laboratory, HPTLC is comparable to LC. Overall analysis time is approximately equal to regular CB analysis time with TLC and densitometry.

(11) D. Park, FDA, Washington, DC, discussed an LC method and a solvent-efficient TLC method for determining aflatoxins which will be tested in a collaborative study (Tarter, E. J., Hanchay, J.-P., & Scott, P. M. (1984) *J. Assoc. Off. Anal. Chem.* **67**, 597–600; and Trucksess, M. W., Brumley, W. C., & Nesheim, S. (1984) *J. Assoc. Off. Anal. Chem.* **67**, 973–975).

(12) T. Romer (Romer Labs, Inc., Washington, MO), discussed some of the results of mycotoxin cases that he has encountered.

(13) Future meetings: FASEB meeting on trichothecenes, July 6–11, 1986, Copper Mountain, CO; Midwest AOAC Regional Section Meeting, June 16–18, 1986, Lincoln, NE; and 4th International Working Conference on Stored-Product Protection, September 21–26, 1986, Tel Aviv, Israel.

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; R. Baldwin; A. S. Y. Chau; R. Fishbeck; J. Guira; J. Lanier; R. Luskin; J. McNeal; E. Meier; D. Runser; S. Sherken; K. Smith; T. Stijve; J. Winter

Jon McNeal was acting chairman when the Committee met on October 29, 1985, in Washington, DC. F. M. Garfield reported on the status of the Quality Assurance (QA) Short Courses for Analytical Laboratories and for Microbiological Laboratories. Since the last annual meeting, 3 short courses have been conducted, one at the 1985 Spring Workshop in Dallas, TX, one this past summer in Arlington, VA, and one just prior to the 1985 AOAC Annual International Meeting in Washington, DC. Attendance at these courses continues to grow; the last Analytical Laboratory QA Short Course had 49 participants. The Microbiology QA Short Course also had 49 registrants. Both courses are scheduled for the 1986 Spring Workshop in Seattle with Jim Dux (Analytical Laboratory QA) and Mike Brodsky (Microbiological QA) as principal instructors. The Analytical Laboratory QA Short Course is also scheduled for the summer of 1986 in Arlington, VA, and for the Annual Meeting in Scottsdale, AZ. If there is enough interest, the Microbiological QA Short Course will also be held in Scottsdale. Tuition for all courses will be increased from $350 to $450 after the 1986 Spring Workshop.

The Committee discussed the possibilities of AOAC conducting QA Short Courses geared specifically to bench level analysts in each discipline, which would stress quality control (QC) aspects over those related to management and administrative considerations. Included would be the QC aspects of instrumentation, analyst documentation responsibilities, discussion of simple QC statistical tools for analysts, etc. Such a course could be scheduled for annual, spring, and/or regional meetings, wherever participation might be at its highest. Questions pertaining to this topic were prepared by the Committee to be included in a proposed written and oral survey of the AOAC constituency.

Last year, the Committee discussed the publication of a book on sampling techniques. At that time, the expense was too great. However, a contract has been awarded by Agriculture Canada for an AOAC Short Course on Sampling to be held in Canada in March 1986. A symposium on this subject is also scheduled for the 1986 meeting in Scottsdale, AZ, to include discussions on the collection of samples, shipping, preparation, storage, stability, etc. The proceedings from this symposium will be published in the *JAOAC* or separately, as appropriate.

The Committee discussed the issue of including critical control points in official AOAC methods. To include all of these in *Official Methods of Analysis* is prohibitive because it would result in a very large increase in the size of that volume. Moreover, the critical control points in methods should be fully described in original journal articles and subsequent collaborative study reports. The Committee recommends that a statement be inserted in the introduction of the book, instructing all users to check the references given in the method for all critical control points and considerations for them in that method.

The Committee prepared questions for the pending AOAC constituency survey regarding bench level QA Short Courses, member expertise and willingness to become instructors of all the AOAC QA Short Courses, member interfacing with other QC-oriented societies and organizations, and needs for certified reference materials for AOAC analytical procedures.

A 5-year plan for the QA Committee activities was discussed. The plan includes the continuation of the current QA courses as long as the interest in student participation continues and funds are sufficient at annual meetings, spring workshops, and if necessary, at summer sessions in Arlington, VA. Also, if the constituency survey shows sufficient interest in AOAC QA courses for bench level technicians, chemists, microbiologists, and toxicologists, the Committee will work toward honoring this need. Future publications by Committee members through AOAC will probably be limited to symposium proceedings unless joint efforts with other QA societies or organizations are generated.

Long Range Planning Committee

ARVID W. MUNSON, *Chairman*
Hazleton Institute, Francais de Toxicologie, L'Arbresle, BP 109 69210 Les Oncins, France

Other Members: C. Andres; W. Bontoyan; G. Boone; B. Borsje; R. Case; A. Celeste; W. Cobb; E. Coffin; R. Ellis; F. Farrell; V. Kadis; J. Minyard; H. Reynolds; M. Wehr; L. Whalen

The Committee met twice during the year to consider revision of the long range plan and other items assigned by the Board of Directors. The Committee submitted a revised long range plan with established priorities in the following categories:

High priority: (1) organization/management; (2) collaborative study and validation process; (3) communication and education; (4) financial support; (5) membership.

Second priority: (6) laboratory management; (7) awards.

Under each priority, subpriorities were established and ranked, using the following system: (A) important; (B) intermediate; (C) less important.

The Board asked the Committee to also consider its Terms of Reference. This was done and revisions were suggested. Presently, the Committee is developing the specific strategies necessary for implementation of the long range plan and action plans for each strategy. These are to be submitted for Board consideration at the January 1986 Board of Directors meeting.

Membership Committee

WARREN R. BONTOYAN, *Chairman*
Environmental Protection Agency, Office of Pesticide Programs, Beltsville, MD 20705

Other Members: W. Cochrane; D. E. Coffin; G. Gentry; A. Hanks; K. R. Hill; D. Kassera; B. Larsen; A. J. Malanoski; C. Opp; D. L. Park; R. Polli; V. Thorpe; J. Williams; R. Young

The Committee met February 6 and October 29, 1985. In February, Article III of the Bylaws pertaining to membership was discussed. It was the consensus of the Committee that the Bylaws are confusing with respect to defining membership and the corresponding rights, privileges, and benefits. After extensive discussion, the following recommendations were presented to the Board of Directors in April 1985:

(1) Amend Article III, Section 1, Classes of Membership, to read: "There shall be three classes of membership in the Association: Members, Sustaining Members, and Honorary Members." In considering this amendment, the current procedures for voting, election of Board Members, etc., need to be adequately defined.

(2) Make the dues for Private and Government Sustaining Members the same, starting at the present $500.00, and increasing as the proposed dues structure for Government Sustaining Members. Existing state and provincial agencies who provided initial support should be given the option of increasing support to the minimum.

(3) Amend Article III, Section 2C, to read: "Any agency of a local, state, provincial, or national government; any firm, business, or organization, with an interest in the development and interlaboratory evaluation of analytical methods shall be eligible to be a Sustaining Member."

(4) Make all Individual and Sustaining Members uniformly entitled to a 10% discount on all publications.

The Board of Directors requested a position paper on (1) status of membership; (2) rights of members, including voting rights; (3) pros and cons of each type of membership.

In October, the Committee discussed membership classes and voting. The Committee felt that the Official Methods Board should decide the acceptance or rejection of methods in a manner which would not require any membership vote. It should then be required that the Official Methods Board consist of approximately equal representation of members from government regulatory bodies, industry, and academia. This would require a change in the Bylaws.

The Committee also felt that the traditional make-up of the Board of Directors should be examined. Present and past representation may be perceived as discriminatory to industry.

Discussion of the procedure for voting with respect to members' rights raised the question of the procedure's legality. The Committee felt that all issues or matters requiring a vote should be voted on by the entire membership. Also discussed was the need to increase membership dues for individuals as well as sustaining members to assure the viability of the Association and ability to provide quality scientific services to members.

As a result of the October meeting, the Committee recommends: (1) Increase individual Member dues from $25 to $45 for 1987. (2) Increase Sustaining Member dues from $500 to $750 for 1987. (3) Amend Bylaws to allow Official Methods Board and appropriate Methods Committee to decide on adoption or rejection of methods, using a mechanism that does not require membership vote. (4) Amend Bylaws so that entire membership votes on all other matters. (5) Require that Official Methods Board and Board of Directors consist of approximately equal representation from industry, academia, and government regulatory bodies. (6) Expand the Board of Directors by at least 2 members.

The Committee will prepare a position paper on these recommendations for the January 1986 Board meeting.

Committee on Regional Sections

RAYMOND H. BOWERS, *Chairman*
General Mills, Inc., James Ford Bell Technical Center, 9000 Plymouth Ave N, Minneapolis, MN 55427

Other Members: G. Boone; A. V. Jain; K. A. McCully; D. Osheim; R. L. Polli; J. Wiskerchen

During the past year the Committee continued work on a set of Regional Section Operations Guidelines. This effort is directed at developing guidelines for establishing and chartering AOAC regional sections, and establishing recommended operating procedures, including recommendations for regional section fiscal operations.

The Committee met October 30, 1985, in Washington, DC, with 6 of 7 members and 8 guests in attendance. The Committee reviewed and revised a draft of the guidelines and set a completion target date of April 1986.

Committee on Safety

ROBERT BIANCHI, *Chairman*
Drug Enforcement Administration, Northeast Laboratory, 555 W 57th St, Suite 1886, New York, NY 10019

Other Members: E. Cole; R. Hall; A. Hofberg; E. Losiewicz; R. Nelson; R. Noel; G. Roach; W. Solomon; W. Yu

The Committee met October 30, 1985, with 5 of 10 members present. Also present was B. Johnson, AOAC staff. Since the last meeting, one inactive member was removed and one new member was added. Additional changes will be made during the coming year. Current committee members represent the federal government, industry, and academia.

The following topics were discussed:

(1) The symposium, "Safety Training of Laboratory Analysts and Managers," was critiqued, and the Committee decided to sponsor another symposium at the 1986 AOAC meeting. Topics addressed will include legal liability and the right to know, laboratory air handling systems, and personal protective equipment.

(2) The Terms of Reference were reviewed and some changes were proposed. The modifications, which will be presented by mid-December, include revision of the Committee's goals, particularly in regard to safety review of all new methods, and adoption of the new AOAC committee membership requirements.

(3) A list of recommended safety references, which covers the areas of chemical, biological, and laboratory safety, was finalized, and is being submitted to the *Referee* for publication. This list will be updated semi-annually and included at the end of the Laboratory Safety chapter of *Official Methods of Analysis*.

(4) The review process for potential safety and health hazards in new and revised methods was a major cause for concern. To assure compliance with the AOAC Handbook regarding safety review of submitted methods, the Committee recommended the adoption of a safety checklist to be developed by the Committee and distributed to the method authors by the reviewers. Use of the checklist will eliminate the need for all proposed methods to be reviewed by the Safety Committee, while placing this responsibility for adequate safety review on the author.

(5) The Committee agreed to establish a list of consultants, with their fields of expertise identified. This list will serve as a safety resource for method authors.

(6) The Committee will prepare a list of recommended audio/visual safety training aids, which will be distributed to the membership through the *Referee*.

Committee on AOAC Scholarship

K. L. MILSTEAD, *Chairman*
4817 Dorset Ave, Chevy Chase, MD 20015

Other Members: D. F. Emery; C. L. Ettinger; D. Firestone; D. E. Root; R. H. Tennyson

The Committee met on October 27, 1985, and considered the following points:

(1) Should the AOAC Scholarship Award be continued?—It was the consensus of the Committee that the award should be continued.

(2) Should the amount of the award be increased?—It was the consensus of the Committee that the award should be increased to $1000 or, as an alternative, that an additional award of $500 annually should be established.

Minority views of the chairman.—These 2 recommendations seem inconsistent. The first one implies that the present award of $500 is inadequate, while the second one implies that the $500 is adequate and additional awards should be established. The Chairman believes that a $500 award makes a significant contribution to the education of deserving students.

(3) Should it be required that a nominee for the award be sponsored by an AOAC member?—There was no consensus on this question. While it was felt that such a requirement could have benefits to the Association, it could also narrow the interest in the award.

(4) How can the costs of administering the award be reduced?—The following suggestions were offered: (a) Require that the sponsor of the applicant be responsible for assembling the application and seeing that it is completed. (b) Require that the applicant submit the required number of copies. (c) Publish the announcement of the award in each issue of the *Journal* and eliminate the mailing of other announcements. (d) Eliminate the awards brochure or revise it to more clearly define the purpose of the award and the requirements for eligibility. (The Committee will be happy to suggest how the brochure should be revised.) (e) Better define the eligibility for the award by possibly limiting it to those who are pursuing a career in analytical chemistry, food science, or microbiology.

(5) Funding the award.—If there is some question about funding the award, it was the consensus of the members present that the Wiley Award should be reduced to its original value of $1000 and the savings should be used to finance and administer the Scholarship Award. Also, the Board may want to consider a program of grants by Associate contributing members to establishing awards in their names as is done by other organizations.

(6) Survey of previous awardees.—It was the consensus of the members present that the cost of such a survey could not be justified.

Comments by the Chairman which are not necessarily shared by other members of the Committee.

I was a member of the long-range planning committee when the Scholarship and Wiley Awards were established and I am familiar with their entire history. It was the intention of Mrs. Wiley in establishing the awards in honor of her husband to help young students in completing their educations who needed financial assistance. While her financial contribution was modest, she hoped that others would contribute and that the fund would grow. This has not occurred.

This does not detract from the original intent of the award, which was clearly to help deserving students without any regard to whether it benefited the Association. It was, of course, hoped that it would in fact increase the awareness of the activities of the AOAC among college students and their sponsors. It was not the intent of the award that it would result in some direct benefit to the Association, and it should not be evaluated on that basis. As indicated above, the purpose of the award was to lend financial assistance to students who needed help in completing their education. I believe it has accomplished this purpose and will continue to do so as long as it is awarded. No scholarship awards of other organizations that I am aware of require the recipients to demonstrate that the organization benefitted directly from the award.

It is sufficient for the organization to know that it assisted deserving students in completing their education. The indirect effects to the organization making the award are difficult to evaluate, but they are considerable.

Committee on Symposia and Special Programs

NICOLE HARDIN, *Chairman*
Food and Drug Administration, 4298 Elysian Fields Ave, New Orleans, LA 70112

Other Members: J. Cherry; D. Firestone; L. Hambleton; J. Karr; A. Malanoski; H. Morris; A. Pohland; D. Stalling

The Committee met in April and October 1985 and finalized symposia programs for the 1985 and 1986 annual international meetings. The Committee has accepted some proposals for the 1987 meeting; however, a limited number of proposals will still be considered for 1987.

The following symposia were presented at the 1985 meeting: Immunotoxicity of Pesticides and Other Toxic Compounds: Its Mechanisms and Up-to-Date Methodology for Evaluation; Critical Analysis of Analytical Methods for Meat Foods; Safety Training of Laboratory Analysts and Managers; Chromatography of Amino Acids: Sample Preparation, GC, LC, and Ion Exchange; Practical Applications of Quality Assurance Principles in the Analytical Laboratory; and Regulatory Round Table: Focus on Pesticide Registration and Regulatory Methodology. The Committee commends the organizers and chairmen of these symposia on their excellent presentations.

In 1986, the Committee will implement the "focus" concept. A focus or theme will be identified for each annual meeting. This focus will provide a forum for soliciting papers and symposia proposals. The focus will not exclude other subject areas, but will help publicize and promote the meeting. The 1986 meeting focus will be sampling. Future focus topics under consideration are robotics (preliminary for 1987), immunochemistry, and computers in science.

The Committee thanks M. Ridgell, AOAC staff, for her invaluable assistance and support in all phases of planning and executing the symposia program.

Also during this past year, the Committee oversaw the Association's short courses, and its planned participation in ISM-10, August 1986, Antwerp, Belgium, and the 1986 IUPAC Pesticide Conference in Canada. The Committee requested that the Board of Directors consider establishing an Education Committee to oversee short courses.

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Codex Committee on Fish and Fishery Products:

Collaborative International Pesticides Analytical Council: Warren R. Bontoyan (Environmental Protection Agency, Office of Pesticide Programs, Beltsville, MD 20705)

Council on Soil Testing and Plant Analysis:

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Federation of Oils, Seeds, and Fats Association, Ltd:

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International Committee on Microbiological Specifications of Food:

International Dairy Federation: Margreet Tuinstra-Laauwars (Langhoven 12, 6721 SR Bennekom, The Netherlands); Robert W. Weik (Food and Drug Administration, Washington, DC 20204)

International Organization for Standardization (ISO): Arthur R. Johnson (Food and Drug Administration, Washington, DC 20204), *Liaison Coordinator*

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Animal Feeding Stuffs (ISO/TC 34/SC 10): Franklin E. Barton II (U.S. Dept of Agriculture, Richard B. Russell Research Center, Box 5677, Athens, GA 30613)

Cereals and Pulses (ISO/TC 34/SC 4): Raymond J. Tarleton (American Association of Cereal Chemists, 3340 Pilot Knob Rd, St. Paul, MN 55121)

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Crude Fiber (ISO/TC 34/WG 3): David O. Holst (University of Missouri, Food Science and Nutrition, Columbia, MO 65211)

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Subcommittee 2, Halogens:

Subcommittee 3, Oxidants and Nitrogen: Richard A. Mandl (Boyce-Thompson Institute, Tower Rd, Ithaca, NY 14853)

Subcommittee 4, Carbon: M. Feldstein (Bay Area Pollution Control District, 939 Ellis St, San Francisco, CA 94109)

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Subcommittee 6, Metals: Michael Kleiman (California College of Medicine Irvine, CA 92717)

Subcommittee 8, Radioactivity:

Subcommittee 9, Laboratory Techniques and Precautions:

Subcommittee 10, Particulates: Howard E. Ayer (University of Cincinnati, Kettering Laboratory, Eden and Bethesda Aves, Cincinnati, OH 45267)

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Pesticide Analysis Committee of Ministry of Agriculture in the United Kingdom:

Dithiocarbamates Panel: Warren R. Bontoyan (Environmental Protection Agency, Office of Pesticide Programs, Beltsville, MD 20705)

Joint Dimethoate Residues Panel:

Pharmaceutical Manufacturers Association Quality Control Vitamin E Committee:

United States Pharmacopeial Convention:

Committee on Pesticide Formulations and Disinfectants

Alan R. Hanks (Office of the Indiana State Chemist, Purdue University, West Lafayette, IN 47907), *Chairman;* Warren R. Bontoyan (Environmental Protection Agency, Office of Pesticide Programs, Beltsville, MD 20705); Alvin L. Burger (Virginia Consolidated Laboratories, 1 N 14th St, Richmond, VA 23219); A. Aner Carlstrom (Chevron Chemical Co., 940 Hensley St, Richmond, CA 94804); Thomas L. Jensen (State Dept of Agriculture, 3703 S 14th St, Lincoln, NE 68502); James J. Karr (Pennwalt Technical Center, 900 First Ave, King of Prussia, PA 19405); James P. Minyard (Mississippi State Chemical Laboratory, PO Box CR, Mississippi State, MS 39762); Richard S. Wayne (American Cyanamid Co., Agriculture Div., Box 400, Princeton, NJ 08540); Richard H. Collier (Purdue University, Dept of Biochemistry, West Lafayette, IN 47907), *Secretary;* Bertram D. Litt (Environmental Protection Agency, 1921 Jefferson Davis Hwy, Arlington, VA 22202), *Statistical Consultant;* James Hansen (Union Carbide Corp, Agricultural Products, PO Box 8361, South Charleston, WV 25303), *Statistical Consultant*

PESTICIDE FORMULATIONS: CARBAMATE AND SUBSTITUTED UREA INSECTICIDES

Referee: Paul D. Jung, Environmental Protection Agency, Office of Pesticide Programs, Beltsville, MD 20705

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Carbofuran and Carbosulfan
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Methiocarb
Stephen C. Slahck, Mobay Chemical Corp., Box 4913, Kansas City, MO 64120

Methomyl
James E. Conaway, Jr, E. I. du Pont de Nemours & Co., Biochemicals Dept; Wilmington, DE 19898

Mexacarbate (Zectran)
William H. McDermott, Union Carbide Corp.. Agricultural Products, Box 12014, Research Triangle Park, NC 27709

Oxamyl
Glenn A. Sherwood, Jr, E. I. du Pont de Nemours & Co., Biochemicals Dept, Wilmington, DE 19898

Pirimicarb
John E. Bagnis, ICI Americas, Inc., Yalding Works, Yalding, Kent, UK ME18 6HN

Propoxur (Baygon)
Stephen C. Slahck, Mobay Chemical Corp., Box 4913, Kansas City, MO 64120

Trimethylphenyl Carbamate Isomers
William H. McDermott, Union Carbide Corp., Agricultural Products, Box 12014, Research Triangle Park, NC 27709

PESTICIDE FORMULATIONS: FUNGICIDES AND DISINFECTANTS

Referee: Peter D. Bland, ICI Americas, Inc., PO Box 208, Goldsboro, NC 27530

Anilazine (Dyrene)
Stephen C. Slahck, Mobay Chemical Corp., Box 4913, Kansas City, MO 64120

Benomyl
Mikio Chiba, Agriculture Canada, Vineland Station, Ontario, Canada L0R 2E0

Carboxin and Oxycarboxin
Milton Parkins, Uniroyal Chemical Co., Crop Protection Analytical Div., Naugatuck, CT 06770

Chlorothalonil
Brian H. Korsch, SCS Biotech, PO Box 348, Painesville, OH 44079

Copper Naphthenate

Dinocap

Dioxins in Pentachlorophenol
Joanne Campbell, Reichold Chemicals, Inc., PO Box 1482, Tacoma, WA 98401

Dithiocarbamate Fungicides
Warren R. Bontoyan, Environmental Protection Agency, Office of Pesticide Programs, Beltsville, MD 20705

Oxythioquinox (Morestan)
Stephen C. Slahck, Mobay Chemical Corp., Box 4913, Kansas City, MO 64120

***o*-Phenylphenol**

Quaternary Ammonium Compounds

Thiram

Triademefon (Bayleton)
Stephen C. Slahck, Mobay Chemical Corp, Box 4913, Kansas City, MO 64120

Triphenyltin (Fentin)
P. Pasma, M&T Chemicals BV, PO Box 70, 4380 AB Vlissingen, Netherlands

Water-Soluble Copper in Water-Insoluble Copper Fungicides

PESTICIDE FORMULATIONS: HERBICIDES I

Referee: Marshall Gentry, State Dept of Agriculture and Consumer Services, Mayo Building, Tallahassee, FL 32301

Chlorophenoxy Herbicides
Robert B. Grorud, North Dakota State Laboratories, Lock Box 937, Bismarck, ND 58502

Dicamba
Benjamin A. Belkind, Velsicol Chemical Corp., 341 E Ohio St, Chicago, IL 60611

Pentachlorophenol

Plant Growth Regulators

PESTICIDE FORMULATIONS: HERBICIDES II

Referee: Dean Hill, Environmental Protection Agency, Denver Federal Center, Denver, CO 80225

Barban
Benjamin A. Belkind, Velsicol Chemical Corp., 341 E Ohio St, Chicago, IL 60611

Benefin, Trifluralin, Ethafluralin, and Pendimethalin
Rodger Stringham, Purdue University, Dept of Biochemistry, West Lafayette, IN 47907

Bensulide
William Y. Ja, Stauffer Chemical Co., 1200 S 47th St, Richmond, CA 94804

Benzoylprop-ethyl

Bromacil and Lenacil
Paul K. Tseng, E. I. du Pont de Nemours & Co., Biochemicals Dept, Wilmington, DE 19898

Chlorosulfuron
Glenn A. Sherwood, E. I du Pont de Nemours & Co., Biochemicals Dept, Wilmington, DE 19898

Dimethyl Tetrachloroterephthalate
Brian Korsch, SDS Biotech, PO Box 348, Painesville, OH 44079

Dinoseb

Fluometuron
Arthur H. Hofberg, Ciba-Geigy Corp., Analytical Section, PO Box 18300, Greensboro, NC 27419

Metasulfuron-methyl
Glenn A. Sherwood, Jr, E. I. du Pont de Nemours & Co., Biochemicals Dept, Wilmington, DE 19898

Methazole
Benjamin A. Belkind, Velsicol Chemical Corp., 341 E Ohio St, Chicago, IL 60611

Naptalam (Alanap)
Milton Parkins, Uniroyal Chemical Co., Crop Protection Analytical Div., Naugatuck, CT 06770

Oryzalin

Substituted Urea Herbicides

Sulfometuron-methyl
Glenn A. Sherwood, Jr, E. I. du Pont de Nemours & Co., Biochemicals Dept, Wilmington, DE 19898

Thiocarbamate Herbicides
William Y. Ya, Stauffer Chemical Co., 1200 S 47th St, Richmond, CA 94804

PESTICIDE FORMULATIONS: HERBICIDES III

Referee: Peter D. Bland, ICI Americas, Inc., PO Box 208, Goldsboro, NC 27530

Alachlor, Butachlor, and Propachlor
David F. Tomkins, Monsanto Co., PO Box 473, Muscatine, IA 52761

Amitrole

Bentazone
Thomas M. Schmitt, BASF Wyandotte Corp., 1609 Biddle Ave, Wyandotte, MI 48192

Bromoxynil
Lawrence J. Helfant, UCAPCO, Inc., PO Box 12014, Research Triangle Park, NC 27709

Cacodylic Acid
Paul J. Brignac, State Chemical Laboratory, Mississippi State, MS 39762

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
A. Dereijke, Duphar BV, Analytical Dept, 1381 CP Weesp, Netherlands

Fluazifop-butyl
Peter D. Bland, ICI Americas, Inc., PO Box 208, Goldsboro, NC 27530

Glyphosate (Isopropylamine Salt *N*-(Phosphoromethyl)Glycine)
Arnolds J. Burns, Monsanto Agricultural Products Co., PO Box 174, Luling, LA 70070

Metolachlor
Arthur H. Hofberg, Ciba-Geigy Corp., Analytical Section, PO Box 18300, Greensboro, NC 27419

Metribuzin
William Betker, Mobay Chemical Corp., Box 4913, Kansas City, MO 64120

Pesticides in Fertilizers
Paul D. Korger, State Dept of Agriculture, Trade and Consumer Protection, Box 7883, Madison, WI 53707

Propanil
Delmas Pennington, Rohm and Haas, PO Box 591, Knoxville, TN 37901

Sodium Chlorate

Triazine Herbicides
Arthur H. Hofberg, Ciba-Geigy Corp., Analytical Section. PO Box 18300, Greensboro, NC 27419

PESTICIDE FORMULATIONS: ORGANOHALOGEN INSECTICIDES

Referee: James Launer, State Dept of Agriculture, Laboratory Services, 635 Capitol St, NE, Salem, OR 97310

Benzene Hexachloride and Lindane
Frederick C. Churchill, Centers for Disease Control, Atlanta, GA 30333

Chlordane
Benjamin A. Belkind, Velsicol Chemical Corp., 341 E Ohio St, Chicago, IL 60611

Chlordimeform
Arthur H. Hofberg, Ciba-Geigy Corp., Analytical Section. PO Box 18300, Greensboro, NC 27419

Dicofol (Kelthane)
Alan M. Rothman, Rohm and Haas Co., Research Laboratories, 727 Norristown Rd, Spring House, PA 19477

Ethylan (Perthane)
Dianne Bradway, Environmental Protection Agency, Denver Federal Center, Denver, CO 80225

Fenvalerate
R. D. Collins, Shell Development Co., PO Box 4248, Modesto, CA 95352

Heptachlor
Benjamin A. Belkind, Velsicol Chemical Corp., 341 E Ohio St, Chicago, IL 60611

Methoxychlor
Rodger Stringham, Purdue University, Dept of Biochemistry, West Lafayette, IN 47907

Methyl Bromide
David McAllister, Great Lakes Chemical Corp., Box 2200, West Lafayette, IN 47906

Toxaphene
William H. Clark, Hercules, Inc., Analytical Div., Research Center, Wilmington, DE 19808

Trichlorfon (Dylox)
Dianne Bradway, Environmental Protection Agency, Denver Federal Center, Denver, CO 80225

PESTICIDE FORMULATIONS: ORGANOTHIOPHOSPHORUS PESTICIDES

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
Stephen C. Slahck, Mobay Chemical Corp., Box 4913, Kansas City, MO 64120

Coumaphos
Linda Ruiz, Bayvet Div. of Cutter Labs, Box 390, Shawnee, KS 66201

Demeton

Demeton-*S*-methyl

Diazinon
Arthur H. Hofberg, Ciba-Geigy Corp., Analytical Section, PO Box 18300, Greensboro, NC 27419

Dimethoate
Richard S. Wayne, American Cyanamid Co., Agriculture Div., Box 400, Princeton, NJ 08540

Dioxathion
William H. Clark, Hercules, Inc., Analytical Div., Wilmington, DE 19808

Encapsulated Organophosphorus Pesticides
James J. Karr, Pennwalt Technological Center, 900 First Ave, King of Prussia, PA 19406

EPN
Benjamin A. Belkind, Velsicol Chemical Corp., 341 E Ohio St, Chicago, IL 60611

Ethoprop
Richard W. Smith, Rhône-Poulenc, Inc., Box 352, Mt Pleasant, TN 38474

Fenitrothion
Dwight L. Mount, Centers for Disease Control, 1600 Clifton Rd, Atlanta, GA 30333

Fensulfothion
Margie Owen, State Chemical Laboratory, Box 329, Auburn, AL 36830
William Betker, Mobay Chemical Corp., PO Box 4913, Kansas City, MO 64120

Fenthion
Willard G. Boyd, Jr, State Chemical Laboratory, Box CR, Mississippi State, MS 39762

Fonophos
William Y. Ja, Stauffer Chemical Co., 1200 S 47th St, Richmond, CA 94804

Isofenfos (Oftanol)
Daniel Terry, Mobay Chemical Corp., Box 4913, Kansas City, MO 64120

Malathion
Richard S. Wayne, American Cyanamid Co., Agriculture Div., Box 400, Princeton, NJ 08540

Methamidophos

Methidathion (Supracide)
Thomas T. Gale, Ciba-Geigy Corp., Box 18300, Greensboro, NC 27419

Oxydemeton-methyl (Metasystox-R)
Stephen C. Slahck, Mobay Chemical Corp., Box 4913, Kansas City, MO 64120

Parathion and Methyl Parathion
Edwin R. Jackson, Mississippi State Chemical Laboratory, Box CR, Mississippi State, MS 39762

Phorate
Roman Grypa, Agway Inc., 978 Loucks Mill Rd, York, PA 17402

Pirimiphos-methyl
Peter D. Bland, ICI Americas, Inc., PO Box 208, Goldsboro, NC 27530

Temephos

***S,S,S*-Tributyl Phosphorotrithioate**
William Betker, Mobay Chemical Corp., Box 4913, Kansas City, MO 64120

PESTICIDE FORMULATIONS: OTHER ORGANOPHOSPHORUS INSECTICIDES

Referee: Marshall Gentry, State Dept of Agriculture and Consumer Services, Division of Chemistry, Tallahassee, FL 32301

Crotoxyphos
Wendy King, State Dept of Agriculture and Consumer Services, Tallahassee, FL 32301

Dichlorvos

Fenamiphos (Nemacur)
Carl Gregg, Mobay Chemical Corp., Box 4913, Kansas City, MO 64120

Methamidophos (Monitor)
James Baird, Mobay Chemical Corp., Box 4913, Kansas City, MO 64120

Mevinphos

Monocrotophos

Naled
A. Aner Carlstrom, Chevron Chemical Co., 940 Hensley St, Richmond, CA 94804

Tetrachlorvinphos

PESTICIDE FORMULATIONS: OTHER INSECTICIDES, SYNERGISTS, AND INSECT REPELLANTS

Referee: James Launer, State Dept of Agriculture, Laboratory Services, 635 Capitol St, NE, Salem, OR 97310

Allethrin
Dean Kassera, McLaughlin Gormley King Co., 8810 Tenth Ave, Minneapolis, MN 55427

Aluminum Phosphide

2,3:4,5-Bis(2-butylene)tetrahydro-2-furaldehyde
Vernon Meinen, McLaughlin Gormley King Co., 8810 Tenth Ave, Minneapolis, MN 55427

Cyhexatin

Cypermethrin
Peter D. Bland, ICI Americas, Inc., PO Box 208, Goldsboro, NC 27530

Cypromazine (Larvadex)
Arthur H. Hofberg, Ciba-Geigy Corp., Analytical Section, PO Box 18300, Greensboro, NC 27419

Dipropyl Isocinchomeronate (MGK 326)
Dave Carlson, McLaughlin Gormley King Co., 8810 Tenth Ave, Minneapolis, MN 55427

Fumigants
Dean Yeaman, Dow Chemical Co., PO Box 1398, Pittsburgh, CA 94565

Nicotine
Rodney J. Bushway, University of Maine, Dept of Food Science, Orono, ME 04469

Permethrin
Hershel F. Morris, State Dept of Agriculture, Box 16390-A, University Station, Baton Rouge, LA 70893

Piperonyl Butoxide and Pyrethrins
Dean Kassera, McLaughlin Gormley King Co., 8810 Tenth Ave, Minneapolis, MN 55427

Resmethrin
Mark Law, Environmental Protection Agency, TSD-Chemical & Biological Investigation, Beltsville, MD 20705

Rotenone and Other Rotenoids
Rodney J. Bushway, University of Maine, Dept of Food Science, Orono, ME 04469

PESTICIDE FORMULATIONS: RODENTICIDES AND MISCELLANEOUS PESTICIDES

Referee: Marshall Gentry, State Dept of Agriculture and Consumer Services, Division of Chemistry, Tallahassee, FL 32301

Brodifacoum
Peter D. Bland, ICI Americas Inc., PO Box 208, Goldsboro, NC 27530

Chlorophacinone

Diphacinone

α-Naphthylthiourea

Sampling

Strychnine

Warfarin

DISINFECTANTS

Referees: Reto Engler, Environmental Protection Agency, Office of Pesticide Programs, Registration Division, Washington, DC 20460
Aram Beloian, Environmental Protection Agency, Benefits and Use Division, Washington, DC 20460

Antimicrobial Agents in Laundry Products
Luther B. Arnold and Jean Vollrath-Vaughn, Vikon Chemical Co., PO Box 1520, Burlington, NC 27215
Jamie McGee, Dow Corning Corp., Midland, MI 48640

Sporicidal Tests
Ted Wendt, Surgikos, Inc., 2500 Arbrook Dr, Arlington, TX 76010

Textile Antibacterial Preservatives
Luther B. Arnold and Jean Vollrath-Vaughn, Vikon Chemical Co., PO Box 1520, Burlington, NC 27215

Tuberculocidal Tests
Joseph Ascenzi, Surgikos, Inc., 2500 Arbrook Dr, Arlington, TX 76010

Use-Dilution Test, Variation and Amendments
Gayle Mulberry, Hill Top Research, Inc., Box 42501, Cincinnati, OH 45242
Eugene C. Cole, University of North Carolina, School of Medicine, Chapel Hill, NC 27514

Virucide Tests
Dale Fredell, Economics Laboratory, Osborne Bldg, St. Paul, MN 55118

Committee on Drugs and Related Topics

Thomas P. Layloff (Food and Drug Administration, National Center for Drug Analysis, 1114 Market St, St. Louis, MO 63101), *Chairman;* Ronald C. Backer (Office of Chief Medical Examiner, 701 Jefferson Rd, South Charleston, WV 25309); William W. Wright (U.S. Pharmacopeial Convention, 12601 Twinbrook Pkwy, Rockville, MD 20857); John E. Zarembo (Revlon Health Care, 1 Scarsdale Rd, Tuckahoe, NY 10707); Ted M. Hopes (Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232); Charles Bibart (The Upjohn Co., 7171 Portage Rd, Kalamazoo, MI 49001); Sal Fusari (22625 St. Joan, St. Clair Shores, MI 48080); Richard A. Baldwin (Food and Drug Administration, 900 Madison Ave, Baltimore, MD 21201); Charles C. Clark (Drug Enforcement Administration, 5205 NW 84th Ave, Miami, FL 33166); Robert C. Livingston (Food and Drug Administration, 5600 Fishers Ln, Rockville, MD 20857); Kaiser Aziz (Food and Drug Administration, Bureau of Medical Devices, 8751 Georgia Ave, Silver Spring, MD 20910); Eric Sheinin (Food and Drug Administration, Washington, DC 20204), *Secretary;* Mordecai Friedberg (National Center for Drugs and Biologics, Rockville, MD 20857), *Statistical Consultant*

BIOCHEMICAL METHODS

Referee: John O'Rangers, Food and Drug Administration, 5600 Fishers Ln, Rockville, MD 20857

Heparin by Non-RIA Procedures Measuring Human Chorionigonadotropin
Lillian Gill, Food and Drug Administration, 900 Madison Ave, Baltimore, MD 21201

Hybridoma-Monoclonal Antibodies
Richard Meyer, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Immunochemical Species Identification of Meat
David Berkowitz, U.S. Dept of Agriculture, Food Safety and Inspection Service, Washington, DC 20250

CLINICAL DIAGNOSTICS AND TEST KITS

Referee: John O'Rangers, Food and Drug Administration, 5600 Fishers Ln, Rockville, MD 20857

Oxylates
Quincy Crider, Sigma Diagnostics, Inc., PO Box 14508, St. Louis, MO 63178

DRUGS I

Referee: James W. Fitzgerald, Food and Drug Administration, Winchester Engineering and Analytical Center, 109 Holton St, Winchester, MA 01890

Acetaminophen in Drug Mixtures
David J. Krieger, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Acetaminophen with Codeine Phosphate
Norlin W. Tymes, Food and Drug Administration, 900 Madison Ave, Baltimore, MD 21201

Allopurinol
Donald Shostak, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Aspirin and Caffeine with Other Drugs
Brihmadesam N. Srinivasan, Food and Drug Administration, 109 Holton St, Winchester, MA 01890

Benzthiazides by LC
Stephen Hauser, Food and Drug Administration, Winchester Engineering and Analytical Center, 109 Holton St, Winchester, MA 01890

Chlordiazepoxide
Stanley E. Roberts, Food and Drug Administration, 109 Holton St, Winchester, MA 01890

Methyldopa
Susan Ting, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Phenothiazine-Type Drugs by TLC
Kurt Steinbrecher, Food and Drug Administration, 909 First Ave, Seattle, WA 98174

Phenothiazines and Tricyclic Antidepressant Formulations
Edward G. Lovering, Health and Welfare Canada, Drug Research Laboratories, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2

Primidone
Stanley E. Roberts, Food and Drug Administration, 109 Holton St, Winchester, MA 01890

Sulfamethoxazole
John W. Robinson, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

DRUGS II

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

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

Epinephrine-Lidocaine Combinations

Epinephrine and Related Compounds by LC-Electrochemical Detectors
John M. Newton, Food and Drug Administration, 50 Fulton St, San Francisco, CA 94102

Ergot Alkaloids
Thomas C. Knott, Food and Drug Administration, 900 Madison Ave, Baltimore, MD 21201

Homatropine Methyl Bromide in Tablets

Morphine Sulfate
Ada C. Bell, Food and Drug Administration, 2nd & Chestnut Sts, Philadelphia, PA 19106

Phenethylamine
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

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

Rauwolfia serpentina
Ugo R. Cieri, Food and Drug Administration, 2nd and Chestnut Sts, Philadelphia, PA 19106

DRUGS III

Referee: Martin Finkelson, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Ampicillin and Amoxicillan
Michel Margosis, Food and Drug Administration, National Center for Drugs and Biologics, Washington, DC 20204

Bisacodyl
Leonard Valenti, Food and Drug Administration, 5600 Fishers Ln, Rockville, MD 20857

Coumarin Anticoagulants
Ella Moore, Food and Drug Administration, New York Regional Laboratory, 850 Third Ave, Brooklyn, NY 11232

Flucytosine
Donald Shostak, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

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

Levodopa
Susan Ting, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Medicinal Gases
Martin Woodhouse, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Meprobamate Tablets
John T. Konecny, Food and Drug Administration, 900 U.S. Customhouse, Philadelphia, PA 19106

Mercury-Containing Drugs
Walter Holak, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Metals in Drug Bulk Powders
Waiter Holak, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Miconazole
Josephine Jee, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Microchemical Tests
Marshall Rabkin, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Penicillins
Barry Mopper, Food and Drug Administration, New York Regional Laboratory, 850 Third Ave, Brooklyn, NY 11232

Protein Nitrogen Units in Allergenic Extracts
Joan May, Food and Drug Administration, National Center for Drugs and Biologics, 8800 Rockville Pike, Bethesda, MD 20014

Salts of Organic Nitrogenous Bases
Samuel Walker, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Trimethobenzamide
Helen Naviasky, Food and Drug Administration, 900 Madison Ave, Baltimore, MD 21201

DRUGS IV

Referee: ———

D- and L-Amphetamines
Irving Wainer, Food and Drug Administration, Division of Drug Chemistry, Washington, DC 20204

Benzodiazepines
Eileen Bargo, Food and Drug Administration, 900 Madison Ave, Baltimore, MD 21201

Diazepam
Michael Tsougros, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Heroin

DRUGS V

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

Chlorpropamide
Richard L. Everett, Food and Drug Administration, 900 Madison Ave, Baltimore, MD 21201

Digitoxin, Automated Individual Tablet Analysis
Benjamin Westenberger, Food and Drug Administration, National Center for Drug Analysis, 1114 Market St, St. Louis, MO 63101

Hydrocortisone
Robert W. Roos, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Beta-Methasone
Susan Lee, C & S Laboratory Consultants, 421 Hudson St, New York, NY 10014

Methocarbamol
Richard L. Everett, Food and Drug Administration, 900 Madison Ave, Baltimore, MD 21201

Prednisolone

Progestins
Larry K. Thornton, Food and Drug Administration, Division of Drug Analysis, 1114 Market St, St. Louis, MO 63101

Steroid Acetates
Linda Ng, Merck, Sharpe & Dohme. Research Laboratories, West Point, PA 19486

Steroid Phosphates
Elaine A. Bunch, Food and Drug Administration, 5003 Federal Office Bldg, Seattle, WA 98174

FORENSIC SCIENCES

Referee: John W. Hicks, FBI Laboratory, 10th & Pennsylvania Ave, Washington, DC 20535

Grouping Tests for Blood and Other Body Fluids
Henry C. Lee, State Police Forensic Science Laboratory, 294 Colony St, Meriden, CT 06450

Electrophoretic Methods
Willard Stuver, Metro-Dade Police Dept Crime Laboratory, Miami, FL

Enzyme-Linked Immunosorbent Assays for Body Fluid Stains

Explosives and Explosives Residues

Gunshot Residue
Donald G. Havekost, Federal Bureau of Investigation, Laboratory Div., Washington, DC 20535

Isoelectric Focusing Methods for Body Fluid Stains
Bruce Budowle, Federal Bureau of Investigation Academy, Quantico, VA

Paints and Other Polymeric Materials

Soils, Geological Analysis
John Wehrenberg, University of Montana, Missoula, MT 89801

Screening and Confirmatory Tests for Dried Bloodstains

Committee on Foods I

Henry 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); Colette P. Levi (General Foods Corp., White Plains, NY 10591); John D. McKinney (Ranchers Cotton Oil, 2691 S Cedar, Fresno, CA 93725); Elmer George (State Dept of Agriculture and Markets, 1220 Washington Ave, Albany, NY 12235); Richard L. Ellis (U.S. Dept of Agriculture, Food Safety and Inspection Service, Washington, DC 20250); Michael E. Knowles (Ministry of Agriculture, Fisheries and Food, Horseferry Rd, London, UK SW1P 2AE); Arthur R. Johnson (Food and Drug Administration, Division of Food Technology, Washington, DC 20204), *Secretary;* Michael W. O'Donnell (Food and Drug Administration, Division of Mathematics, Washington, DC 20204), *Statistical Consultant*

COFFEE AND TEA

Referee: Robert H. Dick, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Ash in Instant Tea
Francis J. Farrell, Thomas J. Lipton, Inc., 800 Sylvan Ave, Englewood Cliffs, NJ 07632

Methyl Xanthines in Coffee and Tea
John M. Newton, Food and Drug Administration, 50 Fulton St, San Francisco, CA 94102

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

Water Extract in Tea

DAIRY PRODUCTS

Referee: ———

Adulteration of Dairy Products with Vegetable Fat
Graham MacEachern, Agriculture Canada, Plant Products, Ottawa, Ontario, Canada K1A 0C5

Babcock Test and Babcock Glassware
Robert L. Bradley, University of Wisconsin-Madison, Food and Science Dept, Madison, WI 53706

Casein and Caseinates
Charles Pynes, Stauffer Chemical Co., Technical Sales and Development Dept, Westport, CT 06880

Chocolate Milk, Fat Test
James T. Marshall, University of Maryland, Dept of Animal Science, College Park, MD 20742

Cryoscopy of Milk
Robert W. Henningson, Clemson University, Office of University Research, Clemson, SC 29631

Fat, Automated Methods
W. Frank Shipe, Cornell University, Dept of Dairy and Food Science, Ithaca, NY 14853

Fat in Milk (AutoAnalyzer)
Raymond L. King, University of Maryland, Dept of Food Science, College Park, MD 20742

Infrared Milk Analyzer (IRMA)
D. A. Biggs, University of Guelph, Dept of Food Science, Guelph, Ontario, Canada N1G 2W1

Iodine
David C. Sertl, Ross Labs, 625 Cleveland Ave, Columbus, OH 43216

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, Dept of Food Science, New Brunswick, NJ 08903
John W. Sherbon, Cornell University, Dept of Dairy and Food Science, Ithaca, NY 14853

Moisture in Cheese
Ronald Case, Kraft Foods, Kraft Ct, Glenview, IL 60025

Nonfat Solids
John W. Sherbon, Cornell University, Dept of Dairy and Food Science, Ithaca, NY 14853

Nitrates in Cheese
James E. Hamilton, Food and Drug Administration, Division of Drug Labeling—Compliance, 5600 Fishers Ln, Rockville, MD 20857

Phosphatase, Rapid Method
Dick H. Kleyn, Rutgers University, Dept of Food Science, New Brunswick, NJ 08903

Phosphatase, Reactivated
Gopala K. Murthy, Food and Drug Administration, Division of Microbiology, 1090 Tusculum Ave, Cincinnati, OH 45226

Phosphorus
Wallace S. Brammell, Food and Drug Administration, Division of Color Technology, Washington, DC 20204

Protein Constituents in Processed Dairy Products
Frederick W. Douglas, Jr, U.S. Dept of Agriculture, Eastern Regional Research Center, Philadelphia, PA 19118

Protein in Milk, Rapid Tests
John W. Sherbon, Cornell University, Dept of Dairy and Food Science, Ithaca, NY 14853

Protein Reducing Substance Tests
Joseph T. Cardwell, Mississippi State University, Dairy Science Dept, Mississippi State, MS 39762

Total Solids and Moisture by Microwave Drying
Judy J. Arey, CEM Corp., PO Box 9, Indian Trail, NC 28079

Vapor Pressure Osmometry
Gary H. Richardson, Utah State University, Dept of Nutrition and Food Science, Logan, UT 84322

Whey Proteins in Nonfat Dry Milk
C. Olieman, Netherlands Institute for Dairy Research, Ede, Netherlands

DECOMPOSITION IN FOODS

Referee: Walter F. Staruszkiewicz, Jr, Food and Drug Administration, Division of Food Technology, Washington, DC 20204

Ammonia in Seafood
Beverly A. Hunter, National Marine Fisheries Service, PO Drawer 1207, Pascagoula, MS 39567

Coprostanol
James F. Stewart, Food and Drug Administration, 3032 Bryan St, Dallas, TX 75204

Crabmeat
Kurt Steinbrecher, Food and Drug Administration, 909 First Ave, Seattle, WA 98174

Diacetyl in Citrus Products
W. S. Hatcher, The Coca-Cola Co., Box 368, Plymouth, FL 32768

Ethanol in Seafoods
Harold R. Throm, Food and Drug Administration, 909 First Ave, Seattle, WA 98174

Gas and Liquid Chromatography
Walter F. Staruszkiewicz, Jr, Food and Drug Administration, Division of Food Technology, Washington, DC 20204

GC Determination of Volatile Amines—TMA and DMA
Ronald C. Lundstrom, National Marine Fisheries Service, Northeast Fisheries Center, Gloucester, MA 01930

Shellfish Decomposition

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

Determination of Fish Content in Coated Products (Breaded or in Batter)
Frederick J. King, National Marine Fisheries Service, Northeast Fisheries Center, Gloucester, MA 01930
H. Houwing, TNO, Division of Nutrition and Food Research, Box 183, 1970 AD, Ijmuiden, The Netherlands

Drained Weight of Block Frozen, Raw, Peeled Shrimp
Michael F. Blattner, Central Analytical Labs, Inc., 2600 Marietta Ave, Kenner, LA 70062

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 Div., Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2

Brominated Oils
James F. Lawrence, Health and Welfare Canada, Food Research Div., Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2

Chloride Titrator
Alfred H. Free, Ames Co., Technical Services, Elkhart, IN 46514

Dilauryl Thiodipropionate

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

Gums

Indirect Additives from Food Packages
Charles V. Breder, Keller and Heckman, 1150 17th St NW, Washington, DC 20036

Nitrates and Nitrites
Jay Fox, U.S. Dept of Agriculture, Eastern Regional Research Center, Philadelphia, PA 19118

Nitrates (Selective Ion Electrode Titration)

Nitrosamines
Nisu P. Sen, Health and Welfare Canada, Food Directorate, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2

Nitrosamines in Food Contact Items
J. T. Gray, Michigan State University, Dept of Food Science and Human Nutrition, East Lansing, MI 48824

Polycyclic Aromatic Hydrocarbons
Frank L. Joe, Food and Drug Administration, Division of Chemistry and Physics, Washington, DC 20204

Polysorbates
Charles F. Smullin, 2402 Heather Rd, Wilmington, DE 19803

Propylene Chlorohydrin

Quinine in Soft Drinks

Sodium Lauryl Sulfate

Sulfiting Agents in Foods

MEATS, POULTRY, AND MEAT AND POULTRY PRODUCTS

Referee: Jon E. McNeal, U.S. Dept of Agriculture, Food Safety and Inspection Service, Washington, DC 20250

Automated Methods
Jon L. Schermerhorn, State Dept of Agriculture and Markets, Food Laboratory, Albany, NY 12235

Bioassay Methods for Meat and Poultry Products

Chemical Antibiotic Methods

Fat in Meat Products
Jon E. McNeal, U.S. Dept of Agriculture, Food Safety and Inspection Service, Washington, DC 20250

Fat and Moisture Analysis, Rapid Methods
Julio D. Pettinati, U.S. Dept of Agriculture, Eastern Regional Research Center, 600 E Mermaid Lane, Philadelphia, PA 19118

Histologic Identification Methods
Albert M. Carey, U.S. Dept of Agriculture, Food Safety and Inspection Service, Beltsville, MD 20705

Identification of Meats, Serological Tests
Arthur P. Marin, State Dept of Agriculture and Markets, State Campus, Albany, NY 12235

LC Methods for Meat and Poultry Products
Sher Ali, U.S. Dept of Agriculture, PO Box 6085, Athens, GA 30604

3-Methyl Histidine
Roger Wood, Ministry of Agriculture, Fisheries and Food, 65 Romney St, London, UK SW1P 3RD

Nitrates and Nitrites

Nitrosamines in Bacon

Nonmeat Protein in Meat
Christopher Hitchcock, Unilever Research, Colworth House, Sharnbrook, Bedford, UK MK44 1LQ

Protein, Crude
David Christians, Hach Co., PO Box 907, Ames, IA 50010

Proteins in Meat
Carolyn Henry, U.S. Dept of Agriculture, Food Safety and Inspection Service, PO Box 5080, St. Louis, MO 63115

Species Identification Methods
Mark E. Cutrufelli, U.S. Dept of Agriculture, Food Safety and Inspection Service, Beltsville, MD 20705

Specific Ion Electrode Applications
Randy Simpson, U.S. Dept of Agriculture, Food Safety and Inspection Service, PO Box 6085, Athens, GA 30604

Steroid Analysis

Temperature, Minimum Processing
James Eye, U.S. Dept 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. Dept 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
Edgar E. Stinson, U.S. Dept of Agriculture, Eastern Regional Research Center, 600 E Mermaid Ln, Philadelphia, PA 19118

Citrinin
David Wilson, University of Georgia, Dept of Plant Pathology, Tifton, GA 31794

Cyclopiazonic Acids
John A. Landsen, U.S. Dept of Agriculture, National Peanut Research Laboratory, Dawson, GA 31742

Emodin and Related Anthoquinones

Ergot Alkaloids
George Ware, Food and Drug Administration, 4298 Elysian Fields Ave, New Orleans, LA 70122

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

Penicillium Islandicum Toxins

Secalonic Acid

Sterigmatocystin
Octave J. Francis, Jr, Food and Drug Administration, 4298 Elysian Fields Ave, New Orleans, LA 70122

Tree Nuts
Vincent P. DiProssimo, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Trichothecenes
Robert M. Eppley, Food and Drug Administration, Division of Chemistry and Physics, Washington, DC 20204

Xanthomegnin and Related Naphthoquinones
Allen S. Carman, Food and Drug Administration, 4298 Elysian Fields Ave, New Orleans, LA 70122

Zearalenone
Glenn A. Bennett, U.S. Dept of Agriculture, Northern Regional Research Center, Peoria, IL 61604

OILS AND FATS

Referee: David Firestone, Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Antioxidants
B. Denis Page, Health and Welfare Canada, Health Protection Branch, Ottawa, Ontario, Canada K1A 0L2

Emulsifiers
H. Bruschweiler, Laboratoire Federal d'Essat des Materiaux, Industrie Genie Civil Arts et Metiers, Unterstrasse 11, 900 St. Gallen, Switzerland

Hydrogenated Fats
Richard A. De Palma, Procter & Gamble Co., Winton Hill Technical Center, 6071 Center Hill Rd, Cincinnati, OH 45224

Lower Fatty Acids
Giovanni Bigalli, Hershey Foods Corp., 1025 Reese Ave, Hershey, PA 17033

Marine Oils
Robert G. Ackman, Nova Scotia Technical College, Box 1000, Halifax, Nova Scotia, Canada B3J 2X4

Olive Oil Adulteration
Enzo Fedeli, Experimental 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, Centre of Science and Medical Sciences for Women, PO Box 22452, Riyadh 11495, Saudi Arabia

Sterols and Tocopherols
Robert J. Reina, Food and Drug Administration, 585 Commercial St, Boston, MA 02109

PLANT TOXINS

Referee: Samuel W. Page, Food and Drug Administration, Division of Chemistry and Physics, Washington, DC 20204

Glucosinolates
Douglas I. McGregor, Agriculture Canada, 107 Science Crescent, Saskatoon, Saskatchewan, Canada S7N 0X2

Pyrrolizidine Alkaloids

Solanaceous Alkaloids

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

Diarrhetic Shellfish Poisons
Takeshi Yasumoto, Tokoku University, Dept of Food Chemistry, Tsutsumidori, Sendai 980, Japan

Neurotoxic Shellfish Poisons
Daniel G. Baden, Rosenstiel School of Marine and Atmospheric Sciences, Div. of Biology & Living Research, Miami, FL 33149

Paralytic Shellfish Poisons (Immunoassay Method)
Patrick Guire, Bio-Metric Systems, Inc., 9932 W 74th St, Eden Prairie, MN 55344

Shellfish Poisons
William L. Childress, Food and Drug Administration, 585 Commercial St, Boston, MA 02109

Tetrodotoxins
Yururu Shimizu, University of Rhode Island, College of Pharmacy, Kingston, RI 02881

Committee on Foods II

Laura L. Zaika (U.S. Dept of Agriculture, Eastern Regional Research Center, 600 E Mermaid Ln, Philadelphia, PA 19118), *Chairman;* Robert A. Martin (Hershey Foods Corp., Hershey Technical Center, 1025 Reese Ave, Hershey, PA 17033); Harry G. Lento (Campbell Soup Co., Campbell Pl, Camden, NJ 08151); V. William Kadis (Alberta Agriculture, Food Laboratory Service Branch, Edmonton, Alberta, Canada T6H 4P2); Earl F. Richter (Hazleton Raltech, PO Box 7545, Madison, WI 53707); George R. Tichelaar (State Department of Food and Agriculture, 3292 Meadowview Rd, Sacramento, CA 95832); Patricia Bulhack (Food and Drug Administration, Division of Color Technology, Washington, DC 20204); Benjamin Krinitz (Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232), *Secretary;* Lois Brown (Food and Drug Administration, Division of Mathematics, Washington, DC 20204), *Statistical Consultant;* John G. Phillips (U.S. Dept of Agriculture, Eastern Regional Research Center, 600 E Mermaid Ln, Philadelphia, PA 19118), *Statistical Consultant*

ALCOHOLIC BEVERAGES

Referee: Randolph H. Dyer, Bureau of Alcohol, Tobacco and Firearms, 1401 Research Blvd, Rockville, MD 20850

Alcohol Content by Oscillating U-Tube Density Meter

Alcohol Content of High Solids Distilled Spirits

Carbon Dioxide in Wine
Arthur Caputi, Jr, E & J Gallo Winery, PO Box 1130, Modesto, CA 95353

Citric Acid in Wine
Leonard Mascaro, Boehringer Mannheim Biochemicals, Box 50816, Indianapolis, IN 46250

Color Intensity for Distilled Alcoholic Products

Ethanol in Wine by GC
Arthur Caputi, Jr, E & J Gallo Winery, PO Box 1130, Modesto, CA 95353

Flavor Compounds in Malt Beverages
George Charalambous, Anheuser-Busch Inc., Technical Center, St. Louis, MO 63118

Glycerol in Wine
Eric N. Christensen, E & J Gallo Winery, Box 1130, Modesto, CA 95353

Hydrogen Cyanide

Malic Acid in Wine
David T. Chia, Letterman Medical Center, Dept of Pathology, San Francisco, CA 94129

Malt Beverages and Brewing Materials
Peter Gales, Anheuser-Busch, Inc., One Bush Pl, St. Louis, MO 63118

Sorbic Acid in Wine
Arthur Caputi, Jr, E & J Gallo Winery, PO Box 1130, Modesto, CA 95353

Sugars in Wine
Guenther Henniger, Boehringer Mannheim GmbH, Bahnhofstrasse 5, D-8132, Tutsing/Obb. Postfach 120, GFR

Sulfur Dioxide in Wine (Ripper Method)
Barry Gump, California State University, Dept of Food Science and Enoiogy, Fresno, CA 93740
Guy Baldwin, Paul Masson Vinyards, PO Box 97, Saratoga, CA 95070

Synthetic Colors
John A. Steele, 13305 Burkhart St, Silver Spring, MD 20904

Tartrates in Wine
Masao Ueda, E & J Gallo Winery, PO Box 1130, Modesto, CA 95353

Thujone
Willie Thurman, Bureau of Alcohol, Tobacco and Firearms, 1401 Research Blvd, Rockville, MD 20850

Vanillin and Ethyl Vanillin
Felipe Alfonso, Bureau of Alcohol, Tobacco and Firearms, 1401 Research Blvd, Rockville, MD 20850

CHOCOLATE AND CACAO PRODUCTS

Referee: H. J. Vos, Populierenlaan, NL 3735LG Bosch en Buin, The Netherlands

Carbohydrates in Chocolate Products
W. Jeffrey Hurst, Hershey Foods Corp., 1025 Reese Ave, Hershey, PA 17033

Moisture in Cacao Products
Robert A. Martin, Hershey Foods Corp., 1025 Reese Ave, Hershey, PA 17033

Nonfat Dry Cocoa Solids

Shell in Cacao Products, Micro Methods
W. Jeffrey Hurst, Hershey Foods Corp., 1025 Reese Ave, Hershey, PA 17033

Triglycerids Composition in Cocoa Butter and Fat from Chocolate

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Total and Solid Fat Content in Chocolate Products by NMR

CEREALS AND CEREAL PRODUCTS

Referee: Ralph H. Lane, University of Alabama, Dept of Food, Nutrition, and Institute Management, University, AL 35486

Iron
James I. Martin, Food and Drug Administration, 60 8th St, NE, Atlanta, GA 30309

Phytates
Barbara F. Harland, Howard University, Dept of Human Nutrition/Food, Washington, DC 20259

COLOR ADDITIVES

Referee: Sandra Bell, Food and Drug Administration, Division of Color Technology, Washington, DC 20204

Arsenic and Heavy Metals
Catherine Bailey, Food and Drug Administration, Division of Color Technology, Washington, DC 20204

Atomic Absorption in Color Analysis

Color in Candy and Beverages
Mary Young, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Color in Cosmetics
Sandra Bell, Food and Drug Administration, Division of Color Technology, Washington, DC 20204

Color in Drugs
Edward Woznicki, Colorcon Inc., Moyer Blvd, West Point, PA 19486

Color in Nonfrozen Dairy Desserts
Desire L. Massart, Vrije 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

Inorganic Salts
Wallace S. Brammell, Food and Drug Administration, Division of Color Technology, Washington, DC 20204

Intermediates, Uncombined, in Certifiable Water-Soluble Azo Colors
Daniel M. Marmion, Allied Chemical Corp., 20 Peabody St, Buffalo, NY 14210

Intermediates in Other Certifiable Colors
Alan Scher, Food and Drug Administration, Division of Color Technology, Washington, DC 20204

Liquid Chromatography
Elizabeth A. Cox, Food and Drug Administration, Division of Color Technology, Washington, DC 20204

Subsidiary Colors in Certifiable Color Additives

X-Ray Fluorescence Spectroscopy
Catherine Bailey, Food and Drug Administration, Division of Color Technology, Washington, DC 20204

FLAVORS

Referee: Kurt Schoen, David Michael & Co., 10801 Decatur Rd, Philadelphia, PA 19154

Additives in Vanilla Flavorings
Sidney Kahan, Kahansultants, Inc., 66 Peachtree Ln, Roslyn Heights, NY 11577

Citral

Essential Oils

Glycyrrhizic Acid and Glycyrrhizic Acid Salts

Imitation Maple Flavors, Identification and Characterization

Organic Solvent Residues in Flavorings

Vanillin and Ethyl Vanillin in Food
Sidney Kahan, Kahansultants, Inc., 66 Peachtree Ln, Roslyn Heights, NY 11577

FRUITS AND FRUIT PRODUCTS

Referee: Frederick E. Boland, Food and Drug Administration, Division of Food Technology, Washington, DC 20204

Adulteration of Apple Juice
Edgar R. Elkins, National Food Processors Association, 1401 New York Ave, Washington, DC 20005

Adulteration of Orange Juice by Pulpwash and Dilution
Donald R. Petrus, Florida Dept of Citrus, Box 1088 AREC, Lake Alfred, FL 33850

Fruit Acids
Elia D. Coppola, Ocean Spray Cranberries, Inc., 225 Water St, Plymouth, MA 02360

Fruit Juices, Identification and Characterization
Ronald E. Wrolstad, Oregon State University, Dept of Food Science and Technology, Corvallis, OR 97331

Moisture in Dried Fruits
Edward Steffan, Dried Fruit Association, Box 86, Fresno, CA 93707

Orange Juice Content
Carl Vandercook, U.S. Dept of Agriculture, Agricultural Research Service, Fruit and Vegetable Chemistry Laboratory, Pasadena, CA 91106

NONALCOHOLIC BEVERAGES

Referee: John M. Newton, Food and Drug Administration, 50 Fulton St, San Francisco, CA 94102

Caffeine and Methyl Xanthines in Nonalcoholic Beverages
John M. Newton, Food and Drug Administration, 50 Fulton St, San Francisco, CA 94102

Caloric Content

Glycyrrhizic Acid Salts in Licorice-Derived Products

Lasiocarpine and Pyrrolizidines in Herbal Beverages

Quinine
Leonard Valenti, Food and Drug Administration, 5600 Fishers Ln, Rockville, MD 20857

Safrole in Sassafras

PRESERVATIVES AND ARTIFICIAL SWEETENERS

Referee: James Nelson, Food and Drug Administration, Import District, 850 Third Ave, Brooklyn, NY 11232

Aspartame, Benzoates, Saccharin, and Caffeine, Liquid Chromatography
Norma G. Webb, State Dept of Agriculture and Consumer Services, 3125 Conner Blvd, Tallahassee, FL 32301

Formaldehyde
Robert J. Reina, Food and Drug Administration, 585 Commercial St, Boston, MA 02109

Organic Preservatives (Thin Layer Chromatography)
Rosella Bigornia, General Foods Corp., White Plains, NY 10591

Sulfites
Walter Holak, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232
Martha Hudak-Roos, National Marine Fisheries Service, 3209 Frederick St, Pascagoula, MS 39568-1207

PROCESSED VEGETABLE PRODUCTS

Referee: Thomas R. Mulvaney, Food and Drug Administration, Division of Food Technology, Washington, DC 20204

Fibrous Material in Frozen Green Beans

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

Sugars in Processed Vegetables by LC

Total Solids by Microwave Moisture Analyzer
Henry B. Chin, National Food Processors Association, 1950 Sixth St, Berkeley, CA 94710

Water Activity in Foods
William H. Stroup, Food and Drug Administration, Food Engineering Branch, 1090 Tusculum Ave, Cincinnati, OH 45226

SPICES AND OTHER CONDIMENTS

Referee: James E. Woodbury, Cal-Compack Foods, Inc., PO Box 265, Santa Ana, CA 92702

Ash and Pungent Principles in Mustard

Bulk Index Methods
Thomas E. Haney, Durkee Foods, 16651 Sprague Rd, Strongsville, OH 44136

Ethylene Oxide and Ethylene Chlorohydrin Residues
Lynn Theiss, R. T. French Co., PO Box 23450, Rochester, NY 14692

Extractable Color in Capsicum Spices and Oleoresins
James E. Woodbury, Cal-Compack Foods, Inc., PO Box 265, Santa Ana, CA 92702

Moisture in Dried Spices
Louis A. Sanna, Santa Maria Chili, Inc., Box 1028, Santa Maria, CA 93456

Monosodium Glutamate in Foods

Piperine in Black Pepper
Ted Lupina, Kalsec, Inc., PO Box 511, Kalamazoo, MI 49005

Pungency of Capsicums and Oleoresins
James E. Woodbury, Cal-Compack Foods, Inc., PO Box 265, Santa Ana, CA 92702

Vinegar
Dana A. Krueger, Krueger Food Labs, 24 Blackstone St, Cambridge, MA 02139

SUGARS AND SUGAR PRODUCTS

Referee: Margaret A. Clarke, Sugar Processing Research, Inc., Box 19687, New Orleans, LA 70179

Chromatographic Methods
Michael Gray, Bio-Rad Laboratories, 32nd & Griffin Ave, Richmond, CA 94804

Color, Turbidity, and Reflectance-Visual Appearance
Margaret A. Clarke, Sugar Processing Research, Inc., Box 19687, New Orleans, LA 70179

Corn Syrup and Corn Sugar
Raffaele Bernetti, CPC International, Box 345, Argo, IL 60501

Enzymatic Methods
Marc Mason, Yellow Springs Instrument Co., Box 279, Yellow Springs, OH 45387

Gas Chromatographic Methods
Mary A. Godshall, Sugar Processing Research, Inc., PO Box 19687, New Orleans, LA 70179

Honey
Jonathan W. White, 217 Hillside Dr, Navasota, TX 77868

Lactose Purity Testing
Janice R. Saucerman, Mead Johnson & Co., Environmental Analytical Services, Evansville, IN 47721

Liquid Chromatographic Methods
W. S. Charles Tsang, Sugar Processing Research Inc., Box 19687, New Orleans, LA 70179

Maple Sap and Syrups
Maria Franca Morselli, University of Vermont, Botany Dept, Burlington, VT 05405

Stable Carbon Isotope Ratio Analysis
Landis W. Doner, U.S. Dept of Agriculture, Eastern Regional Research Center, 600 E Mermaid Ln, Philadelphia, PA 19118

Standardization of Sugar Methods of Analysis
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

Sulfites
Richard Riffer, C&H Sugar, Crockett, CA 94525

Weighing, Taring, and Sampling
Melvin Lerner, U.S. Customs Service, 1301 Constitution Ave, Washington, DC 20229

VITAMINS AND OTHER NUTRIENTS

Referee: Mike J. Deutsch, Food and Drug Administration, Division of Nutrition, Washington, DC 20204

Amino Acids
John P. Cherry, U.S. Dept of Agriculture, Eastern Regional Research Center, 600 E Mermaid Ln, Philadelphia, PA 19118

Automated Nutrient Analysis
Jonathan DeVries, General Mills Inc., 9000 Plymouth Ave, Minneapolis, MN 55427

Biotin
Jacob M. Scheiner, Hoffmann-La Roche, 340 Kingsland St, Nutley, NJ 07110

Carotenoids
Forrest W. Quackenbush, 2911 Browning St, West Lafayette, IN 47906

Dietary Fiber
Leon Prosky, Food and Drug Administration, Division of Nutrition, Washington, DC 20204

Fat in Food by Chloroform–Methanol Extraction
Chester E. Daugherty, Campbell Soups, Campbell Pl, Camden, NJ 08101

Folic Acid
Lynn Hoepfinger, Henkel Corp., Box 191, Kankakee, IL 60901

Iodine
Walter Holak, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

LC Assay for Total A, D, and E Content
James V. Bruno, Waters Associates, 34 Maple St, Milford, MA 01757

Nutrient Assay of Infant Formula
James T. Tanner, Food and Drug Administration, Division of Nutrition, Washington, DC 20204
Stephen A. Barnett, Mead Johnson & Co., 2404 Pennsylvania Ave, Evansville, IN 47721

Pantothenic Acid, Total Activity
Raymond Cooke, Laboratory of the Government Chemist, Food Composition and Nutrition, Cornwall House, Stamford St, London, UK SE1 9NQ

Protein Quality, Evaluation
Phillip H. Derse, DS Associates, 979 Jonathan Dr, Madison, WI 53713

Sodium
Edgar R. Elkins, National Food Processors Association, 1401 New York Ave, NW, Washington, DC 20005

Thiamine Assay, Enzyme and Column Packing Reagents
Wayne Ellefson, Hazleton Laboratories America, Box 7545, Madison, WI 53707

Vitamin A
J. Neville Thompson, Health and Welfare Canada, Health Protection Branch, Ottawa, Ontario, Canada K1A 0L2

Vitamin D
Ellen J. de Vries, Duphar B.V., Research Dept 30, PO Box 2, Weesp, The Netherlands

Vitamin E
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
Stephen A. Barnett, Mead Johnson & Co., 2404 Pennsylvania Ave, Evansville, IN 47721

Committee on Residues

Gerald R. Myrdal (State Dept of Agriculture, Trade and Consumer Protection, PO Box 7883, Madison, WI 53707), *Chairman;* William A. Steller (American Cyanamid Co., PO Box 400, Princeton, NJ 08540); Richard Schmitt (Environmental Protection Agency, Office of Pesticide Programs, Washington, DC 20460); Wendell F. Phillips (Campbell Institute of Technology, Campbell Pl, Camden, NJ 08151); Kenneth W. Boyer (Lancaster Laboratories, Inc., 2425 New Holland Pike, Lancaster, PA 17601); Paul E. Corneliussen (Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204); William G. Fong (State Dept of Agriculture, Division of Chemistry, Tallahassee, FL 32301); 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*

METALS AND OTHER ELEMENTS

Referee: Stephen G. Capar, Food and Drug Administration, Division of Chemical Technology, Washington DC 20204

Arsenic in Animal Tissues and Meat Products by AAS
Randy Simpson, U.S. Dept of Agriculture, Food Safety and Inspection Service, Athens, GA 30604

Atomic Absorption Spectrophotometry (AAS)
Milan Ihnat, Agriculture Canada, Chemistry and Biology Research Institute, Ottawa, Ontario, Canada K1A 0C6

Cadmium and Lead in Earthenware
Benjamin Krinitz, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Emission Spectrochemical Methods
Fred L. Fricke, Food and Drug Administration, 1141 Central Pkwy, Cincinnati, OH 45202

Fluorine
Robert W. Dabeka, Health and Welfare Canada, Health Protection Branch, Ottawa, Ontario, Canada K1A 0L2

Graphite Furnace—Atomic Absorption Spectrophotometry
Robert W. Dabeka, Health and Welfare Canada, Health Protection Branch, Ottawa, Ontario, Canada K1A 0L2

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 Determination After Closed System Digestion
Walter Holak, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Polarography
Susan Hight, Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Tin
Edgar R. Elkins, National Food Processors Association, 1401 New York Ave, NW, Washington, DC 20005

MULTIRESIDUE METHODS (INTERLABORATORY STUDIES)

Referee: Leon D. Sawyer, Food and Drug Administration, 240 Hennepin Ave, Minneapolis, MN 55401

Comprehensive Multiresidue Methodology
Jerry E. Froberg, Food and Drug Administration, 1521 W Pico Blvd, Los Angeles, CA 90015

Fumigants
James L. Daft, Food and Drug Administration, 1009 Cherry St, Kansas City, MO 64106

Low Moisture-High Fat Samples, Extraction Procedure
Leon D. Sawyer, Food and Drug Administration, 240 Hennepin Ave, Minneapolis, MN 55401

Miniaturization
D. Ronald Erney, Food and Drug Administration, 1560 Jefferson Ave, Detroit, MI 48207

Organophosphorus Pesticide Residues
Ronald R. Laski, Food and Drug Administration, 599 Delaware Ave, Buffalo, NY 14202

ORGANOHALOGEN PESTICIDES

Referee: Bernadette McMahon, Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Chlordane
Wilbur Saxton, Food and Drug Administration, 5003 Federal Office Building, Seattle, WA 98174

Chlorinated Dioxins
David Firestone, Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Chlorophenoxy Alkyl Acids

Ethylene Dibromide
Leon D. Sawyer, Food and Drug Administration, 240 Hennepin Ave, Minneapolis, MN 55401

Ethylene Oxide and Its Chlorohydrin

Gel Permeation Chromatographic Cleanup
Timothy Spurgeon, ABC Laboratories, 7200 E ABC Ln, Box 1097, Columbia, MO 65205

Methyl Bromide

Pentachlorophenol
George Yip, Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Pentachlorophenol in Animal and Poultry Tissue
Douglas Gillard, Environmental Protection Agency, 401 M St, SW, Washington, DC 20460

Polychlorinated Biphenyls (PCBs)
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Polychlorinated Biphenyls in Blood
Virlyn Burse, Center for Environmental Health, 1600 Clifton Rd, Atlanta, GA 30333

Tetradifon, Endosulfan, and Tetrasul
Lawrence R. Mitchell, Food and Drug Administration, 60 Eighth St, NE, Atlanta, GA 30309

ORGANONITROGEN PESTICIDES

Referee: W. Harvey 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 Herbicides

Carbamate Insecticides (Liquid Chromatography)
Richard Krause, Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Carbofuran

Chlorothalonil

Daminozide
Hafez Abdel-Kader, Uniroyal Chemical Co., Crop Protection Research and Development, Naugatuck, CT 06770

Dinitro Compounds
Richard Krause, Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

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 Fungicides

Sodium o-Phenylphenate

Substituted Ureas
Ronald Luchtefeld, Food and Drug Administration, 1009 Cherry St, Kansas City, MO 64106

Thiolcarbamate Herbicides

s-Triazines

Trifluralin

ORGANOPHOSPHORUS PESTICIDES

Referee: Keith A. McCully, Health and Welfare Canada, Field Operations Directorate, Ottawa, Ontario, Canada K1A 1B7

Azinphos-methyl

Disulfoton
Sunny Y. Szeto, Agriculture Canada, 6660 NW Marine Dr, Vancouver, British Columbia, Canada V6T 1X2

Extraction Procedures

Fenvalerate
Terry D. Spittler, Cornell University, NY State Agricultural Experiment Station, Geneva, NY 14456

General Method for Organochlorine and Organophosphorus Pesticides

High Fat Samples
Ronald Scharfe, Agriculture Canada, Pesticide Laboratory, Ottawa, Ontario, Canada K1A 0C5

Methamidophos

Monocrotophos

Permethrin

Phorate
James M. Devine, American Cyanamid Co., PO Box 400, Princeton, NJ 08540

Phosphine

Resmethrin

Sweep Codistillation
Barry Luke, State Chemistry Laboratory, 5 MacArthur St, East Melbourne, Victoria 3002, Australia

RADIOACTIVITY

Referee: Edmond J. Baratta, Food and Drug Administration, Winchester Engineering and Analytical Center, Winchester, MA 01890

Cesium-137
Edmond J. Baratta, Food and Drug Administration, Winchester Engineering and Analytical Center, Winchester, MA 01890

Iodine-131
Eugene Easterly, Environmental Protection Agency, PO Box 15027, Las Vegas, NV 89114

Neutron Activation Analysis
William Stroube, National Bureau of Standards, Reactor Building 235, Gaithersburg, MD 20899

Plutonium

Radium-228
Jacqueline Michel, Research Planning Institute, 925 Gervais St, Columbia, SC 29201

Strontium-89 and -90
Joseph A. Hutchinson, State Dept of Health, Radiological Sciences Lab., Albany, NY 12201

Tritium

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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, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Fluorescence Microscopy of Molds, Yeasts, and Spores

***Geotrichum candidum* Morphology**
Sylvia Y. Yetts, Food and Drug Administration, 3032 Bryan St, Dallas, TX 75204

Geotrichum Mold in Canned Fruits and Vegetables
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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
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Howard to Viable Mold Counts of Frozen Fruits and Vegetables, Comparison
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Microscopic Mold Count Methods, Use of Compound Microscope
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Microscopic Mold Counts, Effects of Interfering Plant Material
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Mold in Spices
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Molds and Yeasts in Beverages
Stanley M. Cichowicz, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Standardization of Plant Tissue Concentrations for Mold Counting
Stanley M. Cichowicz, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

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
Ann R. Stack, Food and Drug Administration, Division of Cosmetics Technology, Washington, DC 20204

DRUG AND DEVICE RELATED MICROBIOLOGY

Referee: Gordon Oxborrow, Food and Drug Administration, Minneapolis Center for Microbiological Investigations, 240 Hennepin Ave, Minneapolis, MN 55401

Biological Sterility Indicators
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Chemical Indicators
Marvin L. Hart, 3M Co, Medical Systems and Microbial Products, St. Paul, MN 55144

Limulus Amebocyte Lysate Tests for Endotoxins
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Packaging Integrity for Medical Devices
Ana M. Placencia, Food and Drug Administration, Sterility Research Center, 100 Union St, Minneapolis, MN 55455

Sterility Testing of Medical Devices
Michael J. Palmieri, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Testing and Standardization of Biological Indicators
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FILTH AND EXTRANEOUS MATERIALS IN FOODS AND DRUGS

Referee: John S. Gecan, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Baked Goods with Fruit and Nut Tissues
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Botanical Drugs, Adulteration by Foreign Plant Materials
Frank D'Amelio, Bio Botanica, 75 Commerce Dr, Hauppauge, NY 11788

Botanicals
Marvin Nakashima, Food and Drug Administration, Division of Microbiology, Washington, DC 20204
Joseph A. McDonnell, Food and Drug Administration, 1521 W Pico Blvd, Los Angeles, CA 90015

Canned and Dried Soups
Richard Klein, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Canned Fish and Fish Products
Wilfred A. Sumner, Food and Drug Administration, 50 United Nations Plaza, San Francisco, CA 94102

Chocolate Products
Donald A. Mastrorocco, Jr, Hershey Foods Corp., 19 E Chocolate Ave, Hershey, PA 17033

Cocoa Powder and Press Cake
C. Robert Graham, Lancaster Laboratories, Inc., 2425 New Holland Pike, Lancaster, PA 17601

Dehydrated Vegetable Products
Francis J. Farrell, Thomas J. Lipton, Inc., 800 Sylvan Ave, Englewood Cliffs, NJ 07632

Fecal Sterols
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Food Supplement Tablets
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Grains, Whole, Cracking Flotation Methods
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Insect Excreta in Flour
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Meats, Processed
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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
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Mushroom Products, Dried
Jack L. Boese, Food and Drug Administration, Division of Microbiology, Washington, DC 20204
Alan R. Olsen, Food and Drug Administration, 1521 W Pico Blvd, Los Angeles, CA 90015

Mushrooms, Canned

Particulates in Large-Volume Parenterals
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Performance Evaluation of Methods for Filth
Jack L. Boese, Food and Drug Administration, Division of Microbiology, Washington, DC 20204
James Karpus and Alan Whiteman, Food and Drug Administration, 433 W Van Buren St, Chicago, IL 60607

Rye Bread
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Shrimp
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Soluble Insect and Other Animal Filth
George P. Hoskin and Harriet R. Gerber, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Spices
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Spirulina
John S. Gecan, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Tomatoes and Mushrooms
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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
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***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

Bactoscan Methods
J. D. Cunningham, University of Guelph, Environmental Biology, Guelph, Ontario, Canada N1G 2W1

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
Donald A. Kautter, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Clostridium perfringens
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***Escherichia coli* and Other Coliforms**

Genetic Methods for Detection of Bacterial Pathogens
Walter Hill, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Helium Leaks, Canned Foods

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. Dept of Agriculture, Southern Regional Research Center, Box 5677, Athens, GA 30613

Listeria
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Petrifilm Methods
Roy E. Ginn, Dairy Quality Control institute, 2353 Rice St, St. Paul, MN 55133
Vernal S. Packard, University of Minnesota, Dept of Food Science and Nutrition, St. Paul, MN 55112

Salmonella
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Somatic Cell, Automated Optical Counting Method
Wesley N. Kelly, State Dairy Laboratory, Brookings, SD 57007

Somatic Cell, Fossomatic Counting Method
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Staphylococcus
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Staphylococcus Toxin
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Sugars

***Vibrio cholerae* and Detection of Its Toxins**
Angelo De Paolo, Food and Drug Administration, Gulf Coast Technical Services Unit, Dauphin Island, AL 36528

Virology and Animal Oncology
Edward P. Larkin, Food and Drug Administration, Division of Microbiology, 1090 Tusculum Ave, Cincinnati, OH 45226

Yeasts, Molds, and Actinomycetes
Philip B. Mislivec, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Yersinia enterocolitica
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ANTIBIOTICS

Referee: Stanley E. Katz, Rutgers University, Dept of Biochemistry and Microbiology, New Brunswick, NJ 08903

Affinity Quantitative Determination of Penicillin in Milk
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Bacitracin in Feeds
Carol Harpster, AL Laboratories, 185 LeGrand Ave, Northvale, NJ 07647
John B. Gallagher, International Minerals & Chemicals Corp., PO Box 207, Terre Haute, IN 47808

Bacitracin in Premixes and Feeds (Chemical Method)
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Bambermycins in Feeds
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Chloramphenicol in Animal Tissues
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Chlortetracycline in Feeds

Cup Plate System for Antibiotic Analysis
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Design and Computerization of Microbiological Tests
Peter Kahn, Rutgers University, Dept of Biochemistry and Microbiology, New Brunswick, NJ 08903

Erythromycins

Lasalocid in Feeds (LC Method)
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Lasalocid in Feeds (Microbiological Assay)
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Lincomycin in Feeds
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Monensin in Feeds
Robert E. Scroggs, Elanco Products Co., Box 1750, Indianapolis, IN 46206

Neomycin in Feeds

Oxytetracycline in Feeds
Dorothy M. Brennecke, 3981 Dover Pl, St. Louis, MO 63116

Qualitative Delvo-test for β-Lactam Antibiotic Residues in Milk
Wesley N. Kelley, State Dairy Laboratory, Brookings, SD 57007

Qualitative Determination of β-Lactam Antibiotic Residues in Milk
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Quantitative Determination of β-Lactam Antibiotic Residues in Milk
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Roy Ginn, Dairy Quality Control Institute, Inc., 2353 N Rice St, St. Paul, MN 55133

Screening Procedures for Antibiotics in Feeds
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Statistics of Microbiological Assay

Tetracyclines in Tissues (Chromatographic Assay)
Raymond B. Ashworth, U.S. Dept of Agriculture, Food Safety and Inspection Service, Washington, DC 20250

Tetracyclines in Tissues (Microbiological Assay)
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Tylosin in Feeds
Paul Handy, Eli Lilly & Co., Box 708, Greenfield, IN 46140

Virginiamycin in Feeds
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DRUG RESIDUES IN ANIMAL TISSUES

Referee: Charlie J. Barnes, Food and Drug Administration, Office of New Animal Drug Evaluations, Washington, DC 20204

Benzimidazole
Leon LeVan, Hazleton Raltech, 3301 Kinsman Blvd, Madison, WI 53706

Dimetridazole
Maritza C. Pullano, Food and Drug Administration, U.S. Courthouse, Denver, CO 80202

Estrogenic Compounds

Ipronidazole in Turkey and Swine
Raymond B. Ashworth, U.S. Dept of Agriculture, Food Safety and Inspection Service, Washington, DC 20250

Levamisole
Robert Tondreau, American Cyanamid Co., PO Box 400, Princeton, NJ 08540

Screening Methods
Michael Thomas, U.S. Dept of Agriculture, Food Safety and Inspection Service, Beltsville, MD 20705

Tiamulin (Screening Method)

DRUGS IN FEEDS

Referee: Mary L. Hasselberger, State Dept of Agriculture, Laboratory Div., Lincoln, NE 68502

Amprolium

Arsanilic Acid

Carbadox
Virginia A. Thorpe, State Dept of Agriculture, 1615 Harrison Rd, East Lansing, MI 48823

Ethopabate
Joseph Hildebrandt, Agway, Inc., 777 Warren Rd, Ithaca, NY 14850

Ethylenediamine Dihydroiodide
Gary Ross, North Dakota State Laboratories, 2635 E Main St, Bismarck, ND 58501

Furazolidone and Nitrofurazone
Robert E. Smallidge, Purdue University, Dept 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

Phenothiazine
Chris Foret, West Argo Chemical, 501 Santa Fe, Kansas City, MO 64105

Pyrantel Tartrate
James A. Braswell, Pfizer, Inc., Agriculture Div., 1107 S Missouri St, Lee's Summit, MO 64063

Roxarsone
Glenn M. George, Salsbury Laboratories, 2000 Rockford Rd, Charles City, IA 50616

Sulfa Drug Residues
Robert K. Munns, Food and Drug Administration, 20th and California Sts, Denver, CO 80202

Sulfamethazine and Sulfathiazole (Premix and Finished Feed Levels)
Dwight M. Lowie, State Dept of Agriculture, 4000 Reedy Creek Rd, Raleigh, NC 27607

FEEDS

Referee: Clyde E. Jones, State Dept of Agriculture, 2331 W 31st Ave, Denver, CO 80211

Amino Acid Analysis in Mixed Feeds
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Enzymes and Microbial Additives
William Y. Cobb, Texas A&M University, Box 3160, College Station, TX 77841

Fiber, Crude
David O. Holst, University of Missouri, Food Science and Nutrition, Columbia, MO 65211

Infrared Reflectance Techniques in Mixed Feeds
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Iodine
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Microbial Additives and Enzymes

Minerals
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Non-Nutritive Residues
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Protein, Crude
Peter F. Kane, Purdue University, Dept of Biochemistry, West Lafayette, IN 47907

Sampling and Sample Preparation
Darrel L. Sharpe, State Dept of Agriculture, PO Box 630, Jefferson City, MO 65102

Vitamins

Water by Karl Fischer Method
Raffaele Bernetti, CPC International, Inc., PO Box 345, Argo, IL 60501

FERTILIZERS

Referee: Frank J. Johnson, Tennessee Valley Authority, National Fertilizer Development Center, Muscle Shoals, AL 35660

Biuret
Luis F. C. Folch, Fertilizantes Mexicanos, 12 Avo. Piso, Mexico City DF, Mexico 09430

Boron
James R. Melton, Texas A&M University, Agricultural Analytical Services, College Station, TX 77843

Dicyanodiamide
Michael Rohrl, SKW Trostberg AG, D8223 Trostberg POB1150/1160, GFR

Free and Total Water
Russell D. Duncan, Tennessee Valley Authority, National Fertilizer Development Center, Muscle Shoals, AL 35660

Iron
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Melamine

Nitrogen
Paul R. Rexroad, University of Missouri, Experiment Station Chemical Laboratories, Columbia, MO 65211

Phosphorus
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Potash
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Sampling
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Sample Preparation
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Slow-Release Mixed Fertilizers
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Sodium
Luis F. C. Folch, Fertilizantes Mexicanos, 12 Avo. Piso, Mexico City DF, Mexico 09430

Soil and Plant Amendment Ingredients
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Sulfur
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Water-Soluble Methylene Ureas
Allan Davidson, OM Scott & Sons, Co., Marysville, OH 43041

Zinc
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PLANTS

Referee: ———

Ashing Methods
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Atomic Absorption Methods
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Boron

Chromium
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Emission Spectroscopy
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Fluorine
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Nitrogen, Nonprotein

Selenium
Oscar E. Olson and Ivan S. Palmer, South Dakota State University, Experiment Station, Biochemistry Dept, Brookings, SD 57006

Starch
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Sulfur
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TOBACCO

Referee: Ray Severson, U.S. Dept of Agriculture, Agricultural Research Service, Athens, GA 30613

Alkaloids

Polyphenols

Tar and Nicotine in Cigarette Smoke
Harold C. Pillsbury, Federal Trade Commission, 6th and Pennsylvania Ave, NW, Washington, DC 20580

VETERINARY ANALYTICAL TOXICOLOGY

Referee: P. Frank Ross, U.S. Dept of Agriculture, National Veterinary Services Laboratory, Ames, IA 50010

Animal Serum Thyroxine
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Antibiotic Screening Methods
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Stephen C. Ross, Dept of Agriculture, Animal Disease Lab., Shattuc Rd, Centralia, IL 62801

Arsenic in Animal Tissue
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Atomic Absorption Spectrophotometry
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Chlorinated Phenols in Animal Tissues
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Cholinesterase
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Copper in Animal Tissue
David Osheim, U.S. Dept of Agriculture, National Veterinary Services Laboratory, Ames, IA 50010

Fluoride in Animal Tissue
David Osheim, U.S. Dept of Agriculture, National Veterinary Services Laboratory, Ames, IA 50010

Lead in Animal Tissue
Robert J. Everson, Purdue University, School of Veterinary Medicine, West Lafayette, IN 47907

Monensin

Multielement Analysis by ICP
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Multiple Anticoagulant Screening
John D. Reynolds, Dept of Agriculture, Animal Disease Laboratory, Shattuc Rd, Centralia, IL 62801

Natural Products
George Rottinghaus, University of Missouri, College of Veterinary Medicine, Columbia, MO 65211

Nitrates and Nitrites
Norman R. Schneider and Michael P. Carlson, Veterinary Diagnostic Center, Dept of Veterinary Science, Lincoln, NE 68583

Pesticides in Toxicological Samples
Henry M. Stahr, Iowa State University, College of Veterinary Medicine, Ames, IA 50010

Selenium in Animal Tissue
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Sodium Monofluoroacetate
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Zinc in Animal Tissues
Dana Perry, Veterinary Diagnostic Laboratory, Dept of Veterinary Science, Tucson, AZ 85721

Committee on Hazardous Substances in Water and the Environment

Douglas J. Dube (State Laboratory of Hygiene, 465 Henry Mall, Madison, WI 53706), *Chairman;* Laszlo Torma (State Dept of Agriculture, Montana State University, Bozeman, MT 59717); Nile Frawley (Dow Chemical Co., 574 Building, Midland, MI 48640); Erika Hargesheimer (City of Calgary, Glenmore Waterworks Lab., Calgary, Alberta, Canada T2P 2M5); Ross J. Norstrom (Environment Canada, Canadian Wildlife Service, National Wildlife Research Center, Ottawa, Ontario, Canada K1A DE7); Robert Graves (Environmental Protection Agency, Monitoring and Support Lab., 26 W St. Clair, Cincinnati, OH 45268); Mark F. Marcus (Chemical Waste Management, Inc., 150 W 137th St, Riverdale, IL 60627), *Secretary*

AIR

Referee: ———

BIOMONITORING

Referee: ———

BIOTA

Referee: ———

EFFLUENTS

Referee: ———

HAZARDOUS SUBSTANCES

Referee: ———

Ammonia as a Product Ingredient

Benzene in Consumer Products
Wayne G. Wamer, Food and Drug Administration, Division of Toxicology, Washington, DC 20204

Carbolic Acid (Phenolic) Compounds

Chlorinated Hydrocarbons

Diethylene Glycol and Ethylene Glycol

Flammable Substances in Pressurized Containers

Flash Point of Solids and Semisolids

Formaldehyde

Hazardous Components in Resin Systems

Nitrosamines in Infant Pacifiers
Harold Thompson, Food and Drug Administration, National Center for Toxicological Research, Jefferson, AR 72079

Pentachlorophenol in Toy Paints
Hans E. A. M. Van Langeveld, Food Inspection Services, Florijnruwe 111, 6218 CA Maastricht, The Netherlands

Petroleum Distillates in Mixtures

Selenium

Toxic Metals in Paints
Warren K. Porter, Jr, Consumer Product Safety Commission, 200 C Street, SW, Washington, DC 20204

Turpentine

Viscosity of Liquids

SOILS AND SEDIMENTS

Referee: James Dragun, E. C. Jordan Co., 17515 W Nine Mile Rd, Southfield, MI 48075

Adsorption Isotherms for Volatile Organics
Leverett R. Smith, 622 Clayton Ave, El Cerrito, CA 94530

Distribution Coefficients—Nonvolatile Organics
Danny R. Jackson, Battelle-Columbus Labs, 505 King Ave, Columbus, OH 43201

Distribution Coefficients—Volatile Organics

Soil Column Leaching

WASTE MATERIALS

Referee: David Friedman, Environmental Protection Agency, Office of Solid Waste, Washington, DC 20460

Bioassays
Llewellyn R. Williams, Environmental Protection Agency, Environmental Monitoring Systems Lab., Box 15027, Las Vegas, NV 89114

Inorganic Analytes
Gerald McKee, Environmental Protection Agency, Environmental Monitoring Systems Lab., 26 W St. Clair St, Cincinnati, OH 45268

Organic Analytes
Paul Friedman, Environmental Protection Agency, Office of Solid Waste, Washington, DC 20460

Physical/Chemical Properties
Florence Richardson, Environmental Protection Agency, Office of Solid Waste, Washington, DC 20460

Sampling

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 J. Dube, State Laboratory of Hygiene, 465 Henry Mall, Madison, WI 53706

Ethylene Dibromide
Donald L. Hughes, Hazleton Labs, 3301 Kinsman Blvd, Madison, WI 53707

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

Munitions in Wastewater
Peter Rissell, U.S. Army Toxic and Hazardous Materials Agency, Aberdeen Proving Ground, MD 21010
Thomas Jenkins, Cold Regions Research and Engineering Lab., Hanover, NH 03755-1290

Organohalogen Pesticides in Water
Marie Siewierski, Rutgers University, Cook College, New Brunswick, NJ 08903

Organophosphorus Pesticides in Water

Phenols
Bill Lee, Canada Centre for Inland Waters, Box 5050, Burlington, Ontario, Canada L7R 4A6

Salt

Triazine Herbicides in Water

CHANGES IN OFFICIAL METHODS OF ANALYSIS

The following changes in the methods of the Association become effective, as provided in Article IX, Section 5, of the Bylaws, on the thirtieth day after publication of this *Journal* issue. Section numbers refer to *Official Methods of Analysis*, 14th edition (1984), unless otherwise specified.

Newly adopted methods are numbered in the style of the 14th edition. The first section of the first new method in each chapter is numbered with the chapter number plus **.B01.** Subsequent sections are numbered **.B02, .B03, .B04,** et seq. The **B** signifies that the method was adopted at the 1985 Annual International Meeting, and was published and became official in 1986 in the second supplement to the 14th edition.

"Changes in Methods" is accompanied by an index. Errata and emendations are published under the appropriate chapter, and are indexed. The "Changes in Methods" index is cumulative between editions of *Official Methods of Analysis*.

AOAC committees are considering the development of uniform statistical protocols for the evaluation of collaborative studies and to calculate appropriate statistical performance parameters. After these committees complete this task, AOAC will publish statistical performance parameters on adopted methods according to these protocols.

1. AGRICULTURAL LIMING MATERIALS

No additions, deletions, or other changes.

2. FERTILIZERS

(1) The following final action methods were declared surplus:

(a) Potassium in fertilizers, flame photometric method, **2.108–2.113.**

(b) Potassium in fertilizers, automated flame photometric method, **2.114–2.118.**

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(2) Make the following change in **2.157–2.160,** iron (chelated) in iron chelate concentrates, atomic absorption spectrophotometric method:

In the applicability statement that precedes **2.157,** delete Fe *N,N*-bis(2-hydroxy-5-sulfobenzyl)glycine (DPS) from the list of compounds to which the method is applicable.

3. PLANTS

No additions, deletions, or other changes.

4. DISINFECTANTS

No additions, deletions, or other changes.

5. HAZARDOUS SUBSTANCES

The following gas chromatographic method for determination of *N*-nitrosodibutylamine in latex infant pacifiers was adopted first action:

N-Nitrosodibutylamine in Latex Infant Pacifiers

Gas Chromatographic Method

First Action

5.B01 ***Principle***

Volatile *N*-nitrosamines are extd from cut-up latex pacifier nipples with CH_2Cl_2. Ext is concd and subjected to high temp. purge and trap, and *N*-nitrosamines are eluted from trap and detd by gas chromatgy with thermal energy analysis.

5.B02 ***Reagents***

Use all glass-distd solvs (Burdick & Jackson Laboratories, Inc., or equiv.).

(a) *N-Nitrosamine stock std solns.*—(*1*) *External stock std soln.*—10 μg/mL each of NDMA (*N*-nitrosodimethylamine), NDEA (*N*-nitrosodiethylamine), NDPA (*N*-nitrosodipropylamine), NDBA (*N*-nitrosodibutylamine), NPIP (*N*-nitrosopiperidine), NPYR (*N*-nitrosopyrrolidine), and NMOR (*N*-nitrosomorpholine) in alcohol. (*2*) *Internal stock std soln.*—10 μg NDPA/mL alcohol.

Caution: Volatile *N*-nitrosamines are extremely hazardous compds. Carry out all manipulations involving handling neat liqs or solns in adequately ventilated and filtered fume hood or glove box.

(b) *Mineral oil.*—White, lightwt Saybolt viscosity 125/135 (No. 6358, Mallinckrodt Chemical Works).

(c) *Nitrosation inhibitor.*—10 mg α-tocopherol/mL mineral oil.

(d) *Keeper solns.*—(*1*) *For K-D evaporation.*—80 mg mineral oil/mL CH_2Cl_2. (*2*) *For N evaporation.*—20 mg mineral oil/mL isooctane.

5.B03 ***Apparatus***

(a) *ThermoSorb/N™ cartridges.*—Use as received for quant. trapping of volatile *N*-nitrosamines (Thermedics, Inc., Div. of Thermo Electron Corp., Woburn, MA 01801).

(b) *Variable temperature oil bath.*—Thermostatically controlled, capable of operating at 150 ± 3° and of moving vertically with aid of laboratory jack (The Lab Apparatus Co., PO Box 42070, Cleveland, OH 44142).

(c) *Soxhlet extraction apparatus.*—(Kimble Glass Co.). Allihn condenser with 34/45 ⧩ joint. Extn tube with 34/45 ⧩ upper joint and 24/40 ⧩ lower joint. Extn thimble, 25 × 85 mm borosilicate glass fitted with coarse porosity frit.

(d) *Kuderna-Danish evaporative concentrator.*—(Kontes Glass Co.). 3-ball Snyder column with 24/40 ⧩ joints, 250 mL flask with 24/40 ⧩ joint and 19/22 ⧩ lower joint, and 4 mL graduated concentrator tube with 19/22 ⧩ joint.

(e) *Gas chromatograph.*—Hewlett-Packard Model 5710A, or equiv., equipped with 6 ft × 4 mm id glass column packed with 10% Carbowax 20M/2% KOH on 80–100 mesh Chromosorb WAW (No. 1-1805, Supelco). Condition column overnight at 215°. Operate at temp. program mode from 150 to 190° at 4°/min. Injection port temp. 250°. Carrier gas prepurified Ar at flow rate 40 mL/min. Interface GC app. to thermal energy analyzer, (**f**), via ⅛ in. od stainless steel tube connected to Swagelok fittings and operate at 170°.

(f) *Thermal energy analyzer.*—Model 502, Thermo Electron Corp., or equiv. Operate pyrolysis chamber at 500° in GC mode. O flow to ozonator, 10 mL/min. Keep cold trap at −150° using liq. N/2-methylbutane slush bath. Pressure of reaction chamber, ca 0.9 torr. Record TEA detector response on Hewlett-Packard 3380 integrator.

(g) *Purge and trap apparatus.*—Fig. **5:B1** contains following parts: (*1*) Ar gas cylinder and gauge (Air Products Specialty Gas, Tamaqua, PA 18252); (*2*) metering valve; (*3*) purge gas manifold, 4-position; (*4*) Nalgene needle valve type CPE (No. 6400-0125, Nalge Co., Rochester, NY 14602); (*5*) 18/7 g-g outer joints with pinch clamps (No. 772398, Wheaton Scientific, Millville, NJ 08332); (*6*) impingers, 50 mL graduated glass tubes with 24/40 ⧩ clear-seal, grease-free joints, 18/7 g-g ball joints, and 1 mm id nozzle ca 5 mm above bottom of impinger (No. 753463, Wheaton Scientific); (*7*) variable scale flow-check, calibrated for purge rate in mL Ar/min (No. 7083, Alltech Associates, Inc.). Bubble meter for measuring gas flow rates for GC may be substituted.

Note: Do not use any rubber tubing, gaskets, O-rings, or other items made of rubber in any part of this method.

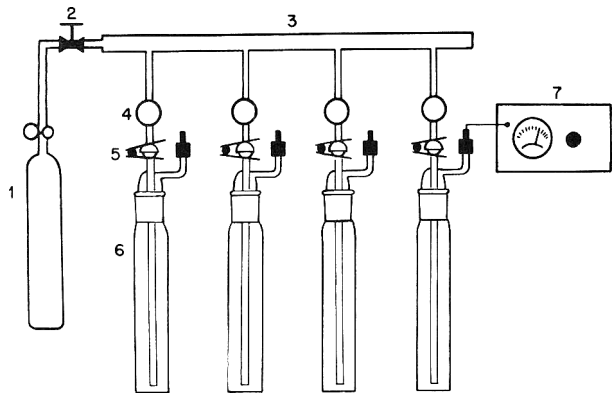

FIG. 5:B1—Diagram of purge and trap apparatus equipped with 4 impinger tubes

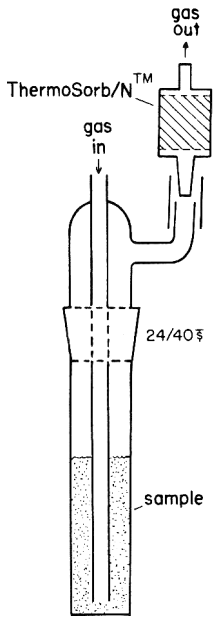

FIG. 5:B2—Close-up diagram of impinger tube fitted with ThermoSorb/N cartridge

5.B04 *Description and Use of Purge and Trap Apparatus*

App. shown in Fig. **5:B1** is designed for high temp. purging and trapping of 7 volatile *N*-nitrosamines from concd sample ext/mineral oil mixt. on 4 samples simultaneously. Cylinder contg prepurified Ar gas equipped with high pressure regulator is used to supply 20 psig to flow-metering valve which regulates final purge flow thru samples. Gas stream is diverted into tubular stainless steel mainfold, 250 × 20 mm od, contg 4 exit tubes spaced 50 mm apart and measuring 40 × 10 mm od. Each of these tubes is coupled using ⅜ in. Tygon tubing to Nalgene needle valves which serve dual purposes: as shut-off valve when less than 4 samples are analyzed; and for making minor adjustments in purge rate due to slight differences in flow characteristics of impinger and cartridges. An 18/7 g-g outer spherical joint is attached to Nalgene valve to permit quick, gas-tight connection to 18/7 g-g ball joint on impinger inlet, using appropriate pinch clamp. As shown in Fig. **5:B2,** impingers are assembled by inserting glass nozzle (1 mm id orifice) into sample mixt. and coupling 24/40 S grease-free male and female joints together to form leak-free seal. Once sealed, Ar gas is allowed to purge thru sample mixt., thru outlet tube of impinger (see Fig. **5:B2**). Tygon tubing is used to connect impinger outlet tube to inlet side marked "AIR IN" of cartridge, which is std male Luer connector. Purged volatile *N*-nitrosamines are then collected on sorbent contained in cartridge with Ar effluent exiting from female Luer connector. Flow rate of Ar is measured directly from cartridge with variable scale flow meter which has been previously calibrated for flow rate of Ar gas (mL/min). Bubble meter can be substituted for variable scale flow meter. Temp. of sample mixt. during purge is controlled by immersing impinger up to sample vol. mark (ca 25 mL line) in thermostatically controlled oil bath capable of operating isothermally up to 150°. Gas manifold, as well as each impinger, is secured by clamps to support grid; therefore, oil bath is moved vertically in and out of position for high temp. purge.

5.B05 *Extraction and Cleanup of Pacifier Samples*

Accurately weigh 5 g from each sample into 250 mL r-b flask and add 100 mL CH_2Cl_2. Dil. internal stock std soln to 50 ng/mL with CH_2Cl_2 and spike contents of flask with 2 mL dild std. Seal flask and let contents stand overnight (16–21 h) at ambient temp.

Transfer ext and rubber pieces to glass extn thimble fitted with coarse porosity glass frit in Soxhlet extn app. Rinse 250 mL r-b flask with 25 mL CH_2Cl_2 and transfer rinse to Soxhlet app. Ext rubber pieces for 1 h in app. at rate of 8 cycles/h.

Let cool and transfer CH_2Cl_2 ext to 250 mL K-D evaporator. Rinse extn flask with two 10 mL portions of CH_2Cl_2 and combine rinses with 125 mL ext. Add 1 mL keeper soln *1* and 2 or 3 boiling chips (Boileezers, Fisher Scientific Co.) to ext. Evap. ext in K-D unit using 3-ball Snyder column on 55° water bath until vol. is reduced to 3–4 mL.

Let K-D unit cool to room temp., allowing excess solv. in Snyder column to rinse down walls of unit into 4 mL K-D tube (total = 3–4 mL). Remove 250 mL reservoir and 3-ball Snyder column, reduce vol. of ext to 2 mL in same K-D tube under gentle stream of N (ca 50 mL/min), and transfer 2 mL ext using disposable Pasteur pipet with two 1 mL mineral oil rinses to 50 mL purge and trap app. contg 20 mL mineral oil and 1 mL of 10 mg/mL of α-tocopherol in mineral oil as nitrosation inhibitor.

Assemble purge and trap app. and connect cartridges to exit tubes with Tygon connector. Adjust Ar flow rate to 400 mL/min thru cartridge ± 5% (i.e., 380–420 mL Ar/min). Note: Check flow rate intermittently during purging, especially within first 15 min because of initial increase in temp. of sample. Immerse purge tubes (up to sample line) or to ca 25 mL mark in 150 ± 3° oil bath for 1.5 h. Remove cartridge and tightly cap. (Note: This is good stopping point; cartridge can be eluted on following day if necessary.)

Elute cartridge using 10 or 20 mL glass Luer-Lok syringe connected to female Luer adapter (air exit side) with 20 mL acetone–CH_2Cl_2 (1 + 1, v/v). Collect eluate in 30 mL culture tube. (Note: 30 mL tube(s) should be scored with file or piece of tape placed at 5 mL vol. mark.)

Evap. ext to ca 5 mL and then transfer with three 1 mL rinses of CH_2Cl_2 to 10 mL graduated tube. Add 0.5 mL keeper soln *2*. Evap. sample (vol. = 8.5 mL) to 2 mL under gentle stream of N. (Note: If 2 mL sample cannot be analyzed same day as evapd, it is advantageous to refrigerate sample at larger vol., i.e., 4–5 mL, and evap. next day before analysis by GC-TEA.)

Analyze 2 mL sample by injecting 8 μL aliquot into GC-TEA.

5.B06 *Quantitation*

Use internal std technic. Dil. external stock std soln with CH_2Cl_2 to 50, 100, and 200 ng/mL to be used as working stds for analysis. Inject 8 μL into GC-TEA to det. responses (peak hts) of NDPA and other nitrosamines for use in internal stdzn calcn. Inject 8 μL of each 2 mL sample ext into GC-TEA. Det. responses (peak hts) of NDPA and any other *N*-nitrosamines detected for use in internal stdzn calcn. Calc. results as follows:

$$\text{ppb } N\text{-Nitrosamine X} = [(PH_X) \times (F_X) \times (100 \text{ ng NDPA})]/[(PH_{NDPA}) \times (F_{NDPA}) \times (\text{g sample})]$$

where PH_X = peak ht in mm of *N*-nitrosamine X in sample; F_X = ng *N*-nitrosamine X/mL in external std soln divided by peak ht in mm of *N*-nitrosamine X in external std soln; 100 ng NDPA = total ng NDPA (internal std) added to sample; PH_{NDPA} = peak ht in mm of NDPA (internal std) in sample; F_{NDPA} = ng NDPA/mL in external std soln divided by peak ht in mm of NDPA in external std soln; g sample = g rubber sample analyzed.

6. PESTICIDE FORMULATIONS

(1) The following first action methods were adopted final action:

(a) CIPAC-AOAC method for gamma-BHC in technical BHC, pesticide formulations, and lindane shampoos and lotions, gas chromatographic method, **6.221–6.226**.

(b) Metribuzin in pesticide formulations, gas chromatographic method, **6.553–6.559**.

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(2) The following gas chromatographic method for determination of cypermethrin (3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylic acid cyano(3-phenoxyphenyl)methyl ester) in pesticide formulations was adopted first action as a CIPAC-AOAC method:

Cypermethrin in Pesticide Formulations
Gas Chromatographic Method
First Action
CIPAC-AOAC Method

6.B01 ***Principle***

Sample is dissolved in methyl isobutyl ketone contg di-(ethylhexyl) phthalate as internal std; 4 cypermethrin isomers, isolated as one peak, are detd by gas chromatgy with flame ionization detection.

6.B02 ***Apparatus and Reagents***

(a) *Gas chromatograph with recorder and integrator.*—With flame ionization detector and 1.0 m × 4 mm (id) glass column packed with 3% OV-101 on 100–120 or 80–100 mesh Chromosorb WHP, capable of on-column injection. Condition newly packed column overnight at 260° with low N flow. Operating conditions: temps—inlet 250°, column 230–240°, detector 250°; carrier gas flow to elute internal std at ca 5.5 min and cypermethrin at ca 11.5 min with ≥30 mm between intercepts of tangents on baseline of std and internal std peaks; adjust H and air for detector as recommended by manuf.; adjust sensitivity to give peak hts 75% full scale.

(b) *Di-(2-ethylhexyl) phthalate (DEHP; dioctyl phthalate).*—Fisher Scientific Reagent, or equiv.

(c) *Methyl isobutyl ketone (MIBK).*—GC quality (J.T. Baker Chemical Co. Reagent, or equiv.).

6.B03 ***Preparation of Standards***

(a) *Internal std soln.*—20 mg DEHP/mL. Weigh ca 10 g DEHP into 500 mL vol. flask, dil. to vol. with MIBK, and mix (soln I). Concn may be varied to accommodate column and instrument differences. If necessary, adjust concn so that peak ht or area of DEHP closely matches peak ht or area of cypermethrin within 10%.

(b) *Cypermethrin std soln.*—4.0 mg/mL. Warm sealed bottle of cypermethrin std (ICI-Americas, Inc.) at 40–50° until no crystals remain; shake bottle. Accurately weigh, in duplicate, ca 0.2 g std into 50 mL vol. flask, and dissolve in 3–4 mL MIBK. Pipet 10.0 mL internal std soln into each flask, dil. to vol. with MIBK, and mix (solns CA, CB). Similarly, weigh ca 0.1 g cypermethrin std into 25 mL vol. flask, dil. to vol. with MIBK, and mix (soln CO).

6.B04 ***Preparation of Sample***

(a) *Technical formulations.*—Proceed as above under cypermethrin std soln, using sample wt contg ca 0.2 g cypermethrin (solns SA, SB, SO).

(b) *Wettable powders.*—Accurately weigh, in duplicate, sample contg 0.2 g cypermethrin into 50 mL vol. flask, pipet in 10.0 mL internal std soln, and add sufficient MIBK to suspend powder. Thoroly shake flask 10 min, dil. to vol. with MIBK, and let powder settle or centrf. until clear. Similarly, prep. soln without internal std, using sample contg ca 0.1 g cypermethrin/25 mL MIBK.

(c) *Ultra-low volume formulations.*—Proceed as above under technical formulations, beginning "Accurately weigh . . .".

(d) *Emulsifiable concentrates.*—Proceed as above under wettable powders.

6.B05 ***System Performance Check and Determination***

Using instrument conditions listed under *Apparatus and Reagents*, inject 1.5 μL portions of solns I, CO, and SO onto column and check for interfering peaks. On-column injection is necessary. Inject std soln CA and adjust parameters to give peak ht ca 75% full scale with peak quality and elution time specified.

Inject 1.5 μL portions of std solns CA and CB until response ratio (area cypermethrin peak/area internal std peak) varies <0.5% of mean. (Area measurements by digital electronic integration are preferred over other methods.) Carry out injections of std and sample solns in following sequence: CA_1, SA_1, SA_2, CB_1, CA_2, SB_1, SB_2, CB_2. Average response ratios for sample and stds that bracket each sample. Successive response ratios should agree ±5% of their mean. If not, repeat analysis.

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

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

CAS-52315-07-8 (cypermethrin)

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(3) The following gas chromatographic method for determination of permethrin (3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylic acid (3-phenoxyphenyl)methyl ester) in pesticide formulations was adopted first action as a CIPAC-AOAC method:

Permethrin in Pesticide Formulations
Gas Chromatographic Method
First Action
CIPAC-AOAC Method

6.B06 ***Principle***

Sample is dissolved in methyl isobutyl ketone contg *n*-octacosane as internal std, and permethrin is detd as total area of 2 isomer peaks by gas chromatgy with flame ionization detection.

6.B07 ***Apparatus and Reagents***

(a) *Gas chromatograph with recorder and integrator.*—With flame ionization detector and 1.0 m × 4 mm (id) glass column packed with 3% OV-210 on 100–120 or 80–100 mesh Chromosorb WHP, capable of on-column injection. Condition newly packed column overnight at 275° with low N flow. Operating conditions: temps—inlet 260°, column 190–220°, detector 250°; carrier gas flow to elute internal std in ca 4.0 min and *trans*-permethrin at ca 9.5 min with ≥30 mm between intercepts of tangents on baseline of internal std and std *cis*- and *trans*-isomer peaks; adjust H and air for detector as recommended by manuf.; adjust sensitivity to give peak hts 75% full scale.

(b) *n-Octacosane std.*—With no peaks at retention times of permethrin isomers (Kodak Laboratory Chemicals, or equiv.).

(c) *Methyl isobutyl ketone (MIBK).*—GC quality (J.T. Baker Chemical Co. Reagent, or equiv.).

6.B08 ***Preparation of Standards***

(a) *Internal std soln.*—1 mg *n*-octacosane/mL. Weigh ca 0.5 g *n*-octacosane into 500 mL vol. flask, dissolve in 300 mL MIBK, dil. to vol. with MIBK, and mix (soln I). Concn may be varied to accommodate column and instrument differences. If necessary, adjust concn so that peak ht of *n*-octacosane closely matches peak ht of permethrin isomers.

(b) *Permethrin std soln.*—4.0 mg/mL. Warm sealed bottle of permethrin std (ICI-Americas, Inc.) at 40–50° until no crystals remain; shake bottle. Accurately weigh, in duplicate, ca 0.1 g std

into 100 mL g-s erlenmeyer. Pipet 25.0 mL internal std soln into each flask and shake until permethrin is dissolved (solns CA, CB). Similarly, weigh ca 0.1 g permethrin std into 25 mL vol. flask, dissolve in 15 mL MIBK, dil. to vol. with MIBK, and mix (soln CO).

6.B09 ***Preparation of Sample***

(a) *Technical formulations.*—Proceed as above under permethrin std soln, using sample wt contg ca 0.1 g permethrin (solns SA, SB, SO).

(b) *Wettable and dustable powders (suspendibility >50%).*—Accurately weigh, in duplicate, sample contg 0.1 g permethrin into 100 mL g-s erlenmeyer. Pipet 25.0 mL internal std soln into flask, stopper, and shake thoroly 10 min. Let settle, filter thru Whatman No. 54 paper into g-s flask, and use filtrate for analysis. Similarly, prep. soln without internal std, using sample contg ca 0.1 g permethrin/25 mL MIBK.

(c) *Emulsifiable concentrates.*—Proceed as above under wettable and dustable powders.

(d) *Water-dispersible granules.*—Grind ca 20 g sample to fine powder and thoroly mix. Accurately weigh, in duplicate, sample contg 0.1 g permethrin into 100 mL g-s erlenmeyer. Pipet 25 mL internal std soln into flask and place in ultrasonic bath 10 min. Proceed as above under wettable and dustable powders, beginning "Let settle . . . ".

6.B10 ***System Performance Check***

Using instrument conditions listed under *Apparatus and Reagents*, inject 3 or more 1.5 μL portions of soln CA onto column and adjust parameters to give peak ht ca 75% full scale with peak quality and elution time specified. On-column injection is necessary. Inject 1.5 μL solns I, CO, and SO and check for interfering peaks.

6.B11 ***Determination***

Inject 1.5 μL std solns CA and CB until response ratio (total area of *cis*- and *trans*-permethrin peaks/area internal std peak) varies <0.5% of mean. (Area measurements by digital electronic integration are preferred over other methods.) Carry out injection of std and sample solns in following sequence: CA_1, SA_1, SA_2, CB_1, CA_2, SB_1, SB_2, CB_2. Average response ratios for sample and stds that bracket each sample. Successive response ratios should agree ±5% of their mean. If not, repeat analysis.

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

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

CAS-52645-53-1 (permethrin)

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(4) The following gas chromatographic method for determination of butachlor (*N*-(butoxymethyl)-2-chloro-*N*-(2,6-diethylphenyl)acetamide) in pesticide formulations was adopted first action as an AOAC-CIPAC method:

Butachlor in Pesticide Formulations

Gas Chromatographic Method

First Action

AOAC-CIPAC Method

6.B12 ***Principle***

Sample is dissolved in acetone contg triphenyl phosphate as internal std, analyzed by gas chromatgy with flame ionization detection, and measured by comparison with internal std on the basis of integrated relative peak areas.

6.B13 ***Safety***

LD_{50} of butachlor has been found to be 4600 mg/kg in rat acute oral studies (Monsanto). Avoid excessive exposure by wearing protective clothing.

6.B14 ***Apparatus***

(a) *Gas chromatograph.*—With flame ionization detector and on-column injection ports. Temps—column oven 250°, injection port 280°, detector 300°; gas flows (mL/min)—He carrier gas 30, H 34, air 430; sample size 1.0 μL; run time 25 min.

(b) *Column.*—6 ft × 2 mm (id) glass column (on-column configuration) packed with 10% SP-2250 on 100–120 mesh Supelcoport (Supelco, Cat. No. 1-2132), or equiv. SP-2250 is methyl–phenyl silicone (50 + 50). Precondition overnight at 250° before use. Retention times of butachlor and internal std are ca 5.9 and 18.5 min, resp.

6.B15 ***Reagents***

(a) *Acetone.*—Pesticide grade (Fisher Scientific Co., or equiv.).

(b) *Triphenyl phosphate internal std soln.*—Gold Label (Aldrich Chemical Co., Inc.). Weigh 6.4 g into 1 L vol. flask. Dissolve in and dil. to vol. with acetone.

(c) *Butachlor std soln.*—99.7% (recrystd from hexane at −40°; Monsanto Chemical Co.). Accurately weigh 0.2 g into small flask. Add by pipet 25 mL internal std soln and shake to dissolve.

6.B16 ***Determination***

Accurately weigh sample contg ca 0.2 g butachlor into small flask. Add by pipet 25.0 mL internal std soln and shake ≥5 min to ext butachlor. For emulsifiable concs, use ca 0.3000 g.

Make replicate 1 μL injections of butachlor std soln and measure response ratio, R (area butachlor peak/area internal std peak) for each injection. Repeat until consecutive response ratios agree ±0.5%.

Make duplicate injections of sample soln and det. av. R. Follow with injection of butachlor std soln; average R' for std before and after sample injection.

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

where R and R' = av. response ratios for sample and std, resp.; W and W' = wt (g) of sample and std, resp.; P = % purity of std.

CAS-23184-66-9 (butachlor)

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(5) The following gas chromatographic method for determination of propachlor (2-chloro-*N*-(1-methylethyl)-*N*-phenylacetamide) in pesticide formulations was adopted first action as an AOAC-CIPAC method:

Propachlor in Pesticide Formulations

Gas Chromatographic Method

First Action

AOAC-CIPAC Method

6.B17 ***Principle***

Sample is dissolved in acetone contg diisobutyl phthalate as internal std, analyzed by gas chromatgy with flame ionization detection, and measured by comparison with internal std on basis of integrated peak areas.

6.B18 ***Safety***

LD_{50} of propachlor has been found to be 1800 mg/kg in rat acute oral studies (Monsanto). Material is classified as slightly toxic. Avoid excessive exposure by wearing protective clothing.

6.B19 ***Apparatus***

(a) *Gas chromatograph.*—With flame ionization detector and on-column injection ports. Temps—column oven 200° (isothermal), injection port 250°, detector 250°; gas flows (mL/min)—He carrier gas 30, H 34, air 430; sample size 1.0 μL; run time 25 min.

(b) *Column.*—6 ft × 2 mm (id) glass column (on-column configuration) packed with 10% SP-2250 on 100–120 mesh Supelcoport (Supelco, Cat No. 1-2132), or equiv. SP-2250 is methyl–phenyl silicone (50 + 50). Precondition overnight at 250° before use. Retention times for propachlor and internal std are ca 5.29 and 11.51 min, resp.

6.B20 ***Reagents***

(a) *Acetone.*—Pesticide grade (Fisher Scientific Co., or equiv.).

(b) *Diisobutyl phthalate internal std soln.*—Accurately weigh 6.4 g diisobutyl phthalate (Eastman Kodak Co.) into 1 L vol. flask. Dissolve in and dil. to vol. with acetone.

(c) *Propachlor std soln.*—Accurately weigh 0.2 g propachlor (recrystd from MeOH; Monsanto Chemical Co.) into small flask. Add by pipet 25 mL internal std soln and shake to dissolve.

6.B21 ***Determination***

Accurately weigh sample contg ca 0.2 g propachlor into small flask. Add by pipet 25.0 mL internal std soln and shake ≥5 min to ext propachlor. For flowable formulations, use 0.5 g sample; for Ramrod®/Atrazine flowable formulations, use 0.6 g sample; for granular formulations (20 G), use 1.0 g sample. Thoroly mix solns by mech. shaking. Vigorously mix granular samples 15 min.

Make replicate 1 μL injections of propachlor std soln and measure response ratio, R (area propachlor peak/area internal std peak), for each injection. Repeat until consecutive response ratios agree ±0.5%.

Make duplicate injections of sample soln and det. av. R. Follow with injection of propachlor std soln; average R' for std before and after sample injection.

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

where R and R' = av. response ratios for sample and std, resp.; W and W' = wt (g) of sample and std, resp.; P = % purity of std.

CAS-1918-16-7 (propachlor)

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(6) The following interim liquid chromatographic method for determination of dicofol (4-chloro-α-(4-chlorophenyl)-α-(trichloromethyl)benzenemethanol) in pesticide formulations was adopted first action:

Dicofol in Pesticide Formulations

Liquid Chromatographic Method

First Action

6.B22 ***Principle***

Dicofol is dissolved in MeOH, sepd by liq. chromatgy, and detd by comparison of peak hts of stds and samples.

6.B23 ***Apparatus***

(a) *Liquid chromatograph.*—Provided with pulseless, const flow pump and 15 μL sample loop or auto-injector. Operating conditions: mobile phase flow rate 2.0 mL/min; detector sensitivity 0.5 AUFS; temp., 30°.

(b) *Detector.*—UV spectrophtr or fixed wavelength UV detector at 254 nm.

(c) *Recorder.*—Range to match output of LC detector.

(d) *Liquid chromatographic column.*—Analytical: stainless steel, 250 × 4.6 mm, packed with Zorbax C8, 6 μm spherical particles (DuPont Co., Instruments Div., Concord Plaza, Wilmington, DE 19898). Guard: stainless steel, 50 × 4.6 mm, packed with LiChrosorb RP-18, 10 μm particle size (Merck, MC/B Manufacturing Chemists, Inc.).

(e) *Filter.*—Millex-HV disposable filter assembly, 0.45 μm (Millipore Corp., or equiv.).

6.B24 ***Reagents***

(a) *Mobile phase.*—MeOH–H_2O–HOAc (75 + 25 + 0.2), components individually filtered thru 0.45 μm filter. Mix 750 mL MeOH, 250 mL H_2O, and 2 mL glacial HOAc, and degas.

(b) *Dicofol std soln.*—Accurately weigh ca 35 mg pure 1,1-bis(4-chlorophenyl)-2,2,2-trichloroethanol and ca 7.5 mg pure 1-(2-chlorophenyl)-1-(4-chlorophenyl)-2,2,2-trichloroethanol into 1 oz vial and add 20 mL MeOH by pipet.

6.B25 ***System Performance Check***

Dissolve, in 1 oz vial, ca 50 mg Kelthane tech. in 20 mL MeOH. Sample is very plastic; heat at 60° to liquefy.

Inject 15 μL onto liq. chromatgc column and det. sepn factor (α) for dicofol and *p,p'*- and *o,p'*-DDE isomers as follows:

$$\alpha = k'2/k'1$$

where $k'2$ and $k'1$ are column capacity ratios of isomer pairs, defined as follows:

$$k' = (t_r - t_o)/t_o$$

where t_r = elution time of retained component and t_o = dead vol. elution time. Sepn factors are 1.62 for dicofol *o,p'*- and *p,p'*-isomers, and 1.38 for DDE isomers. Sepn factors of 1.55 for dicofol pair and 1.25 for DDE pair would be approx. limits for proper performance. Performance check should also confirm that key components are adequately resolved. DDT isomer pairs should be resolved from DDE isomers and should fit between DDE isomers. Example chromatogram is shown in Fig. **6:B1.** Fig. **6:B2** is example of unacceptable resolution where DDT isomer pair is not resolved from DDE isomers.

6.B26 ***Preparation of Sample***

(a) *Kelthane MF.*—Accurately weigh ca 100 mg MF formulation in 1 oz vial and add 20 mL MeOH by pipet. Shake to dissolve. Filter thru 0.45 μm filter.

(b) *Kelthane EC.*—Accurately weigh ca 250 mg EC formulation in 1 oz vial and add 20 mL MeOH by pipet. Shake to dissolve. Filter thru 0.45 μm filter.

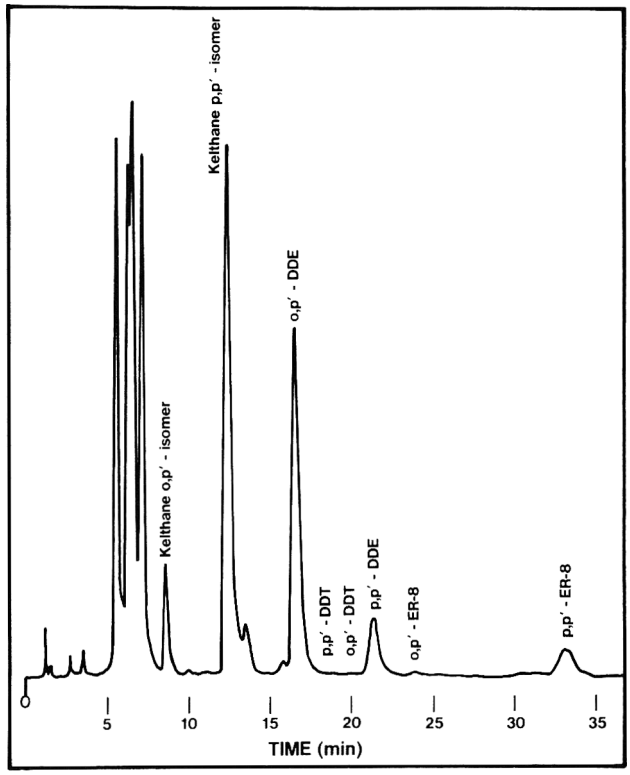

FIG. 6:B1—LC chromatogram of Kelthane technical

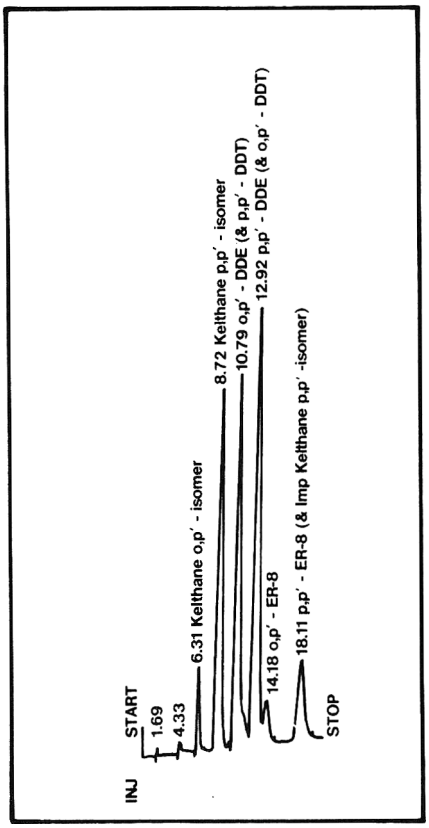

FIG. 6:B2—LC chromatogram of Kelthane standards

6.B27 ***Determination***

Inject 15 μL dicofol std soln. Det. peak hts of *p,p'*- and *o,p'*-dicofol. Inject 15 μL sample soln.

$$\%\ p,p'\text{-dicofol} = (PH/PH') \times (W'/W) \times 100$$

$$\%\ o,p'\text{-dicofol} = (PH/PH') \times (W'/W) \times 100$$

$$\%\ \text{Active ingredient} = \%\ p,p'\text{-dicofol} + \%\ o,p'\text{-dicofol}$$

where PH and PH' = peak ht of isomer in sample and std solns, resp.; W and W' = wt of sample and std injected, resp. Wt sample injected is calcd by wt = $15 \times W_v/20$, where W_v = wt of sample in vial, μg.

CAS-115-32-2 (dicofol)

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(7) The following gas chromatographic method for determination of fensulfothion (phosphorothioic acid *O,O*-diethyl *O*-[4-(methylsulfinyl)phenyl] ester) in pesticide formulations was adopted first action:

Fensulfothion in Pesticide Formulations

Gas Chromatographic Method

First Action

(Method is suitable for tech. and liq. formulations of fensulfothion.)

6.B28 ***Principle***

Sample is dissolved in CH_2Cl_2 contg 4-chlorophenyl sulfoxide as internal std, and fensulfothion is detd by gas chromatgy.

6.B29 ***Apparatus***

(a) *Gas chromatograph.*—Equipped with flame ionization detector (FID). Temps—column 225°, injection port 250°, detector 250°; carrier gas 30–40 mL/min (either He or N); air and H flows as recommended for FID; sample size 2.0 μL; retention times (min)—internal std 4.0, fensulfothion 5.5. Adjust parameters to cause fensulfothion to elute in 5–6 min, but do not use column temp. >240°. If internal std and fensulfothion peaks are not completely sepd, repack column.

(b) *Column.*—0.9 m (3 ft) or 1 m × 2 mm (id) glass column packed with 5% OV-330 on 80–100 mesh Chromosorb WHP (Supelco). Condition newly packed columns 8–16 h at 240° before use.

6.B30 ***Reagents***

(a) *4-Chlorophenyl sulfoxide.*—Aldrich Chemical Co., Cat. No. 12,104-5, or equiv. that contains no impurities eluting at retention time of fensulfothion.

(b) *Internal std soln.*—Weigh 1.0 g 4-chlorophenyl sulfoxide, dissolve in 1 L CH_2Cl_2, and mix well. Keep tightly stoppered.

(c) *Fensulfothion reference std soln.*—Accurately weigh amt of ref. std (Mobay Chemical Corp.) contg ca 100 mg fensulfothion into ca 100 mL glass bottle. Add by pipet 50.0 mL internal std soln. Stopper and mix well.

6.B31 ***Preparation of Sample***

Accurately weigh sample contg ca 100 mg fensulfothion into glass bottle (ca 100 mL). Pipet in 50.0 mL internal std soln. Stopper and mix well.

6.B32 ***Determination***

Make repetitive 2 μL injections of fensulfothion ref. std soln until response is stable and ratios of fensulfothion peak area to internal std peak area for successive injections agree within 1% of their mean. Peak ht may be substituted for peak area.

Make duplicate 2 μL injections of each sample. Response ratios (R) for fensulfothion internal std for 2 sample injections must agree ±1% of their mean. If not, repeat detn, starting with std injections. After every 4–6 sample injections and after last sample injection, make 2 injections of fensulfothion std soln. Av. std soln ratios preceding and following sample must be ±1.0% of mean; otherwise, repeat series of injections.

6.B33 ***Calculation***

Calc. ratios for each injection. Average 2 sample ratios and 4 std ratios (std injections immediately before and after sample injections).

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

where R and R' = av. sample and std ratios (fensulfothion peak/internal std peak), resp.; W and W' = mg sample and std, resp.; and P = % purity of fensulfothion std.

CAS-115-90-2 (fensulfothion)

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(8) The following liquid chromatographic method for determination of oxythioquinox (6-methyl-1,3-dithiolo[4,5-b]quinoxalin-2-one) in pesticide formulations was adopted first action as an AOAC-CIPAC method:

Oxythioquinox in Pesticide Formulations

Liquid Chromatographic Method

First Action

AOAC-CIPAC Method

(Method is suitable for tech. oxythioquinox and formulations with oxythioquinox as only active ingredient.)

6.B34 ***Principle***

Sample with 1-phenyl-1-pentanone internal std is extd with CH_3CN, and oxythioquinox is detd by reverse phase liq. chromatgy.

6.B35 ***Apparatus and Reagents***

(a) *Liquid chromatograph.*—Able to generate >10 MPa (>1430 psi) and measure *A* at 280 nm. Operating conditions: column temp. ambient; flow rate 2 mL/min (ca 5 MPa); chart speed 0.5 cm/min; injection vol. 10 μL; *A* range 0.320 AUFS; retention times: 1-phenyl-1-pentanone ca 3.1 min, oxythioquinox ca 5.4 min. Pump LC mobile phase thru column until system is equilibrated (flat baseline). *Allow each injection* ca 7 min run time, then pump CH_3CN ca 4 min to remove impurities. Pump LC mobile phase ca 4 min, allowing system to re-equilibrate before next injection.

(b) *Chromatographic column.*—250 × 4.6 mm id packed with ≤10 μm C18 bonded silica gel.

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

(d) *Chloroform.*—Spectrophtric grade or equiv.

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

(f) *1-Phenyl-1-pentanone (n-valerophenone) internal std soln.*—1 g/100 mL $CHCl_3$.

(g) *Reference std oxythioquinox.*—Mobay Chemical Corp.

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

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

6.B36 ***Preparation of Standard***

Accurately weigh ca 100 mg ref. std into 100 mL vol. flask. Pipet 10 mL internal std soln into flask and swirl to mix. Add ca 50 mL CH_3CN, sonicate 4 min, dil. to vol. with CH_3CN, and mix well. Filter portion of soln for LC analysis.

6.B37 ***Preparation of Sample***

Accurately weigh amt of sample contg ca 100 mg oxythioquinox into 100 mL vol. flask. Pipet 10 mL internal std soln into flask, and swirl to mix. Add ca 50 mL CH_3CN, sonicate 4 min, dil. to vol. with CH_3CN, and mix well. Filter portion of soln for LC analysis.

6.B38 ***Determination***

Adjust operating parameters to elute oxythioquinox in 5.0–5.9 min. Adjust injection size and attenuation to give largest possible on-scale peaks. Make repetitive injections of ref. std soln and calc. response ratios (R) = oxythioquinox peak area (or ht)/internal std peak area (or ht). Response ratios must agree ±1%. Average duplicate response ratios obtained with ref. std soln.

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

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

6.B39 ***Calculation***

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

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

CAS-2439-01-2 (oxythioquinox)

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(9) The following interim liquid chromatographic method for determination of bendiocarb (2,2-dimethyl-1,3-benzodioxol-4-yl methylcarbamate) in pesticide formulations was adopted first action:

Bendiocarb in Technical and Wettable Powder Pesticide Formulations

Liquid Chromatographic Method

First Action

(Applicable to tech. bendiocarb and its 20 and 80% wettable powder formulations)

6.B40 ***Principle***

Bendiocarb is extd from sample with fixed vol. of CH_3CN contg 0.1% v/v propiophenone internal std. Soln is filtered and chromatographed on reverse phase column with CH_3CN–H_2O (40 + 60) mobile phase. Compd is quantitated by comparison of response ratio for bendiocarb/propiophenone in sample and std.

6.B41 ***Apparatus and Reagents***

(a) *Liquid chromatograph.*—Const vol. pump, UV detector, injector. Injection system may be manual or automatic. Response may be measured by peak ht or peak area. Column: 250 × 4.6 mm id, 6.4 mm od, type 316 stainless steel, slurry-packed with Partisil 10 ODS 2 (Whatman Ltd). Mobile phase: Mix 800 mL CH_3CN with H_2O and dil. to 2 L with H_2O. Degas by applying reduced pressure until solv. just boils. Maintain this pressure 10 min.

Set UV detector to 254 nm. At 2 mL/min, pump 50 mL CH_3CN thru column, followed by 50 mL CH_3CN–H_2O (75 + 25). Change to mobile phase, pump 50 mL to waste, then connect system to recycle mobile phase.

(b) *Internal std soln.*—0.1% v/v propiophenone in CH_3CN.

(c) *Bendiocarb reference std soln.*—FBC Ltd, Hauxton, Cambridge, UK. Weigh 0.49–0.51 g bendiocarb into 100 mL g-s conical flask. Add, by pipet, 25.0 mL internal std soln. Inject 5 μL into system. Adjust flow rate to give bendiocarb peak at 3–5 min and internal std peak at 1.5 times elution time of bendiocarb.

6.B42 ***Preparation of Sample***

Accurately weigh amt of sample contg ca 0.50 g bendiocarb into 100 mL g-s conical flask. Use 0.49–0.51 g for tech. material, 0.61–0.64 g for 80% formulation, and 2.5–2.55 g for 20% formulation.

Using same pipet as for std, add 25.0 mL internal std soln. Swirl to dissolve. Filter soln, which contains suspended solids, thru suitable filter and use filtrate for liq. chromatgy.

6.B43 ***Determination***

It is necessary to establish that LC system has achieved stability and that it remains stable. After any period of idling, whether pump is running or not, make at least 3 injections of std soln. For each injection, measure bendiocarb and internal std peaks and calc. response ratio, bendiocarb/internal std. Re-run stds until response ratio achieves acceptable repeatability, then inject sample soln in duplicate. Follow duplicate sample injections with std. Average sample responses. Use as std response ratio the mean of those which occur on either side of sample injections.

6.B44 ***Calculation***

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

where R and R' = av. response ratios for sample and std, resp.; W and W' = wt (g) of sample and std, resp.; and P = % purity of std.

CAS-22781-23-3 (bendiocarb)

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(10) The following interim liquid chromatographic method for determination of carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranol methylcarbamate) in pesticide formulations was adopted first action:

Carbofuran in Pesticide Formulations

Liquid Chromatographic Method

First Action

6.B45 ***Principle***

Carbofuran is extd from sample with MeOH contg acetophenone as internal std. Soln is centrfgd and chromatographed on reverse phase column with MeOH–H_2O (50 + 50) mobile phase. Compd is quantitated by comparison of response ratio for carbofuran/acetophenone in sample and std.

6.B46 ***Apparatus and Reagents***

(a) *Liquid chromatograph.*—High pressure pump, capable of 5000 psi up to desired flow rate; sensitivity of 0.2 AUFS; UV detector with 8–12 μL flow-thru cells, operating at 280 nm; guard column (optional), Brownlee RP-18, No. 140–200 and guard cartridge No. 180-GU (Rheodyne, Inc., Berkeley, CA), ambient temp.; column, 150–300 × 3–5 mm id C18 (typically 250 × 4.1 mm), ca 40°; injector, Waters Associates Model U6K, Rheodyne Model 7120 or 7125, or Micromeritics Model 725, or equiv.; recorder, 10 in. 10 mV full scale at 0.5 cm/min.

(b) *Mobile phase.*—Use distd or distd deionized H_2O and distd in glass MeOH. Use prefiltered solvs or filter thru submicron filters, 0.5 μm (Millipore or Gelman). Measure H_2O and MeOH (50 + 50) and combine; do not add one to the other to det vol. Alternatively, use 2-pump liq. chromatgc system with sep. metering for each solv. Flow rate 1 mL/min.

(c) *Internal std soln.*—0.5 mg acetophenone (≥98%, Parrish Chemical Co., Aldrich Chemical Co., Pfaltz and Bauer, or equiv.)/mL MeOH, distd in glass.

(d) *Carbofuran reference std soln.*—0.5 mg carbofuran (FMC Corp., Agricultural Chemical Group, Group Quality Assurance, 100 Niagara St, Middleport, NY 14105)/mL MeOH. Also contg 0.5 mg/mL of 7-hydroxycarbofuran (FMC Corp.).

6.B47 ***Preparation of Sample***

Use balance capable of 0.01 mg resolution, or increase all wts and vols by factor of 10. Accurately weigh amt of sample contg ca 8–10 mg carbofuran into 100 mL g-s conical flask. Add 20 mL internal std soln. Swirl to dissolve. Ext ca 30 min on reciprocating shaker or 2 min on vortex mixer. Centrf. and use supernate for liq. chromatgy.

6.B48 ***Determination***

Optimize column as follows: Set column temp. to 40°. Det. retention time of carbofuran (preferably 12–15 min). Alter mobile phase if necessary. Det. retention time and resolution of acetophenone relative to carbofuran. If acetophenone is not resolved at baseline from carbofuran, alter mobile phase by reducing MeOH. This may result in retention time >15 min for carbofuran on some columns.

Det. retention time of 7-hydroxycarbofuran. If it interferes with carbofuran, further optimization is required: Increase or decrease column temp., in 5° increments, and alter mobile phase composition to maintain the resolution and retention times of carbofuran and acetophenone const. Note retention time and resolution of 7-hydroxyfuran peak. When it is well resolved from carbofuran, impurity interferences are minimized and sample analysis can begin.

Inject 10 μL sample. Det. response ratio R for peak ht (or area) of carbofuran/peak ht (or area) of internal std.

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

where R' and R = av. response ratio for std and sample solns, resp.; W' and W = wt (mg) of carbofuran std and sample, resp.; and P = % purity of carbofuran std.

On some high resolution columns, flow of 2 mL/min can be used to reduce analysis time. If signs of decomposition are noted, 3–5 drops of H_3PO_4/L may be added to mobile phase.

CAS-1563-66-2 (carbofuran)

7. ANIMAL FEED

No additions, deletions, or other changes.

8. BAKING POWDERS AND BAKING CHEMICALS

No additions, deletions, or other changes.

9. BEVERAGES: DISTILLED LIQUORS

No additions, deletions, or other changes.

10. BEVERAGES: MALT BEVERAGES AND BREWING MATERIALS

No additions, deletions, or other changes.

11. BEVERAGES: WINES

No additions, deletions, or other changes.

12. BEVERAGES: NONALCOHOLIC AND CONCENTRATES

No additions, deletions, or other changes.

13. CACAO BEAN AND ITS PRODUCTS

No additions, deletions, or other changes.

14. CEREAL FOODS

The following ion-exchange method for determination of phytate in foods was adopted first action:

Phytate in Foods

Anion-Exchange Method

First Action

14.B01 ***Principle***

Phytate is extd from duplicate samples of dried foods using dil. HCl. Ext is mixed with EDTA/NaOH soln and placed on an ion-exchange column. Phytate is eluted with 0.7M NaCl soln and wet-digested with mixt. of concd HNO_3/H_2SO_4 to release P, which is measured colorimetrically. Amt of phytate in original sample is calcd as hexaphosphate equiv.

14.B02 ***Apparatus***

(a) *Glass barrel columns.*—0.7 × 15 cm, equipped with valve (Econo-columns, Bio-Rad Laboratories, or equiv.).

(b) *Anion exchange resin.*—AG1-X4, 100–200 mesh, chloride form (Bio-Rad Laboratories). Check resin (according to method below) by measuring recovery of purified Na phytate.

(c) *Micro-Kjeldahl flasks.*—100 mL, or 25 × 200 mm digestion tubes.

(d) *Micro-Kjeldahl digestion rack.*

(e) *Spectrophotometer.*—To read at 640 nm.

14.B03 ***Reagents***

(a) *HCl.*—2.4%. Add 54 mL HCl to 1 L vol. flask and dil. to vol. with H_2O.

(b) *NaCl solns.*—0.1 and 0.7M.

(c) *Phosphate std soln.*—80 μg/mL. Weigh 0.350 g dried, desiccated K acid phosphate (primary std) into 1 L vol. flask, add ca 500 mL H_2O and 10 mL 10N H_2SO_4, and dil. to vol. with H_2O.

(d) *Molybdate soln.*—2.5% ammonium molybdate in 1N H_2SO_4. Dissolve 12.5 g ammonium molybdate in 200 mL H_2O. Transfer to 500 mL vol. flask, add 50 mL 10N H_2SO_4, and dil. to vol. with H_2O. Stable.

(e) *Sulfonic acid reagent (1-amino-2-naphthol-4-sulfonic acid).*—Dissolve 0.16 g 1-amino-2-naphthol-4-sulfonic acid, 1.92 g Na_2SO_3, and 9.60 g $NaHSO_3$ in 90 mL H_2O. Quant. transfer to 100 mL vol. flask. Heat to dissolve if necessary. Dil. to vol. with H_2O. (Store in brown bottle in refrigerator. Prep. fresh weekly.)

(f) *Na_2EDTA–NaOH reagent.*—In 250 mL flask, stir 10.23 g Na_2EDTA (0.11M) and 7.5 g NaOH (0.75M). Dil. to vol. with H_2O. Stable.

14.B04 ***Preparation of Phosphate Standard Curve***

Adjust spectrophtr to 640 nm, and equilibrate ≥15 min.

Pipet 1.0, 3.0, and 5.0 mL P std soln into 50 mL vol. flasks. Add ca 20 mL H_2O. Mix thoroly. Add 2 mL molybdate soln. Mix well.

Add 1 mL sulfonic acid soln. Mix well. Dil. to vol. with H_2O. Mix well. Wait 15 min, and read in spectrophtr at 640 nm. Calcns for typical std curve:

Std soln, 80 μg P/mL			
mL std	μg P	*A*	Concn/*A* (*K*)
1.0	80	0.1805	443.21
3.0	240	0.516	465.12
5.0	400	0.852	469.48
"Mean *K*"			459.27

14.B05 ***Determination***

Accurately weigh ca 2.0000 g sample and place in 125 mL erlenmeyer. Add 40 mL 2.4% HCl (20 mL of 2.4% HCl/g sample.) Cover flask and shake vigorously 3 h at room temp.

Meanwhile, prep. columns. Add 3 mL H_2O to empty mounted column and then pour H_2O slurry of 0.5 g resin into column. After resin bed has formed, wash column with 15 mL of 0.7M NaCl. Wash column with 15 mL H_2O.

Remove sample from shaker and filter with vac. thru Whatman No. 1 paper. Sample ext is stable at least 1 week if refrigerated.

Prep. blank by mixing 1 mL 2.4% HCl with 1 mL Na_2EDTA–NaOH reagent, dil. to 25 mL with H_2O, and pour mixt. onto column.

Pipet 1.0 mL filtrate into 25 mL g-s graduate. Add 1.0 mL Na_2EDTA–NaOH reagent. Dil. to 25 mL with H_2O. Mix and quant. transfer to column; discard eluate. Elute with 15 mL H_2O; discard eluate. Elute with 15 mL 0.1M NaCl; discard eluate. Elute with 15 mL 0.7M NaCl; collect this 0.7M fraction in digestion vessel. Add 0.5 mL H_2SO_4 and 3.0 mL HNO_3 to flask. Add 3 glass beads. Before adding next sample to column, pour 15 mL H_2O thru column. After 1 week or 3 samples, discard old resin and replace with fresh resin.

Digest under hood on micro-Kjeldahl rack over medium heat until active boiling ceases, and cloud of thick yellow vapor fills neck of flask. Heat contents 5 min more on medium heat, 5 min on low heat, then turn off burner.

When flask is cool, add ca 10 mL H_2O, swirl, or heat flask on low temp. setting if necessary to dissolve salt. Continue heating flask on low temp. 10 min. Let soln cool. Quant. transfer soln to 50 mL vol. flask. Add 2.0 mL molybdate soln; mix well. Add 1.0 mL sulfonic acid reagent; mix well. Dil. to vol., mix well, let stand 15 min, and read *A* at 640 nm. Calc. phytate concn in food or feed:

$$\text{Phytate, mg/g sample} = \text{"mean } K\text{"} \times A \times 20/(0.282 \times 1000)$$

where A = absorbance; "mean K" = std P (μg)/A/n (stds); phytate = 28.2% P.

15. COFFEE AND TEA

No additions, deletions, or other changes.

16. DAIRY PRODUCTS

(1) The first action quantitative *Bacillus stearothermophilus* disc method for determination of beta-lactam antibiotics in milk, **16.135–16.139,** was editorially revised to include a statement that in the case of any dispute or controversy, method **16.163** is the conclusive method for the quantitative determination of penicillin in milk. (Ref.: JAOAC **65,** 389(1982).)

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(2) The following technique for sampling barrel cheese was adopted first action:

16.B01 ***Sampling Technic—First Action***

Sampling by means of trier.—Insert 30.5 cm (12 in.) blade length trier 7 cm (2.75 in.) from edge of cheese and toward nearest outside edge of barrel at 11° from vertical. Center of plug hole shall be 7 cm from edge of cheese. Trier guide fixed at 11° angle is available and may be used as aid (NCI, 699 Prince St, Alexandria, VA 22314).

If cheese barrel is full, i.e., not >2–3 cm headspace, it is possible to draw reliable sample thru bung or sample port in cover which permits insertion of trier at 7 cm point. If headspace is >3 cm, remove cover; otherwise, point of trier insertion will be distorted. In no instance should barrel contg cheese from more than 1 vat be selected as sample for moisture analysis.

For reliable sample, insert trier to draw full 27.9–30.5 cm (11–12 in.) plug from full container. If plug breaks short of 25.4 cm (10 in.), draw another plug from different location 7 cm from edge. For plug between 25.5 and 30.5 cm, remove top 11.4 cm (4.5 in.) for sealing plug hole. Transfer next 10.2 cm (4 in.) portion to sample container. Discard remaining bottom portion of plug.

Ref. JAOAC **68,** 718(1985).

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(3) A provision to use the 5% Gercock milkfat test bottle was adopted first action as an addition in **16.065**:

16.B02 ***Alternative Apparatus—First Action***

Alternative Gercock-style milkfat test bottle.—5%, 162 ± 3 mm high. Bottom of bottle is flat, and axis of flat graduated tube is vertical when bottle stands on level surface. Quantity of milk charge is 18 g; 18 g is acid-etched and pigmented in clear space above 5% graduated line. Specifications:

(*1*) *Bulb.*—Capacity of bulb to junction with neck must be ≥45 mL. Shape of bulb is cylindrical, 36 ± 0.5 mm od. Wall thickness 1.4 ± 0.2 mm. Word "Sealed", manufacturer's number or name, and matted marking spot must be legibly and permanently marked on surface.

(*2*) *Side filling tube.*—8 mm od, 0.1 mm wall thickness. To enter bulb below junction of bulb and neck of flat graduated tube along curved portion of bulb and with min. angle extend to center of bulb and maintain vertical axis for 40 mm to ht of 3 ± 2 mm above bottom of bottle.

(*3*) *Neck.*—Graduated portion of flat tube is uniform cross section ≥4 mm beyond extremes of graduated scale. Length of graduated portion of flat neck ≥75 mm. Graduated at whole %, 0.5%, 0.1%, and 0.05%. Graduations are acid-etched, with black pigment annealed to graduation. Graduation widths ≤0.2 mm. Each whole % and 0.5% line is numbered on left above line and these lines extend across flat surface. Smallest graduated line is ≥2.5 mm. Intermediate graduation line extends 1 ± 0.2 mm equally on both sides of smallest graduated line.

(*4*) *Vertical line.*—Vertical line is acid-etched and pigmented on right side of graduated flat tube. This line touches intermediate lines at their extreme right end and extends beyond 0 and 0.5% lines ≥3 mm. Width of vertical lines ≤0.2 mm.

(*5*) *Error.*—Max. error of total graduation or any part thereof must not exceed vol. of smallest unit of graduation (=0.01 mL).

(*6*) *Gercock-style pipet—raw milk.*—To conform to following specifications:

Total length	≤280 nm
Suction tube diam.	7 ± 0.2 mm od
Wall thickness	0.9 ± 0.04 mm
Suction tube length	130 mm
Delivery tube diam.	5 ± 0.2 mm od
Distance of graduation mark above bulb	15–45 mm
Length of nozzle	15–20 mm
Delivery time	5–8 s
Graduation	To deliver 17.6 mL at 20° when bottom of meniscus coincides with mark on suction tube
Max. error	≤0.05 mL
Permanent markings on bulb	"Sealed", mfgr's No. or name, TD 17.6 mL at 20°, tolerance 0.05 mL, 5–8 s

(7) *Gercock-style pipet—processed milk.*—To conform to following specifications:

Total length	≤280 mm
Suction tube diam.	7 ± 0.2 mm od
Wall thickness	0.9 ± 0.04 mm
Length of suction tube	130 mm
Delivery tube diam.	5 ± 0.2 mm od
Distance of graduation mark above bulb	15–45 mm
Length of nozzle	15–20 mm
Delivery time	5–8 s
Graduation	To deliver 17.6 mL at 38° when bottom of meniscus coincides with mark on suction tube
Max. error	≤0.05 mL
Permanent markings on bulb	"Sealed", mfgr's No. or name, TD 17.6 mL at 38°, tolerance 0.05 mL, 5–8 s

(Glassware is available from Forcoven Products, Inc., PO Box 1556, Humble, TX 77338.)

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(4) An alternative rapid sample preparation for determination of residual phosphatase in casein was adopted first action as an addition in **16.127–16.129**:

Phosphatase (Residual) in Casein

16.B03 *Alternative Rapid Sample Preparation—First Action*

Place ca 25 mL 7.5% butyl alcohol, **16.127(i)**, in 100 mL vol. flask, add 3.00 g casein, and shake 1 min. Add 10 mL carbonate buffer, **16.121(a)**, and shake 1 min. Let stand 1 min. Dil. to vol. with 7.5% butyl alcohol. Invert and shake flask until casein dissolves, or use ultrasonic bath to hasten dissolution of casein. Store casein soln in refrigerator overnight.

17. EGGS AND EGG PRODUCTS

No additions, deletions, or other changes.

18. FISH AND OTHER MARINE PRODUCTS

The following headspace gas chromatographic method for determination of ethanol in canned salmon was adopted first action:

Ethanol in Canned Salmon

Headspace Gas Chromatographic Method

First Action

18.B01 *Principle*

Liq. from canned salmon is sepd into oil and aq. phases. Aq. phase is analyzed for EtOH by headspace gas chromatgy and flame ionization detection. Ratios of peak areas of EtOH to internal std in sample and std are compared.

18.B02 *Apparatus*

(a) *Gas chromatograph.*—Model 5880A, equipped with flame ionization detector (Hewlett-Packard, Avondale, PA), or equiv. (Equiv. system must include electronic data system or integrator capable of measuring peak areas for off-scale peaks.) Representative operating conditions: Temps—injector 200°, detector 250°, column 150°; gas flows—N carrier gas, 50 mL/min, H 45 mL/min, air 500 mL/min. Suggested sensitivity: Choose attenuation so that injection of 5 mL headspace from ca 11 ppm headspace std (contains ca 11 ppm EtOH and 4.2 ppm *tert*-BuOH; see prepn of headspace stds) gives *tert*-BuOH peak ≥50% <100% FSD. EtOH peak will then be ≥25% FSD.

(b) *Gas chromatographic column.*—6 ft × 4 mm id glass, packed with 80–100 mesh Super Q (Alltech Associates, Inc., Deerfield, IL). With column in place and connected to detector, purge with N carrier gas at 50 mL/min at 33° (ambient temp.) for 30 min. Increase temp. at 3°/min to 225° and hold for 2 h. Decrease to operating temp., let column stabilize, adjust carrier gas flow to 50 mL/min, and let column further stabilize overnight.

System check.—For operating conditions given in (a), retention times are ca 3.5–3.8 min for EtOH; and ca 10.4–11.5 min for *tert*-BuOH. However, adjust column temp. for adequate resolution between EtOH and *tert*-BuOH peaks, and any significant product peaks. For some products, small peak may occur at retention time ca 8.4–8.8 min; this should not significantly overlap *tert*-BuOH peak.

(c) *Syringes.*—Gas-tight, Hamilton No. 1005-LTN (5.0 mL capacity) or No. 1010-LTN (10.0 mL capacity) (The Anspec Co., Inc., Ann Arbor, MI).

(d) *Headspace vials.*—Glass, Kimble Cat. No. 60910-L, 23 × 85 mm, 6 dram (ca 22 mL) capacity (Ace Glass, Inc.) fitted with perforated screw cap (Cat. No. 95053) with Teflon-faced liner (Cat. No. 9522) (both screw cap and liner from Alltech Associates, Inc.).

(e) *Continuously adjustable digital microliter pipet.*—Pipetman Model P-200, 20–200 μL range (Gilson, Cat. No. P-200), equipped with disposable microliter pipet tips (Rainin Cat. No. RC-20) (both from Rainin Instrument Co., Inc., Woburn, MA), or equiv.

18.B03 *Reagents*

(a) *Sodium chloride crystal.*—Baker Analyzed Reagent (J.T. Baker Chemical Co.).

(b) *tert-Butanol internal std solns.*—(*1*) *Stock soln.*—Approx. 6000 ppm. Into tared 250 mL vol. flask, pipet 2.0 mL liq. *tert*-BuOH (99.5%, Aldrich Chemical Co., Inc.). Reweigh stoppered flask and its contents to det. wt of *tert*-BuOH (ca 1.50 g). Dil. to vol. with H_2O. (*2*) *Working soln.*—Approx. 108 ppm. Pipet 9.0 mL stock soln into 500 mL vol. flask and dil. to vol. with H_2O.

(c) *Ethanol std solns.*—(*1*) *Stock soln.*—Approx. 15 600 ppm. Into tared 100 mL vol. flask, pipet 2.0 mL absolute alcohol (USP, U.S. Industrial Chemicals Co., New York, NY). Reweigh stoppered flask and its contents to det. wt of EtOH (ca 1.56 g). Dil. to vol. with H_2O. (*2*) *Intermediate soln.*—Approx. 1090 ppm. Pipet 7.0 mL stock soln into 100 mL vol. flask and dil. to vol. with H_2O. (*3*) *Working solns.*—Solns A, B, C, and D, ca 22, 44, 76, and 109 ppm, resp. Pipet 2.0, 4.0, 7.0, and 10.0 mL intermediate soln into sep. 100 mL vol. flasks and dil. each to vol. with H_2O. Soln E, ca 11 ppm. Pipet 10.0 mL soln D (ca 109 ppm) into 100 mL vol. flask and dil. to vol. with H_2O.

Prep. fresh EtOH std solns weekly.

18.B04 *Check for Possible Contamination*

(a) *Air.*—Using gas-tight syringe, inject 5 mL air (in location where aliquots will be withdrawn from headspace stds and samples) into GC app. to ensure that syringe is not contaminated and that air does not contain compds that significantly interfere with EtOH and *tert*-BuOH peaks in analysis.

(b) *Blank.*—Pipet 5.0 mL H_2O into glass headspace vial. Add 3.0 g NaCl and seal. Hold vial in vertical position so as not to wet Teflon-faced liner while swirling. Swirl contents vigorously 2 min, and then let stand ≥5 min. Withdraw 5 mL aliquot from headspace into gas-tight syringe by withdrawing plunger in single, slow, continuous action; then inject it into the GC app. to det. any significant interference with EtOH and *tert*-BuOH peaks in analysis. This should be done in same location where air is analyzed for contamination, (a).

When air contains low levels of interfering compds, analysis of blank may reveal that levels are too low to cause significant interference with headspace analysis. However, analysis of blank may indicate that headspace must be withdrawn from headspace vials where air is free from interfering compds. If air in room is contaminated with EtOH, headspace stds and samples can be prepd in that room, but headspace must be withdrawn in area free of EtOH contamination. If it is then brought back into contaminated

room and immediately injected into GC app., analysis will not be contaminated with EtOH.

18.B05 ***Preparation of Headspace Standards***

Pipet 5.0 mL EtOH working std soln into glass headspace vial. Then add 200 μL *tert*-BuOH working internal std soln (ca 108 ppm), using adjustable microliter pipet (Model P-200) to give *tert*-BuOH concn of ca 4.2 ppm. Gently mix 3–4 s, add 3.0 g NaCl, and seal vial. Holding vial in vertical position so as not to wet Teflon-faced liner while swirling, swirl contents vigorously 2 min, and then let stand ≥5 min. Inject ca 5 mL aliquot of headspace into GC app. (see below).

18.B06 ***Preparation of Calibration Curve***

Prep. headspace stds as previously described using EtOH working std solns A, B, C, D, and E (ca 11–109 ppm). Analyze each headspace std as follows: Withdraw 5 mL aliquot of headspace from vial into gas-tight syringe by withdrawing plunger in single, slow, continuous action; then inject it into GC app. If for any reason a second injection is required, prep. new headspace std. Between injections, pump syringe ≥10–15 times to eliminate gases and H_2O vapor from previous injection to avoid contamination.

Use peak areas only for quantitation. To det. peak area, use tangent skimming for EtOH peak, on tail of air peak. For each headspace std, calc. peak area ratio, R = area EtOH peak/area *tert*-BuOH peak. Prep. calibration curve as follows: For each headspace std, plot R against concn of EtOH working std soln used to prep. that std. Draw best curve that fits points on graph, or use automated curve fitting or multi-level calibration if instrument is so equipped.

18.B07 ***Canned Salmon Aqueous Phase***

Open can and drain liq. into 250 mL beaker while pressing lid against contents. Retain salmon for sensory analysis if appropriate. Transfer liq. to 250 mL separator and let oil and aq. phases sep. Drain aq. phase into g-s cylinder and store until analysis.

18.B08 ***Preparation of Headspace Samples***

Use same procedure given for prepn of headspace stds, except transfer 5.0 mL sample soln into headspace vial with accurate 5 mL Mohr-style pipet. For sample soln, use either undild canned salmon aq. phase, or, when necessary for GC analysis (see below), canned salmon aq. phase accurately dild with H_2O.

18.B09 ***Analysis of Headspace Samples***

Use same procedure given for analysis of headspace stds (see prepn of calibration curve) and inject 5 mL aliquot of headspace into GC app. Analysis time for samples is ca 38 min because of late-eluting peak at ca 35 min.

Calc. peak area ratio, R, and det. EtOH concn in sample soln from calibration curve.

If EtOH concn is higher than that of most concd std, accurately dil. original canned salmon aq. phase with H_2O to give concn within calibration limits, and reanalyze dild sample. Multiply by diln factor to obtain concn for original undild sample.

19. FLAVORS

No additions, deletions, or other changes.

20. FOOD ADDITIVES: DIRECT

No additions, deletions, or other changes.

21. FOOD ADDITIVES: INDIRECT

No additions, deletions, or other changes.

22. FRUITS AND FRUIT PRODUCTS

(1) The following liquid chromatographic method for determination of quinic, malic, and citric acids in apple juice and cranberry juice cocktail was adopted first action:

Quinic, Malic, and Citric Acids in Cranberry Juice Cocktail and Apple Juice

Liquid Chromatographic Method

First Action

22.B01 ***Principle***

Juice is eluted thru disposable cartridge to remove interferences, filtered, and injected into liq. chromatgc system. Quinic, malic, and citric acids are sepd by using 2 reverse phase LC columns in tandem with UV detection at 214 nm. Compds are quantitated by comparison with external stds.

22.B02 ***Apparatus and Reagents***

(a) *Liquid chromatograph.*—System equipped with Model U6K injector, Model 450 variable wavelength detector operable at 214 nm, 0.1 AUFS (Waters Associates, Inc.), and computing integrator (Hewlett-Packard Integrator 3390, or equiv.).

(b) *Analytical columns:*—(*1*) Supelcosil LC-18, or equiv., 5 μm particle size, 25 cm × 4.6 mm, in tandem with and followed by (*2*) Radial-Pak C18 cartridge (Waters Associates, Inc.), 5 μm particle size, 10 cm long, used with Radial Compression Module. Radial-Pak C18 cartridge can be substituted by any std 25 or 30 cm stainless steel reverse phase C18 column with 10 μm particle size. Connect Bio-Rad reverse phase micro-guard column (ODS-10) ahead of column 1. Mobile phase: phosphate buffer at 0.8 mL/min; sensitivity 0.1 AUFS.

(c) *Disposable cartridges.*—Sep-Pak C18 (Waters Associates, Inc.).

(d) *Chemicals.*—Potassium phosphate monobasic (KH_2PO_4), 85% H_3PO_4, MeOH, CH_3CN. Filter all solvs thru aq. or org. 0.45 μm filter.

(e) *LC mobile phase.*—Phosphate buffer, 0.2M KH_2PO_4, pH 2.4. Weigh 27.2 g KH_2PO_4 in beaker. Add H_2O to 950 mL. Using pH meter and H_3PO_4, adjust to pH 2.4. Pour into 1 L graduate and adjust to vol. with H_2O; filter.

22.B03 ***Preparation of Sample and Standards***

(a) *Working std solns.*—Accurately weigh, to nearest 0.1 mg, 0.200 g ACS grade quinic, malic, and citric acids. Dil. combined acids to 100 mL in vol. flask with H_2O; filter.

(b) *Sample solns.*—Condition cartridge by eluting 10 mL CH_3CN–H_2O (50 + 50) thru 10 mL Luer-Lok syringe. Remove syringe and pass 10 mL air thru cartridge. Elute 10 mL sample thru conditioned cartridge. Discard first 4–5 mL and collect next 4–5 mL. Filter sample for LC analysis.

22.B04 ***Determination***

Condition system with 100% MeOH (or MeOH–H_2O (70 + 30)) followed by H_2O and then phosphate buffer. Reverse order at end of working day; *never let MeOH come into contact with phosphate buffer*. Operating conditions: flow rate 0.80 mL/min; 214 nm detector; temp. ambient; sensitivity 0.1 AUFS. Column system is satisfactory when baseline sepn is achieved between sugar front peak and quinic acid peak in cranberry juice cocktail and/or apple juice. Inject 5–20 μL (with U6K) std soln after each 2 sample injections to check linearity. Inject 5 μL sample soln. Use av. of 2 injections for std and sample response. Elution pattern: quinic, malic, citric, fumaric.

22.B05 ***Calculations***

Quinic, malic, citric, or fumaric acid, %

$$= (PA/PA') \times (V'/V) \times C$$

where PA and PA' = peak area of sample and std, resp.; V and V' = vol. of sample and std. resp.; and C = concn of std, %.

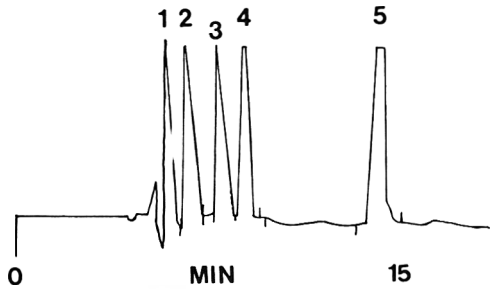

FIG. 22:B1—LC chromatogram of cranberry juice cocktail sample: (1) solvent and sugar front (2) quinic acid; (3) malic acid; (4) ascorbic acid and some shikimic acid; (5) citric acid

Note: Malic stds (L-malic or D-L-malic) will yield 2 peaks, the second of which is fumaric acid. Amt of fumaric acid present in malic acid std is so small that std is ≥99.9% fumaric acid-free. Impurity does not affect analysis. Cranberry juice cocktail, fortified with vitamin C, will exhibit large peak appearing after malic. (This is due to coelution of ascorbic acid + shikimic acid and is not quantified.) For details about this sepn see Fig. **22:B1.**

CAS-77-92-9 (citric acid)
CAS-6915-15-7 (malic acid)
CAS-77-95-2 (quinic acid)

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(2) The following interim UV/vis and fluorescence spectrophotometric method for detection of adulteration of processed Florida orange juice was adopted first action:

Adulteration of Processed Florida Orange Juice
UV/Vis and Fluorescence Spectrophotometric Method
First Action

(Applicable only to processed Florida orange juice; method is qualitative only.)

22.B06 ***Principle***

Orange juice produced or packed in Florida cannot contain pulpwash solids. Adulteration by diln and/or addn of pulpwash is qualitatively detected by comparison of UV/vis absorption and fluorescence excitation and emission spectra, by measuring sum of *A* at 443, 325, and 280 nm, and by ratios of *A* at 443/325 nm.

22.B07 ***Apparatus***

(a) *UV/vis spectrophotometer.*—Coleman Model 124 UV/vis recording spectrophtr (Perkin-Elmer Corp.) or equiv. with capability to scan from 600 to 200 nm with spectral bandwidth of 2 nm, and interfaced to recorder having scale expansion capabilities.

(b) *Fluorescence spectrophotometer.*—Farrand Mark I ratio spectrofluorometer or equiv. instrument stdzd with solid Pyrex std with excitation at 315 nm and emission at 355 nm, range setting 0.3, sample-ratio switch on sample, and fluorescence intensity adjusted with gain to 0.180 μamp

22.B08 ***Orange Juice and Pulpwash Standards***

Authentic frozen concentrated orange juice (FCOJ) and pulpwash samples with ref. UV/vis and fluorescence spectra may be obtained from State of Florida Dept of Citrus, 700 Experiment Station Rd, Lake Alfred, FL 33850. Use authentic products and ref. spectra for method familiarization and to verify instrumental performance. Reconstitute FCOJ and pulpwash stds to 11.8° Brix as in **22.026.**

22.B09 ***Reagents***

Alcohol.—Absolute EtOH (U.S. Industrial Chemicals or equiv.). Verify absence of fluorescence background at wavelengths of interest (**22.B11(b)**).

22.B10 ***Preparation of Sample***

Concentrated orange juice.—Reconstitute com. frozen concd orange juice according to product label directions, using graduated cylinder for vol. measurements; mix thoroly, and det. total sol. solids (°Brix) as in **22.026.** Dil. reconstituted samples further with equal vol. of H_2O. Take 5 mL of this sample, dil. to 50 mL with absolute EtOH, and place in dark at room temp. for <24 h. Following storage, centrf. sample at 1500 rpm for 5 min to sep. flocculent ppt from serum (soln). Use serum directly for UV/vis spectrophotometry; mix equal vols of serum and 90% EtOH for fluorescence characterization.

Single-strength orange juice.—Det. total sol. solids (°Brix) of single-strength juice by **22.026.** Prep. single-strength juice sample by dilg thoroly mixed juice with equal vol. of H_2O. From this point, prepn procedure is same as for concd orange juice, viz., "Take 5 mL of this sample . . .".

22.B11 ***Analysis***

(a) *UV/vis analysis.*—Transfer aliquot of clear serum into 10 mm quartz cuvet; scan (spectral bandwidth of 2 mm) serum from 600 to 200 nm at 60 nm/min; use appropriate *A* range settings for different portions of spectrum: 600–370 nm, 0–0.5 absorbance; 370–300 nm, 0–2 absorbance; 300–200 nm, 0–5 absorbance. Record sample *A* at 443, 325, and 280 nm and sum (443 + 325 + 280); also calc. and record 443/325 nm *A* ratio. Multiply *A* sum of sample by 1.085 (ratio of 12.8/11.8° Brix) to give adjusted absorbance sum (AAS). AAS value is required to relate results to data in JAOAC **63**, 1317(1980), which were obtained during Florida's 12.8° Brix reconstitution requirement.

$$1.085 \times (443 + 325 + 280)_{sample} = AAS$$

(b) *Fluorescence analysis.*—Det. fluorescence excitation and emission spectra of EtOH-dild serum sample at room temp. by using following parameters:

Excitation, nm	*Emission, nm*	*Range setting*
340	423	0.1
302	353	0.1
290	343	0.1
283	333	0.1
270	333	0.1
230	310	0.03

Fluorescence spectra are qual. and confirmatory in combination with UV/vis spectra. Fluorescence spectra confirm adulteration of orange juice by pulpwash addn or H_2O diln.

22.B12 ***Interpretation***

To distinguished types of adulteration, description of spectral shifts and intensity is outlined:

Adulteration by dilution.

(a) *UV/vis spectra.*—Simple diln causes noticeable shift of 223 nm peak to shorter wavelength; dild sample will register peak in region extending from 223 to 206 nm; diln causes adjusted *A* (AAS) to occur near or below lower end of distribution range for authentic samples (2.002–2.992; 2.410 ± 0.164).

(b) *Fluorescence spectra.*—Diln causes reduction in intensity of 340 nm (excitation) and 423 (emission) peaks; nondild orange juice samples show fluorescence intensity of 0.06–0.07 for both these wavelengths.

Adulteration by addition of pulpwash.

(a) *UV/vis spectra.*—Pulpwash addn causes the following: reduction in 443/325 nm ratio, normal range of orange juice is 0.09–0.23 (0.144 ± 0.026); increased AAS; lack of resolution for 443 and 425 nm peaks; increased resolution of 280 nm peak; shift in 223 nm peak toward 230 nm; *A* >1.0 for 325 nm peak strongly indicates adulteration.

(b) *Fluorescence spectra.*—Excitation and emission characterization of orange juice and pulpwash:

Excitation, nm	Emission, nm	Range setting	Excitation spectra	
			Orange juice	Pulpwash
340	423	0.1	strong	much stronger
302	353	0.1	inflection	strong max.
290	343	0.1	strong max.	min. or inflection
283	333	0.1	strong max.	min.
270	333	0.1	inflection	shoulder or max.
230	310	0.03	weak	weaker

Adulteration by addition of pulpwash and by dilution.

(a) *UV/vis spectra.*—Adulteration causes the following: decrease in 443/325 nm ratio and lack of resolution of 443 nm peak; *A* at 325 and 280 nm (indicative of polyphenols and flavonoids) may appear normal because of combined effects of pulpwash addn and diln; increased resolution of 280 nm peak; pulpwash addn alone causes upward shift in 223 nm peak toward 230 nm; diln alone causes shift downward toward 206 nm; suspected pulpwash-contg sample with 223 nm peak indicates diln also.

(b) *Fluorescence spectra.*—Dild, pulpwash-added juice shows deformities in excitation-emission curves (see table).

Complete substitution of pulpwash for orange juice.

(a) *UV/vis spectra.*—Adulteration causes the following: AAS ranges from 2.617 to 4.991 (3.781 ± 0.473); 443/325 ratio ranges from 0.017 to 0.112 (0.048 ± 0.020); poor resolution of 443 and 425 nm peaks; absorbance at 325 and 280 nm is stronger and more resolved than for orange juice.

(b) *Fluorescence spectra.*—See table on differences between orange juice and pulpwash; see also JAOAC **63,** 1317(1980).

Ref.: JAOAC **68,** 1202(1985).

23. NUTS AND NUT PRODUCTS

No additions, deletions, or other changes.

24. MEAT AND MEAT PRODUCTS

An editorial revision was made in the method for determination of salt (chlorine as sodium chloride) in meat, **24.010.**

In **24.010,** change the last sentence to read: "Det. as in **18.035,** beginning "and titr. with . . .".".

25. METALS AND OTHER ELEMENTS

(1) The following first action method was adopted final action:

Cadmium and lead in cookware, hot leach atomic absorption method, WHO-AOAC method, **25.016–25.023.**

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(2) The following first action method was declared surplus:

Tin in food, atomic absorption spectrophotometric method, **25.161–25.163.**

26. MYCOTOXINS

(1) Make the following revision:

Reinstate **26.054** (1980) 13th edition, *Preparation of Sample,* in order to provide a sample preparation procedure for whole cottonseed or kernels for use in the first action thin layer and liquid chromatographic methods, aflatoxins in cottonseed products, **26.052–26.060.** The section is as follows:

26.B01 *Preparation of Sample*

Grind whole seed or kernels in Wiley mill, or equiv., to pass No. 10 sieve. For seed contg lint, screen ground sample on 4/64 in. screen to remove coarse lint. Grind meals to pass No. 18 sieve. Quarter or riffle ground sample to obtain 50–100 g anal. sample.

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(2) The following liquid chromatographic method for determination of aflatoxins M_1 and M_2 in fluid milk was adopted first action:

Aflatoxins M_1 and M_2 in Fluid Milk
Liquid Chromatographic Method
First Action

26.B02 *Principle*

Aflatoxins M_1 and M_2 are extd from milk on C18 cartridge, eluted with ether onto silica column, eluted with CH_2Cl_2–alcohol, and derivatized with trifluoroacetic acid. Liq. chromatgc peaks are detected fluorometrically and compared with std-TFA derivatives.

26.B03 *Reagents*

(a) *Solvents.*—Distd in glass CH_3CN, CH_2Cl_2, and isopropyl alcohol; reagent grade alcohol, ether (0.01% EtOH preservative), hexane, MeOH, trifluoroacetic acid, and H_2O (deionized, filtered thru 0.45 μm filter).

(b) *Water–acetonitrile wash soln.*—95 + 5.

(c) *Methylene chloride–alcohol elution soln.*—95 + 5.

(d) *Mobile phase.*—Prep. H_2O–isopropyl alcohol–CH_3CN (80 + 12 + 8). Degas in ultrasonic bath, or equiv. Alternative solv. proportions may be used to give optimum resolution (i.e., 84 + 11 + 5).

(e) *Aflatoxin std solns.*—Aflatoxin M_1 (Eureka Laboratories, Sacramento, CA 95816) and aflatoxin M_2 (Sigma Chemical Co.). Prep. stock solns (ca 200 μg M_1/mL and 100 μg M_2/mL) in CH_3CN and det. concns according to **26.004–26.011,** using extinction coefficients of 19 850 and 21 400 for M_1 and M_2, resp., in CH_3CN. Make working std soln contg 0.50 μg M_1 and 0.10 μg M_2/mL in CH_3CN–benzene (1 + 9) for use in prepg M_1-TFA derivative.

(f) *Dichlorodimethylsilane (DDS).*—5% in toluene. Add 5 mL DDS (99%) (Aldrich Chemical Co., or equiv.) to toluene and dil. to 100 mL. Store in g-s flask in cold. (*Caution:* DDS is a lachrymator and is flammable.)

26.B04 *Apparatus*

(a) *Silica gel cleanup columns.*—0.8 × 4.0 cm polypropylene Econo-Column with Luer tip, 35 μm, porous polypropylene bed support disk, and 10 mL reservoir (Bio-Rad Laboratories, Cat. No. 731-1550).

(b) *Silica gel cleanup column packing and preparation.*—Dry silica gel 60, particle size 0.040–0.063 mm (E. Merck, No. 9385) in 105° oven for 1 h. Cool and add 1% H_2O by wt. Shake in sealed container and equilibrate overnight before use. Assemble polypropylene column and 250 mL vac. flask fitted with 1-hole stopper as shown in Fig. **26:B1.** Fill column to ca 2 mL mark with silica gel (ca 1 g). Pull gentle vac. to pack bed and add ca 1 g anhyd. Na_2SO_4 to top of silica gel bed.

(c) *Extraction cartridges.*—C18 Sep-Pak sample prepn cartridges (Waters Associates, Inc.).

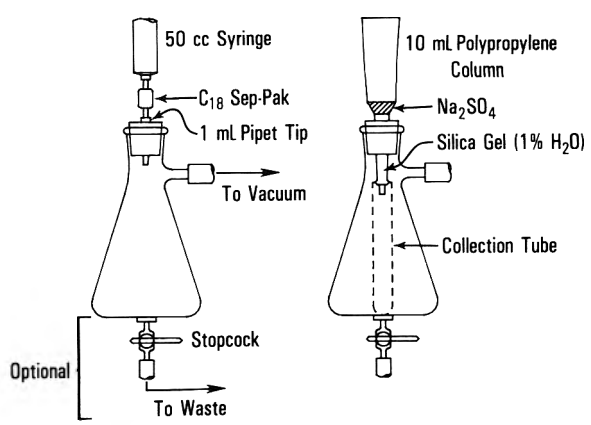

FIG. 26:B1—Diagram of apparatus for extraction and cleanup of milk extracts

(d) *Disposable pipet tips.*—50 and 200 μL Eppendorf or equiv.

(e) *Liquid chromatograph.*—Any pulse-free or pulse-dampened liq. chromatgc system which includes pump(s), injector, and compatible recorder.

(f) *Fluorescence detector.*—Any fluorescence detector capable of providing 365 nm excitation and >400 nm emission wavelengths and sensitivity of 50–100% full-scale response for 1 ng M_1-TFA derivative (e.g., Kratos-Schoeffel FS 970).

(g) *LC analytical column.*—Any 0.4 × 25 cm column contg spherical 5 μm particle size C18 bonded silica gel (e.g., DuPont ODS, Spherisorb 5 ODS II).

(h) *Vacuum regulator.*—Any com. or custom device capable of creating and controlling partial and full vac. with side arm vac. flask.

(i) *Silylated vials for aflatoxin std solns.*—Fill 1 or 1½ dram glass vials nearly full with 5% DDS and heat ca 40 min at 45–55°. Discard soln, and rinse vials 3 times with toluene and then 3 times with MeOH. Heat vials in oven at 75° for 20–30 min to evap. MeOH. Cap vials (with Teflon liners) and store for aflatoxin std solns.

26.B05 *Extraction*

Attach inlet (longer) stem of C18 cartridge to Luer tip of 30–50 mL syringe. Assemble syringe, cartridge, and vac. flask as shown in Fig. **26:B1.** Adjust vac. to pull solvs thru cartridge in fast *dropwise* manner (ca 5 mm Hg). Prime cartridge by adding 5 mL MeOH, then 5 mL H_2O (do not pull cartridge dry; leave small excess H_2O in stem). Discontinue vac. and remove cartridge-syringe assembly from stopper to prevent loss of prime.

Warm sample to room temp. Gently invert sample ≥10 times to evenly distribute cream in nonhomogenized samples. Transfer 20 mL milk to graduate *contg* 20 mL hot (ca 80°) H_2O. (If necessary, more hot H_2O may be used to thin milk soln.)

Replace cartridge-syringe assembly in stopper. Pour entire 40 mL warm, dild sample into syringe and gently pull sample thru cartridge at flow rate ca 30 mL/min (very fast drops). *Caution:* Too fast a flow will not allow sufficient time for aflatoxin to adsorb, resulting in low recoveries. Add 10 mL H_2O–CH_3CN wash soln to syringe and pull thru. Plug syringe barrel with stopper and pull hard vac. on cartridge for ca 30 s to remove as much wash soln as possible from packing. Remove cartridge and dry inside of both stems with cotton swab or tissue paper to eliminate any remaining wash soln. Reprime cartridge by adding 150 μL CH_3CN to inlet bed support disk and let solv. soak into packing for 30 s. Attach cartridge to dry glass or plastic 10 mL Luer tip syringe, retaining same stem as inlet.

Insert silica gel cleanup column into 250 mL vac. flask fitted with 1-hole rubber stopper (Fig. **26:B1**). Wash column with 5 mL ether. Add 7 mL ether to syringe-cartridge positioned above silica gel cleanup column. With plunger, slowly force ether thru cartridge (in portions), collecting eluate in column reservoir. Pull ether slowly thru silica cleanup column, using vac. to maintain flow rate ca 10 mL/min (fast drops). Rinse silica column with 2 mL addnl ether, continuing to use vac. Discard ether.

Remove column and stopper from flask and place 16 × 125 mm collection tube in flask to catch eluate from column. Add 7 mL elution solv. (CH_2Cl_2–alcohol) to column reservoir. Pull solv. thru column with vac. at ca 10 mL/min flow rate, collecting eluate in tube.

Discontinue vac. and remove collection tube from assembly. Evap. eluate just to dryness under N stream, using heat to keep collection tube near room temp. or under vac. at <35°.

Transfer residue to 1 dram vial with CH_2Cl_2 and evap. to dryness under N on steam bath or in heating block <50°. (Do *not* overheat dry sample.) Save for derivative prepn.

26.B06 *Liquid Chromatography*

Prep. derivative of sample exts by adding 200 μL hexane and 200 μL trifluoroacetic acid to dry residue in vial. Shake on vortex mixer ca 5–10 s. Let mixt. sit 10 min at 40°, in heating block or bath; then evap. to dryness under N on steam bath or heating block (<50°). Add 2 mL H_2O–CH_3CN (75 + 25) to vial to dissolve residue and shake well in vortex mixer for LC analysis. For derivative of std M_1, add 200 μL hexane and 50 μL trifluoroacetic acid to silylated vial and mix. Add 50 μL M_1–M_2 working std soln *directly* into hexane–TFA mixt. and shake on vortex mixer 5–10 s. Treat as described for sample derivative. Stabilize instrument and detector for suitable period at flow rate of 1.0 mL/min with H_2O–isopropanol–CH_3CN (80 + 12 + 8). Adjust detector attenuator so that 50–100 μL injection of std (0.625–1.25 ng M_1, 0.125–0.25 ng M_2) gives 50–75% full-scale recorder pen deflection for aflatoxin M_1. Inject LC std 2–3 times until peak hts are const. Prep. std curve from either peak hts or peak areas to ensure linear relationship. Inject sample exts (typically 50–100 μL) with std injections interspersed to ensure accurate quantitation. Retention times of M_1 (as TFA derivative) and M_2 are ca 4–5 min and ca 7 min, resp.

Calc. aflatoxin concn:

$$\text{ppb } (M_1 \text{ or } M_2) = (H \times C' \times VI' \times V)/(H' \times VI \times W)$$

where H and H' = peak ht of sample and std, resp.; C' = concn of std (ng/μL); VI' and VI = vol. injected of std and sample, resp.; V = final total sample vol. (μL); and W = vol. of milk represented by final ext (typically 20 mL). Sep. calc. concn for M_1 and M_2.

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(3) The following interim thin layer chromatographic method for determination of deoxynivalenol in wheat at levels ≥300 ng/g was adopted first action:

Deoxynivalenol in Wheat

Thin Layer Chromatographic Method

First Action

(Applicable at levels ≥300 ng/g)

26.B07 *Apparatus*

(a) *Grinder.*—Centrifugal grinding mill equipped with 2 mm sieve (Retsch Model ZM-1, available from Brinkmann, or equiv.).

(b) *Chromatographic tube.*—Polypropylene (10 mm id × 50 mm) equipped with plastic filter disk and reservoir (QS-Q, California Scientific, 410 Martin Ave. Santa Clara, CA 95050).

(c) *Filter flask.*—125 mL fitted with rubber stopper having 7/16 in. (11 mm) diam. single hole to hold chromatgc tube.

(d) *TLC plates.*—Precoated 20 × 20 cm silica gel 60 plates (No. 5763, E. Merck, Darmstadt, FRG, or equiv.). Predipped plates: Dip plates in 15% $AlCl_3$ soln (see *Reagents* (e) (2)) and let stand in vertical position 5 min to drain. Remove residual $AlCl_3$ from back of plate with wet paper towel. Air-dry 2 h and activate 1 h at 105°. Store in dust-tight cabinet.

(e) *Viewing cabinet.*—Chromato-Vue C-6 (Ultra-Violet Products, Inc., San Gabriel, CA 91778), or equiv., fitted with longwave UV lamp.

(f) *Fluorodensitometer.*—Optional. Camag Model 76511 (Muttenz, Switzerland). Excitation 366 nm, emission filter cutoff 400 nm, with SP 4100 electronic integrator (Spectra Physics, San Jose, CA 95134), or equiv.

26.B08 *Reagents*

(a) *Activated charcoal.*—Darco G-60 (J.T. Baker Chemical Co.).

(b) *Alumina.*—Neut., activated, 80–200 mesh No. 9296 (MCB Manufacturing Chemists, Inc., South Plainfield, NJ 07080), or equiv.

(c) *Diatomaceous earth.*—Acid-washed Celite 545.

(d) *Glass wool.*

(e) *Aluminum chloride soln.*—(*1*) *Spray reagent.*—Dissolve 20 g $AlCl_3.6H_2O$ in 100 mL alcohol–H_2O (1 + 1). (*2*) *Dip reagent.*—Mix 15 g $AlCl_3.6H_2O$ with 15 mL H_2O, and add 85 mL alcohol; then mix and warm on steam bath until dissolved.

(f) *Deoxynivalenol (DON) std solns.*—*Stock soln.*—0.5 mg/mL. Weigh 5.0 mg DON into 10 mL g-s vol. flask, dil. to vol. with ethyl acetate–MeOH (19 + 1), and shake vigorously to dissolve. *TLC std soln.*—20 μg/mL. Pipet 1.0 mL DON stock soln into 25 mL vol. flask and dil. to vol. with ethyl acetate–MeOH (19 + 1).

26.B09 *Sample Preparation*

Grind 5–10 lb (2–4 kg) sample to pass U.S. 20 mesh sieve and blend.

26.B10 ***Extraction***

Weigh 50 g ground, blended wheat into 500 mL g-s erlenmeyer. Add 200 mL CH_3CN–H_2O (84 + 16); secure stopper with masking tape. Vigorously shake 30 min on shaker. Filter thru Whatman No. 2 paper. Collect 20 mL filtrate in 25 mL graduated cylinder.

26.B11 ***Column Chromatography***

Secure chromatgc tube on 125 mL filter flask. Place small ball of glass wool in bottom of tube and add ca 0.1 g Celite.

Weigh 0.7 g charcoal, 0.5 g alumina, and 0.3 g Celite. Place in 50 mL beaker and thoroly mix with spatula. Add mixt. to chromatgc tube. Or prep. mixt. of charcoal–alumina–Celite (7 + 5 + 3) in g-s erlenmeyer in quantities enough for number of columns needed. Mix well. Weigh 1.5 g mixt. for each column. Keep mixt. from sepg by occasionally mixing.

Tap tube lightly to settle packing. Apply suction and place ball of glass wool on top. Columns may be prepd ahead of time and stored in upright position in beaker covered with Al foil for use as needed.

Apply 20 mL ext to column and apply vac. Flow rate should be 2–3 mL/min with 20 cm Hg vac. As soln reaches top of packed bed, rinse cylinder with 10 mL CH_3CN–H_2O (84 + 16) and then add rinse to column and continue aspiration until flow stops. Do *not* let column go dry between addn of ext and addn of wash soln. Cover vac. nipple tightly with small piece of Al foil and evap. solv. slowly to dryness on steam bath. (Caution: Do not contaminate sample with H_2O from condensing steam.) It is essential that no H_2O droplets remain in flask on cooling. Add 3 mL ethyl acetate to residue and heat to boiling on steam bath; then remove from bath and gently swirl to dissolve DON. Transfer ext soln to 2 dram vial and rinse with three 1.5 mL portions of ethyl acetate. Evap. ext to dryness on steam bath under stream of N. Retain dry ext for TLC. Final ext represents 5 g sample.

26.B12 ***Quantitation***

Dissolve residue in vial in 100 μL $CHCl_3$–CH_3CN (4 + 1). Apply 5 and 10 μL aliquots of sample soln alongside 1, 2, 5, 10, and 20 μL working std soln (20 ng DON/μL) spots on scored TLC plate (1 cm channels). Develop plate with $CHCl_3$–acetone–isopropanol (8 + 1 + 1) in unequilibrated tank (development time is ca 1 h). Remove plate from tank and let solv. evap. from plate at room temp. in well ventilated hood ≥10 min. Residual solv. can result in fading of DON spots during subsequent operations. For undipped plates, spray evenly with $AlCl_3$ soln until layer just appears wet. Before heating, briefly examine plate under longwave UV light for possible blue fluorescing interferences. Heat plate 7 min in upright position in 120° convection oven. Caution: Analyst may need to optimize time and temp. settings for particular plates used. Place plate on cool surface in dark 1 min. Observe DON as blue fluorescent spot under longwave UV light at R_f ca 0.6. Spot should be well resolved from background fluorescent spots; 20 ng DON std spot should be discernible from background of TLC plate. Lower limit of detection should be detd for other com. brands of TLC plates when substituted.

Quantitate DON by comparing fluorescence intensity of sample spot with those of std DON spots visually or densitometrically. When using densitometer, scan spots from top to bottom, parallel to direction of development. Densitometric responses *must* be confirmed by visual inspection, especially for levels ≤200 ng/g. Response must be linear relative to std concn, at least for 100, 200, and 400 ng spots; 20 and 40 ng spots can be used to obtain est. of concns ≤100 ng/g. For quantitation at these lower concns. rechromatograph sample, using more sample ext. After scanning all channels of plate, repeat scan for first spot measured. Values for the 2 scans should agree within instrument replication capabilities as previously detd. Ozone produced by instruments equipped with Xe lamps may fade DON spots. Fading can be prevented by covering TLC plate before and during densitometric scanning or by venting ozone.

Calc. amt of DON in sample, using following formula:

$$\text{DON, ng/g sample (ppb)} = S \times (C/X) \times (V/W)$$

where S = μL std equal to sample; C = concn of std soln (20 μg/mL); X = μL sample spot that had fluorescence intensity equal to std spot; V = final vol. of sample (μL); and W = amt of sample represented by final ext (5 g).

When dilns are required, ext soln equiv. to 0.75 g sample is used for initial detn; therefore, ext soln equiv. to 4.25 g sample remains in vial.

Ref.: JAOAC **69**, 37(1986).

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(4) The following gas chromatographic method for determination of deoxynivalenol in wheat at levels ≥350 ng/g was adopted first action:

Deoxynivalenol in Wheat

Gas Chromatographic Method

First Action

(Applicable at levels ≥350 ng/g.)

26.B13 ***Apparatus***

(a) *Gas chromatograph.*—Hewlett-Packard Model 5880A, or equiv., equipped with ^{63}Ni electron capture detector and 183 cm × 2 mm id glass tube packed with 3% OV-101 on 80–100 mesh Chromosorb WHP.

(b) *Centrifuge.*—IEC EXO centrf. for Class 1, Group D, for Hazardous Locations (International Equipment Co., Damon Corp., Needham Heights, MA 02194).

(c) *Column for sample cleanup.*—Disposable polypropylene column fitted with plastic filter disk and 12 mL extension funnel, Quick-Sep Code QS-Q and QS-R (Bio Lab Products, San Jose, CA 95128).

(d) *Disposable test tube.*—125 × 16 mm id (Kimble Products).

(e) *Vortex tube mixer.*—Vortex-Genie Model K-550G (Scientific Industries, Inc., Bohemia, NY 11716), or equiv.

(f) *Dry heating block.*—Reacti-Therm, equipped with Reacti-Block T-1 (Pierce Chemical Co.), or equiv.

26.B14 ***Reagents***

(a) *Deoxynivalenol (DON) stock soln.*—100 ng/μL. Dissolve 1.0 mg DON (Myco-Lab Co., Box 321, Chesterfield, MO 63107) in 10 mL MeOH. Store in freezer. Caution: DON may decompose in MeOH after ca 30 days.

(b) *Heptafluorobutyric acid anhydride (HFBAA).*—Pierce Chemical Co.

(c) *Silica gel.*—E. Merck silica gel H, medium particle size (10–40 μm) (Brinkmann Instruments, Inc.). Heat 25 g silica gel 3 h at 110°, cool to room temp. in desiccator, add 1.5 mL H_2O, shake until thoroly mixed, and store overnight in air-tight container.

(d) *Solvents.*—MeOH, absolute EtOH, acetone, CH_3CN, toluene, *n*-hexane, CH_2Cl_2, and $CHCl_3$, all distd in glass (Burdick & Jackson Laboratories, Inc., or equiv.). (*Caution:* $CHCl_3$ is possible carcinogen.)

(e) *Catalyst.*—4-Dimethylaminopyridine (4-DMAP), 99% min. purity (Aldrich Chemical Co.). Prep. catalyst soln contg 2 mg/mL by dissolving 100 mg 4-DMAP in 50 mL toluene–CH_3CN (95 + 5).

26.B15 ***Sample Extraction***

Grind wheat sample to pass 2 mm screen. Weigh 25 g sample into 250 mL g-s flask, add 10 mL H_2O and 125 mL $CHCl_3$–MeOH (8 + 2), and shake 60 min, using wrist-action shaker set at fast rate. Filter sample thru fluted paper. Transfer 10 mL sample filtrate to 4 dram vial, and conc. to dryness on dry heating block under stream of N. Save sample residue for column cleanup.

26.B16 ***Column Cleanup***

Prep. silica gel slurry by shaking 25 g silica gel and 100 mL CH_2Cl_2. Insert 16 × 125 mm test tube into each metal centrf. shield. Insert Quick-Sep column into each test tube. Pipet 5 mL silica gel slurry into each Quick-Sep column and centrf. 2 min at 1000 rpm. Discard liq. collected in each test tube. (All elutions referred to in

sample cleanup procedure are accomplished by centrfg column assemby at 1000 rpm for 2 min.) Dissolve sample residue in 3 mL CH_2Cl_2, using vortex tube mixer, and transfer to column. Rinse vial with 2 mL CH_2Cl_2 and add rinse to column. Centrf. column assembly at 1000 rpm for 2 min. Discard liq. in test tube. In similar manner, wash column with 10 mL toluene–acetone (8 + 2) and discard liq. in test tube. Insert column into clean test tube. Elute DON from column with 8 mL CH_2Cl_2–MeOH (95 + 5). Quant. transfer sample eluate to 4 dram vial and conc. to dryness on dry heating block at 60°, under stream of N. Final ext represents 2 g sample.

26.B17 ***Derivatization***

Transfer 10 μL DON stock soln to 3 dram vial and evap. to dryness. Treat sample residue from column cleanup step and std identically. Transfer 1.0 mL 4-DMAP catalyst soln to vial and add 50 μL HFBAA. Firmly cap vial and warm 20 min on dry heating block at 60°. Let derivatized reaction mixt. cool to room temp. Add 1.0 mL 3% aq. $NaHCO_3$ soln to vial, mix 2 min on tube mixer, and let stand until layers are fully sepd. Transfer 100 μL org. (upper) layer by syringe to 2 dram vial contg 900 μL hexane. Final concn of std soln, expressed as DON equiv. wt/μL, is 0.1 ng/μL. Final concn of sample soln, expressed as underivatized sample equiv. wt/μL, is 0.0002 g/μL.

26.B18 ***Gas Chromatography***

Complete GC analysis on same day as derivatization.

Operating conditions.—Carrier gas CH_4–Ar (5 + 95); flow rate 60 mL/min; chart speed 0.5 cm/min; attenuation adjusted to give 10% FSD for 100 pg std; injection port 200°; oven temp. program: initial temp. 175°, initial time 10 min, program rate 10°/min, final temp. 250°, final time 5 min.

Std curve.—Inject 1–5 μL derivatized DON std directly onto column to obtain peak response. Construct std curve by plotting amt of derivatized DON vs detector response for 100–500 pg range. Detector response (peak area) for 100–500 pg range varies linearly. Retention time for DON derivative under operating conditions listed is ca 6.5 min.

Determination.—Inject 2 μL sample ext into gas chromatograph under same conditions used for prepg std curve. Calc. amt of DON in sample by comparing peak area of sample with peak area of derivatized DON std as follows:

$$\text{DON, ng/g} = (C'/C) \times (V'/V) \times (PA/PA')$$

where C' = concn of DON std (ng/μL); V' = vol. of DON std injected (μL); PA = peak area of sample; PA' = peak area of std; C = concn of sample (0.0002 g/μL, if 25 g sample is used); and V = vol. of sample ext injected (μL).

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(5) Make the following correction in **26.002,** general reagents for determination of mycotoxins.

Change **26.002**(a) to read:

(a) *Benzene–acetonitrile mixture.*—98 + 2; prep. . . . .

27. NUTS AND NUT PRODUCTS

No additions, deletions, or other changes.

28. OILS AND FATS

(1) Make the following revisions:

(a) In the method for melting point of fats and fatty acids, Wiley method, **28.012–28.013,** change **28.013,** third paragraph, first line, to read:

Place 30 × 3.5–3.8 cm id test tube, contg . . . .

(b) In the first action method for total *trans* fatty acid isomers in margarines, gas chromatographic method, **28.A06–28.A13,** add the precision statement shown in **28.068.**

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(2) The following gas chromatographic method for determination of triglycerides in fats and oils was adopted first action as an IUPAC-AOAC method:

Triglycerides in Fats and Oils
Gas Chromatographic Method
First Action
IUPAC-AOAC Method

28.B01 ***Principle***

Triglyceride groups having same C number are sepd by direct gas chromatgy of soln of oil or fat, under temp.-programmed conditions, and identified by ref. to std triglyceride soln. Triglycerides having same C number are not detd individually. Content is detd by peak area ratio.

28.B02 ***Apparatus and Reagents***

(a) *Gas chromatograph.*—With facilities for on-column injection, oven temp. programming up to 350°, and, preferably, electronic integration. All-glass system is preferable.

(b) *Column.*—Glass, ca 0.5–0.6 m long, and 2–4 mm id, filled with 3% (or less) methyl polysiloxane on acid-washed silanized support (OV-1 is suitable). Carrier gas flow 50 mL/min. He is recommended as carrier gas but N may be used with some loss in resolution. Condition column prior to use at 350° for ≥36 h with carrier gas flow rate of ca 5 mL/min.

(c) *Triglycerides.*—Purity 99%. Prep. std soln in CH_2Cl (or diisopropyl ether) contg ca 10 mg/mL each of tricaprin, tricaprylin, trilaurin, trimyristin, tripalmitin, and tristearin.

28.B03 ***Determination of Triglycerides Correction Factors***

With 2 μL microsyringe, inject ca 1 μL triglycerides std soln with injection and detector temps set at ca 375° and initial oven temp. ca 220°. Immediately commence programming oven temp. to increase at rate of ca 4–5°/min (but not >5°) and continue analysis until temp. reaches ca 350°. Maintain this temp. until all triglycerides have eluted from column.

Assume that trilaurin is completely recd from column and calc. correction factor, f_i, for each remaining triglyceride from

$$f_i = (C_{si}/C_L) \times (A_L/A_{si})$$

where A_L = peak area for trilaurin; A_{si} = peak area for std triglyceride i; C_L = concn (mg/mL) of trilaurin; C_{si} = concn (mg/mL) of std triglyceride i.

Det. f from ≥2 injections of std soln. Plot graph of av. values for f for each triglyceride against corresponding C number. Correction factor >1.1 is unsatisfactory. Decrease stationary phase loading or increase carrier gas flow rate to achieve acceptable correction factors.

Plot values of retention time for each std triglyceride peak against corresponding C number. Straight line should be obtained from which expected retention times for other triglycerides can be detd.

28.B04 ***Preparation of Sample Solution***

Warm sample as necessary to completely liquefy. Homogenize liq. sample by gently shaking container. Prep. 50 mg/mL soln of sample in CH_2Cl (or diisopropyl ether). For example, transfer ca 1.25 g liq. sample to 25 mL graduated flask using pipet and dissolve sample (while still liq.) in 2–3 mL solv. Dil. with same solv., and mix. If sample is known to contain significant amts of mono- or diglycerides or free fatty acids, remove these according to **28.144–28.149** before proceeding with analysis as described for detn of triglycerides correction factors.

28.B05 ***Determination***

Identify each peak by using identification graph. Det. peak areas of each group of triglycerides. Calc. corrected peak areas by using correction factors detd either by calcn or by interpolation from graph of correction factors obtained for std triglycerides. Det. quantity of each group of triglycerides having same C number

expressed as percentage relative to total triglycerides content by formula:

$$(A_{TGi}/A_T) \times 100$$

where A_{TGi} = corrected peak area of triglycerides group i; A_T = total corrected peak area of triglycerides groups contained in sample ($A_T = \Sigma A_{TGi}$).

Difference between results of 2 detns carried out on same day by same analyst using same app. for same test material and for triglycerides present >10% should not exceed 1% absolute. For triglycerides present at levels of <10%, difference should not exceed 0.5% absolute. Triglycerides present at <5% are detd less accurately.

Ref.: Pure Appl. Chem. **57**, 1515(1985).

29. PESTICIDE RESIDUES

(1) The following first action methods were adopted final action:

(a) Organochlorine and organophosphorus pesticide residues, gas chromatographic method, **29.A01–29.A04.**

(b) Applicability of **29.037–29.043,** determination of organochlorine pesticide residues in poultry fat, to determination of the same residues in beef and swine fats.

(c) *N*-Methylcarbamate insecticide and metabolite residues, liquid chromatographic method, **29.A05–29.A13.**

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(2) The following gas chromatographic method for determination of ethylene dibromide in grains and grain products was adopted first action:

Ethylene Dibromide in Grains and Grain Products

Gas Chromatographic Method

First Action

29.B01 *Principle*

Whole grains and intermediate grain-based products are extd by soaking in acetone–H_2O; ready-to-eat products are extd by hexane co-distn. Portions of exts are dried and analyzed by gas chromatgy with electron capture detection.

29.B02 *Reagents*

(a) *Solvents.*—2,2,4-Trimethylpentane (isooctane), acetone, and hexane (all pesticide quality). Check for interferences by injecting 5 μL into GC system operated as described under *Apparatus.*

(b) *Calcium chloride.*—Analyzed reagent grade, anhyd., 8 mesh.

(c) *Sodium sulfate.*—Analyzed reagent grade, anhyd., granular.

(d) *Sulfuric acid (concd).*—Analyzed reagent grade.

(e) *Std solns.*—(*1*) *Stock soln.*—Prep. in 50 mL vol. flask equipped with Teflon-lined screw cap. Add ca 40 mL isooctane to flask and weigh flask + isooctane to nearest 0.1 mg. Introduce 20 μL pure ref. std (EPA Ref. Std P480) into isooctane and re-weigh to det. wt of EDB. Dil. to vol. with isooctane and calc. concn in μg/mL. Store in freezer. (*2*) *Working std soln.*—Prep. in hexane by serial diln of stock soln to final concn of ca 4 pg/μL. Store in glass container with Teflon-lined screw cap. Store in refrigerator or freezer when not in use.

29.B03 *Apparatus*

(a) *Volumetric flasks.*—50 mL with Teflon-lined screw caps (A.H. Thomas Co., No. 4995-C18).

(b) *Soaking vessels.*—250 mL erlenmeyers with Teflon-lined screw caps (A.H. Thomas Co., No. 4903-K22) or 250 mL media bottles with Teflon-lined screw caps (Wheaton Glass Co., No. 219627).

(c) *Teflon liners.*—For erlenmeyers (A.H. Thomas Co., No. 2390-H82).

(d) *Test tubes.*—15 mL with Teflon-lined screw caps (A.H. Thomas Co., No. 9212-K44).

(e) *Centrifuge.*—For use with test tubes.

(f) *Distilling trap.*—Barrett, 20 mL graduated, 24/40 (A.H. Thomas Co., No. 7133-K44).

(g) *Condenser.*—Friedrich, 24/40 (Fisher Scientific Co., No. 07-744-5).

(h) *Heating mantle.*—To fit 500 mL r-b flask.

(i) *Variable electric transformer.*—0–120 V (Powerstat Model 1168, or equiv.).

(j) *Magnetic stirrer.*—Thermolyne Model 7200, or equiv., with mag. stirring bar, 1 × 5/16 in.

(k) *Gas chromatograph.*—Equipped with 1.8 m × 4 mm glass column packed with 10% SP-1000 on 80–100 mesh Supelcoport and const current ^{63}Ni electron capture detector (Hewlett-Packard 5730 or equiv.) operated under following conditions: temps—injector 200°, oven 115°, detector 350°; CH_4–Ar (5 + 95) carrier gas 40 mL/min. Adjust attenuator to give ½ FSD for 20 pg EDB. Retention time of EDB is ca 4 min. Condition new GC column by holding at 60° for 2 h with 40 mL/min carrier gas flow. Slowly increase temp. to 200° and hold overnight. Cool to 115°, let equilibrate, and check EDB response. If proper sensitivity is not achieved, longer conditioning at 200° may be required.

29.B04 *Extraction and Cleanup*

Store all samples in freezer until just before analysis.

(a) *Whole grain and intermediate processed grain products.*—Weigh 50 g into appropriate soaking vessel. Add 150 mL acetone–H_2O (5 + 1) and seal with Teflon-lined screw cap. Swirl; let whole grains soak 48 h at 20–25°, with intermittent swirling. Follow same procedure for intermediate products, except soak 16 h. Using disposable pipet, transfer ca 10 mL supernate into 15 mL test tube, add 1–2 g anhyd. 8-mesh $CaCl_2$, secure Teflon-lined screw cap, and shake 2 min. If all $CaCl_2$ dissolves, add more and shake again. Let stand 30 min or centrf. Proceed with *Determination.*

(b) *Ready-to-eat products.*—Weigh 20 g into 500 mL r-b flask. Add 150 mL H_2O and stirring bar. While cooling flask under stream of cold tap H_2O or swirling in ice-H_2O bath, slowly add 25 mL concd H_2SO_4. Stopper flask to prevent loss of EDB. Pipet 10.0 mL hexane into flask immediately before co-distn. Connect r-b flask to Barrett trap and Friedrich condenser. Place flask in heating mantle on top of stirrer and turn on stirrer. Co-distil hexane and EDB into Barrett trap, using heating mantle with transformer set at 75% full power. Continue distn until 1–2 mL H_2O is collected. Remove flask from heating mantle to prevent further H_2O distn. Drain lower H_2O layer and discard. Drain hexane into 15 mL test tube, add 2–3 g anhyd. Na_2SO_4, secure Teflon-lined screw cap, and shake vigorously 1 min. Let stand 30 min or centrf. Proceed with *Determination.* When flask is cool to touch, pipet second 10.0 mL hexane portion into flask and distil as before. Repeat distn third time.

Calculation of equivalent sample weight.—(*1*) Whole grains and intermediate processed grain products:

$$\text{mg sample equiv./}\mu\text{L final ext} = 50/125 = 0.4$$

where 50 and 125 = g sample weighed and mL acetone added (H_2O is removed), resp.

(*2*) Ready-to-eat products:

$$\text{mg sample equiv./}\mu\text{L final ext} = 20/10 = 2$$

where 20 and 10 = g sample weighed and mL hexane added (not recovered), resp. Each distn uses same calcn.

29.B05 *Determination*

Inject 5 μL dried ext into gas chromatograph operated as specified under *Apparatus.* Quantitate EDB by comparison of peak hts or integrator counts from sample and appropriate std. If EDB response is >100% FSD, quant. dil. sample with hexane to achieve appropriate on-scale response. Sum EDB amts found in each distn of ready-to-eat products to obtain total. Limits of quantitation are 2 ppb EDB for whole grains and intermediate products, 0.4 ppb EDB for ready-to-eat products.

CAS-106-93-4 (ethylene dibromide)

30. SPICES AND OTHER CONDIMENTS

The following interim distillation method for determination of moisture in spices was adopted first action:

Moisture in Spices
Distillation Method
First Action

30.B01 ***Apparatus***

Connect 500 or 1000 mL shortneck r-b flask by means of Bidwell-Sterling 5 mL capacity moisture receiver to 400 mm West-type condenser. Clean distg tube receiver and condenser with chromic acid soln, rinse thoroly with H_2O and then with ca 0.5N alc. KOH, and drain 10 min. If alc. KOH soln contains carbonates, follow with alc. rinse. Calibrate receiver by distg 1 ± 0.01 mL H_2O with 100 mL solv. into receiver. Cool; repeat until calibration is complete.

30.B02 ***Reagents***

(a) *Toluene.*—For most spices.

(b) *Hexane.*—For capsicums, onions, garlic, and other spices contg large amts of sugar.

30.B03 ***Determination***

Place 40 g spice (or enough to yield 2–5 mL H_2O) in distg flask. Add enough solv. to cover sample completely (never <75 mL). Fill receiving tube with solv., pouring thru top of condenser. Insert loose cotton plug in top of condenser to prevent condensation of atm. moisture in tube. Bring to boil and distil slowly, ca 2 drops/s, until most of H_2O distils over; then increase rate of distn to ca 4 drops/s. Continue distg until 2 consecutive readings 15 min apart show no change. Dislodge any H_2O held up in condenser with brush or wire loop. Rinse condenser carefully with ca 5 mL toluene. Continue distn 3 to 5 min; cool receiver to room temp. (ca 25°), allowing it to stand in air or immersing it in H_2O. Solv. and H_2O layers should now be clear; if not, let stand until clearing occurs. Read vol. of H_2O, estg to nearest 0.01 mL, and calc. to percent.

31. SUGARS AND SUGAR PRODUCTS

(1) The following first action method was adopted final action:

Color classification of honey, instrumental method II, **31.A01–31.A03.**

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(2) The following first action tables were adopted final action:

(a) Table **31:A1,** Commercial Baumé table for dry substance in corn syrup and high fructose corn syrup.

(b) Table **31:A2,** Commercial Baumé table of refractive index of corn syrups and high fructose corn syrups at 45°.

(c) Table **31:A3,** Refractive index table for dry substance in corn syrups and high fructose corn syrups.

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(3) Make the following correction in method **31.245–31.253:**

Change the first line of the title to read:

Saccharides (Major) in Corn Sirup

32. VEGETABLE PRODUCTS, PROCESSED

No additions, deletions, or other changes.

33. WATERS; AND SALT

The following liquid chromatographic method for determination of TNT, RDX, HMX, and 2,4-DNT, in wastewater and groundwater was adopted first action:

TNT, RDX, HMX, and 2,4-DNT in Wastewater and Groundwater
Liquid Chromatographic Method
First Action

33.B01 ***Principle***

Presence of 2,4,6-trinitrotoluene (TNT), 1,3,5-trinitro-1,3,5-triazacyclohexane (RDX), 1,3,5,7-tetranitro-1,3,5,7-tetraazacyclooctane (HMX), and 2,4-dinitrotoluene (DNT) in wastewater from munitions manufacturing and processing facilities and in groundwater is identified and measured by comparison of liq. chromatgc peak areas and retention times. Analytical detection limits for TNT, RDX, HMX, and 2,4-DNT are estd to be 14, 22, 26, and 10 μg/L, resp.

33.B02 ***Apparatus***

(a) *Liquid chromatograph.*—With high pressure pump and 254 nm fixed wavelength UV detector, or variable wavelength detector set at 254 nm, strip chart recorder, stand-alone digital or computer-controlled integrator, and 100 μL sample loop injector.

Operating conditions: 25 cm × 4.6 mm (5 μm) LC8 reverse phase column (Supelco), operated at room temp. Mobile phase H_2O–MeOH–CH_3CN (50 + 38 + 12, v/v), prepd in graduates, not vol. flasks. Prep. 750–1000 mL, vac.-filter thru solv.-washed Whatman glass microfiber filter to remove particulate matter, and degas. Prep. fresh solv. daily. Flow rate 1.5 mL/min. Set integrator threshold low enough to avoid neg. intercept in working curve and high enough to avoid pos. intercept. Set chart speed at 0.2 in./min.

(b) *LC syringe.*—Liq.-tight syringe of 0.5–1.0 mL capacity (Hamilton 750, or equiv.).

(c) *Filters.*—(*1*) Nuclepore syringe filter, 25 mm diam. (*2*) 0.4 μm Nuclepore polycarbonate, 25 mm diam., or Millex-SR 0.5 μm filter unit.

(d) *Filtration syringe.*—25 mL, glass or polyethylene (e.g., Plastipak, Becton, Dickinson and Co., or equiv.).

(e) *Scintillation vials.*—20 mL glass with polyethylene, not Al, cap insert. Use as received.

Soak all vol. glassware overnight in detergent, scrub briefly, rinse well with hot tap H_2O, rinse with acetone, rinse with deionized H_2O, oven-dry at 105°, and rinse with appropriate soln before filling.

33.B03 ***Reagents***

(a) *LC solvents.*—H_2O, MeOH, CH_3CN, all LC grade.

(b) *Methanol–acetonitrile diluent.*—MeOH–CH_3CN (76 + 24). Use throughout as diluent for all H_2O samples. Prep. using graduates, not vol. flasks. (Diln with this mixt., rather than MeOH alone, eliminates neg. peak which elutes just before HMX and may affect integration.)

33.B04 ***Calibration Standards***

(a) *Individual stock std solns.*—Vac.-dry anal. ref. stds of DNT, TNT, RDX, and HMX (US Army Toxic and Hazardous Materials Agency, Aberdeen Proving Ground, MD 21010) at room temp. to const wt (±1 mg). Use vac. desiccator or vac. oven attached to H_2O aspirator or vac. pump. For RDX and HMX, remove most of isopropanol by Pasteur pipet, air-dry for 3–4 h, then vac.-dry. Store std anal. ref. materials in desiccator over dry $CaCl_2$ or Drierite and place in dark when not in use.

Accurately weigh ca 0.1 g dried std onto weighing paper, transfer carefully to 250 mL vol. flask, and reweigh weighing paper. Record mass to 0.1 mg.

For DNT and TNT, dissolve and dil. to vol. with MeOH. For HMX and RDX, add 100 mL CH_3CN to dissolve, then dil. to vol. with MeOH.

Wrap stoppered joint with Parafilm to protect against evapn. Calc. concn exactly in mg/L and label flasks. Store at ca 4° (not <0°).

(b) *Combined analyte stock std soln.*—Remove stock std solns from refrigerator and let warm to room temp. (>30 min but not overnight). Invert flasks several times to mix. Into 1 L vol. flask, pipet 10.0 mL each of DNT and TNT stock std solns and 25.0 mL each of RDX and HMX stock std solns. Dil. to vol. with MeOH.

This std soln contains ca 4.0 μg DNT and TNT/mL and ca 10.0 μg RDX and HMX/mL.

Calc. concns exactly in μg/mL; label and date flask. Wrap stoppered joint with Parafilm and store flask in refrigerator when not in use. Combined stock std soln can be used 1 week.

(c) *Working std solns.*—Prep. fresh each day as needed. Remove combined analyte stock std soln from refrigerator and let warm to room temp. (>30 min but not overnight). Invert flask several times to mix. Transfer 2.00, 5.00, 10.00, and 20.00 mL by pipet to four 250 mL vol. flasks, resp. Fill to mark with MeOH–CH_3CN diluent. Stopper and invert 10 times to mix. Calc. concns exactly in μg/L; label and date flasks.

(d) *Injection std solns.*—For each working std soln, pipet 10.0 mL into scintillation vial. Add 10.0 mL H_2O by pipet, cap, and shake to mix. Prep. blank by combining 10.0 mL MeOH–CH_3CN diluent with 10.0 mL LC grade H_2O in vial. Cap and mix. Label all vials appropriately.

Solns contain following concns in 10 mL aliquot:

Aliquot vol. combined std, mL	*Approx. concns, μg/L*	
	For DNT and TNT	*For RDX and HMX*
2	32	80
5	80	200
10	160	400
20	320	800

These values represent concns before addn of H_2O. Actual concns are half as large. Solns are treated similarly: 1-to-1 diln is made by adding 10.0 mL MeOH–CH_3CN diluent to 10.0 mL aq. sample. Thus, anal. results derived from working curve do not need to be corrected for this extra diln.

Make 10.0 mL MeOH–CH_3CN/H_2O mixts in scintillation vials rather than in vol. flasks because of slight vol. contraction which would cause systematic error. Because std solns would be dild. with H_2O to vol., and samples would be dild with org. solv. to vol., vol. contraction would lead to samples richer in org. solv. than are std solns. Take care at this step to pipet these 10.0 mL vols accurately because significant error at this stage is compounded when peak areas are measured.

33.B05 *Liquid Chromatography*

Initial conditioning.—Follow procedure below for instrument warmup, except pass ≥30 void vols (ca 60 mL) mobile phase thru column. Continue until UV detector baseline is level when set to greatest sensitivity.

Calc. plate no. as follows:

Take 1 mL aliquot from combined working stock std soln and dil. to 100 mL in vol. flask with MeOH–CH_3CN. Use proper sample prepn and injection procedure described to obtain chromatogram. All 4 analytes should elute within 10 min. Use conditions described in *Apparatus*, but select chart speed that spreads peaks abnormally wide (such that widths at half ht are ≥2.0 cm). Est. peak width at half ht to nearest 0.1 mm. Calc. no. of theoretical plates (N) on column from each peak:

$$N = 5.54 \times (t_r/t_{0.5})^2$$

where t_r = retention time and $t_{0.5}$ = width of peak at half ht, both in min.

Average results for all 4 analytes. If av. value is <3000 plates, carefully recheck calcn. If no error is apparent, let another 30 void vols of mobile phase wash thru column and repeat experiment. If calcd value of N still does not exceed 3000, column is not performing to specification and should be replaced.

Warmup procedure.—Turn on all electronic equipment and let warm up >30 min. Pass >15 void vols of mobile phase thru column (20 min at 1.5 mL/min) and continue until UV detector baseline is level when set to greatest sensitivity. Make sure pumps are not experiencing vapor lock as indicated by large pressure fluctuations. Check system thoroly for leaks.

Sample injection procedure.—Fill analytical syringe with MeOH–CH_3CN and discharge to waste. Repeat twice more to remove traces of previous sample. Rinse syringe 3 times with sample. Fill syringe with sample to >500 μL and pass most of this thru sample loop, avoiding introducing air bubbles. Overfilling loop in this manner assures that sample injected is not dild by solv. in loop.

33.B06 *Preparation of Working Curve*

Obtain chromatograms of 4 working stds and blank in duplicate (10 injections total). Sequence injections randomly. Plot peak area vs concn for each of the 4 analytes. Do not average duplicates before plotting. Inspect plot for gross deviations from linearity. Analytical response should be linear from 10 μg/L to 20 mg/L for DNT and TNT and from 25 μg/L to 50 mg/L for RDX and HMX. Significant deviation from linearity is evidence for systematic bias. Calc. regression line for each analyte.

33.B07 *Analysis of Water Samples*

Remove combined analyte working stock std solns from refrigerator and let warm to room temp. (>30 min but not overnight). Warm up instrument and condition LC column. Pipet 10.0 mL sample into scintillation vial. Add 10.0 mL MeOH–CH_3CN diluent by pipet. Attach cap tightly. Shake vigorously. Let stand >15 min before filtration.

Load new Nuclepore (or Millex-SR) filter into filter holder. Rinse 25 mL filtration syringe with MeOH–CH_3CN diluent, then fill to ca 10 mL with sample. Filter sample and discard filtrate. Fill syringe with remaining sample. Filter into new scintillation vial. Label vial appropriately.

Using proper procedure, inject these solns into liq. chromatograph. Typical retention times for HMX, RDX, TNT, and 2,4-DNT are 3.2, 4.1, 7.0, and 7.8 min, resp.

33.B08 *Calculations*

Prep. working curve for each anayte in the form, $y = b_0 + b_1x$, where x = analyte concn in μg/L and y = peak area. Det. concn of each analyte in H_2O sample by substituting measured peak area into calcn.

Ref.: U.S. Army Cold Regions Research and Engineering Laboratory (1984) CRREL Report 84-29, National Technical Information Service, Springfield, VA. Anal. Chem. **58**, 170(1986); **58**, 176(1986).

34. COLOR ADDITIVES

No additions, deletions, or other changes.

35. COSMETICS

No additions, deletions, or other changes.

36. DRUGS: GENERAL

The following first action methods were adopted final action:

(a) Fluoride in drug tablets and solutions, fluoride-selective electrode method, **36.063–36.069.**

(b) Amitriptyline in drug tablets and injectables, liquid chromatographic method, **36.207–36.211.**

37. DRUGS: ACIDIC

(1) The following first action method was adopted final action:

Sulfisoxazole in drug tablets, solutions, and ointments, liquid chromatographic method, **37.166–37.172.**

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(2) The following interim method was adopted first action:

Primidone in drug tablets, liquid chromatographic method, **37.A17–37.A22.**

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(3) The following liquid chromatographic method for determination of chlorpropamide in drug tablets was adopted first action:

Chlorpropamide in Drug Tablets
Liquid Chromatographic Method
First Action

37.B01 ***Principle***

Chlorpropamide is dissolved in mobile phase and detd by liq. chromatgy with UV detection at 240 nm.

37.B02 ***Apparatus***

(a) *Liquid chromatograph.*—Equipped with sampling valve capable of introducing 20 μL injections. UV detector capable of operating at 240 nm, and recorder/integrator.

(b) *Column.*—Zorbax ODS, 5–6 μm diam. spherical particles, 4.6 mm × 25 cm (E.I. Dupont, or equiv.).

(c) *Filters.*—Millipore type HVLP, 0.45 μm porosity (Millipore Corp.), or equiv.

37.B03 ***Reagents***

(a) *Mobile phase.*—52/48 ratio of aq./org. phases: (*1*) *Aqueous.*—Acetic acid–H_2O (1 + 99). (*2*) *Organic.*—LC grade CH_3CN.

(b) *Chlorpropamide std soln.*—Transfer ca 50 mg, accurately weighed, USP Chlorpropamide RS to 100 mL vol. flask and dissolve in mobile phase. Dil. quant. to final concn of ca 0.05 mg/mL in mobile phase.

(c) *Resolution soln.*—Chlorpropamide + *p*-chlorobenzenesulfonamide (PCBS) (ca 0.05 mg/mL of each) in mobile phase.

37.B04 ***Preparation of Sample***

Transfer accurately weighed portion of finely ground tablets equiv. to 45–55 mg chlorpropamide to 100 mL vol. flask. Add ca 70–80 mL mobile phase and shake thoroly 6–8 min (or sonicate 3–4 min) and dil. to vol. with mobile phase. Dil. quant. to final concn ca 0.05 mg/mL in mobile phase. Filter portion thru 0.45 μm filter for LC analysis.

37.B05 ***System Suitability***

Set mobile phase at flow rate ca 1.5 mL/min. Retention time for chlorpropamide should not be <4.0 min. Adjust flow rate and/or solv. ratio (do not exceed 50% CH_3CN) for desired retention time. Column should conform to following performance parameters: theoretical plates (n) not <1500; tailing factor (T) not > 1.5; resolution (R) between chlorpropamide and PCBS not <2.0. Relative std deviation for 4 consecutive std injections should be <2.0%.

37.B06 ***Determination***

Make 20 μL injections of std and samples. Det. peak responses (area or ht) obtained and calc. amt of chlorpropamide:

$$\text{Chlorpropamide, mg/tab.} = (r/r') \times (C/W) \times \text{DF} \times \text{ATW}$$

where r and r' = responses for sample and std, resp.; C = concn of chlorpropamide std soln, mg/mL; W = sample wt, g; DF = diln factor for sample, mL; ATW = av. tablet wt, g/tab.

CAS-94-20-2 (chlorpropamide)

38. DRUGS: ALKALOIDS AND RELATED BASES

The final action protein nitrogen unit precipitation method for determination of allergenic extracts in drugs, **38.205–38.207,** was amended to include a procedure for determination of nitrogen:

Nitrogen Determination

38.B01 ***Reagents***

(a) *Sulfuric acid.*—Sp. gr. 1.84, N-free.

(b) *Copper sulfate.*—$CuSO_4.5H_2O$, N-free. Prep. satd aq. soln.

(c) *Acid soln.*—Add ca 40 mL satd aq. $CuSO_4$ to 9 lb bottle of H_2SO_4 in 10 mL portions with thoro mixing. After several days, excess anhyd. $CuSO_4$ crystallizes and supernatant acid is ready for use ($CuSO_4$–H_2SO_4 soln).

(d) *Potassium sulfate.*—N-free.

(e) *Sodium hydroxide soln.*—50%.

(f) *Boric acid soln.*—2%.

(g) *Indicator soln.*—Me red–bromocresol green soln. Mix 1 part 0.1% alc. Me red soln with 5 parts 0.1% alc. bromocresol green soln.

(h) *Hydrochloric acid.*—0.01N. Prep. as in **50.011** and stdze as in **50.015** or **50.017,** or use 0.01000N HCl (purchased as std).

38.B02 ***Apparatus***

See **47.022.**

38.B03 ***Determination***

Pipet 9 mL prepd sample into 30 mL digestion flask. Add ca 500 mg K_2SO_4, 3 boiling stones, and 2 mL $CuSO_4$–H_2SO_4 soln. Place flask in digestion rack. Heat carefully and digest sample until soln turns colorless. Continue digestion for addnl ½ h. Cool and place thin film of pet. jelly on rim of flask. Transfer digest and boiling chips to distn app. and rinse flask 5 or 6 times with 1–2 mL portions of H_2O. Place 125 mL erlenmeyer contg 5 mL 2% H_3BO_3 soln and 5 drops of indicator under condenser with tip extending below surface of soln. Add ca 6 mL NaOH (50% w/w) to still. If distn app. uses steam distn, distil at rate of 5 mL/min and collect ca 50 mL. If app. does not introduce steam into distg flask, collect 10–15 mL distillate and dil. to ca 50 mL with H_2O. Titr. distillate with 0.01N HCl to end point (pinkish purple). Perform blank detn in same manner, using H_2O in place of sample.

38.B04 ***Calculation***

$$\text{mg N/mL} = [(\text{mL HCl} - \text{mL HCl blank}) \times \text{normality} \times 14.007 \times 10/9]/\text{mL sample}$$

39. DRUGS: STEROIDS AND HORMONES

The following liquid chromatographic method for determination of prednisolone in tablets and bulk drugs was adopted first action:

Prednisolone in Tablets and Bulk Drugs
Liquid Chromatographic Method
First Action

39.B01 ***Principle***

Prednisolone is detd by normal phase liq. chromatgy, using silica column and UV detection.

39.B02 ***Apparatus***

(a) *Liquid chromatograph.*—Equipped with Model 6000A solv. delivery system, Model 440 UV detector, and Model 730 data module (Waters Associates, Inc.) or equiv. system. Operating conditions: flow rate 1.5 mL/min; 254 nm detector, 0.20 AUFS; temp. ambient; 10–15 μL injection.

(b) *LC column.*—25 cm × 4.6 mm id, packed with 5–6 μm porous spherical silica particles (DuPont Instruments, Wilmington, DE 19898), or equiv. column that passes system suitability tests.

(c) *Filters.*—Polytetrafluoroethylene membrane filters Type FH and Type LS, pore sizes 0.5 and 5.0 μm, resp. (Millipore Corp.).

39.B03 ***Reagents***

(a) *Solvents.*—Distd in glass (OmniSolv, EM Industries, Gibbstown, NJ 08027).

(b) *LC mobile phase.*—Mix 60 mL 95% MeOH with 1.0 mL acetic acid and dil. to 1 L with H_2O-washed 1,2-dichloroethane. Filter thru Type FH filter and degas 5 min.

(c) *Internal std soln.*—1.0 mg/mL. Transfer 100 mg fluoxymesterone to 100 mL vol. flask. Dissolve in 5 mL MeOH and dil. to vol. with CH_2Cl_2.

(d) *Prednisolone std soln.*—(*1*) *Stock std soln.*—0.5 mg/mL. Accurately weigh ca 25 mg USP Ref. Std Prednisolone into 50 mL vol. flask. Add 2 mL MeOH and dil. to vol. with CH_2Cl_2. (*2*) *Working std soln.*—0.05 mg/mL. Pipet 10.0 mL stock std soln and 5.0 mL internal std soln into 100 mL vol. flask. Add 6 mL MeOH and dil. to vol. with CH_2Cl_2.

39.B04 ***Preparation of Samples***

Bulk drugs.—Accurately weigh ca 50 mg sample (dried in vac. 3 h at 105°) into 100 mL vol. flask. Add 4 mL MeOH and dil. to vol. with CH_2Cl_2. Pipet 10.0 mL sample soln and 5.0 mL internal std soln into 100 mL vol. flask. Add 6 mL MeOH and dil. to vol. with CH_2Cl_2.

Tablets.—Grind tablets to pass No. 60 sieve. Transfer accurately weighed portion of powder contg 5 mg prednisolone to 100 mL vol. flask. Add 6 mL MeOH and place flask in ultrasonic bath 2 min. Add ca 50 mL CH_2Cl_2 and return to ultrasonic bath 1 min. Add 5.0 mL internal std soln and dil. to vol. with CH_2Cl_2. Shake flask vigorously and filter portion of soln thru Type LS filter into 25 mL g-s flask.

Individual tablet assay (content uniformity).—Place tablet in 125 mL g-s flask and add 200 μL H_2O. Let stand until tablet disintegrates and add 1 mL MeOH for each mg prednisolone declared. Place flask in ultrasonic bath until tablet is dispersed. Add 1.0 mL internal std soln for each mg prednisolone declared and dil. with CH_2Cl_2 to ca 0.05 mg/mL. Shake flask vigorously and filter portion of soln thru Type LS filter into 25 mL g-s flask.

39.B05 ***Determination***

Let LC system equilibrate with 1.5 mL/min flow rate. Inject 10–15 μL prednisolone working std soln. Retention times of fluoxymesterone and prednisolone should be ca 6 and 9 min, resp., with R (resolution) value ≥6. Inject 5 replicate aliquots of prednisolone working std soln and calc. response ratios. CV will be ≤2.0% in suitable system. Proceed with sample analysis, injecting same amt of sample soln.

39.B06 ***Calculations***

Calc. results, using response ratios (A and A') relative to internal std:

Bulk drugs:

$$\text{Prednisolone, \%} = (A/A') \times (C/S) \times 100$$

Tablets (composite):

$$\text{Prednisolone, mg/tab.} = (A/A') \times C \times (W/S) \times 100$$

Tablet (individual):

$$\text{Prednisolone, mg/tab.} = (A/A') \times C \times (T/D)$$

where A and A' = response ratios for sample and std solns, resp.; C = mg prednisolone/mL working std soln.; W = av. tablet wt (g); S = sample wt (g); T = labeled amt (mg) of prednisolone in tablet; and D = concn (mg/mL) of prednisolone in tablet soln, based on labeled amt/tab. and diln.

Ref.: JAOAC **67**, 674(1984).

CAS-50-24-8 (prednisolone)

40. DRUGS: ILLICIT

No additions, deletions, or other changes.

41. DRUGS AND FEED ADDITIVES IN ANIMAL TISSUES

No additions, deletions, or other changes.

42. DRUGS IN FEEDS

The following interim atomic absorption spectrophotometric method for determination of roxarsone in feeds was adopted first action:

Roxarsone in Feeds

Atomic Absorption Spectrophotometric Method

First Action

(Method dets total As and is not specific for roxarsone. Applicable range is 0–50 ppm 4-hydroxy-3-nitrobenzene arsonic acid.)

42.B01 ***Principle***

Sample is extd with aq. ammonium carbonate soln and analyzed by furnace AAS for total As content which is converted by factor to roxarsone concn in finished feed.

42.B02 ***Reagents***

(a) *Water.*—Super Quality from Millipore Super Q system.

(b) *Nitric acid.*—Mallinckrodt, ACS grade.

(c) *Argon.*—Linde purified.

(d) *Nickel nitrate.*—$Ni(NO_3)_2.6H_2O$ (Mallinckrodt AR).

(e) *Nickel nitrate soln.*—Approximately 2000 ppm Ni. Dissolve 10.0 g $Ni(NO_3)_2.6H_2O$ in H_2O and dil. to 1 L with H_2O.

(f) *Ammonium carbonate.*—Powder, purified (Matheson, Coleman & Bell).

(g) *Methanol.*—Anhyd., ACS (Matheson, Coleman & Bell).

(h) *Diluting soln.*—Add 5 mL concd HNO_3 and 150 mL anhyd. MeOH to 1 L vol. flask, dil. to vol. with H_2O, and mix.

(i) *Tantalum pentoxide.*—99.99% Ta_2O_5 (Aldrich Chemical Co.).

(j) *Tantalum pentoxide soln.*—Suspend 2.0 g in 10 mL H_2O.

(k) *Roxarsone std soln.*—1250 ppm roxarsone (356 ppm As). Accurately weigh 625.0 mg roxarsone ref. std (Salsbury Laboratories, Inc.) into 500 mL vol. flask. Dissolve and dil. to vol. with 2% ammonium carbonate soln. *Caution:* Wear protective clothing and avoid breathing dust.

(l) *Dilute roxarsone std soln.*—12.5 ppm roxarsone (3.56 ppm As). Dil. 10.0 mL roxarsone std soln to 1 L with H_2O.

(m) *Control feed extract.*—Using typical nonmedicated poultry or swine ration, prep. feed ext as described under *Sample Preparation*. Test suitability of control feed ext by dilg 1 mL aliquot with dilg soln used in sample prepn. Set up AAS system and furnace conditions as described in procedure. It is not necessary to perform calibration for this test; absorbance reading is satisfactory. Zero spectrophtr on 20 μL injection of dilg soln and measure A on 20 μL control feed ext. Absorbance reading ≤0.010 indicates suitability.

(n) *Working std soln.*—Transfer 1.0 mL dil. roxarsone std soln to 10 mL vol. flask. Dil. to vol. with control feed ext. Transfer 1.0 mL aliquot of this soln to 25 mL erlenmeyer and add 9.0 mL dilg soln. Twenty μL working std soln = 50 ppm roxarsone in feed sample for wts and vols used in procedure.

42.B03 ***Apparatus***

(a) *Atomic absorption spectrophotometer.*—Perkin-Elmer Model 5000, or equiv., with heated graphite atomizer furnace, autosampler, and printer sequencer.

(b) *Mechanical shaker.*—Wrist-action.

(c) *Pipets.*—Eppendorf: 10, 20, 50, and 1000 μL.

(d) *Dispensing pipet.*—Lab Industries Repipet, or equiv., 10 mL capacity set to deliver 9.0 mL dilg soln.

42.B04 ***Sample Preparation***

Grind sample in Wiley mill to pass 20 mesh sieve and thoroly mix. Weigh 5.0 g ground sample into 250 mL vol. flask or 300 mL erlenmeyer. Add 2.0 g ammonium carbonate powder and 200.0 mL H_2O, place on mech. shaker, and shake vigorously 5 min at room temp. Remove flask from shaker and let suspended feed particles settle 15–30 min. Transfer 1.0 mL aliquot of feed ext to 25 mL erlenmeyer and add 9.0 mL dilg soln. Mix thoroly. Repeat this step

on reagent blank and on std-fortified control feed ext equiv. to 50 ppm roxarsone in feed. Samples and std are now ready for furnace AAS analysis.

42.B05 ***AAS Conditions***

Set up graphite furnace and spectrophtr according to following conditions and allow 30 min warm-up time. Operating conditions: lamp, As EDL operated at 8 watts, properly aligned; lamp current, 0 ma; wavelength, 193.7 nm; slit, 0.7 nm bandpass, low position; readtime, 5 s; mode, AA-BG; readout, concn; signal, peak ht on instrument display and A on recorder if used; std 1, S1 = 50.0 ppm (μg/g) roxarsone (use 3 digits), do not use S2 and S3.

Install furnace assembly in AAS system and align as in manufacturer's instructions.

42.B06 ***Furnace Tube Coating Procedure***

Prep. 20% Ta_2O_5 aq. suspension. Shake suspension vigorously, introduce 50 μL aliquot into pyrolytically coated graphite furnace tube, and perform following sequence of operations: H_2O flow, 1–2 L/min to cool furnace; Ar pressure, 35 psi; on/off switch, on; gas control, on.

Step 1 (drying): temp., 100°; ramp time, 10 s; hold time, 90 s.

Step 2 (charring): temp., 1000°; ramp time, 10 s; hold time, 30 s.

Step 3 (atomizing): temp., 2700°; ramp time, 5 s; hold time, 10 s; stop flow, on.

Repeat coating procedure twice (3 applications). Tube is now ready for furnace AAS use.

(With initial coating, some material may flake after approx. 35 firings. If this happens, pass small brush or Kimwipe thru tube to remove loose tantalum and apply single recoating.)

42.B07 ***Furnace Conditions for Sample Assay***

Step 1 (drying): temp., 100°; ramp time, 10 s; hold time, 50 s.

Step 2 (charring): temp., 1000°; ramp time, 10 s; hold time, 30 s.

Step 3 (atomizing): temp., 2300°; ramp time, 0 s; hold time, 5 s; read, on; stop flow, on.

Step 4 (burnout): temp., 2400°; ramp time, 0 s; hold time, 5 s; read, off; flow, 300 mL/min (stop flow, off).

42.B08 ***Autosampler Conditions***

Install autosampler assembly in furnace and align as per manufacturer's instructions. Operating conditions: power switch, on, and let autosampler go thru count down; program sequence, press standby key to bring programmer into operating mode; method number, enter 1 and press method # key; recalibrate, for full tray recalibrate at 9A, 18B, and 27C; last sample, program number for last sample in sample tray; sample vol., enter 20 μL and press sample vol. key; alternate vol., enter 10 μL and press alternate vol. key ($Ni(NO_3)_2.6H_2O$ soln, 2000 ppm Ni); instrument program, enter 1 and press instrument program key; HGA program, enter 1 and press HGA program key.

Use sample tray 1 with method 1 in autosampler sequence. This method uses external std technic for instrument calibration. In this procedure, only position S1 is used because calibration is based on single point std with calibration std equiv. to 50.0 ppm roxarsone in finished feeds.

42.B09 ***Sample Analysis***

Load sample tray 1 as follows: Place blank of reagents (dilg soln (**h**)) in AZ location of tray; place 50.0 ppm roxarsone feed std in S1 location and at position 1 (check sample), then load samples in sequence around tray, starting at position 2. Place 2000 ppm Ni soln in reagent container and place in appropriate location for alternate sample in autosampler. Cover sample tray with cover provided to minimize evapn; these solns contain MeOH.

Instrument and samples are now ready for calibration and sample analysis. Press start/stop key to start program in sampling cycle. AZ and S1 calibration should be done in duplicate. Observe A for duplicate S1 values. These values should be within reasonable agreement (±5%) before program is allowed to proceed with samples. Instrument is recalibrated by autosampler in setup instructions which will monitor calibration for any changes and update calibration as time progresses.

42.B10 ***Calculations***

Instrument is programmed to calc. sample ppm on basis of single point std equiv. to 50 ppm roxarsone. For wts or vols other than those specified, manual calcn is required.

CAS-121-19-7 (roxarsone)

43. VITAMINS AND OTHER NUTRIENTS

(1) The following first action methods were adopted final action:

(a) Calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc in infant formula, inductively coupled plasma emission spectroscopy method, **43.292–43.296.**

(b) Sulfur amino acids in food and feed ingredients, ion exchange chromatographic method, **43.A08–43.A13.**

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(2) The first action method for determination of total dietary fiber in foods, enzymatic-gravimetric method, **43.A14–43.A20,** was adopted final action with the following changes and corrections:

(a) **43.A14,** *Principle,* line 1: Change to read ". . . >10% fat, . . ."

(b) **43.A14,** *Principle,* line 4: Change to read ". . . protein and starch. When analyzing mixed diets, always ext fat prior to detg total dietary fiber. Four vols . . ."

(c) **43.A15,** *Apparatus* (**h**): Change to read ". . . to 0.1 mg."

(d) **43.A16,** *Reagents* (**b**): Add as a last sentence, "One vol. H_2O mixed with 4 vols 95% EtOH will also give 78% EtOH final concn."

(e) **43.A16,** *Reagents* (**d**), line 3: Change to read ". . . 6.05 g Na phosphate . . ."

(f) **43.A16,** *Reagents* (**g**), line 4: Change to read, ". . . from Sigma Chemical Co., Kit No. TDF-100."

(g) **43.A18,** *Sample Preparation,* line 1: Change to read, ". . . fiber on dried sample. Homogenize . . ."

(h) **43.A20,** *Calculations:* Change to read as shown below:

$$\text{Blank} = \text{mg blank residue} - \left(\frac{\%\text{ protein in blank residue} + \%\text{ ash in blank residue}}{100}\right) \times \text{mg blank residue}$$

$$\text{TDF}, \% = \left[\frac{\text{mg residue} - \left(\frac{\%\text{ protein in residue} + \%\text{ ash in residue}}{100}\right) \times \text{mg residue} - \text{av. blank}}{\text{mg sample}}\right] \times 100$$

(3) The first action method for determination of nutrients in milk-based infant formula, **43.A21–43.A40,** was amended as follows:

In **43.A27,** *Assay,* change line 5 to read:

. . . bring vol. to 5 mL/tube. Add 5 mL basal medium stock soln, (j), to all tubes for total vol. of 10 mL/tube. Cap all tubes . . .

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(4) The following methods for nutrient analysis of milk-based infant formula were adopted first action:

Proximate Analysis of Milk-Based Infant Formula

First Action

43.B01 ***Ash***

See **16.035.**

43.B02 ***Fat***

See **16.187(a), 16.192,** and **16.064,** using sample size 4–5 g.

43.B03 ***Protein***

See **16.036, 2.055–2.057,** except use 12 g sample and do not increase amt H_2SO_4 for each g sample.

43.B04 ***Total Solids***

See **16.032.**

43.B05 ***Carbohydrate***

Det. by difference:

Carbohydrate = total solids − (protein + fat + ash)

Chloride in Milk-Based Infant Formula

Potentiometric Method

First Action

43.B06 ***Principle***

Product is dispersed with H_2O and acidified; sol. chlorides are titrd potentiometrically with $AgNO_3$.

43.B07 ***Apparatus***

See **32.035.**

43.B08 ***Reagents***

(a) *Nitric acid, dilute.*—(1 + 49). Dil. 20 mL HNO_3 to 1 L with H_2O.

(b) *Silver nitrate std soln.*—0.0856N. Dissolve 14.541 g $AgNO_3$ in H_2O and dil. to 1 L in vol. flask. Stdze as in **32.037.** Store in pyrex container out of direct sunlight. Soln is stable in room light.

(c) *Sodium chloride std soln.*—0.0856N. Dissolve in H_2O 5.000 g NaCl previously dried 2 h at 110°, and dil. to 1 L in vol. flask.

(d) *Water.*—Distd or deionized, halogen-free, by following test: Add 1 mL ca 0.1N $AgNO_3$ and 5 mL HNO_3 (1 + 4) to 100 mL of the H_2O. No more than slight turbidity should be produced.

43.B09 ***Standardization***

Proceed as in **32.037,** except in second paragraph, calc. normality from $AgNO_3$ soln used; do not adjust to 0.0856N.

43.B10 ***Determination***

Pipet 50 mL ready-to-feed infant formula into 250 mL beaker. Add 50 mL HNO_3 (1 + 49). Titr. as in **32.037,** using 10 mL buret (graduated in 0.05 mL).

mg Cl/L = [(mL $AgNO_3$) × (N $AgNO_3$) × (35.453 mg/meq) × (1000 mL/L)]/50 mL

Cobalamin (Vitamin B_{12} Activity) in Milk-Based Infant Formula

Turbidimetric Method

First Action

(Thruout all stages, except where otherwise noted, protect solns from undue exposure to light.)

43.B11 ***Stock Solutions for Basal Media***

(a) *Adenine–guanine–uracil soln.*—Dissolve 1.0 g each of adenine sulfate, guanine.HCl, and uracil in 50 mL warm HCl (1 + 1), cool, and dil. with H_2O to 1 L.

(b) *Asparagine soln.*—Dissolve 10 g L-asparagine.H_2O in H_2O and dil. to 1 L.

(c) *Polysorbate 80 soln.*—Dissolve 25 g polysorbate 80 (polyoxyethylene sorbitan monooleate) in alcohol to make 250 mL.

(d) *Salt soln A.*—Dissolve 50 g anhyd. KH_2PO_4 and 50 g anhyd. K_2HPO_4 in H_2O, dil. to 1 L, and add 10 drops of HCl.

(e) *Salt soln B.*—Dissolve 20 g $MgSO_4.7H_2O$, 1 g NaCl, 1 g $FeSO_4.7H_2O$, and 1 g $MnSO_4.H_2O$ in H_2O, dil. to 1 L, and add 10 drops of HCl.

(f) *Vitamin soln I.*—Dissolve 25 mg riboflavin, 25 mg thiamine.HCl, 0.25 mg biotin, and 50 mg niacin in 0.02N HOAc to make 1 L.

(g) *Vitamin soln II.*—Dissolve 50 mg *p*-aminobenzoic acid, 25 mg Ca pantothenate, 100 mg pyridoxine.HCl, 100 mg pyridoxal.HCl, 20 mg pyridoxamine.2HCl, and 5 mg folic acid in 25% alcohol to make 1 L.

(h) *Xanthine soln.*—Suspend 1.0 g xanthine in 150–200 mL H_2O, heat to ca 70°, add 30 mL NH_4OH (2 + 3), and stir until solid dissolves. Cool, and dil. to 1 L with H_2O.

(i) *Acid-hydrolyzed casein.*—Available as HY CASE AMINO from Humko Sheffield Chemical Div., PO Box 398, Memphis, TN 38101. Other com. sources of vitamin-free, acid-hydrolyzed casein have been found satisfactory.

43.B12 ***Culture and Suspension Media***

(a) *Liquid culture medium.*—Dissolve 15 g peptonized milk, 5 g H_2O-sol. yeast ext, 10 g anhyd. glucose, and 2 g anhyd. KH_2PO_4 in ca 600 mL H_2O. Add 100 mL filtered tomato juice, and adjust to pH 6.5–6.8 with NaOH soln. Add, with mixing, 10 mL polysorbate 80 soln and dil. with H_2O to 1 L. (Difco Lactobacilli Broth AOAC, Difco Laboratories, has been found satisfactory.) Dil. measured amt with equal vol. of H_2O contg 1 ng vitamin B_{12}/mL. Add 10 mL portions to 20 × 150 mm screw-cap test tubes, sterilize 15 min in autoclave at 121–123°, cool rapidly, and store in refrigerator.

(b) *Agar culture medium.*—To 500 mL liq. culture medium, (a), add 5.0–7.5 g agar, and heat with stirring on steam bath until agar dissolves. Add ca 10 mL portions of hot soln to test tubes, cover to prevent contamination, sterilize 15 min in autoclave at 121–123°, and cool tubes in upright position as rapidly as practicable to keep color formation at min. Store in dark at ca 10°. (Difco agar culture medium for AOAC microbiological assays (Lactobacilli Agar AOAC) has been found satisfactory.)

(c) *Suspension medium.*—Dil. measured vol. appropriate basal medium stock soln, Table **43.03,** with equal vol. H_2O. Add 10 mL portions dild medium to test tubes, cover to prevent contamination, sterilize 15 min in autoclave at 121–123°, and cool tubes as rapidly as practicable to keep color formation at min. Store in dark at ca 10°.

43.B13 ***Stock Culture of Test Organism***

For *Lactobacillus leichmannii,* ATCC No. 7830, prep. stab culture in 1 or more tubes of agar culture medium, **43.B12(b).** Incubate 6–24 h at 37° held const to within 0.5°, and finally store in dark at ca 10°. Before using new culture in assay, make several successive transfers of culture in 1–2 week period. Prep. fresh stab culture at least once a week and do not use for prepg inoculum if >1 week old.

Activity of slow-growing culture may be increased by daily or twice-daily transfer of stab culture, and is considered satisfactory when definite turbidity in liq. inoculum can be observed 2–4 h after

inoculation. Slow-growing culture seldom gives suitable response curve and may cause erratic results.

43.B14 ***Assay Tubes***

Meticulously cleanse by suitable means (Na lauryl sulfate USP has been found satisfactory as detergent), hard-glass test tubes, ca 20 × 150 mm, and other necessary glassware. (The test organism is highly sensitive to minute amts of growth factors and to many cleansing agents. Therefore, it may be preferred to follow cleansing by heating 1–2 h at ca 250°.)

Prep. tubes contg std soln as follows: To test tubes add, in duplicate (or replicate), 0.0 (for uninoculated blanks), 0.0 (for inoculated blanks), 1.0, 2.0, 3.0, 4.0, and 5.0 mL, resp., of std soln. Prep. tubes contg assay soln as follows: To similar test tubes add, in duplicate (or replicate), 1.0, 2.0, 3.0, and 4.0 mL, resp., of assay soln.

To each tube of std soln and assay soln, add H_2O to make 5.0 mL. Then add 5.0 mL basal medium stock soln and mix. Cover tubes suitably to prevent bacterial contamination, and sterilize 10 min in autoclave at 121–123°, reaching this temp. in not >10 min. Cool as rapidly as practicable to keep color formation at min. Take precautions to keep sterilizing and cooling conditions uniform thruout assay. Too close packing of tubes in autoclave, or overloading of it, may cause variation in heating rate.

Aseptically inoculate each tube, except 1 set of duplicate (or replicate) tubes contg 0.0 mL std soln (uninoculated blanks), with 1 drop of inoculum. Incubate for 16–24 h at 37° held const to within 0.5°. Contamination of assay tubes with any foreign organism invalidates assay.

43.B15 ***Calibration of Photometer***

Using inoculum, **43.B20,** and std stock soln, **43.B19(a),** proceed as directed below. Aseptically add 1 mL inoculum to ca 300 mL sterile suspension medium contg 1.0 mL std stock soln, and incubate mixt. for same period and at same temp. to be employed in detn, **43.B16.** After incubating, centrf. and wash cells 3 times with ca 50 mL portions of 0.9% NaCl soln; then resuspend cells in the NaCl soln to make 25 mL.

Evap. 10 mL aliquot of cell suspension on steam bath, and dry to const. wt at 110° in vac. oven. Correcting for wt of NaCl, calc. dry wt of cells in mg/mL of suspension.

Dil. second measured aliquot of cell suspension with 0.9% NaCl soln so that each mL is equiv. to 0.5 mg dry cells. To test tubes add, in triplicate, 0.0 (for blanks), 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, and 5.0 mL, resp., of this dild cell suspension. To each tube add 0.9% NaCl soln to make 5.0 mL. Then add 5.0 mL basal medium stock soln, mix (1 drop of suitable antifoam agent may be added; 1–2% soln of Dow Corning Antifoam AF Emulsion or Antifoam B has been found satisfactory), and transfer to optical cell. With blanks set at 100%*T*, measure %*T* of each tube under same conditions to be used in assay. Prep. curve by plotting %*T* readings for each level of dild cell suspension used against cell content (mg dry wt) of resp. tubes.

Repeat calibration step at least twice more for photometer to be used in assay. Draw composite curve, best representing 3 or more individual curves, relating %*T* to mg dried cell wt for photometer under conditions of assay. Once curve for particular instrument is established, all subsequent relationships between %*T* and cell wt are detd directly from this curve. Assay limits expressed as mg dried cell wt/tube are so detd.

43.B16 ***Determination***

Incubate tubes 16–24 h until max. turbidity is obtained, as demonstrated by lack of significant change during 2 h addnl incubation period in tubes contg highest level of std soln. Det. *T* of tubes as follows: Thoroly mix contents of each tube (1 drop of suitable antifoam agent soln may be added; 1–2% soln of Dow Corning Antifoam AF emulsion or Antifoam B has been found satisfactory), and transfer to optical cell. Agitate contents, place cell in photometer set at any specific wavelength between 540 and 660 nm, and read %*T* when steady state is reached. Steady state is observed 3–4 s after agitation when galvanometer reading remains const for >30 s. Allow ca same time interval for reading on each tube.

With *T* set at 100% for uninoculated blank level, read %*T* of inoculated blank level. If this reading corresponds to dried cell wt >0.6 mg/tube, disregard results of assay. Then with *T* reset at 100% for inoculated blank level, read %*T* for each of remaining tubes. Disregard results of assay if %*T* observed at 5.0 mL level of std soln (against inoculated blank) is equiv. to that for dried cell wt <1.25 mg/tube.

Prep. std concn-response curve by plotting %*T* readings for each level of std soln used against amt of ref. std contained in resp. tubes.

Det. amt of vitamin for each level of assay soln by interpolating from std curve. Discard any observed *T* values equiv. to <0.5 mL or >4.5 mL, resp., of std soln. Proceed as in **43.B17.**

43.B17 ***Calculation***

For each level of assay soln used, calc. vitamin content/mL of assay soln. Calc. av. of values obtained from tubes that do not vary by >10% from this av. If number of acceptable values remaining is <⅔ of original number of tubes used in 4 levels of assay soln, data are insufficient for calcg potency of sample. If number of acceptable values remaining is ≥⅔ of original number of tubes, calc. potency of sample from av. of them.

43.B18 ***Basal Medium Stock Solution***

For each liter of vitamin B_{12}-free, double-strength basal medium to be prepd, add the following in order listed: 20 mL adenine–guanine–uracil soln, 20 mL asparagine soln, 20 mL salt soln A, 20 mL salt soln B, 40 mL vitamin soln I, 40 mL vitamin soln II, 20 mL xanthine soln, and 20 mL polysorbate 80 soln. Add H_2O to total vol. ca 800 mL and mix well to prevent the following solids from pptg when added. Then add the following in order listed: 10 g acid-hydrolyzed casein, 0.4 g L-cystine and 0.4 g D-L-tryptophan dissolved together in min. vol. of dil. HCl (236 mL concd HCl/L), 40 g dextrose, 33.2 g Na acetate trihydrate, and 4 g ascorbic acid. Do not agitate soln vigorously after adding ascorbic acid. Swirl gently until all solids are dissolved. Adjust pH to 6.0 with either HCl or NaOH, using pH meter. Dil. to vol. Add polysorbate soln after pH adjustment when indicator is used because indicator is absorbed by polysorbate. Some com. media have been found satisfactory.

43.B19 ***Cyanocobalamin Standard Solutions***

(a) *Stock soln.*—100 ng/mL. Accurately weigh, in closed system, USP Cyanocobalamin Ref. Std equiv. to 50–60 μg cyanocobalamin, that has been dried to const wt and stored in dark over P_2O_5 in desiccator. Dissolve in 25% alcohol, and dil. with addnl 25% alcohol to make cyanocobalamin concn exactly 100 ng/mL. Store in dark at ca 10°.

(b) *Intermediate soln.*—1 ng/mL. Dil. 10 mL stock soln with 25% alcohol to 1 L. Store in dark at ca 10°.

(c) *Working solns.*—Dil. an aliquot of intermediate soln with H_2O to 500 mL. This primary std soln is equiv. to 0.01–0.04 ng/mL and is ca equal to assay concn of sample prepn. Similarly, prep. 2 addnl secondary stds, one higher and one lower (e.g., 0.010 and 0.020 ng/mL) to provide wider working range for vitamin B_{12} concns in samples.

43.B20 ***Inoculum***

Make transfer of cells from stock culture of *Lactobacillus leichmannii,* **43.B13,** to 2 sterile tubes contg 10 mL liq. culture medium. **43.B12(a).** Keep all transfers as sterile as possible. Incubate 6–24 h at 37° held const to 0.5°. Under aseptic conditions, centrf. culture and decant supernate. Wash cells with 3 ca 10 mL portions of sterile 0.9% NaCl soln or sterile suspension medium, **43.B12(c).** Resuspend cells in 10 mL sterile 0.9% NaCl soln or sterile suspension medium. Dil. aliquot with sterile 0.9% NaCl soln or sterile suspension medium to give *T* equiv. to that for dried cell wt (as described in **43.B15**) of 0.50–0.75 mg/tube when read against suspension medium set at 100%*T*. Cell suspension so obtained is inoculum.

43.B21 ***Assay Solution***

Prep. aq. extg soln just before use contg, in each 100 mL, 1.3 g anhyd. Na_2HPO_4, 1.2 g citric acid.H_2O, and 1.0 g anhyd. Na metabisulfite, $Na_2S_2O_5$. Take amt of sample contg 50–100 ng vitamin B_{12} and transfer to beaker contg amt of extn soln to contain not >0.03 mg $Na_2S_2O_5$/mL at final assay concn between 0.01 and 0.04 ng vitamin B_{12}/mL.

Disperse sample soln evenly in liq. Wash down sides of beaker with H_2O and cover with watch glass. Autoclave mixt. 10 min at 121–123° and cool. If lumping occurs, agitate mixt. until particles are evenly dispersed. Adjust mixt. to pH 4.5 with vigorous agitation. Dil. mixt. with H_2O to measured vol. to obtain approx. vitamin B_{12} concn of 0.2 ng/mL and filter.

Take aliquot of filtrate and check for dissolved protein by adding dropwise, first dil. HCl, and if no further ppt forms, then with vigorous stirring, NaOH soln. Proceed as follows with aliquot:

(a) If no further pptn occurs, add with vigorous stirring, NaOH soln to pH 6.0, dil. with H_2O to final measured vol. contg vitamin B_{12} activity equiv. to ca 0.014 ng/mL.

(b) If further pptn occurs, adjust mixt. to point of max. pptn (ca pH 4.5), dil. with H_2O to measured vol., and filter. Take aliquot of clear filtrate and proceed as under (a).

43.B22 ***Assay***

Using std soln, **43.B19(c)**, assay soln, **43.B21**, basal medium stock soln, **43.B18**, and inoculum, **43.B20**, proceed as in **43.B14**, **43.B16**, and **43.B17**.

CAS-68-19-9 (cobalamin; cyanocobalamin; vitamin B_{12})

Phosphorus in Milk-Based Infant Formula
Spectrophotometric Method
First Action

43.B23 ***Principle***

P is detd spectrophtric. on ashed sample by complexing with molybdovanadate reagent.

43.B24 ***Apparatus***

(a) *Spectrophotometer.*—Capable of operation at 400 nm.

(b) *Muffle furnace.*—Equipped with pyrometer and controller.

(c) *Ashing dishes.*—Silica or porcelain.

43.B25 ***Solutions***

(a) *Dilute hydrochloric acid.*—(1 + 3). Add 250 mL HCl to 750 mL H_2O.

(b) *Molybdovanadate reagent.*—Dissolve 20 g ammonium molybdate in 200 mL hot H_2O and cool. Dissolve 1.0 g ammonium metavanadate in 125 mL hot H_2O, cool, and add 160 mL HCl. Gradually add, with stirring, molybdate soln to vanadate soln and dil. with H_2O to 1.0 L.

(c) *Phosphorus std soln.*—(*1*) *Stock std soln.*—2 mg P/mL. Weigh 8.7874 g KH_2PO_4, dried 2 h at 105°. Quant. transfer to 1 L vol. flask and add ca 750 mL H_2O to dissolve. Dil. to vol. with H_2O. Store in refrigerator. (*2*) *Working std soln.*—0.1 mg P/mL. Dil. 50 mL stock std soln with H_2O to 1 L. Store in refrigerator. Prep. fresh on day of analysis.

43.B26 ***Preparation of Sample***

Accurately weigh 10.0 g sample (ca 4.0 mg P) into ashing dish and evap. to dryness on hot plate or steam bath. Ignite sample in muffle furnace at max. temp. of 600° until free of C (3–4 h). Cool and add 40 mL HCl (1 + 3) and several drops of HNO_3 and bring to boil on hot plate. Cool, transfer quant. to 100 mL vol. flask, and dil. to vol. with H_2O.

43.B27 ***Determination***

Transfer aliquots of working std soln of 0.0, 5.0, 8.0, 10.0, and 15.0 mL to resp. 100 mL vol. flasks. These represent 0.0, 0.5, 0.8, 1.0, and 1.5 mg P. Pipet 20.0 mL sample soln into 100 mL vol. flask. To each std and sample flask, add 20.0 mL molybdovanadate reagent, dil. to vol. with H_2O, and mix well. Let flasks stand 10 min for complete color development.

Det. *A* of stds and samples in 1 cm cells at max. near 400 nm, using 0.0 mg std to zero spectrophtr. Use linear regression of std *A* vs mg P of stds to det. mg P for each sample.

43.B28 ***Calculations***

$$\text{P, mg/L} = \text{mg P} \times 5000 \times \text{sample density/g sample}$$

Sample density should be ca 1.03 g/mL for ready-to-feed formula.

Thiamine (Vitamin B_1) in Milk-Based Infant Formula
Fluorometric Method
First Action

43.B29 ***Principle***

Thiamine content of ext is estd by oxidizing thiamine to thiochrome and measuring fluorescence. Intensity of fluorescence is proportional to thiamine concn.

43.B30 ***Apparatus***

(a) *Photofluorometer.*—Equipped with narrow transmittance range filters with input filter max. at ca 365 nm and output filter max. at ca 435 nm.

(b) *Chromatographic tubes.*—Glass chromatgc tubes (ca 275 mm overall length with reservoir capacity ca 60 mL) consisting of 3 parts described below fused together and having approx. measurements listed (inside diam.): Top: reservoir 95 mm long and 30 mm diam., converging into middle part. Middle part: adsorption tube 145 mm long and 6 mm diam., converging into lower part. Lower part: tube drawn into capillary 35 mm long and of such diam. that, when tube is charged, rate of flow will not be >1 mL/min.

43.B31 ***Reagents***

(a) *Chromatographic resin.*—Add 300 mL 2N HCl to 50 g Bio-Rex 70 (H form) (Bio-Rad Laboratories), stir 15 min, decant, and repeat. Add 300 mL H_2O, stir 1 min, decant, and repeat until pH of H_2O is 4.5–7.0. H_2O should be free of suspended resin when allowed to settle 15 s. If not, repeat H_2O washing until clear.

(b) *Sodium acetate, 2N.*—Dissolve 272 g NaOAc.$3H_2O$ in enough H_2O to make 1 L.

(c) *Bromcresol green indicator.*—Dissolve 0.1 g indicator by triturating in agate mortar with 2.8 mL 0.05N NaOH, and dil. to 200 mL with H_2O.

(d) *Enzyme soln, 10% w/v.*—Dissolve 10 g Takadiastase (Pfaltz and Bauer) in H_2O and dil. to 100 mL. Prep. fresh daily.

(e) *Potassium chloride soln, 25% w/v.*—Dissolve 250 g KCl in sufficient H_2O to make 1 L.

(f) *Acid potassium chloride soln.*—Add 8.5 mL HCl to 1 L neut. KCl soln.

(g) *Potassium ferricyanide soln, 1%.*—Dissolve 1 g $K_3Fe(CN)_6$ in sufficient H_2O and dil. to 100 mL. Prep. fresh daily.

(h) *Oxidizing reagent.*—Mix 4.0 mL 1% $K_3Fe(CN)_6$ soln with sufficient 15% NaOH soln to make 100 mL. Use this reagent within 4 h after prepn.

(i) *Isobutyl alcohol.*—Redistd in all-glass app.

(j) *Quinine sulfate stock soln.*—Use quinine sulfate soln to monitor reproducibility of fluorometer. Prep. stock soln by dissolving 10 mg quinine sulfate in 0.1N H_2SO_4 sufficient to make 1 L. Store stock soln in red or amber containers.

(k) *Quinine sulfate std soln.*—Dil. 5.0 mL quinine sulfate stock soln with sufficient 0.1N H_2SO_4 to make 200 mL. This soln fluoresces to about same degree as does isobutyl alcohol ext of thiochrome obtained from 1 μg thiamine HCl. Store this soln in red or amber containers.

(l) *Acidified alcohol, 20% w/v.*—Dil. 250 mL alcohol with H_2O to make 1 L. Add HCl dropwise to adjust to pH 3.5–4.3.

(m) *Acetic acid, 3%.*—Dil. 30 mL glacial acetic acid with H_2O to 1 L.

43.B32 *Tube Preparation*

Prep. chromatgc tubes for use as follows: With aid of glass rod, place pledget of fine glass wool over upper end of capillary. Taking care to wash down all resin from walls of reservoir, add H_2O suspension of 1.0–2.0 g resin to adsorption tube. To keep air out of adsorption tube, keep layer of liq. above surface of resin during adsorption process. Place rubber cap filled with H_2O to avoid inclusion of air, over lower end of capillary to prevent tube from draining.

43.B33 *Preparation of Thiamine HCl Standard Solutions*

(a) *Stock std soln, 100 μg/mL.*—Accurately weigh 50.0 mg USP Thiamine HCl Ref. Std previously dried by storing over P_2O_5 in desiccator. Since ref. std is hygroscopic, take precautions to avoid moisture absorption during weighing. Transfer 50.0 mg std to 500 mL vol. flask. Dissolve in acidified 20% alcohol, and dil. to 500.0 mL with addnl acidified alcohol. Store in red or amber g-s bottle in refrigerator. Soln is stable several months.

(b) *Working std soln, 3 μg/mL.*—Dil. 3.0 mL stock std soln with 0.1N HCl to make 100 mL. Prep. fresh daily.

43.B34 *Preparation of Sample*

(a) Pipet into 100 mL centrf. tube (or 125 mL erlenmeyer) amt of sample estd to contain ca 15 μg thiamine HCl. Add 65 mL 0.1N HCl. Continue as in (c) below.

(b) Pipet 5 mL working std soln (3 μg/mL) into 100 mL centrf. tube (or 125 mL erlenmeyer). Add 65 mL 0.1N HCl. Continue as in (c) below.

(c) Evenly disperse any solid material in liq. If lumping occurs, agitate vigorously so that all particles come in contact with liq. Digest 30 min at 95–100° in steam bath or in boiling H_2O with frequent mixing, or autoclave mixt. at 121° for 30 min.

Cool, and, if lumping occurs, agitate mixt. until particles are evenly dispersed.

43.B35 *Enzyme Hydrolysis*

Quant. transfer cooled sample and std exts to resp. 100 mL vol. flasks. Adjust pH of each soln to 4.0–4.5 with ca 5 mL 2N NaOAc soln using bromcresol green indicator on spot plate. Add 5 mL enzyme soln, mix, and incubate 3 h at 45–50°. Cool, dil. to 100 mL with 0.1N HCl, and filter thru paper known not to absorb thiamine. (Ash-free paper has been found satisfactory.)

43.B36 *Purification*

Pass 10 mL aliquot of filtered std and sample solns (contg ca 1.5 μg thiamine HCl) thru resp. prepd chromatgc tubes. Then wash each tube with three 5 mL portions of almost-boiling H_2O, taking care to prevent surface of liq. from falling below surface of resin.

Elute thiamine from resin by passing five 4.0–4.5 mL portions of almost-boiling acid KCl soln thru each tube. Take care to prevent surface of liq. from falling below surface of resin until final portion of acid KCl soln has been added. Collect eluate thus obtained from hydrolysis and purification of std in 25 mL vol. flask. Cool and dil. to vol. with acid KCl soln. Designate this as *std soln*. Collect eluate thus obtained from hydrolysis and purification of aliquot of sample in 25 mL vol. flask. Cool and dil. to vol. with acid KCl soln. Designate this as *assay soln*.

43.B37 *Oxidation of Thiamine to Thiochrome*

Precision and accuracy of results depend on uniform technic in conducting following oxidation procedure:

To each of 4 or more 40 mL tubes or reaction vessels, add ca 1.5 g NaCl or KCl and 5 mL std soln. (Protect these solns from light; thiochrome is light-sensitive.)

Use pipet that delivers 3 mL in 1–2 s for addn of oxidizing reagent. Place tip of pipet, contg oxidizing reagent, in neck of tube and hold it so that stream of oxidizing reagent does not hit side of tube. Swirl tube gently to produce rotary motion in liq. and immediately add 3 mL oxidizing reagent. Remove pipet and swirl tube again to ensure adequate mixing. Let stand 9.0 min. *Immediately* add 13 mL redistd isobutyl alcohol, stopper tube, and shake vigorously 15 s. Treat one or more addnl tubes similarly. Treat each of 2 or more of remaining tubes (std blanks) in same manner except replace oxidizing reagent with 3 mL 15% NaOH soln.

To each of 4 or more similar tubes, add 5 mL assay soln. Treat these tubes in same manner as directed for tubes contg std soln (including addn of 1.5 g NaCl or KCl).

After isobutyl alcohol has been added to all tubes shake again ca 2 min. Centrf. tubes at low speed until clear supernate can be obtained from each. Pipet or decant ca 10 mL isobutyl alcohol ext (upper layer) from each tube into cell for measurement of thiochrome fluorescence.

43.B38 *Thiochrome Fluorescence Measurement*

Measure fluorescence of isobutyl alcohol ext from oxidized assay soln and call this reading "U". Measure fluorescence of ext from assay soln which has been treated with 3 mL 15% NaOH soln (assay blank) and call this reading "b". Measure fluorescence of ext from std soln which has been treated with 3 mL 15% NaOH soln (std blank) and call this reading "d".

Thiamine HCl, mg/L "as fed" formula

$$= [(U - b)/(S - d)] \times [15/(A \times 1000)] \times R$$

where U = reading of assay sample; b = reading of assay sample blank; S = reading of std sample; d = reading of std sample blank; R = reconstitution value expressed as wt or vol. of sample required to make 1 L "as fed" formula; A = amt of sample, in g or mL.

43.B39 *Notes and Precautions*

Thiochrome is stable in isobutyl alcohol but it is desirable to make readings promptly. Ordinarily, delays up to 20 min will not alter results, providing solns are not exposed to bright daylight.

Duplicate samples simultaneously carried thru assay procedure should give values agreeing ±2.5% of mean. Assays of homogeneous material made on different days should agree ±5% of mean.

Between readings of thiochrome samples, check photofluorometer with working quinine soln.

CAS-67-03-8 (thiamine HCl)
CAS-59-43-8 (thiamine)

44. EXTRANEOUS MATERIALS: ISOLATION

(1) The following alkaline phosphatase detection method for mammalian feces in corn meal was adopted first action:

Mammalian Feces in Corn Meal
Alkaline Phosphatase Detection Method
First Action

44.B01 *Principle*

Intestinal tract of most mammals contains alk. phosphatase enzyme. Enzyme at test pH and temp. splits phosphate radicals from substrate/pH indicator phthln diphosphate to produce light pink to red-purple color from free phthln.

44.B02 *Apparatus*

(a) *Hot water bath.*—Maintained at 42° ± 1°.

(b) *Hot plate stirrer and 41 mm ovoid stirring bar.*—Fisher 1451158A or equiv.

(c) *Petri dishes.*—Plastic disposable, 150 × 20 mm or 150 × 15 mm (Falcon 1058 or plastic/glass equiv.).

(d) *Weighing boats.*—8.1 × 8.1 × 1.9 cm, 100 mL capacity (Fisher Scientific Co., Cat. No. 02210B, or approx. size equiv.).

44.B03 ***Reagents***

(a) *Magnesium chloride soln.*—Dissolve 0.203 g $MgCl_2.6H_2O$ and dil. to 500 mL with H_2O. Indefinite shelf life.

(b) *Stock test reagent.*—Dissolve 19.0 g borax ($NaB_4O_7.10H_2O$) and 6.28 g anhyd. Na_2CO_3 in 1 L H_2O with stirring. Add 0.94 g phthln diphosphate and stir while adding 2 mL $MgCl_2$ soln. Prepn is stable ca 4 months at room temp. Soln should be colorless and ca pH 9.5. Discard if not colorless. Degraded phthln diphosphate produces pink color in reagent. Store phthln diphosphate in desiccator below 0°. (Phthln diphosphate, Sigma P 9875.)

(c) *Liquid test agar.*—Prep. fresh before using, 150 mL per 10 g sample to be analyzed. Measure equal vol. of stock test reagent, (b) (half of total test agar vol. needed), and H_2O into sep. appropriate size beakers. Beaker for H_2O must be large enough to accommodate 2 times vol. of H_2O. Reserve stock test reagent. Place beaker of H_2O on hot plate stirrer, add stirring bar (ovoid 41 mm), and, with rapid stirring, add sufficient agar to H_2O to yield 2% agar soln (1.5 g agar/75 mL H_2O). Continue stirring, and heat to boil (watch for foam-over). Cover beaker with cover glass to prevent heat loss. When agar begins to foam, add reserved stock test reagent, pouring reagent down side of beaker to prevent agar from coming out of soln. Stir rapidly with heat ca 1 min.

44.B04 ***Determination***

Weigh 10 g corn meal into weighing boat from each well mixed subsample. Prep. appropriate amt of liq. test agar, (c). Cool boiled test agar by placing beaker of test agar into larger beaker of cold H_2O. Continually *stir* test agar and *maintain* temp. check until soln is 55°. Pour test agar into petri dish, ca 150 mL per dish. Immediately distribute monolayer of corn meal onto surface of test agar. This is accomplished by gently tapping weighing boat held so that corn meal flows over one side, not from corner, while tilting and moving boat above agar surface as corn meal flows. Let corn meal become wet with test agar and sink before adding another layer. Continue in this manner until entire 10 g sample has been added. Distribution time should be ca 1 min per 10 g corn meal sample. Best sepn of corn meal and excreta occurs while test agar is hot. Multiple samples can be added to resp. dishes, one at a time or a little of each sample to its resp. dish sequentially, until all of each sample has been distributed.

Let test agar gel (requires ≥20 min). Agar is gelled when no agar flows when dish is slightly tipped. (Caution: Take care not to disturb dispersed material in liq. test agar. If particles are moved, color concn around particles will be diffused and pos. spots will be missed.) When gelling is complete, check for pink spots, viewing plate against white background. Mark spots on lid of dish, using grease pencil. Mark lid and bottom of dish, using H_2O-proof marker, so that lid can always be placed in same position.

Incubate petri dish at 42° in H_2O bath 10 min. Submerge plate in H_2O bath just enough to cover agar level in dish. When incubating several dishes at one time, place plates in H_2O in pairs, staggering times so that reading delays are avoided and small, rapidly diffusing pink spots are not missed. Remove plate from H_2O bath after 10 min. Wipe inside lid to remove fog and hold lid so that bottom edge of lid is 2–3 mm above top edge of petri dish base while reading plate. Replace lid and repeat 10 min incubation 2 times, marking addnl pink spots on the petri dish lid after each period. Tally and record number of spots as fecal particles/10 g sample. Spots which appear and then are not seen on subsequent checks and spots which are seen on bottom of petri dish with corn meal are to be counted in tally.

44.B05 ***Positive Control for Feces and Test Agar Medium***

Scatter some ground known rodent feces on petri dish of liq. test agar in place of corn meal sample and continue with method. One control plate is needed for each batch of test agar prepd.

44.B06 ***Response***

Amt, intensity, and range of color (light pink to red-purple) observed will vary depending on size of fecal particles, species source, and diet of animal. Particles as small as 250 μm can be identified.

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(2) An improved thin layer chromatographic visualization reagent and color development technique for uric acid was adopted to replace **44.186**(c) and **44.188**(c), respectively, in method **44.186–44.188**, excrement (bird and insect) on food and containers, thin layer chromatographic method for uric acid. The entire method is printed here.

Excrement (Bird and Insect) on Food and Containers
Thin Layer Chromatographic Method for Uric Acid
First Action

(Applicable to suspect material not suitable for detn by **44.185** and/or to confirmation of **44.185** when adequate material is available.)

44.B07 ***Apparatus and Reagents***

(a) *Thin layer cellulose plates.*—*See* **44.176**. E. Merck cellulose plates, 0.10 mm, EM No. 5757-7 (EM Science, Cherry Hill, NJ 08034) have also been found satisfactory.

(b) *Cellulose powder.*—*See* **44.003**(g).

(c) *Detection spray.*—(*1*) *Soln A.*—1% $K_3Fe(CN)_6$. (*2*) *Soln B.*—2% $FeCl_3$ (calcd as anhyd.). Refrigerate both solns. Protect soln A from light. Solns are stable ca 2 weeks. (*3*) *Spray reagent.*—To 18 mL H_2O, add 1 mL each of solns A and B; mix. Prep. *immediately* before use.

(d) *Developing solvent.*—*n*-BuOH–MeOH–H_2O (4 + 4 + 3). Measure vols sep. and mix well to form stable single phase. To 30 mL of this soln, add 1 mL HOAc; mix well. Prep. fresh daily.

(e) *Dye mixture.*—Dissolve 16 mg amaranth (formerly FD&C Red No. 2) and 32 mg FD&C Yellow No. 6 in 50 mL H_2O; mix well.

(f) *Lithium carbonate soln.*—1 mg/mL.

(g) *Uric acid std soln.*—(*1*) *Stock soln.*—1 mg/mL. Dry 105 mg uric acid in 100° oven overnight and cool to room temp. in desiccator. Accurately weigh 60 mg Li_2CO_3 and transfer to 100 mL vol. flask. Accurately weigh 100 mg cool uric acid and transfer quant. to the 100 mL flask with ca 50 mL H_2O. Place in 60° H_2O bath and agitate until soln clears. Cool immediately under tap H_2O to room temp. and dil. to vol. with H_2O. For short term use (<3 days), store in refrigerator; for extended use, place portions in small containers and store hard-frozen. (*2*) *Working soln.*—100 μg/mL. Pipet 10 mL stock soln into 100 mL vol. flask and dil. to vol. with H_2O. Prep. fresh daily.

44.B08 ***Preparation of Sample***

(a) *Insect excreta.*—Transfer material to small test tube, crush with glass rod, and add 0.05–0.10 mL Li_2CO_3 soln, (f). Let soak ca 10 min and centrf. Obtain clear supernate and proceed as in **44.188**.

(b) *Paper bags or cartons.*—Cut 5–6 mm diam. portion from suspect area. Cut another 5–6 mm portion from nearby unstained area as neg. control. Place individually in small test tubes. Add ca 0.1 mL Li_2CO_3 soln, (f), to each tube; agitate with small stirring rod. Let soak ca 10 min and proceed as in **44.188**.

(c) *Other suspect material.*—Transfer small portion to test tube, add ca 0.1 mL Li_2CO_3 soln, (f), and stir with glass rod. Let soak ca 10 min; centrf. Obtain clear supernate and proceed as in **44.188**.

44.B09 ***Determination***

(a) *Spotting of plates.*—Place coated plate on heated metal slab reading ca 87° on surface thermometer or 70° on 3 in. (76 mm) immersion thermometer inserted through hole in stopper until tip touches bottom of 250 mL conical flask contg 125 mL glycerol. *Caution:* Plates tend to crack, particularly prescored plates, unless heated evenly. Place infrared lamp or forced hot air source (e.g., hair dryer) above plate to speed drying of spots. Spot 1 μL uric acid working std soln, (g)(*2*), at each edge and at center of plate ca 15–20 mm from bottom. Spot 1 μL dye mixt., (e), to side of each working std spot. These dyes serve as visual markers during development, with R_f for amaranth at 0.38–0.40; uric acid, 0.41–

0.43; and Yellow No. 6, 0.65, using Analtech plate and sandwich chamber. Merck plates have lower R_f values, with R_f for amaranth approx. equal to that of uric acid. Spot samples and neg. controls along same line at ≥10 mm intervals. Keep spots at min. size by drying well between successive small addns.

(b) *Development of plates.*—Scribe horizontal line, ca 1 mm wide, across plate exactly 10 cm above origin, completely removing cellulose layer. Develop to this line in conventional satd tank without pre-equilibration or, alternatively, form sandwich chamber with uncoated plate [See **44.175(b)** and **44.177(c)** and **(f)**] and develop. Dry plate on heated metal slab or in forced draft oven ca 5 min at 75–80°.

(c) *Examination with UV light.*—Observe plate under shortwave (254 nm) UV light in darkened room, marking each quenching (dark) spot with penciled dots at top, bottom, left, and right edges. Shortwave lamps in fluorescent tube style have integral filters with transmission characteristics that change with use. Some UV viewing cabinets have label attached calling attention to this fall-off of transmittance of 254 nm. High levels of uric acid should appear as dark spots at R_f = 0.40 ± 0.05, depending on conditions of development.

(d) *Color development.*—Spray plate evenly in hood, concentrating on horizontal zone between upper (yellow) dye spots and ca 2 cm below lower (red) dye spots, only until blue uric acid spots clearly appear at R_f stated in (c). Immediately outline spots with soft (No. 1) pencil, marking weakest spots first. Continue spraying *only until background begins to darken.* Immediately outline any addnl spots which appear (again, weakest ones first). (*Caution:* Excessive spraying accelerates plate darkening.)

Refs.: JAOAC **61**, 903(1978); **66**, 394(1983).

CAS-69-93-2 (uric acid)

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(3) In the method for mold in ground spices, Howard mold count, **44.213**, correct the applicability statement to read: "... powder, and other ground capsicums)"

45. FORENSIC SCIENCES

No additions, deletions, or other changes.

46. MICROBIOLOGICAL METHODS

(1) The following first action method was adopted final action:

Salmonella detection in foods, hydrophobic grid membrane filter method, **46.A06–46.A11**.

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(2) The following hydrophobic grid membrane filter method for aerobic plate count in foods was adopted first action:

Aerobic Plate Count in Foods
Hydrophobic Grid Membrane Filter Method
First Action

46.B01 ***Principle***

Hydrophobic grid membrane filter (HGMF) uses membrane filter imprinted with hydrophobic material in grid pattern. Hydrophobic lines act as barriers to spread of colonies, thereby dividing membrane filter surface into sep. compartments of equal and known size. Number of squares occupied by colonies is enumerated and converted to most probable number value of organisms by using formula given below.

46.B02 ***Apparatus, Culture Media, and Reagents***

(a) *Hydrophobic grid membrane filter (HGMF).*—Membrane filter has pore size of 0.45 μm and is imprinted with nontoxic hydrophobic material in grid pattern. ISO-GRID (available from QA Laboratories, Ltd, 135 The West Mall, Toronto, Ontario, Canada M9C 1C2) or equiv. meets these specifications.

(b) *Filtration units for HGMF.*—Equipped with 5 μm mesh prefilter to remove food particles during filtration. One unit is required for each sample. ISO-GRID (available from QA Laboratories, Ltd) or equiv. meets these specifications.

(c) *Pipets.*—1.0 mL serological with 0.1 mL graduations, 1.1 or 2.2 mL milk pipets are satisfactory. 5.0 mL serological with 0.1 mL graduations.

(d) *Blender.*—Waring Blendor, or equiv. multispeed model, with low-speed operation at 10 000–12 000 rpm, and 250 mL glass or metal blender jars with covers. One jar is required for each sample.

(e) *Vacuum pump.*—H_2O aspirator vac. source is satisfactory.

(f) *Manifold or vacuum flask.*

(g) *Peptone/Tween 80 (PT) diluent.*—Dissolve 1.0 g peptone (Difco 0118) and 10.0 g Tween 80 in 1 L H_2O. Dispense enough vol. into diln bottles to give 90 ± 1 mL or 99 ± 1 mL after autoclaving 15 min at 121°.

(h) *Tryptic soy-fast green agar (TSFA).*—15.0 g tryptone, 5.0 g phytone (or soytone), 5.0 g NaCl, 0.25 g fast green FCF (CI No. 42053), and 15.0 g agar dild to 1 L with H_2O. Heat to boiling. Autoclave 15 min at 121°. Temper to 50–55°. Aseptically adjust pH to 7.3 ± 0.1. Dispense ca 18 mL portions into 100 × 15 mm petri dishes. Surface-dry plated medium before use.

(i) *Tris buffer.*—1.0M. Dissolve 121.1 g tris(hydroxymethyl)amino methane in ca 500 mL H_2O. Adjust soln to desired pH with concd HCl and dil. to 1 L with H_2O. Store at either room temp. or 4–6°.

(j) *Acetate buffer.*—1.0M. Dissolve 60 mL glacial acetic acid in ca 500 mL H_2O. Adjust soln to desired pH with 5M NaOH and dil. to 1 L with H_2O. Store at 4–6°.

(k) *Amylase stock soln.*—Dil. 10 g α-amylase (Sigma No. A1278 or equiv.) to 100 mL with tris buffer, pH 7.0. Warm to 35° if necessary to aid soln. Filter thru Whatman No. 1 paper (or equiv.) to remove insoluble material; then filter-sterilize using 0.45 μm membrane filter. Store up to 1 week at 4–6° or up to 3 months at −18°.

(l) *Cellulase stock soln.*—Dil. 10 g cellulase (Sigma No. C7502 or equiv.) to 100 mL with acetate buffer, pH 5.0. Warm to 35° if necessary to aid soln. Filter thru Whatman No. 1 paper (or equiv.) to remove insoluble material; then filter-sterilize using 0.45 μm membrane filter. Store up to 1 week at 4–6° or up to 3 months at −18°.

(m) *Diastase stock soln.*—Dil. 10 g diastase (Sigma No. A6880 or equiv.) to 100 mL with tris buffer, pH 7.0. Warm to 35° if necessary to aid soln. Filter thru Whatman No. 1 paper (or equiv.) to remove insoluble material; then filter-sterilize using 0.45 μm membrane filter. Store up to 1 week at 4–6° or up to 3 months at −18°.

(n) *Hemicellulase stock soln.*—Dil. 10 g hemicellulase (Sigma No. H2125 or equiv.) to 100 mL with acetate buffer, pH 5.5. Warm to 35° if necessary to aid soln. Filter thru Whatman No. 1 paper (or equiv.) to remove insoluble material; then filter-sterilize using 0.45 μm membrane filter. Store up to 1 week at 4–6° or up to 3 months at −18°.

(o) *Trypsin stock soln.*—Dil. 10 g trypsin (Difco No. 0152 or equiv.) to 100 mL with tris buffer, pH 7.6. Warm to 35° if necessary to aid soln. Filter thru Whatman No. 1 paper (or equiv.) to remove insoluble material; then filter-sterilize using 0.45 μm membrane filter. Store up to 1 week at 4–6° or up to 3 months at −18°.

(p) *Lecithinase (phospholipase A_2) stock soln.*—Dil. com. enzyme soln (Sigma No. P9139 or equiv.) to 25 units/mL with tris buffer, pH 8.0. Filter-sterilize using 0.45 μm membrane filter. Store up to 1 week at 4–6° or up to 3 months at −18°.

(q) *Pectinase stock soln.*—Use com. enzyme soln of pectinase from *Aspergillus niger*, contg 3–6 units/mg protein, dissolved in 40% glycerol (Sigma No. P5146 or equiv.). Filter-sterilize using 0.45 μm membrane filter. Store up to 1 week at 4–6° or up to 3 months at −18°.

(r) *Protease stock soln.*—Use com. enzyme soln of protease from *Bacillus subtilis*, containing 7–15 units/mg protein (Biuret) in aq. soln (Sigma No. P8775 or equiv.) Filter-sterilize using 0.45 μm membrane filter. Store up to 1 week at 4–6° or up to 3 months at −18°.

46.B03 ***Sample Preparation***

(a) *Liquid egg.*—Thoroly mix sample with sterile spoon or spatula and prep. 1:10 diln by aseptically weighing 11 g egg material into sterile wide-mouth g-s or screw-cap bottle; add 99 mL PT diluent, (g), and 1 tablespoonful of sterile glass shot. Thoroly agitate 1:10 diln to ensure complete soln or distribution of egg material in diluent by shaking each container rapidly 25 times, each shake being up-and-down movement of ca 30 cm, time interval not exceeding 7 s. Let bubbles escape. Transfer representative portion from 1:10 diln for higher serial dilns as needed. If enzyme treatment is needed (see Table **46:B1**), combine 5 mL of 1:10 diln with 1 mL enzyme stock soln. Incubate 20–30 min at 35–37° in H_2O bath. Correct for addnl diln factor by filtering 1.2 mL of enzyme-treated sample.

(b) *Other liquid samples.*—Mix contents of sample container thoroly. To prep. 1:10 diln, aseptically transfer 10 mL sample into 90 mL PT diluent, (g). Mix by shaking bottle 25 times thru 30 cm arc in 7 s. Transfer representative portions from 1:10 diln for higher serial dilns as needed. If enzyme treatment is needed (see Table **46:B1**), combine 5 mL of 1:10 diln with 1 mL enzyme stock soln. Incubate 20–30 min at 35–37° in H_2O bath. Correct for addnl diln factor by filtering 1.2 mL of enzyme-treated sample.

(c) *Whole egg powder.*—Thoroly mix sample with sterile spoon or spatula and prep. 1:10 diln by aseptically weighing 11 g egg material into sterile wide-mouth g-s or screw-cap bottle; add 99 mL PT diluent, (g), and 1 tablespoonful of sterile glass shot. Thoroly agitate 1:10 diln to ensure complete soln or distribution of egg material in diluent by shaking each container rapidly 25 times, each shake being up-and-down movement of ca 30 cm, time interval not exceeding 7 s. Let bubbles escape. Transfer representative portion from 1:10 diln for higher serial dilns as needed. If testing 1:10 diln is necessary, prep. 1:100 diln and combine 10 mL of 1:100 diln with 1 mL trypsin stock soln, (o). Incubate 20–30 min at 35–37° in H_2O bath. Filter entire 11 mL vol. to test 1:10 diln.

Table 46:B1. Enzyme treatments for foods[a]

Food	Enzyme
Skim milk	none
Raw milk	none
Fluid dairy products other than skim milk	trypsin
Ice cream: without stabilizers	trypsin
contg gums	hemicellulase
contg cellulose derivatives	cellulase
Spray-dried milks	trypsin
Cheeses	trypsin
Spray-dried cheese powders	cellulase or protease[b]
Sour cream	diastase
Yoghurt	trypsin
Butter	none
Margarine	none
Egg: liq. or powder	trypsin
Raw beef, pork, poultry	trypsin
Cooked meat or poultry	trypsin
Flour	none
Rice	none
Chocolate	amylase
Breakfast cereals	cellulase
Cake mixes	amylase
Fruit puree (e.g., fig paste)	pectinase
Raw vegetables	none
Lecithin	lecithinase
Food colorings	none
Gums	hemicellulase
Citrus juices	pectinase
Infant formula	trypsin
Sodium caseinate	protease
Nut meats	none
Shrimp	none
Oysters	trypsin

[a] Based on analysis of 1 mL of 1:10 diln. Foods tested at dilns of 1:100 or higher do not usually need enzyme treatment.
[b] Varies, depending on individual product.

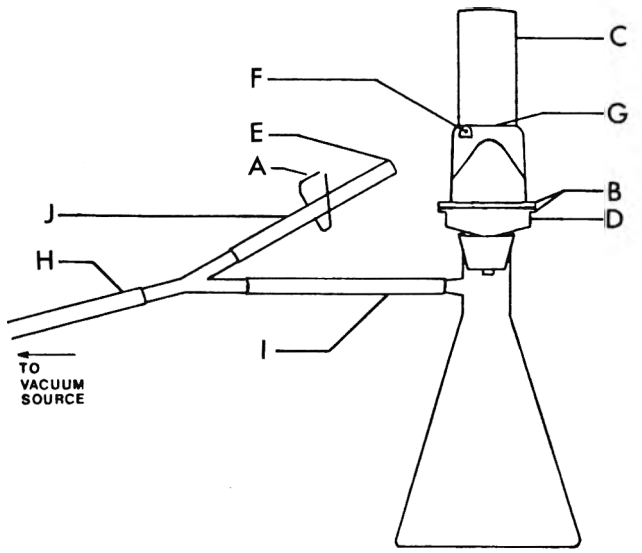

FIG. 46:B1—Filtration unit

(d) *Other foods.*—To prep. 1:10 diln, aseptically weigh 10 g sample into sterile blender jar. Add 90 mL PT diluent, (g), and blend 2 min at low speed (10 000–12 000 rpm). Transfer representative portion from 1:10 diln for higher serial dilns as needed. If enzyme treatment is needed (see Table **46:B1**), combine 5 mL of 1:10 diln with 1 mL enzyme stock soln. Incubate 20–30 min at 35–37° in H_2O bath. Correct for addnl diln factor by filtering 1.2 mL of enzyme-treated sample.

46.B04 ***Analysis***

Select appropriate diln for analysis, depending on desired counting range. Ordinarily, 1:100 diln is satisfactory, producing counting range of 100/g or mL to 500 000/g or mL. Use 1:10 diln if very low counts are expected.

(See Figs **46:B1** and **46:B2.**) Turn on vac. source. Place sterile filtration unit on manifold or vac. flask. Open clamp A. Rotate back funnel portion C. Aseptically place sterile HGMF on surface of base D. Rotate funnel forward. Clamp shut by sliding jaws L of stainless steel clamp over entire length of flanges B extending from both sides of funnel C and base D, and rotating moving arm K into horizontal (locked) position.

Aseptically add ca 15–20 mL sterile H_2O to funnel. Pipet required vol. of appropriate diln into funnel. Apply free end of vac. tubing E to suction hole F to draw liq. thru prefilter mesh G. Aseptically add addnl 10–15 mL sterile H_2O to funnel and draw thru mesh as before. Close clamp A to direct vac. to base of filtration unit and draw liq. thru HGMF.

Open clamp A. Rotate moving arm K of stainless steel clamp into unlocked (ca 45° angle) position and slide jaws L off of flanges B. Rotate back funnel C. Aseptically remove HGMF and place on surface of pre-dried TSFA (h) plate. Avoid trapping air bubbles between filter and agar.

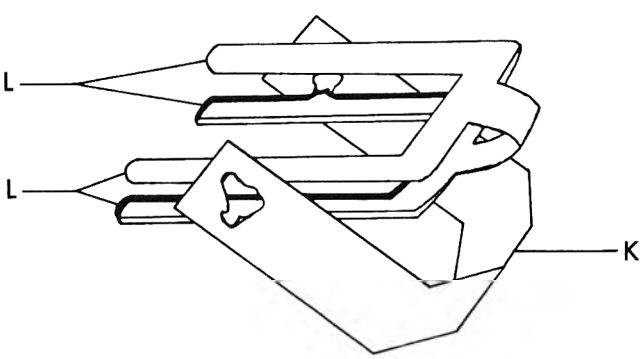

FIG. 46:B2—Filtration unit clamp

(a) *Raw milk, pasteurized milks and creams, and egg powders.*—Incubate 48 ± 3 h at 32°. Colonies will be various shades of green. Count all squares contg one or more colonies (pos. squares) except that if a single colony has clearly spread to adjacent squares, count it as one pos. square. Convert pos. square count to MPN with the formula, MPN = $[N \log_e (N/(N - x))]$, where N = total number of squares and x = number of pos. squares. Multiply by reciprocal of diln factor and report as MPN of total bacteria/g or mL.

(b) *Liquid egg.*—Incubate 3 days (72 ± 3 h) at 32°. Proceed as in (a).

(c) *All other foods.*—Incubate 48 ± 3 h at 35°. Proceed as in (a).

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(3) The following interim dry rehydratable film methods for bacterial and coliform counts in milk were adopted first action:

Bacterial and Coliform Counts in Milk
Dry Rehydratable Film Methods
First Action

46.B05 ***Principle***

Method uses bacterial culture plates of dry medium and cold H_2O-sol. gel. Undild or dild samples are added directly to plates at rate of 1.0 mL per plate. Pressure, when applied to plastic spreader placed on overlay film, spreads sample over ca 20 sq. cm growth area. Gelling agent is allowed to solidify and plates are incubated and then counted. Either pipet or plate loop continuous pipetting syringe can be used for sample addn for bacterial count analyses.

46.B06 ***Apparatus***

(a) *Std method plates.*—Plates contain std methods media nutrients, **46.005**(g), cold H_2O-sol. gelling agent coated onto film base, overlay film coated with gelling agent, and 2,3,5-triphenyltetrazolium chloride indicator. Circular growth area of single plate contains ca twenty 1 cm squares outlined on film base. Petrifilm SM Plates® (available from Medical-Surgical Division/3M, 225-5S 3M Center, St. Paul, MN 55144) or equiv. meets these specifications.

(b) *Violet red bile plates.*—Plates contain violet red bile nutrients conforming to APHA standards as given in Compendium of Methods for the Microbiological Examination of Foods, 2nd ed., 1984 (American Public Health Association, 1015 18th St, NW, Washington, DC 20005), cold H_2O sol. gelling agent, and 2,3,5-triphenyltetrazolium chloride. Petrifilm VRB Plates® (available from Medical-Surgical Division/3M), or equiv. meets these specifications.

(c) *Plastic spreader.*—Provided with Petrifilm plates, consists of concave side and smooth flat side, designed to spread milk sample evenly over plate growth area.

(d) *Pipets.*—Calibrated for bacteriological use or plate loop continuous pipetting syringe to deliver 1.0 mL.

(e) *Colony counter*—Std app., Quebec model preferred, or one providing equiv. magnification and visibility.

46.B07 ***Analysis***

(a) *Bacterial colony count.*—Use Petrifilm SM or equiv. plates. Place plate on flat surface. Lift top film and inoculate 1 mL sample onto center of film base. Carefully roll top film down onto inoculum. Distribute sample over prescribed growth area with downward pressure on center of plastic spreader device (recessed side down). Leave plate undisturbed 1 min to permit gel to solidify. Incubate plates 48 ± 3 h at 32° ± 1°.

In incubator, place plates in horizontal position, clear side up, in stacks not exceeding 10 units. Count plates promptly after incubation period. If impossible to count at once, store plates after required incubation at 0–4.4° for not >24 h. This should be avoided as a routine practice.

Use std colony counter for counting purposes. Magnifier-illuminator may also be used to facilitate counting. Colonies stain in various shades of red. Count all colonies in countable range (30–300 colonies).

To compute bacterial count, multiply total number of colonies per plate (or av. number of colonies per plate if counting duplicate plates of same diln) by reciprocal of diln used. When counting colonies on duplicate plates of consecutive dilns, compute mean number of colonies for each diln before detg av. bacterial count. Estd counts can be made on plates with >300 colonies and should be reported as estd counts. In making such counts, circular growth area can be considered to contain ca twenty 1 cm squares. To isolate colonies for further identification, lift top film and pick colony from gel.

(b) *Coliform count.*—Use Petrifilm VRB or equiv. plates. Proceed as in (a), but distribute sample over plate by using plastic spreader, flat side down. Incubate plates 24 ± 2 h at 32° ± 1°. Count as in (a), but count only red colonies that have one or more gas bubbles associated (within 1 colony diam.) with them. Count all colonies in countable range (15–150 colonies). Red colonies without gas bubbles are not counted as coliform organisms.

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(4) The following DNA colony hybridization method for detection of enterotoxigenic *Escherichia coli* was adopted first action:

Enterotoxigenic *Escherichia coli*
DNA Colony Hybridization Method Using Synthetic Oligodeoxyribonucleotides and Paper Filters
First Action

(*Caution:* This procedure uses radioactive compd. Personnel must receive adequate training and monitoring and have proper facilities available for handling this substance.)

46.B08 ***Principle***

Chemically synthesized pieces of DNA (oligodeoxyribonucleotides) that code for regions of genes detg bacterial virulence can be used to identify pathogenic strains of bacteria. These oligomers are radioactively labeled in vitro and hybridized with colonies of bacterial cells that have been lysed and fixed to paper filters. Colonies contg same region of a gene will bind labeled DNA and become radioactive. Such colonies can be detected by autoradiography.

46.B09 ***Reagents***

(Prep. all media according to manufacturer's instructions and use analytical grade materials whenever possible. Note: DNA often adheres to unsiliconized glass. When working with solns contg DNA, use siliconized glassware or disposable plasticware unless otherwise specified.)

(a) *Lysis mixture A.*—Combine 50 mL 10N NaOH, (s), 300 mL 5.0M NaCl, (u), and 650 mL H_2O.

(b) *Lysis mixture B.*—Combine 50 mL 2.0M Tris, pH 7.0, (v), 400 mL 5.0M NaCl, (u), and 500 mL H_2O.

(c) *Hybridization mixture.*—Combine in plastic tube or beaker: 28.9 mL H_2O, 15.0 mL 20X SSC, (d), 5.0 mL 50X Denhardt's soln, (e), and 0.1 mL 0.5M EDTA soln, pH 8.0, (f). Final vol. is 49 mL. Use immediately.

(d) *20X std saline citrate soln (SSC).*—Dissolve 175.4 g NaCl and 88.2 g Na citrate in final vol. of 1 L H_2O.

(e) *50X Denhardt's soln.*—Dissolve 2.0 g Ficoll (av. molecular wt 400 000), 2.0 g polyvinyl pyrrolidone (av. molecular wt 360 000), and 2.0 g bovine serum albumin in 200 mL H_2O. Store at −20° in 5.0 mL aliquots.

(f) *0.5M Disodium ethylenediamine tetraacetate soln, pH 8.0.*—Dissolve 186.12 g Na_2EDTA in 800–900 mL H_2O. Adjust to pH 8.0 with 10N NaOH, (s). Dil. to 1 L with H_2O.

(g) *Sonicated calf thymus DNA.*—Dissolve 1 g purified calf thymus DNA in 100 mL H_2O by stirring 3–4 h. Sonicate until av. molecular wt is 300 000–500 000, which may be detd by electrophoresis with appropriate stds such as 123-base ladder (Bethesda Research Laboratories (BRL), Gaithersburg, MD). Store in 1 mL

portions in 13 × 100 mm screw-cap tubes. Glass may be used in this instance only.

(h) *6X SSC soln.*—Combine 300 mL 20X SSC, (d), with 700 mL H_2O.

(i) *2X SSC soln.*—Combine 100 mL 20X SSC, (d), with 900 mL H_2O.

(j) *Synthetic DNA stock soln.*—Approx. 150–350 μg/mL. (A_{260} = 5–10 units.) Soln of 22-base, single stranded DNA molecules [STH (human) and STP (porcine) oligodeoxyribonucleotide probes for enterotoxin genes] will have concn ca 20–50μM. Store at −20°.

(k) *Synthetic DNA working soln.*—Dil. stock soln, (j), in H_2O to 10μM. Store at −20°.

(l) *2.0M Tris soln, pH 7.6.*—Dissolve 242.28 g Tris in ca 800 mL H_2O. Adjust to pH 7.6 with concd HCl. Dil. to 1 L with H_2O.

(m) *1.0M $MgCl_2$ soln.*—Dissolve 9.52 g $MgCl_2$ in final vol. of 100 mL H_2O.

(n) *0.5M Dithiothreitol soln.*—Weigh 0.77 g dithiothreitol and combine with H_2O to final vol. of 10.0 mL. Store at 4°.

(o) *10mM Spermidine soln.*—Dissolve 14.5 mg spermidine in final vol. of 10.0 mL H_2O. Store at −20°.

(p) *10X Kinase buffer.*—Combine 2.5 mL 2.0M Tris, pH 7.6, (l), 1.0 mL 1.0M $MgCl_2$, (m), 1.0 mL 0.5M dithiothreitol, (n), 1.0 mL 10mM spermidine, (o), 20 μL 0.5M EDTA, (f), and 4.5 mL H_2O. Store at 4°.

(q) *(γ-^{32}P) ATP.*—Aq. soln of adenosine triphosphate, specific activity 3000–7000 Ci/mmole. ("Crude" prepn from ICN Pharmaceuticals, Inc., Irvine, CA 92713, or equiv.). Store at −70° if possible.

(r) *Bacteriophage T4 polynucleotide kinase.*—20 units/μL (BRL or equiv.).

(s) *10N NaOH soln.*—Dissolve 400 g NaOH in final vol. of 1 L H_2O.

(t) *2.0M Tris soln, pH 8.0.*—Follow instructions for (l) but adjust pH to 8.0.

(u) *5.0M NaCl soln.*—Dissolve 292.2 g NaCl in final vol. of 1 L H_2O.

(v) *2.0M Tris soln, pH 7.0.*—Follow instructions for (l) but adjust pH to 7.0.

(w) *Glycerol freezing soln.*—Combine 50.0 mL glycerol and 50.0 mL H_2O. Dispense 0.5 mL aliquots into 1 dram vials. Sterilize by autoclaving 15 min at 121°.

(x) *NACS PREPAC column loading buffer.*—Dissolve 308.4 g ammonium acetate in final vol. of 1 L H_2O.

(y) *NACS PREPAC column eluting buffer.*—Dissolve 19.3 g ammonium acetate in final vol. of 1 L H_2O.

(z) *Brain heart infusion or trypticase soy broth and agar.*—For microbial growth.

(aa) *Scintillation fluid.*—Dissolve 5.0 g 2,5-diphenyloxazole in 1 L toluene.

(bb) *ST probe soln.*—Combine equal vols of STH and STP working soln, (k).

(cc) *Phosphoramidite soln.*—0.5 g (Applied Biosystems, Inc., Foster City, CA 94404; American BioNuclear, Emeryville, CA 94608; or equiv.), reagent grade (≥95%), made up to 0.1M using anhyd. CH_3CN, (nn), and glass syringe transfer procedures with protection from atm. H_2O. Vortex mix until dissolved.

(dd) *Thiophenol soln.*—Mix 80 mL *p*-dioxane (≤0.01% H_2O), 80 mL triethylamine (99+%), and 40 mL thiophenol (99+%) ("Gold Label," Aldrich Chemical Co., or equiv.).

(ee) *1H-Tetrazole soln.*—Add 300 mL anhyd. CH_3CN, (nn), to 10 g resublimed tetrazole, (oo), with protection from atm. H_2O, and sonicate until dissolved. Warm (30–40°), if necessary.

(ff) *Ammonium hydroxide soln.*—28–30% NH_3, as supplied.

(gg) *Acetic anhydride soln.*—Combine 160 mL tetrahydrofuran (≤0.01% H_2O), 20 mL 2,6-lutidine ("Spectro Grade," Eastman Kodak Co., or equiv), and 20 mL acetic anhydride (99+%).

(hh) *4-Dimethylaminopyridine soln.*—Dissolve 13 g recrystd 4-dimethylaminopyridine, (pp), in 200 mL tetrahydrofuran (≤0.01% H_2O).

(ii) *Trichloroacetic acid soln.*—Weigh 125 g trichloroacetic acid (Aldrich "Gold Label" or equiv., 99+%) in beaker with min. exposure to atm. moisture and transfer to storage container using 4 L CH_2Cl_2 (≤0.006% H_2O).

(jj) *Iodine soln.*—Combine 320 mL tetrahydrofuran, 80 mL 2,6-lutidine, and 10.2 g I crystals. Sonicate until dissolved. Add 8.0 mL H_2O, dropwise, with stirring.

(kk) *Dimethoxytrityl (DMT) assay soln.*—Dissolve 19 g *p*-toluenesulfonic acid monohydrate in 1 L LC grade CH_3CN (0.1M).

(ll) *Triethylammonium acetate (TEAA) buffer*—With const stirring, add 28 mL triethylamine, (qq), to 1.8 L H_2O followed by 10 mL glacial acetic acid. Titr. slowly with more acid to pH 7.0 and then vac. filter thru type HA 0.45 μm filter (Millipore Corp. or equiv.).

(mm) *Detritylation soln.*—Add 3 mL glacial acetic acid to 97 mL H_2O.

(nn) *Anhydrous acetonitrile.*—Store 1 L LC grade CH_3CN (≤0.007% H_2O, Burdick & Jackson Laboratories, Inc., or equiv.) over type 4A molecular sieves ≥24 h.

(oo) *Resublimed 1H-tetrazole.*—Sublime 20 g 1*H*-tetrazole (99+%, Aldrich "Gold Label" or equiv.) in std sublimation app. at ≤0.25 torr and 130–140°. (Yields ca 15 g sublimate.)

(pp) *Recrystallized 4-dimethylaminopyridine.*—Dissolve 200 g 4-dimethylaminopyridine in ca 1 L hot (50–60°) tetrahydrofuran contg 20 g decolorizing charcoal. Filter while still hot thru glass fiber paper (Grade 934AH, "Reeve Angel," Whatman, Inc., or equiv).

(qq) *Triethylamine*—99+% (Aldrich "Gold Label" or equiv. LC grade).

46.B10 *Apparatus and Materials*

(a) *Labware.*—100 × 15 mm glass petri plates; plastic beakers and tubes to contain up to 100 mL; 100 × 15 or 20 mm plastic petri plates; plastic conical tubes to contain up to 500 μL; plastic pipets to cover range 1–10 mL; variable vol. micropipettors and tips to cover range 1–1000 μL.

(b) *Incubators.*—(*1*) Capable of maintaining 37 ± 1°; (*2*) capable of maintaining 40 ± 1°; (*3*) capable of maintaining 50 ± 1°; (*4*) H_2O bath or dry block capable of maintaining 37 ± 1°.

(c) *UV spectrophotometer.*—To measure DNA concn at 260 nm. (1 A_{260} unit is 50 μg/mL for double stranded DNA and 33 μg/mL for single stranded DNA.)

(d) *Ultralow temperature freezer.*—Capable of maintaining −70° is preferred, but freezer (not frost-free) at −20° may be substituted.

(e) *Freezer.*—Capable of maintaining −20° (not frost-free).

(f) *Cellulose filters.*—No. 541 (Whatman), 82–85 mm diam.

(g) *Absorbent filters.*—Whatman No. 1 or similar, ca 85 mm diam.

(h) *NACS PREPAC column.*—DNA binding resin (BRL or equiv.).

(i) *Scintillation counter.*—Or Geiger-Mueller counter if calibrated in cpm.

(j) *X-ray film and developing chemicals.*—8 × 10 in. is convenient size. Kodak XAR X-ray film or equiv.

(k) *Darkroom.*—Facilities for X-ray film development with appropriate safelight.

(l) *X-ray film holder cassette.*—With intensifying screens (Kodak regular, Eastman Kodak Co.; Dupont Cronex Lightening Plus, E.I. Dupont de Nemours & Co.; or equiv.).

(m) *Centrifuge.*—Capable of spinning 500 μL conical plastic tubes (Eppendorf Model 5412, Brinkmann Instruments, Inc., or equiv.).

(n) *Vacuum desiccator.*—Needed only if prepd colony hybridization filters must be stored 1 week.

(o) *DNA synthesizer.*—Manual or automated synthesis system (i.e., Applied Biosystems synthesizer Model 380A; other synthesis systems providing equiv. results are also acceptable).

(p) *Synthesis ("reaction") columns.*—1 μmol long chain alkylamine-functionalized controlled pore glass, either prepacked or hand-packed (Applied Biosystems or equiv.).

(q) *Fraction collector.*—To collect fractions from automated synthesis system. Should have auxilliary signal input.

(r) *Liquid chromatographic system.*—App. with gradient elution capability, UV detection at 254 or 260 nm, and μBondapak® C_{18}, 7.8 mm × 30 cm column (Waters Associates, Inc., or equiv.).

(s) *Rotary vacuum centrifuge.*—To conc. LC-purified oligodeoxyribonucleotides (Savant, Hicksville, NY 11801, or equiv.).

(t) *Glass syringes.*—Capacity up to 10 mL for transfer of anhyd. CH_3CN with protection from atm. moisture.

(u) *Type HV, 0.45 μm filters.*—To remove LC column particulates (Millipore or equiv.).

46.B11 *Colony Hybridization Filter Preparation*

Transfer candidate cultures to 5 mL brain heart infusion or trypticase soy broth and incubate 18–24 h at 37°. If culture must be stored before analysis can be performed, aseptically add 2.0 mL culture to 0.5 mL freezing soln, (w). Store at −70° if possible. (Note: Frost-free freezers will decrease culture viability and may result in loss of virulence determinants. If cultures must be stored at −20°, use non-frost-free unit. This precaution holds for all frozen material in this procedure.)

Aseptically inoculate 5 mL rich broth with portion of frozen bacterial culture. Sterile cotton swabs are well suited for this purpose. Always include known pos. and neg. control cultures on every filter (see below). (If culture is not thawed, it may be reused innumerable times.) Incubate culture 18–24 h at 37°. At same time, aseptically prepare 100 × 15 mm petri plates contg either brain heart infusion or trypticase soy agar and dry inverted 18–24 h at 37°. After inoculating cultures in orderly array and ensuring that resulting colonies will not ultimately merge while growing, inoculate agar plates with test cultures, using sterile microbiological needle, toothpick, cotton swab, or replicator; 9–10 mm is convenient distance between cultures. Record location of each culture; it is vital that culture patterns and resulting autoradiogram(s) can be oriented unambiguously. Prep. multiple plates and concomitant filters because hybridization procedure may have to be repeated and number of steps to be repeated is thereby lessened. Incubate plates inverted 18–24 h at 37°. Mark cultures failing to grow; otherwise, false-neg. results may be reported.

Label Whatman No. 541 cellulose filters, (f), 82–85 mm diam., using soft lead pencil, and also mark filter so it can be oriented unambiguously after replication. (Note: Other manufacturers make filters with physical properties equiv. to Whatman No. 541. However, DNA binding abilities of such filters are not always suitable for use in DNA hybridization.) Apply filter so that side with pencil markings faces colony array on agar surface of plate contg colonies. Wetting initial edge of filter paper and rolling to opposite edge usually eliminates formation of air pockets. If air bubbles are entrapped between filter and agar plate, remove by applying gentle pressure with glass spreader. This maneuver also ensures more efficient attachment of cultures to filter paper, but care must be taken to avoid spreading colonies because of excessive pressure. Filters may be peeled from plate immediately, but more definitive reactions are usually obtained if filter remains situated 1–2 h. (Note: Colony array on filter is now mirror image of array originally applied to agar plate.)

Lyse colonies replicated onto filters by transferring filters with colony side up onto absorbent cellulose filters (such as Whatman No. 1 or S & S No. 597, ca 85 mm diam.) contained in glass 100 × 15 mm petri plates and previously wetted with 1.5–2.0 mL lysis mixt. A, (a). Be sure that no air is entrapped between filters. Heat filters in glass plate for 3–5 min in steam. Transfer steamed filters to glass petri plates contg absorbent cellulose filters previously wetted with 1.5–2.0 mL lysis mixt. B (b). Again, be sure that no air pockets result. Maintain filters in horizontal position when transferring so that lysed colonies (DNA) will not become confluent. Let filters become completely neutralized by remaining situated 5–10 min.

If filters are not to be used immediately, air-dry on absorbent paper at room temp. and store under vac. between filter papers. Such filters have been kept ca 1 year without noticeable change in results.

46.B12 *Oligodeoxyribonucleotide Synthesis*

(Note 1: A number of companies will custom-synthesize oligodeoxyribonucleotides. Also, several oligodeoxyribonucleotide synthesis systems are com. available, both automated and manual. Results are generally satisfactory if manufacturer's instructions are followed. This method uses one of com. available, automated synthesizers and procedure described below is meant to serve only as example.)

(Note 2: All solns for prepn and isolation of synthetic oligodeoxyribonucleotides should be prepd in deionized H_2O passed thru 0.2 μm filter ("Versacap Filter Unit," Gelman Sciences, Ann Arbor, MI 48106, or equiv.).)

According to manufacturer's instructions, use Applied Biosystems, "fast" cycle but with following modifications of step times: trichloroacetic acid to column detritylation step, 75 s (retained in fraction collector); CH_3CN to column post-detritylation step, 50 s (also retained and pooled with above in fraction collector); CH_3CN to column, pre-coupling step, 120 s; coupling step, 180 s; capping step, 120 s. Synthesis is ended with dimethoxytrityl (DMT) group retained at 5′ terminus. Automated cleavage from support is achieved with concd NH_4OH at room temp. for 1 h. Dil. delivered NH_4OH soln with 1 mL concd NH_4OH, heat 10 h at 60° in 3.7 mL vial with Teflon-lined screw cap (Supelco, or equiv.). Let cool to room temp. Add 50 μL triethylamine (qq). Evap. NH_3 with N stream to ca 2 mL.

46.B13 *Quantitation of Coupling Yield*

To det. isolated product yield (see below) and ensure satisfactory coupling at each addn, theoretical yields of product must be calcd. Dil. each collected fraction (from detritylation and post-detritylation steps above) to 5 mL with DMT-assay soln (kk). Mix each fraction well and read *A* at 530 nm. Use assay soln (kk) as reference std. Compare *A* with that of previous fraction to det. coupling efficiency of each step (generally 97–99%). To det. overall theoretical yield, multiply all individual step-yields.

46.B14 *Oligodeoxyribonucleotide Purification and Isolation*

To det. chromatgc properties of prepn, perform anal. run. Set detector for 0.1 AUFS. Inject 10 μL soln evapd to 2.0 mL. In ambient temp. column, start 20–30% gradient (at 1%/min) of CH_3CN in triethylammonium acetate buffer, (ll). Continue at 30% CH_3CN after 10 min. Generally, major DMT-product elutes at 10 ± 3 min. After elution time is detd, repeat chromatgy on preparative scale (inject 100 μL crude soln, 1.0 AUFS). Collect center position of major peak.

46.B15 *Oligodeoxyribonucleotide Processing*

Before synthetic oligonucleotide can be used as substrate for polynucleotide kinase, LC solvs and DMT group must be removed. Conc. collected LC fraction using N ca 10–20 min to remove most CH_3CN. Conc. sample to dryness using rotary vac. centrf., (s). Add 1 mL 3% (v/v) acetic acid to remove DMT protecting group. Vortex-mix to dissolve. After 5–10 min at room temp., freeze in crushed dry-ice and conc. using vac. centrf., (s). Dissolve residue in 1 mL H_2O. Add 1 mL anal. grade ethyl acetate to ext org. impurities and vortex-mix thoroly. Let org. layer sep. from aq. layer contg DNA and possible LC column particulates. (Centrf. if necessary.) Remove org. layer with Pasteur pipet and discard. If insoluble LC column particulates are present, syringe-filter DNA soln thru type HV, 0.45 μm filter (u). Let DNA soln gravity-filter and collect residual soln by rapidly depressing syringe plunger. Remove 50 μL aliquot from 1 mL filtered DNA soln for *A* measurement. Conc. both remaining sample and *A* aliquot to dryness. Dissolve aliquot in 1 mL H_2O and measure *A* at 260 nm. Since 1/20 of sample has been removed, multiply reading by 19 to obtain *A* units in total purified sample. Discard *A* aliquot. Multiply *A* in total purified sample by 10 (because only 10% of total synthesis reaction was purified) to obtain *A* units of entire isolable product. Compare this yield with calcd value (1 μmole × theoretical yield [see above] × molar *A* of oligonucleotide synthesized × 10^{-3}) to det. yield of isolable product. Molar *A* is calcd by adding number of purines (dA plus dG) times 14 000 plus number of pyrimidines (dC plus T) times 7000. These factors are molar extinction coefficients and 10^{-3} is used to convert molar *A* to μmoles/mL which is a millimolar concn.

46.B16 ***End-Labeling of Synthetic DNA***

Synthetic oligodeoxyribonucleotides are rehydrated to ca 5–10 A_{260} units (ca 150–350 μg/mL) to serve as stock soln (j). One A_{260} unit corresponds to ca 33 μg/mL single-stranded DNA. Molecular wt of 22-base, single-stranded DNA molecule is ca 7260. Prep. 10 μM working soln for each DNA probe (10 pmoles/μL, 72.6 μg/μL). If desired, STH and STP synthetic DNA probes can be combined into single soln, 5μM in each probe (**bb**).

Mix 5 μL DNA probe soln, (**bb**), 2.5 μL 10X kinase buffer, (**p**), 15 μL H_2O, 1.5 μL (γ-^{32}P) ATP, (**q**), and 1 μL T4 kinase, (**r**), in 500 μL plastic conical centrf. tube, (**a**), on ice. Add kinase, (**r**), last and return enzyme immediately to −20° because it is quite heat-labile. Centrf., (**m**), 2–3 s to adequately mix reagents. Incubate at 37° in H_2O bath or dry block heater, (**b**), 1 h. Add 2 μL 0.5M EDTA, (**f**), to terminate reaction. Add 1.6 μL 4.0M ammonium acetate soln, (**y**), to bring ammonium acetate concn to 0.25M before applying sample to NACS PREPAC column.

Unincorporated ^{32}P is removed by binding DNA to NACS PREPAC column, (**h**). Equilibrate column with 0.25M ammonium acetate, (**x**), 2 h. Load reaction mixt. onto column and wash, using gravity or very gentle pressure, with ca 4 mL loading buffer, (**x**), to remove free ATP. Elute bound DNA with 200 μL aliquots of eluting buffer, (**y**). Do not force liq. thru column rapidly. Collect three 200 μL fractions in 500 μL plastic tubes, (**a**). Spot 2 μL of each fraction onto ca 2 × 2 cm paper (e.g., Whatman 3MM), dry, add ca 5 mL scintillation fluid, (**aa**), and assay radioactivity by scintillation counting. Geiger-Mueller counter, (i), may suffice if properly calibrated and used. Most labeled DNA is eluted from column in fractions 1 and 2. Pool fractions and count triplicate 2 μL portions as described above. Est. total vol. of prepn by carefully drawing into plastic 1 mL pipet. Calc. total amt of radioactivity recovered in prepn. Usually, 1–2 × 10^8 cpm is obtained if specific activity of ATP, (**q**), is 3000–7000 Ci/mmol. Store at −20°.

46.B17 ***Colony Hybridization***

Freshly prep. 50 mL hybridization mixt., (c). Boil 1.0 mL sonicated calf thymus DNA, (**g**), 5 min in H_2O bath and add to hybridization mixt., (**c**). Dispense 10 mL sonicated calf thymus DNA-hybridization mixt. into 100 × 15 or 20 mm *plastic* petri dish and insert cellulose filter contg lysed colony array. To use std amt of probe for each hybridization, det. vol. of probe DNA soln required to contain 1 × 10^6 cpm after correcting for 14.2 day half-life of ^{32}P. Add 1 × 10^6 cpm probe DNA to soln contg filter. Mix briefly and incubate plate overnight at 40°.

Wash hybridized filters free of ^{32}P-labeled DNA not specifically bound to DNA from colonies on filter by removing filter from hybridization mixt. and rinsing 5–10 s in plastic petri dish contg 10 mL 6X SSC, (h). Drain and recover filter with 6X SSC. Incubate 1 h at 50°. Again, drain plate, recover with 6X SSC, and incubate 1 h at 50°. Finally, rinse filter 5–10 s at room temp. in 2X SSC, (**i**). Air-dry on absorbent paper at room temp. to prevent curling. Mount filter to 8 × 10 in. stiff paper (e.g., Whatman 3MM) using small pieces of tape. Cover with plastic or glassine sheet (such as document or neg. holder) to prevent contamination of intensifying screens in X-ray film holders.

46.B18 ***Autoradiography***

Exposure time is dictated by amt of radioactive DNA bound to filter. If increase above background exceeds 10 cps when Geiger-Mueller counter is held over filter, it is likely that pos. reaction will be visible after 4 h exposure at room temp. However, if increase of 2–3 cps is observed, enclose loaded film cassette in sealed plastic bag and expose film overnight, preferrably at −70° or at least −20°. If −70° is not available, cassette can be sandwiched between slabs of dry ice to reduce exposure time.

In darkroom, place X-ray film onto plastic-covered filter in cassette film holder with intensifying screens. Expose film for appropriate length of time as detd above. After exposure, let cassette equilibrate at room temp. (to prevent moisture accumulation) before removing plastic bag. Develop X-ray film by following manufacturer's instructions. If spots are too faint or too intense for analysis, expose new film for appropriate length of time.

46.B19 ***Reporting of Results***

Lysed colonies of *E. coli* strains contg DNA coding for heat-stable enterotoxins will bind radioactively labeled oligonucleotide probe for ST. These radioactive lysed colonies will expose X-ray film and dark spots will be evident after development. Det. if each unknown culture is pos. or neg. by comparing spot intensity to that of pos. and neg. culture controls. However, many factors can influence quality of these results: size of colonies, amt of cellular debris, amt of DNA per lysed colony, hybridization and washing temps, hybridization time, specific activity of probe, and length of autoradiogram exposure. Well documented pos. and neg. controls must be present on every filter to ensure that the procedure has been performed correctly and that compensation for nonspecific binding of labeled probe DNA (neg. colonies that may be seen as faint spots) has been made.

If neg. control cultures exhibit faint spots, and pos. culture spots are intense, re-wash filter(s) in 6X-SSC, (**h**), at 52–55° twice for 1 h each time. Dry filters and re-expose autoradiogram. Take care because thermal stability of oligonucleotide hybrids is much less than that of longer DNA molecules.

46.B20 ***Troubleshooting***

Unsatisfactory autoradiograms can result from several factors, some of which have been listed in the previous section. False-neg. results can be due to spontaneous loss of plasmids, especially when strains are cultivated excessively under nonselective laboratory conditions (i.e., re-isolation or further subculture). Also, hybridization and/or washes at excessively high temps can result in decreased DNA probe binding which in turn can lead to neg. observation. Occasionally, very large colonies do not become affixed to filters and cellular material is lost from hybridization filters. False-pos. results can be observed if either hybridization or washing temp. is too low. Nonspecific DNA probe binding will occur. Autoradiogram exposures of excessive time can result in overemphasis of limited, nonspecific binding of probe to neg. cultures; this may be falsely reported as pos. results. Other possible sources of error and their remedies have been discussed (**46.048**; JAOAC **67**, 801(1984)).

Finally, it is essential to note that resulting autoradiogram spot arrays are mirror images of plate inoculation patterns. This is not the case with **46.035–46.048**. Results are accurately read if autoradiograms are reversed (left to right) before interpretation. Films must be marked so that they can be unambiguously oriented with recorded location of each test culture.

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(5) The following enzyme immunoassay screening method for detection of *Salmonella* in foods was adopted first action:

Salmonella in Foods
Enzyme Immunoassay Screening Method
First Action

Method is screening procedure for presence of *Salmonella* in all food types; it is not a confirmatory test because monoclonal antibodies used in test may cross-react with small percentage of non-*Salmonella*.

Enrichment broths and M-broth from samples pos. by EIA method must be streaked on selective media as in **46.118** and typical or suspicious colonies must be identified as in **46.119–46.128**.

Detn of pos. result is objective and must be performed using filter photometer having 405–420 nm filter. Pos. result is valid only when neg. and pos. controls possess acceptable optical density readings.

46.B21 ***Principle***

Detection of *Salmonella* antigens is based on solid phase immunoassay and uses mag. force to transfer solid phase from one reaction mixt. to another. Monoclonal antibodies to *Salmonella*

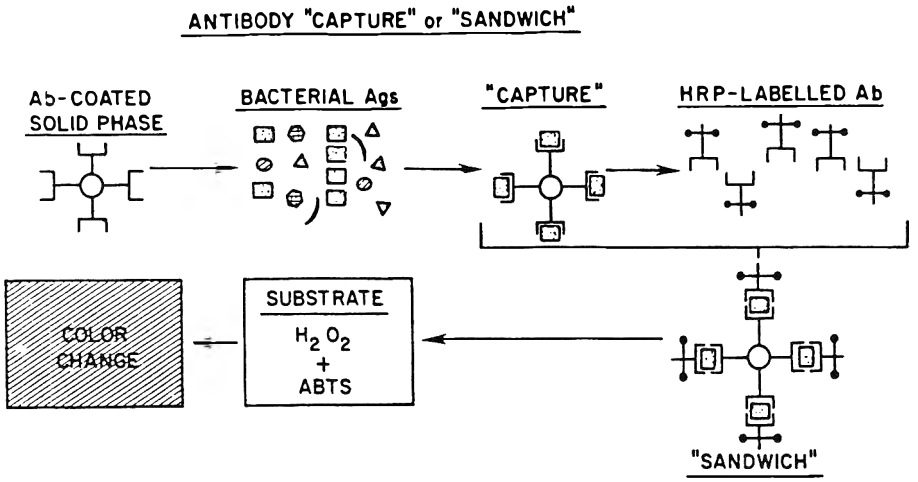

FIG. 46:B3—Antibody "capture" or "sandwich"

Ab = antibody
Ag = antigen
HRP = horseradish peroxidase
H_2O_2 = hydrogen peroxide
ABTS = 2,2'-azino-di[3-ethylbenzthiazoline-sulfonate(6)]

antigen are bound to surface of beads made of ferrous metal. Beads are placed in sample to be assayed. If *Salmonella* antigens are present in sample, they will attach to specific antibody on beads. Beads are washed and then released into reaction mixt. contg peroxidase-conjugated anti-*Salmonella* immunoglobulins. Conjugate will bind to *Salmonella* antigens if they are attached to antibody molecules on surface of beads. Beads are washed to remove unbound conjugate and then placed in substrate soln. Appearance of color indicates presence of *Salmonella* antigen in sample. Fig. **46:B3** shows schematic representation.

46.B22 *Method Performance*

For all foods:

Results	*Percent*	*95% Confidence Range (Approx.)*
Agreement[1]	96.9	95.7–98.1
False neg. (BAM)[2]	1.5	0.4– 2.5
False neg. (EIA)[3]	3.4	1.8– 5.0

[1] This rate reflects no. of samples read identically between AOAC/BAM (Bacteriological Analytical Manual (1984) 6th ed., AOAC, Arlington, VA) culture method and EIA.

[2] This rate reflects no. of samples found to be pos. by EIA but detd to be neg. by AOAC/BAM culture method.

[3] This rate reflects no. of samples found to be pos. by AOAC/BAM culture method but detd to be neg. by EIA.

Of 21 laboratories, 10 (48%) had complete agreement between culture method and EIA (153/153); 17 laboratories (81%) showed agreement on ≥96% of samples; 20 (95%) showed agreement on ≥93% of samples.

46.B23 *Reagents*

Items (a)–(m) are available as Salmonella Bio-EnzaBead Screen Kit (Organon Teknika Corp., 2020 Bridge View Dr, Charleston, SC 29405).

(a) *Antibody-coated beads.*—Monoclonal antibodies to *Salmonella*, 2 vials (48 beads/vial). Store bead vials tightly capped at 2–8°. Beads are stable 14 days after opening.

(b) *Control antigens.*—Pos. control (heat-treated *S. javiana*) which reacts with antibodies to *Salmonella*, 1 vial; neg. control which is nonreactive with antibodies to *Salmonella*, 1 vial. Reconstituted control antigens are stable 28 days when stored at 2–8°.

(c) *Conjugate diluent.*—1 vial (24 mL/vial). Contains 1% bovine serum in phosphate-buffered saline contg 0.05% Tween 20 and 0.01% Thimerosal as preservative.

(d) *Reagent water.*—1 bottle (125 mL/bottle). Store at room temp. or warm to room temp. before use.

(e) *Phosphate-buffered saline.*—PBS, pH = 7.5 ± 0.2; 1 bottle (125 mL/bottle). Contains 1.2 g Na_2HPO_4, 0.22 g $NaH_2PO_4.H_2O$, and 8.5 g NaCl/L H_2O.

(f) *Peroxidase-conjugated antibodies to Salmonella.*—1 vial (lyophilized). When reconstituted, conjugate is stable 28 days when stored at 2–8°.

(g) *Wash solution (50×).*—1 vial (2.5 mL). Contains 2.5% surfactant.

(h) *ABTS substrate.*—2 vials (lyophilized). After reconstitution, each vial contains 0.03% 2,2'-azino-di(3-ethyl-benzthiazoline-sulfonate). Reconstituted substrate is stable 14 days when stored tightly capped at 2–8°. Let reconstituted substrate warm to room temp. before dispensing.

(i) *Substrate diluent for ABTS.*—2 vials (30 mL/vial). Contains H_2O_2.

(j) *"Stop" soln.*—1 vial (5 mL/vial). Contains 1.25% NaF. *Caution:* Avoid contact with skin. If contact occurs, wash area with H_2O.

(k) *Microtitration plates.*—Plate (3⅝ × 5 in.) possessing 96 wells, each having capability of holding >0.3 mL fluid. These must be designed in 8 × 12 format which will fit into mag. transfer device. Spaces between wells should be hollowed out, and not filled in with plastic coming to top of well. Available as "Accessory Package" (Organon Teknika Corp.), or equiv. may be used. *Caution:* Not all microtitrn plates meet these criteria.

(l) *Package insert.*

(m) *Data record sheets.*

(n) *M-broth.*—5.0 g yeast ext, 12.5 g tryptone, 2.0 g D-mannose, 5.0 g Na citrate, 5.0 g NaCl, 5.0 g K_2HPO_4, 0.14 g $MnCl_2$, 0.8 g $MgSO_4$, 0.04 g $FeSO_4$, 0.75 g Tween 80. Suspend ingredients in 1 L H_2O, and heat to boiling for 1–2 min. Dispense 10 mL portions into 16 × 125 mm screw-cap test tubes. Cap tubes loosely and autoclave 15 min at 121°. Tighten caps securely for storage. Final pH should be 7.0.

(o) *Diagnostic reagents.*—Necessary for cultural confirmation of presumptive pos. EIA tests; see **46.116**.

46.B24 *Apparatus*

Items (a)–(e) are available from Organon Teknika Corp.

(a) *Magnetic transfer device.*—Mag. app. which houses microtitrn plates and is used to transfer metal beads from one reagent to another as well as to wash metal beads.

(b) *Incubator.*—37° with 100 rpm agitator.

(c) *Enzyme immunoassay reader.*—Photometer with 405–420 nm screening filter which will read thru microtitrn plates. Must be able to be set to zero while reading thru unreactive substrate well (blank). Reader should be equipped with printer so that records of analysis

can be kept. Semiautomated Organon Teknika 30 or equiv. meets these specifications.

(d) *Bead dispenser.*—Either single bead dispenser or 96-well bead dispenser, or suitable alternative. Places beads into wells of microtitrn plate.

(e) *Micropipet.*—Capable of delivering accurate amts in range 50–300 μL. Micropipets capable of delivering these vols to multiple wells simultaneously (multichannel) or individually (single channel) are needed.

(f) *Centrifuge.*—Having min. capacity to spin centrf. tubes (≤20 mm diam.) at 1500 × *g* in swinging bucket rotor or 3000 × *g* in fixed angle rotor for 20 min. IEC Centra 7 tabletop centrf. with IEC 216 horizontal rotor (available from International Equipment Co., 300 Second Ave, Needham Heights, MA 02194), or equiv., meets these criteria.

(g) *Boiling water bath.*—Able to attain and maintain 100°. Must be able to hold centrf. tubes upright. Microwave or autoclave set at 100° is acceptable alternative, as are generators of flowing stream. *Caution:* H_2O baths which do not maintain boiling conditions are unacceptable.

(h) *Vortex mixer.*—Capable of vigorous agitation of centrf. tube, such that pellet at bottom of conical tube can be resuspended. S/P mixer (available from Scientific Products, Inc.) or equiv. meets these criteria.

46.B25 *General Instructions*

Include pos. control antigen and duplicate neg. controls with each group of test samples. All controls must function properly for test to be valid. One addnl well per group of test samples should be left empty initially. This well, filled with substrate, will be used to "blank" assay reader. See sample data record sheet (Fig. **46:B4**).

Use data record sheets to identify location of each test sample.

Do not use mag. transfer device without top and bottom plate in position. Always insert plates in device with notched side facing operator.

Do not reuse wells of a plate or the beads.

Use sep. pipets for each sample and kit reagent to avoid cross-contamination. Take care not to contaminate substrate with conjugate.

The components and procedures of this test kit have been standardized for use in Bio-EnzaBead procedure. Use of components or procedures other than those supplied by Organon Teknika Corp. may yield unsatisfactory results.

46.B26 *Preparation of Sample*

(a) *Pre-enrichment.*—Pre-enrich product in non-inhibitory broth to initiate growth of salmonellae. Methods used may vary with product, and should be performed as indicated in **46.117**, or in Bacteriological Analytical Manual, 6th ed., AOAC, Arlington, VA, Chap. 7, section C, with the following exception:

Raw or highly contaminated products.—Aseptically weigh 25 g sample into sterile blender jar. Add 225 mL sterile lactose broth. Blend 2 min at high speed (ca 20 000 rpm). Cap jars securely and let stand at room temp. 60 min. Mix well by shaking and det. pH with test paper. Adjust pH to 6.8 ± 0.2, if necessary, using sterile 1N NaOH or HCl, capping jar securely and mixing well before detg final pH. Aseptically transfer contents of each jar to sterile wide-mouth, screw-cap 500 mL jar. Loosen jar caps ¼ turn and incubate 24 ± 2 h at 35°.

(b) *Selective enrichment.*—Transfer 1 mL incubated pre-enrichment mixts to selenite cystine broth and 1 mL into tetrathionate broth as in **46.118**(a). (For dried active yeast, substitute lauryl sulfate tryptose broth **46.115**(u) for selenite cystine broth.) Incubate 18–24 h at 35°.

(c) *Post-enrichment.*—Remove selective broths from incubation and mix by hand or by vortex mixer. Remove 0.5 mL from tetrathionate tube and transfer to 10 mL tube of sterile M-broth which has been warmed to 35°. Also remove 0.5 mL from selenite cystine tube and transfer to same tube of M-broth and vortex-mix well. Incubate M-broth tube 6 h at 35°. Return tetrathionate and selenite cystine tubes to 35° incubator for addnl 6 h.

(d) *Centrifugation and preparation of sample for EIA analysis.*—Remove M-broth from incubation and mix tube by hand or vortex mixer. Pipet 10 mL into centrf. tube (≤20 mm diam.) and label tube. Refrigerate (2–8°) remaining M-broth and tetrathionate and selenite cystine tubes from (c) above for cultural confirmation of any enzyme immunoassay pos. samples. These broths may be refrigerated, if necessary, for ≤18 h at 2–8°. Centrf. M-broth at *min.* speed of 1500 × *g* (swinging bucket rotor) or 3000 × *g* (fixed angle rotor) for 20 min. Suction off supernate from tube using trap flask filled with disinfectant. Resuspend pellet with 1 mL PBS. Vortex-mix tube to mix well. Heat resuspended pellet in boiling H_2O bath or in flowing steam 20 min. Cool heated exts to 25–37°

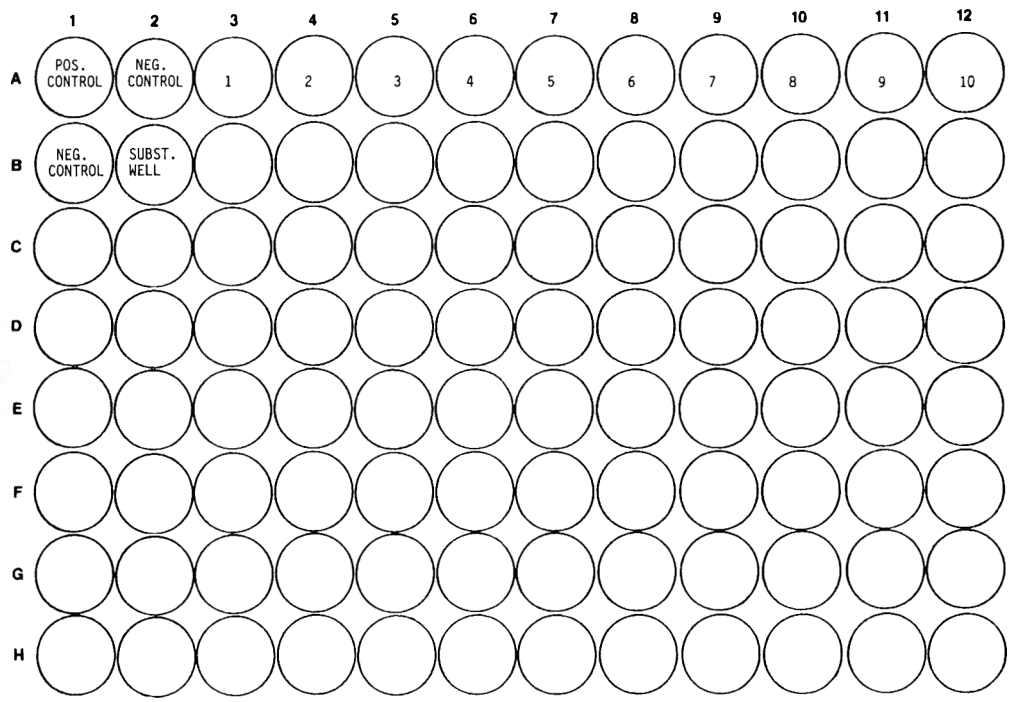

FIG. 46:B4—Data record sheet for identifying location of test samples

prior to analysis by EIA. *Caution:* Heated exts which are not cooled to this temp. can destroy monoclonal antibodies on metal beads.

46.B27 ***Enzyme Immunoassay***

(*1*) Reconstitute control antigens by adding 2.5 mL PBS to each vial. Swirl gently to dissolve. Following record sheet, add 0.2 mL aliquot of neg. and pos. control antigens and 0.2 mL aliquot of test samples into designated wells of 96-well plate. Note: Use 2 neg. controls and one pos. control for each group of samples. Label plate "antigen".

(*2*) Using bead dispenser or forceps, place antibody-coated beads in wells of one of empty plates according to record sheet. If using forceps, remove beads from vials by rolling beads into cap or onto gauze pad. Do *not* put bead in substrate blanking well.

(*3*) To start assay, simultaneously transfer beads to "antigen" plate by using mag. transfer device as follows:

(a) Invert 96-well plate and insert it with notched end facing operator into top slot until it snaps into position. Magnet should be in UP position.

(b) Slide plate contg beads (with notched end facing operator) into lower slot until it snaps into position, centering it under inverted top plate.

(c) To remove beads from bottom plate, lower magnet to full DOWN position (all beads should now be in inverted plate). Without disturbing top plate, remove lower plate (save for later step) and slide plate contg samples under inverted plate.

(d) Raise magnet to allow all beads to drop into wells of "antigen" plate.

(*4*) Remove bottom plate contg samples and beads. Incubate plate with agitation (10–100 rpm) for 20 min at 37°. During incubation period, proceed to steps 5 and 6.

(*5*) If entire plate is being used, prep. 1× wash soln by adding 1.5 mL 50× wash soln to 75 mL reagent water in clean glass or plastic screw-cap bottle. Mix by inverting bottle several times. Add 0.3 mL 1× wash soln into appropriate wells of 96-well plate previously saved. Label plate "wash 1". Similarly, fill second plate with 1× wash soln and label "wash 2". If entire plate is not being used, calc. amt of wash soln required by multiplying number of tests by 0.6 and prep. amt of 1× wash soln required based on 0.5 mL 50× wash soln to 25 mL reagent water.

(*6*) Prep. conjugate soln by adding 24 mL 1× conjugate diluent to lyophilized material in vial. Mix gently by inverting bottle several times. Date vial. Add 0.2 mL conjugate into appropriate wells of sep. plate. Label plate "conjugate".

(*7*) Following 20 min incubation (above), wash beads as follows:

(a) Assure that 96-well plate (from step 3a) is inverted in top slot of mag. transfer device and that magnet is in UP position.

(b) Slide "antigen" plate contg beads under top plate and lower magnet to DOWN position (all beads should now be in inverted plate).

(c) Remove bottom "antigen" plate without disturbing top plate and place it in container for proper disposal.

(d) Slide "wash 1" under top plate and wash beads 12 times by raising and lowering magnet to extreme UP and full DOWN positions (count UP and DOWN as 1 wash).

(e) With magnet in DOWN position (beads in top inverted plate), remove wash plate without disturbing top plate and save for step 11c. *Caution:* Proceed immediately with next step. Do not let beads dry in top plate.

(*8*) Slide "conjugate" plate under top plate and raise magnet to let beads drop into wells.

(*9*) Remove bottom plate contg conjugate and beads and incubate with agitation (10–100 rpm) for 20 min at 37°.

(*10*) While beads are incubating, reconstitute ABTS substrate by adding contents of substrate diluent vial to lyophilized substrate. Mix gently by inverting bottle several times. Date substrate. Add 0.2 mL room temp. substrate into each appropriate well of unused plate. Also, put 0.2 mL into extra well which will be used to "blank" EIA reader.

(*11*) Following conjugate incubation period, wash beads as follows:

(a) Assure that 96-well plate (from step 3a) is still inverted in top slot of mag. transfer device and that magnet is in UP position.

(b) Slide "conjugate" plate contg beads under top plate and lower magnet to DOWN position (all beads should now be in inverted plate).

(c) Remove bottom plate and slide "wash 1" under top plate. Raise magnet to extreme UP position, allowing beads to fall into "wash 1".

(d) Remove top plate and *replace with unused top plate*. Lower magnet to remove beads from "wash 1". Wash beads twice in "wash 1" by raising and lowering magnet to its extreme UP and full DOWN positions (count UP and DOWN as 1 wash).

(e) With magnet in DOWN position (beads in top plate), remove "wash 1" and insert "wash 2".

(f) Wash beads 9 times by raising and lowering magnet to extreme UP and full DOWN positions.

(g) With magnet in UP position (beads in "wash 2"), *replace top inverted plate with unused plate. Caution:* This change must be made to avoid contamination of substrate with conjugate.

(h) Lower magnet and remove bottom "wash 2" without disturbing top plate.

(*12*) Immediately slide "substrate" plate under top plate and raise magnet to let all beads drop into the wells.

(*13*) Remove bottom plate contg substrate and beads and incubate uncovered at room temp. (20–25°) for 10 min. *Do not agitate plate.*

(*14*) After 10 min, add 0.025 mL (25 μL) "stop" soln to each well including substrate blanking well. Gently swirl beads in plate to disperse colored reaction product forming at surface of beads. Remove beads (be sure that 96-well plate is inverted in top slot of mag. transfer device) by placing "substrate" plate in mag. transfer device and lowering magnet.

(*15*) Remove "substrate" plate and release beads into used plate by sliding plate into mag. transfer device and raising magnet. Mix contents of substrate blanking well with pipet tip.

(*16*) Read results on EIA reader.

(*17*) Sterilize all used plates, tubes, etc., prior to disposal. Tightly close and return unused reagents to 2–8° storage.

46.B28 ***Reading***

Insert 405 nm filter and bring reader to zero (blank reader) on well contg only substrate and "stop" soln. Then read each individual control and sample well. Average optical density readings of the 2 neg. control wells. For test to be valid, pos. control should read ≥0.200 and av. of neg. controls should read ≤0.120. Record optical density (OD) of each well on data sheet. Samples reading ≥0.200 should be considered pos. Samples reading <0.200 should be considered neg.

46.B29 ***Confirmation of Positive EIA Samples***

Pos. EIA reading indicates that *Salmonella* may be present. However, since antibodies may cross-react with a few other organisms, cultural confirmations should be performed by streaking HE, XLD, and BS plates from tetrathionate broth, selenite cystine broth, and M-broth tubes as described in **46.118**, and typical or suspicious colonies should be identified as in **46.119–46.128**.

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(6) In the method for detection of *Salmonella* in foods, **46.117–46.119**, change **46.117(f)** to read:

(f) *Instant nonfat dry milk (NFDM).*—Aseptically . . .

47. MICROCHEMICAL METHODS

No additions, deletions, or other changes.

48. RADIOACTIVITY

The final action gamma-ray spectroscopic method for determination of iodine-131, barium-140, and cesium-137 in milk, **48.025–48.029**, was extended to include determination

of iodine-131 and cesium-137 in other foods. The following first action changes are required:

(a) Change the title to read:

Iodine-131, Barium-140, and Cesium-137 in Milk and Other Foods

Gamma-Ray Spectroscopic Method

First Action

(b) Modify the first paragraph of **48.025** as follows:

48.B01 ***Principle***

Applicable to ^{131}I, ^{140}Ba, and ^{137}Cs in fluid milk preserved with HCHO, and I and Cs in foods. Because of the nature of gamma-emitting radionuclides, attenuation of gamma-rays in food slurries or mixt. would be similar to that of milk or H_2O. Unlike in milk samples, other radionuclides might be present in foods. Therefore, before performing calcn, gamma-ray energy spectrum should be inspected. Since cessation of above-ground weapons testing in 1960s, no other radionuclides have been observed or detected. (Should any be detected, matrix technic should be expanded using std source for suspected radionuclide to det. matrix coefficient.)

Known vol. is placed in counting vessel positioned over and around right cylinder scintillation crystal detector NaI (Tl) of multichannel gamma spectrometer. Gamma radiation is counted for given time. Accumulated pulses from selected photon energy range are sepd from other gamma-emitting radionuclides and background radiation by simultaneous equations. ^{40}K is always present as natural contaminant and may contribute counts in 1 or more of photopeak ranges. Mutual interferences among these 4 photopeaks are eliminated by applying matrix technic to sep. activities of the 4 nuclides. Measurement of one std source of each nuclide provides the matrix coefficients.

(c) Add the following new section:

48.B02 ***Preparation of Sample***

No special preparation is needed for milk samples. For other foods, do not include inedible material such as bone, apple cores, nut shells, and egg shells as part of sample. Homogenize sample in blender or mech. homogenizer. Dietary samples prepd for consumption do not require blending, but must be sufficiently mixed to ensure representative sample.

49. VETERINARY ANALYTICAL TOXICOLOGY

(1) The following first action method was adopted final action:

Copper in serum, atomic absorption spectrophotometric method, **49.003–49.007**.

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(2) The following interim spectrophotometric method for determination of arsenic in liver tissue was adopted first action:

Arsenic in Liver Tissue

Spectrophotometric Method

First Action

49.B01 ***Principle***

Liver tissue is dry-ashed overnight at 500°, ash is dissolved, and portion is reacted with Zn metal to evolve arsine gas. Arsine is trapped and As is detd spectrophtric.

49.B02 ***Apparatus and Reagents***

(a) *Hydrochloric acid.*—3N.

(b) *Copper sulfate.*—Anhyd., powd (J.T. Baker Chemical Co., or equiv.).

(c) *Magnesium oxide–magnesium nitrate slurry.*—Suspend 7.5 g MgO and 10.5 g $Mg(NO_3)_2.6H_2O$ in enough H_2O to make 100 mL. Agitate vigorously before adding to sample.

(d) *Stannous chloride soln, 20% (w/v).*—Dissolve 20 g As-free $SnCl_2.2H_2O$ in HCl and dil. to 100 mL with HCl.

(e) *Silver diethyldithiocarbamate (AgDDC) soln.*—Dissolve 0.50 g AgDDC salt in pyridine and dil. to 100 mL with pyridine. Mix and store in amber bottle. Reagent is stable several months at room temp. (Fisher Scientific Co., Cat. No. S-666, or equiv.).

(f) *Arsenic std solns.*—(*1*) *Stock soln.*—500 μg/mL. Accurately weigh 0.660 mg NBS Ref. Std As_2O_3, or equiv., dissolve in 25 mL 2N NaOH, and dil. to 1 L with H_2O. (*2*) *Intermediate soln.*—10 μg/mL. Transfer 2 mL stock soln to 100 mL vol. flask, and dil. to vol. with H_2O. (*3*) *Working soln.*—2 μg/mL. Transfer 10 mL intermediate soln to 50 mL vol. flask and dil. to vol. with H_2O.

(g) *External control.*—Std Ref. Material (SRM) 1566 Oyster Tissue (13.4 ± 1.9 mg As/kg) or equiv.

(h) *Potassium iodide soln, 15% (w/v).*—Dissolve 15 g KI in H_2O and dil. to 100 mL.

(i) *Zinc.*—Shot contg <0.00001% As (Fisher Scientific Co., No. Z-12).

(j) *Distillation apparatus.*—See **41.009(e)**. Use 125 mL erlenmeyer instead of 250 mL. Use narrow test tube as receiver and submerge delivery tube in AgDDC soln.

49.B03 ***Preparation of Standard Curve***

Transfer 0.5, 1.0, 3.0, 6.0, and 10.0 mL aliquots of working soln corresponding to 1, 2, 6, 12, and 20 μg As to sep. 125 mL erlenmeyers. Dil. to 50 mL with 3N HCl. Carry these solns thru distn procedure. Plot *A* at 540 nm on ordinate vs μg As on abscissa. Det. best fitting straight line, using all 5 points, by method of least squares.

49.B04 ***Preparation of Sample***

Blend tissue in high-speed blender until completely homogeneous. Accurately weigh 2.00 g tissue into 30 mL Coors crucible. Analyze one external control with each set of 10 samples or fraction thereof. Add 5 mL well mixed $MgO/Mg(NO_3)_2.H_2O$ slurry and mix thoroly with stirring rod. Prep. blank by adding 5 mL well mixed slurry to sep. crucible and carrying it thru subsequent steps in procedure. Dry samples, controls, and blank to apparent dryness on hot plate or in drying oven at <100°. Cover each crucible with watch glass and place in cold muffle furnace. Set furnace temp. at 250° for 3 h; then gradually increase temp. to 500° and leave overnight.

Cool crucibles to room temp., moisten residue with 5 mL H_2O, and transfer quant. to 50 mL vol. flask with 3N HCl. Dil. to vol. with 3N HCl and mix well. Transfer 25 mL aliquot to 125 mL erlenmeyer and dil. to 50 mL with 3N HCl.

49.B05 ***Distillation***

Add 2 mL 15% KI soln and swirl. Add 1 mL $SnCl_2$ soln and swirl. Cool flasks in freezer or ice bath 45 min or until samples reach 4°. Pipet 6 mL AgDDC soln into narrow receiver test tube, one for each std, external control, sample, and blank. Have all parts of distn app. ready for immediate assembly. Quickly add 10 g Zn shot and pinch of Cu_2SO_4 to erlenmeyer, assemble app., and distil 1 h at room temp. Det. *A* at 540 nm for blank, external control, sample, and std AgDDC solns in suitable spectrphtr. Subtract blank reading from sample and control, and det. mg As/kg directly from std curve. External control results must fall within accepted range (95% confidence limit) for all results to be valid.

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(3) The following interim potentiometric method for determination of nitrate in forages was adopted first action:

Nitrate in Forages

Potentiometric Method

First Action

49.B06 ***Principle***

Nitrate is extd from sample into aq. $Al_2(SO_4)_3$ soln and detd potentiometrically. Lower limit of detection is 0.50% KNO_3.

49.B07 ***Apparatus***

(a) *Drying oven.*—Forced air, capable of heating to 100°.

(b) *Nitrate-specific ion electrode.*—Model 93-07, Orion Research, Inc., 840 Memorial Dr, Cambridge, MA 02139, or equiv. Monitor performance by assuring absolute value of std curve per decade slope ≥54 mV.

(c) *Reference electrode.*—Double junction (Model 90-02, Orion Research, Inc., or equiv.). Use extg soln as outer filling soln.

(d) *pH meter.*—Capable of measuring electrode potentials to nearest mV.

49.B08 ***Reagents***

(a) *Preservation soln.*—Dissolve 0.1 g phenylmercuric acetate in 20 mL dioxane (*Caution:* May form dangerous peroxides; see **51.070**) and dil. to 100 mL with H_2O.

(b) *Extracting soln.*—Dissolve 15.76 g $Al_2(SO_4)_3.18\ H_2O$, 70.0 mg oven-dried KNO_3 (dry 2 h at 100°), and 1.0 mL preservation soln, (a), in 500 mL H_2O. Dil. to 1 L with H_2O.

(c) *Nitrate std solns.*—(*1*) *Stock std soln.*—100 000 mg KNO_3/L. Weigh 20.00 g KNO_3 (dried 2 h at 100°) into 200 mL vol. flask, dissolve in 100 mL H_2O, and dil. to vol. with H_2O. (*2*) *Intermediate std soln.*—10 000 mg KNO_3/L. Dil. 20.0 mL soln (*1*) to 200 mL with extg soln. (*3*) *Working std solns.*—Dil. 1.00, 2.00, 4.00, and 10.0 mL soln (*2*) to 200 mL with extg soln to make 120, 170, 270, and 570 mg KNO_3/L solns. (Caution: Prep. all std solns from same lot of extg soln.)

49.B09 ***Preparation of Standard Curve***

Det. potential of blank (use extg soln as blank, equiv. to 70 mg KNO_3/L) and each working std soln while mag. stirring. Plot potential against nitrate concn (mg KNO_3/L) on semilog paper with concn on log scale. Det. std curve per decade slope. Per decade slope specification of electrode manuf. should be met or exceeded. (Note: Measure potential only after sample and stds are at same temp.)

49.B10 ***Preparation of Sample***

Dry sample in 60° forced air oven to const wt. Grind dried sample to pass 2 mm screen and thoroly mix. Ext 1.00 g mixed ground sample with 100 mL extg soln by shaking 15 min. Keep ext sealed in container until potential is measured.

49.B11 ***Quality Control***

Analyze external control samples of known nitrate concn or spiked samples (at least one for every 10 samples or fraction thereof). Results should indicate acceptable accuracy.

Hold suspect any analyses with unacceptable external control results and unacceptable std curve per decade slopes.

49.B12 ***Determination***

Det. potential of unfiltered ext at same temp. that std potentials are measured, stirring ext at same rate used for stds. Record potential after reading becomes stable or 1 min after insertion, whichever comes first.

49.B13 ***Calculation***

Calc. forage nitrate concn (% KNO_3 on dry wt basis):

$$\%\ KNO_3 = C_u = (C_g - 70) \times 0.010$$

where C_u = forage nitrate concn and C_g = nitrate concn (mg KNO_3/L) of ext obtained from std curve. If desired, convert nitrate concn expressed as % KNO_3 to ppm NO_3-N and ppm NO_3^- by multiplying by 1386 and 6133, resp.

Ref.: JAOAC **69,** March (1986).

50. STANDARD SOLUTIONS AND CERTIFIED REFERENCE MATERIALS

No additions, deletions, or other changes.

51. LABORATORY SAFETY

No additions, deletions, or other changes.

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Entries are located by section number. First action methods are designated by an asterisk: **5.B01***, which shows that the method was adopted first action at the 1985 meeting and became official on publication in "Changes in Methods" in the March/April 1986 issue of *J. Assoc. Off. Anal. Chem.* as part of the second or B supplement to the current (14th) edition of *Official Methods of Analysis*.

Actions on present official methods are identified as in the following examples: first action method adopted final action, **36.207** (final 1986); revision in an official method, **26.052** (rev. 1986). Other actions such as repeals or deletions are similarly shown with the action and year of publication of the action in "Changes in Methods," **28.006** (repealed 1985).

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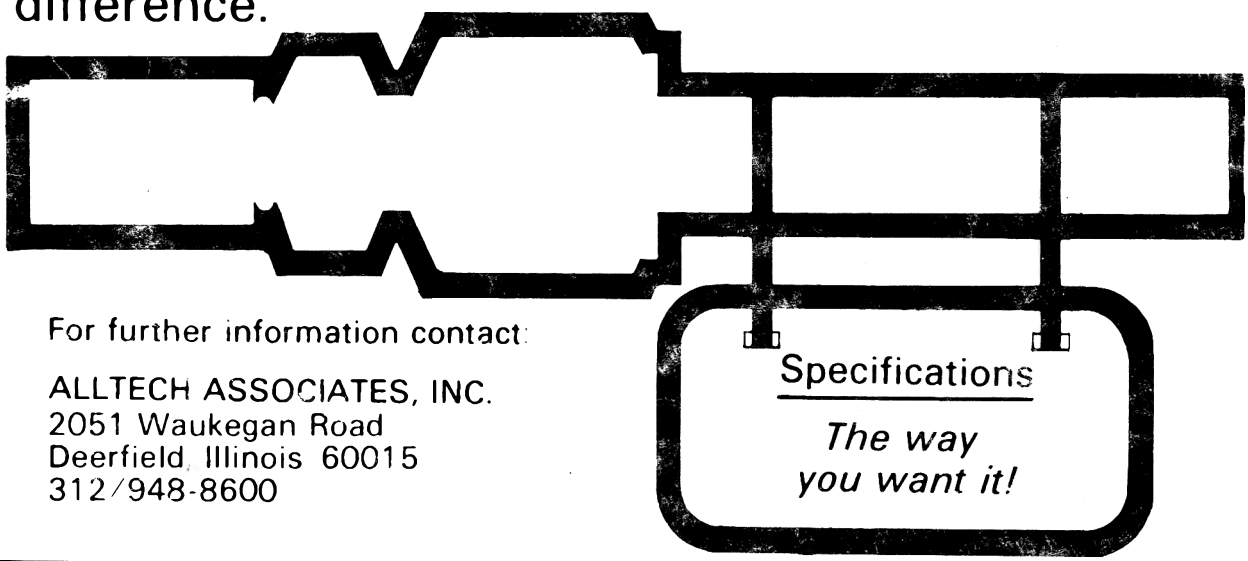

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