January / February 1993 Volume 76 Number 1

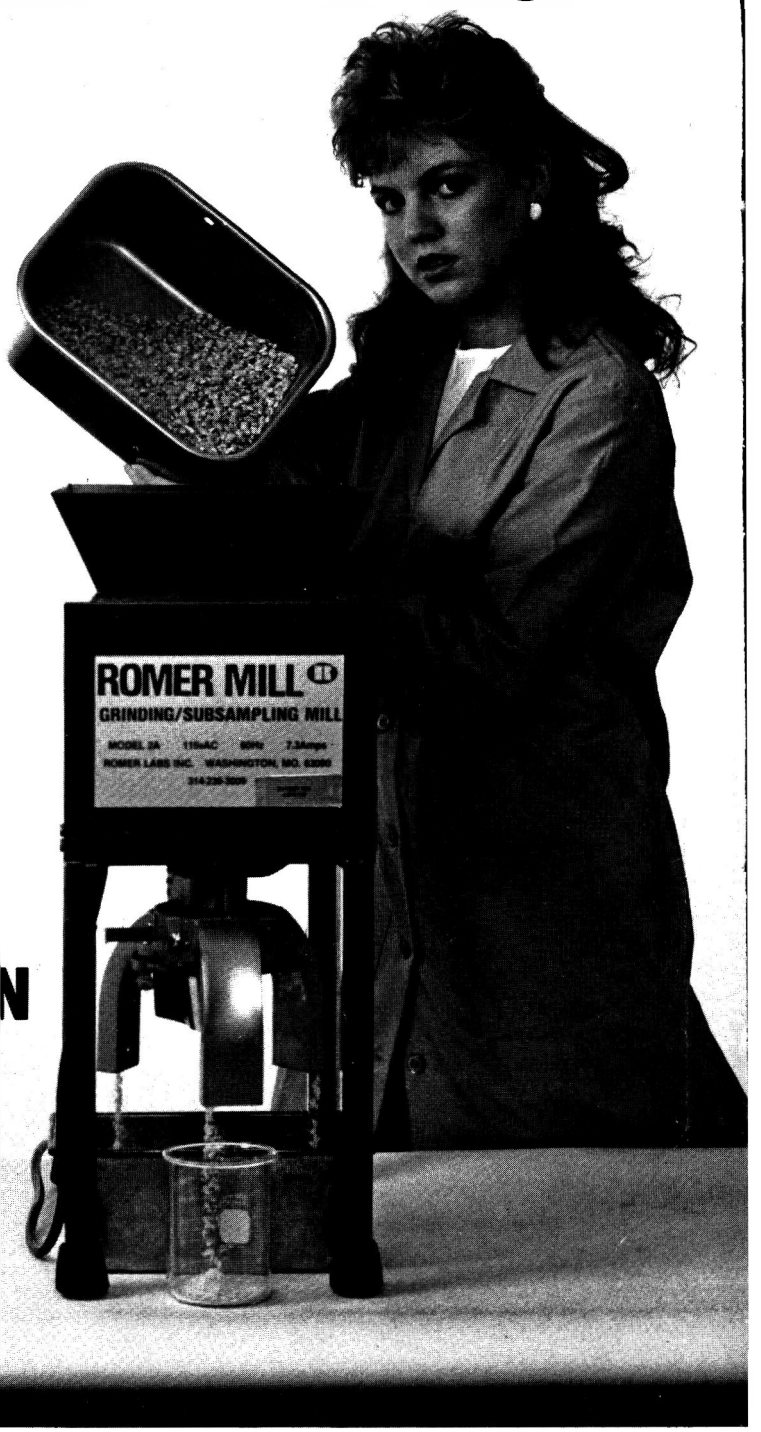
JOURNAL OF AOAC INTERNATIONAL

The Scientific Association Dedicated to Analytical Excellence

AOAC INTERNATIONAL

JAINEE 76(1) 1-256 (1993)
ISSN 1060-3271

NO MORE RIFFLING WITH A ROMER MILL



FROM TWO POUNDS OF GRAIN TO A REPRESENTATIVE ANALYTICAL SAMPLE IN ONE MINUTE

- **EFFICIENT:** Simultaneously Grinds & Subsamples 2 lbs. Per Minute
- **ACCURATE:** Prepares **25 g.** Representative Analytical Sample From **5 Lb.** of Aflatoxin Contaminated Corn
- **VERSATILE:** Can Be Used For Mycotoxins, Pesticides, Fumigants, Antibiotics, Drugs, Vitamins, Mineral & Proximate Analyses

EASY TO CLEAN: Can Be Thoroughly Cleaned in One Minute

FLEXIBLE: Will Grind High Moisture Corn

DUST FREE: Little or No Dust Generated

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

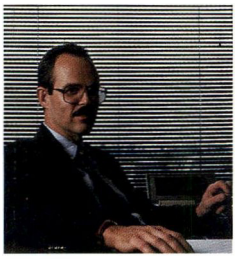
CAPACITY: 100 g to 15 Lb.

ROMER LABS, INC.
1301 Stylemaster Drive
Union, MO 63084
(314) 583-8600 Fax (314)583-6553

CIRCLE 74 ON READER SERVICE CARD

Electrochemical Analysis— Think of it as a mountain bike for the mind.

On being a scientist. I was one of those kids who was serious about everything I did, and I did what I mistook for serious science when I was still in high school. With some free parts from two uncles, a bottle of mercury and a helical potentiometer donated by Beckman Instruments, I managed to build a simple polarograph for a science fair. So when people say scientists are "different," I don't object. Sure, it's a stereotype, but if you checked out the empty casinos the last time Pittcon played Atlantic City, you can understand why it exists.



Pete Kissinger

On advertising to scientists. Granting the stereotype, I don't know why our journals are cluttered with ads that seem to have been designed with no thought to their audience. The people I know avoid them like the plague.

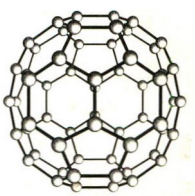
Look at it this way: most scientists do their best work by the time they're 35 years old. If we want to reach the top, we had better get there fast. So we don't have time for the brag-and-boast of advertising, we need to know if the product can help us reach our destination.

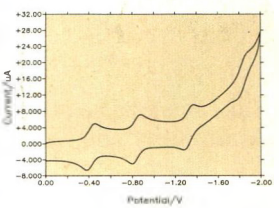
On choosing an analytical technique. Success in analytical chemistry depends on selecting the right tool for the measurement challenge. Many still try to hit nails with a saw or cut wood with a hammer, when it would be so much easier to do the reverse. Even then, it is too limiting to restrict the choice to the two most generally useful tools—spectroscopy and chromatography.

Electrochemistry? You may remember the technique from undergraduate days as little more than a curiosity (the way the liberal arts majors remember you). Today, electrochemical analysis can do some things no other technique can do, and it does others better . . . or faster . . . or to lower detection levels.

The instrumentation has all been brought up to the state of the art. You can focus on results, not the complexity of the technique. Computer control (even Windows™) provides storage and manipulation capabilities.

On where electrochemistry fits in. If you're researching buckyballs, metal complexes, room temperature superconductors, heterogeneous catalysis . . . if your work involves determining formal redox potentials, obtaining electron transfer numbers, measuring the reaction of intermediates and unstable species . . . hey, there's lots more. And they're all destinations to which electrochemistry can take you, quickly, flawlessly, like a mountain bike through an Alpine forest.





On knowing when to shut up. If you're interested in learning more about electrochemical analysis in its modern form, the sponsor of this message is eager to talk to you. As for me, I've probably said enough. I don't want to risk sounding like I'm advertising something.

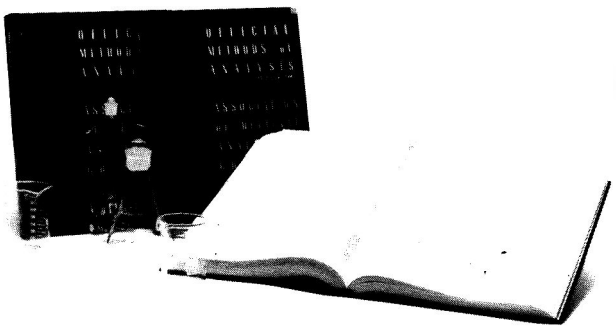
Serious science for serious scientists

BAS
Bioanalytical Systems, Inc.

BAS Japan • 36-4, 1-Chome • Oshiage, Sumida-ku • Tokyo 131, Japan • PH (81) 33 624 0367 • FAX (81) 33 624 3387
BAS Europe • Fruithoflaan, 18, B-2500 Lier (KH) Belgium • PH (32) 3 482 1376 • FAX (32) 3 482 4636
Bioanalytical Systems, Inc. • 2701 Kent Ave • West Lafayette, IN USA 47906 • PH (317) 463-4527 • FAX (317) 497-1102

CIRCLE 82 ON READER SERVICE CARD

Be Sure Your Laboratory Uses the Best Most Current Analytical Methods Available..



Official Methods of Analysis of the AOAC, 15th Edition

Save Time and Money, Achieve More Accurate Analysis, and Improve Your Laboratory's Reputation

The demonstrated reliability of AOAC methods prompts their use whenever a need for analysis arises in regulatory, research, and quality control activities. Chemists, microbiologists, and other scientists depend upon Official Methods of Analysis of the AOAC because they know the reliability and reproducibility of AOAC methods have been demonstrated through thorough testing by interlaboratory collaborative studies in government, industry, and university laboratories.

The authority of the methods is further enhanced by AOAC collaboration with other worldwide organizations.

Take Advantage of AOAC Methods Availability and Ease-of-Use

Over 1,800 AOAC methods are ready for your immediate use. The easy-to-follow, step-by-step format specifies all reagents and apparatus. And alternative methods are often provided to accomodate a wide range of laboratory capabilities.

Each method is referenced for background and development material, and contains Chemical Abstracts Service (CAS) numbers where applicable. Both chemical and common names are provided for all drugs and pesticides.

Comply More Easily with Government Regulations!

Since their inception in 1884, AOAC methods have been accorded preferred status in the courts and are often referred to in national, state, and provincial regulations and governmental and commercial specifications.

Be Confident You're Using the Best, Most Current Methods Available

As analytical needs change and as knowledge and techniques advance, new and revised methods are continually being validated by AOAC. Between editions, these are published in annual supplements which are sent, at no additional cost to purchasers of the most recent edition.

Thus, AOAC's OFFICIAL METHODS OF ANALYSIS not only brings you up to date, it keeps you there!

CONTENTS

Volume I—Agricultural Chemicals, Contaminants, Drugs

Agricultural Liming Materials
Fertilizers
Plants
Feeds
Drugs in Feeds
Disinfectants
Pesticide Formulations
Hazardous Substances
Metals & Other Elements
Pesticide & Industrial Chemicals
Waters; Salt
Microchemical Methods
Radioactivity
Veterinary Analytical Toxicology
Cosmetics
Extraneous Materials
Microbiological Methods
Drugs
Drug & Feed Additives in Animal Tissues
Forensic Methods

Volume II—Food Composition

Baking Chemicals
Distilled Liquors
Malt Beverages
Wines
Nonalcoholic Beverages
Coffee & Tea
Cacao Bean
Cereal Foods
Dairy Products
Eggs & Egg Products
Fish & Other Marine Products
Flavors
Fruits & Fruit Products
Gelatin, Dessert Preparations
Meat & Meat Products
Nuts & Nut Products
Oils & Fats
Vegetable Products
Spices & Other Condiments
Sugars & Sugar Products
Vitamins
Color Additives
Food Additives, Direct & Indirect
Natural Poisons

In Volumes I & II:

Appendixes: Standard Solutions and Certified Reference Materials, Laboratory Safety, Reference Tables. Subject and Method Number Indexes.

AOAC—For over 100 years, the mission of the Association of Official Analytical Chemists has been to provide the analytical science community with fully validated standard methods for the chemical and biological analysis of foods, drugs, cosmetics, pesticides, fertilizers, feeds, hazardous substances, air, water, soil, and any other products and substances affecting the public health and safety, the economic protection of the consumer, or the quality of the environment.

1200 Pages. 1990. 2 Volumes. Hardbound, 185 illus. ISBN 0-935584-42-0.
Binders for supplements are shipped with the first supplement. $281.00 in continental North America (USA, Canada, Mexico). $312.00 outside continental North America. Members: subtract 10% discount. Includes 5 annual supplements plus a looseleaf binder for their storage.

To obtain your copy of OFFICIAL METHODS OF ANALYSIS, send your order with the following: your name and address, a check or Mastercard, VISA, or Diners credit card information (name of card, card number, and expiration date) and your signature to

AOAC INTERNATIONAL

at AOAC-J, 1970 Chain Bridge Road, Dept. 0742, McLean, VA 22109-0742
(703) 522-3032, FAX (703) 522-5468

All orders must be prepaid. Please make checks payable to AOAC, U.S. funds on U.S. banks only. Credit card orders may be placed by mail, telephone, or FAX.

JOURNAL OF AOAC INTERNATIONAL

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

Table of Contents

January/February 1993 VOL. 76, NO. 1 JAINEE 76(1)1–256 (1993)

The *Journal of AOAC International* (ISSN 1060–3271) is published bimonthly by AOAC International, Suite 400, 2200 Wilson Blvd, Arlington, VA 22201-3301 USA. **Subscriptions** for Vol. 76 (1993) are: Members, $120.00 in U.S., $158.00 outside U.S.; Nonmembers, $175.00 in U.S., $213.00 outside U.S. Second class postage paid at Arlington, VA, and additional mailing offices. Abstracted or indexed in AGRICOLA, Analytical Abstracts, Biological Abstracts, Cambridge Scientific Abstracts, Chemical Abstracts, Current Advances in Ecological Sciences, Current Contents, and others. Printed in USA.
POSTMASTER: Send address changes to AOAC International, Suite 400, 2200 Wilson Blvd, Arlington, VA 22201-3301 USA.

RECOMMENDATIONS FOR OFFICIAL METHODS

EXECUTIVE, OFFICER, AND COMMITTEE REPORTS

AOAC INTERNATIONAL

OFFICIAL METHODS OF ANALYSIS

New Products

Empore Brand Extraction Disk for Water-Soluble Analytes

A Empore brand extraction disk containing poly (styrenedivinylbenzene) (SDB) is highly effective for adsorption of water-soluble polar analytes in water matrixes. The disk is available through J.T. Baker, Inc. and Varian Sample Preparation Products. Application notes for use with EPA method 625 analytes in various water matrixes are available on request. With the new disks, users can extract 1 L samples of waste water in 20 min or less using a standard 90 mm glass filter apparatus. The convenient and easy-to-use disks can replace slower standard liquid/liquid extraction (LLE) techniques while generating higher sample throughput and reducing solvent usage. The high sample processing rates and excellent recovery capability of these disks let technicians process more samples per hour, compared with standard LLE equipment. 3M.
Circle No. 301 on reader service card.

Ultradense Bonded Layer Proven Superior for Silica Chromatography

A new type of chromatographic phase, functionally equivalent to monomeric bonded phases on silica, is much more resistant to damage by acids and bases due to bonding chemistry that creates an ultrahigh density surface within the first few angstroms above the silica substrate. The coating shows unprecedented stability to hydrolysis, and tests at both high and low pH prove its superiority to conventional monomeric bonded phases. Research Corporation Technologies.
Circle No. 302 on reader service card.

Datalogger for Portable Ambient Air Analyzers

The Model dl3200F datalogger is designed to collect and store data from any Century OVA or portable Miran ambient air analyzer. It provides intelligent datalogging, with powerful graphics for quick data analysis. The dl3200F can be set up on any IBM compatible PC in a simple prompting format and includes a full graphic software package, offering a powerful analysis tool for data reduction and presentation. The unit is compact—only 4 keys—and user-friendly. The Foxboro Co.
Circle No. 303 on reader service card.

Pelleted Catalysts Introduced by Environmental Products Group

Johnson Matthey's Environmental Products Group announced the introduction of a pelleted catalyst that is particularly appropriate for new or replacement situations where catalysts are used. The catalyst is able to withstand higher temperatures than most pellets. In addition, these high activity pellets increase cost effectiveness by requiring smaller amounts to achieve conversion. Some typical applications for pelleted catalysts include converting processes; metal finishing; chemical processes; rubber, wood, and paper waste treatment; and a number of others involving volatile organic compound emissions. Johnson Matthey.
Circle No. 304 on reader service card.

Vacuum Pump Inlet/Exhaust Filters Eliminate Oil Mist and Smoke

Balston vacuum pump inlet filters are designed to remove relatively large quantities of solids and liquids from the inlet stream at 90% for 0.1 μm particles, permitting recovery of valuable or hazardous materials, and preventing costly damage to the pump and downtime. Models are available for service from 2 torr down to 10^{-6} torr. The filters remove heavy concentrations of extremely fine oil droplets from the vacuum pump exhaust. The exhaust gas may be recycled to the process or into a clean work area, eliminating the need to run ductwork and exhaust pipes outside. Expensive lubricating oils are also captured and filtered for re-use. Balston Inc.
Circle No. 305 on reader service card.

New Detector Option for Gow-Mac Chromatograph

The Option 223 discharge ionization detector is an upgrade option for the Gow-Mac Series 580 isothermal gas chromatograph. The detector is sensitive in the 1–10 ppm range making it an extremely useful took in trace gas analysis. It is equipped with a heater, metering valve, and column connections with VCR fittings. The accompanying electronic control unit includes an electrometer, high voltage and polarization power supply, digital display for dc voltage and discharge current (mA), and detector temperature control with digital readout. Non-radioactive, universal and concentration dependent, the detector is a valuable tool in gas analysis. Gow-Mac Instrument Co.
Circle No. 306 on reader service card.

24-Port SPE Vacuum Manifolds Allow Multiple Extraction of Samples

Two larger versions of Supelco's Visiprep vacuum manifold for solid phase extraction double the extracting capability of the original model. The Visiprep 24-port vacuum manifold and the Visiprep-DL 24-port manifold let the user extract up to 24 samples simultaneously, while still providing the ability to control the flow at each individual port with an integral flow control valve. Both are constructed of rugged chemical-resistant materials for durability. The Visiprep-DL model features disposable liners, virtually eliminating the possibility of cross-contamination when consec-

utive samples are extracted using the same manifold port. A number of replacement parts and accessories for these products can be purchased individually, as well. Supelco.
Circle No. 307 on reader service card.

In-Situ Chemical Reaction Analysis Systems

New FTIR-based technology is designed for use in chemical product and process development to study reactions in-situ, under actual conditions. ReactIR reaction analysis systems permit rapid, quantitative analysis of reactants, intermediates, and products without distributing reaction conditions. Reaction pathways, mechanisms, and kinetics can be determined in real-time, and this information can be used to improve product quality and process performance. The systems are engineered to provide safety and performance under a wide range of temperatures, pressures, and reaction conditions. The systems can be configured to meet the specific requirements of each reaction engineering application. Applied Systems, Inc.
Circle No. 308 on reader service card.

Custom Hand Blown And Hand Pressed Glass Production Capabilities

Woods' Glass, a leading manufacturer of hand blown and hand pressed soda lime glass products and custom components, is offering a new brochure on the company's manufacturing capabilities. Woods' Glass produces a variety of glassware manufactured to customer specifications for use in chromatography and other laboratory applications. Desiccaters, ranging in diameter from 4 to 10 in.; cultures dishes up to 12 in. in diameter, glass domes to heights of 20 in. and diameters to 12 in.; specialty items requiring sturdy, uniform walls; and staining jars, dishes, and trays are some of the products produced to customer design requirements. Woods' Glass.
Circle No. 309 on reader service card.

Protect Operator and Environment from Toxic Vapors, Gases, Fumes, and Particles

Safelab Airone ducted or ductless fume hoods are specially designed so that they may be operated without external ducting using internal filters. In this mode, they can be used in virtually any laboratory location, thus avoiding expensive ducting and increased utility costs. The hoods feature automatic fan speed control, ensuring constant face velocity; low air flow alarm and LED visual indicator; automatic vertically opening glass sash; available in a 6 foot model for use with dual operators; worktops available in stainless steel, vitrosteel, monolithic stone, epoxy resin, trespa, and plastic laminate. Safelab Systems.
Circle No. 310 on reader service card.

Collapsible Fabric Tank with Replaceable Liner

ZipTank, a revolutionary concept in collapsible storage tank design, provides high tech solutions for manufacturers' temporary process storage problems. A unique, 2-component system, ZipTank features a high strength fabric outer restraint and a chemically resistant, inexpensive, replaceable inner liner. When not in use, ZipTank folds compactly for storage and, thus, provides the manufacturer the freedom necessary to quickly address temporary storage needs. ZipTank's high strength outer restraint results in a storage tank with a significantly smaller footprint than found in the common "pillow" tank. ILC Dover Inc.
Circle No. 311 on reader service card.

I-Chem Updates Performance-Based Specifications

I-Chem has added 14 additional pesticides and PCBS to its updated *Performance-Based Specifications* document, which lists the analytes and quantitation limits for the company's sample containers. This document is available free and compares side-by-side I-Chem's analyte list and quantitation limits to U.S. EPA Superfund specifications. I-Chem.
Circle No. 312 on reader service card.

Pre-Welded Sanitary Sampling Port

A pre-welded sampling port has been added to the existing line of Ionpure ports designed for sterile sampling of high purity water systems. These sanitary sampling ports are ideal for the electronic, pharmaceutical, food and beverage, and health care industries where monitoring bacterial levels is critical. The pre-welded sampling port is available in 4 in. × 2.0 in. od and 4 in. × 3.0 in. od lengths of sanitary 316L stainless steel pipe with tri-clamp connections, allowing easy installation into sanitary systems, eliminating the need for on-site welding and polishing. Ionpure.
Circle No. 313 on reader service card.

Seamless Formed Vessels, Tubing, Liners, Cones, Skirts, and Nozzles

An advanced forming technique produces seamless vessels, tubing, liners, cones, skirts, and nozzles from refractory and reactive metals, including tantalum, niobium (columbium), molybdenum, zirconium, vanadium, and their alloys. A combination of deep draw, spinning, and machining processes are used to produce these high quality formed parts with wall thicknesses from 0.003 in. and up, and height-to-diameter ratios of more than 20:1. B-J Enterprises, Inc.
Circle No. 314 on reader service card.

Version 3.0 of GLOBAL LAB Data Acquisition for DOS Software

Version 3.0 of Data Translation's GLOBAL LAB Data Acquisition for DOS, a high performance, real-time, mouse-driven software package, offers extensive display (including XY plotting), analysis, and customized printing capabilities, while fully supporting gap-free data transfers to 416,000 samples/s. Version 3.0 adds many features and support for the newest hardware products from Data Translation: DT2839 high speed, high channel board with throughput to 416kHz, analog thresholding triggering, and expanded entry channel-gain list, on up to 32 single ended or 16 differential channels; DT2833 high speed, simultaneous sample and hold board with 12-bit simultaneous sampling at rates to 250 kHz on 8 channels.
Circle No. 315 on reader service card.

Two and Four Liter EZE-SEAL Stirred Autoclaves

New 2 and 4 L models of the user-friendly EZE-SEAL stirred autoclaves and pressure vessels feature a metallic Double Delta seal ensuring an easy-to-operate low torque vessel closure. The new models accommodate larger reaction volumes and reduce scale-up efforts to process conditions. Autoclave Engineers.
Circle No. 316 on reader service card.

Stereomicrosope Offers High Optical Performance

The Carl Zeiss GSZ zoom stereomicroscope offers high optical performance, stability, and versatility. This multi-purpose instrument is ideal for industrial testing, scientific research, and education, where high-quality low magnification is needed at an economical price. Based on the Greenough principle, the GSZ offers excellent resolution and contrast. The GSZ features a built-in magnification changer with a zoom range of 1:5. In addition, front lenses can be used to alter the range of magnification. The GSZ stereomicroscope provides up to 132 mm of free working distance, allowing ample room for manipulation. Carl Zeiss, Inc.
Circle No. 317 on reader service card.

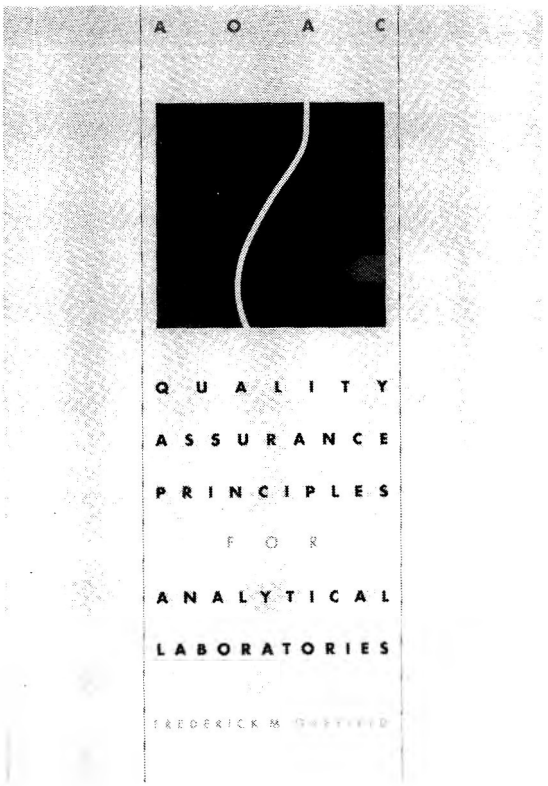
Capillary Electrophoresis Software

New software for the SpectraPHORESIS automated instruments for CE displays critical run parameters in real-time, permanently stores data, and provides unique audit trails for each run. Current, voltage, and temperature are critical parameters in capillary electrophoresis. The software displays one of these parameters in real-time. When the operator uses voltage or temperature programming, the real-time trace confirms that actual reach set values. Monitoring the current is especially useful for detecting plugged capillaries, and confirms successful recondition of the capillary. The real-time trace is stored with the data file for a permanent record of a critical, run parameter. Spectra-Physics.
Circle No. 318 on reader service card.

Personal Oxygen Monitor

The Gasman-Ox is a shirt-pocket size personal gas monitor for oxygen. The unit has a back lighted digital display and is supplied with adjustable audible and visual alarms preset at 19 and 23%. The monitors are rugged and waterproof. They are RF shielded, weigh under 9 ounces, and are powered by either disposable or rechargeable AA batteries. CEA Instruments Inc.
Circle No. 319 on reader service card.

ESSENTIAL FOR YOUR QUALITY ASSURANCE PROGRAM . . .



*E*ssential for any lab wanting to improve or initiate a quality assurance (QA) program. Material was extensively revised for this new edition which features new information and concepts developed since the handbook was last published. An important addition is a chapter on utilizing statistical applications and analytical control charting techniques. Appendixes, too, have been revised and one added on laboratory accreditation criteria – criteria which can be used for self-evaluation of lab QA programs and operations management procedures.

Each chapter offers recommendations for developing and operating a QA program. The book also provides solid justification for commitment of resources to a quality assurance program.

Contents: Quality Assurance Planning. Statistical Applications and Control Charts. Personnel Considerations. Management of Equipment and Supplies. Sample and Record Handling. Sampling and Sample Analysis. Proficiency and Check Samples. Audit Procedure. Design and Safety of Facilities. Laboratory Accreditation.

Appendixes: Typical Contents of a Quality Manual for Testing Laboratories. Forms Used by U.S. Federal Agencies. Instrument Performance Checks. FDA Audit Measure Procedures. Proficiency and Check Sample Programs. Accreditation Criteria.

192 pages. 2nd edition. May 1991. Softbound. ISBN 0-935584-46-3.

$63.00 In Continental North America (USA, Canada, Mexico)

$69.00 Outside Continental North America

To order: Send your name and address and payment. AOAC International accepts checks (US funds on US banks only, please) and VISA, MasterCard or Diners credit cards. When paying by credit card please include: type of credit card, card number, expiration date and your signature.

Send to: AOAC International - J
1970 Chain Bridge Road
Dept. 0742
McLean, VA 22109-0742

Credit card orders may also be placed by phone +1 (703) 522-3032, or FAX +1 (703) 522-5468.

Members: subtract 10% discount

For Your Information

Meetings

March 23–25, 1993: AOAC Board of Directors Meeting, AOAC, Arlington, VA. Contact: Nora Petty, AOAC, 2200 Wilson Blvd, Suite 400, Arlington, VA 22201-3301, telephone 703/522-3032.

March 24, 1993: AOAC MidAtlantic USA Section Meeting. Contact: David B. MacLean, 6422 Alloway Ct, Springfield, VA 22152, telephone 703/451-1578.

March 29–30, 1993: AOAC Europe Section Meeting, Barcelona, Spain. Contact: Juan Sabater, Laboratorio Dr. J. Sabater Tobella, Calle de Londres 6, 08029 Barcelona, Spain, telephone 343-322 88 06.

May 10–12, 1993: AOAC Northeast Section Meeting, Guelph, Ontario, Canada. Contact: Cathy Burchat, Ontario Ministry of Agriculture and Foods, Bldg No. 43, McGilvray St, University of Guelph, Guelph, ON, N1G 2W1, Canada, telephone 519/823-8800.

June 7–9, 1993: AOAC Midwest Section Meeting, W. Des Moines, IA. Contact: P. Frank Ross, USDA National Vet Serv Lab Toxicol Lab, PO Box 844, 1800 Dayton Rd, Ames, IA 50010, telephone 515/239-8542.

June 23–25, 1993: AOAC Pacific Northwest Section Meeting, Evergreen State College, Olympia, WA. Contact: Andrew Held, MEI Charlton, Inc., 2233 SW Canyon Rd, Portland, OR 97301, telephone 503/228-9663.

July 22–23, 1993: Fifth International Symposium on the Harmonization of Internal Quality Assurance Schemes for Analtyical Laboratories, Washington, DC. Contact: George Heavner, AOAC, 2200 Wilson Blvd, Suite 400, Arlington, VA 22201-3301, telephone 703/522-3032.

July 25–29, 1993: 107th AOAC International Annual Meeting and Exposition, Washington, DC. Contact: Margaret Ridgell, AOAC, 2200 Wilson Blvd, Suite 400, Arlington, VA 22201-3301, telephone 703/522-3032.

Cincinnati Highlight: Board Names Jensen Official Methods Board Chair

Thomas L. Jensen, Nebraska Department of Agriculture Laboratory Administrator, has been named Chair of the AOAC Official Methods Board. The Official Methods Board develops polices affecting the approval of official methods of analysis. It assists in training methods volunteers, advises on disputes over conflicting recommendations, and acts as final review for first action approval.

Jensen has been Agriculture Laboratory Administrator in Lincoln, NE, since 1979. Previously a chemist in Nebraska's Feed and Chemical Laboratory, he has served as an Associate Referee on Disulfoton, a General Referee in the Pesticide Formulations area, a member and chair of the Committee on Pesticide Formulations and Disinfectants, and member of the Official Methods Board (1986–1990). He has also been active in the AOAC Midwest Section, as an executive board member and chairman of the section in 1985–1986, and has served as a member of the State and Provincial Participation Committee and the Meetings, Symposia, and Educational Programs Committee.

When asked about his plans for the Official Methods Board, Jensen responded, "My goal will be to continue to work with the Board of Directors to streamline the method validation process and help determine the role to be played by the Official Methods Board in relation to the Validated Methods Task Force, and the AOAC Research Institute.

"I also plan to continue to foster communication with the various committees and boards within AOAC International, especially the volunteers working under the direction of the Official Methods Board. This organization does not just pack up and go home after the annual meetings. There are a myriad of activities that must continue to take place during the year by countless volunteers in order for the Association to remain vital.

"I'm excited to again be part of this effort, and in working with both staff and the volunteers for the next 3 years."

Jensen succeeds Michael Brodsky, Ontario Ministry of Health. During his 3-year term, Brodsky presided over many changes in the official methods program, from a period of interim method approval and voting by a small portion of members at an AOAC annual meeting to the present system of standardized review, Official Methods Board approval of first action methods, and membership vote by mail ballot for final action methods.

Wiley Award Symposium Focuses on Fumonisins

A number of research papers on fumonisins, including the 1992 AOAC Wiley Award Address, were presented as a symposium during the 1992 AOAC International Annual Meeting, July 31–September 2, in Cincinnati, OH.

The Wiley Award Address keynoted this symposium and was presented by the Wiley Award recipient and expert on the occurrence and analysis of fumonisins, P. Frank Ross, U.S. Department of Agriculture. Ten papers were organized around Ross' theme. They detailed the research on fumonisins by Canadian, European, and several U.S. federal and state agencies and the need for rapid, worldwide dissemination of information on these mycotoxins.

Ross reviewed the widespread fumonisin-related animal health problems in the United States after the 1989–1990 corn harvest season. Subsequent corn crops have not been associated with such problems, but survey data, according to Ross, have clearly demonstrated that fumonisins can be detected in corn

every year. Similar findings have been reported from other regions of the world where corn is grown.

Ross reported on the toxicity, analytical behavior, and occurrence of fumonisins from work spearheaded by state and federal scientists in many disciplines. As analytical capabilities improved, the scientific community concluded that fumonisins are widespread in corn and corn products. Typically, fumonisins B_1 (FB_1), B_2 (FB_2), and B_3 (FB_3) are always present at constant ratios. Also, basic research on toxicity confirmed long held suspicions that equine species are the most fumonisin-sensitive animals. Epidemiological and experimental data suggest that FB_1 concentrations of ≥5 ppm in horse feed is of concern.

Because fumonisins are carcinogenic or at least promoters of carcinogenicity, new carcinogenicity studies and additional toxicological information on fumonisins are needed. To this end, large quantities of purified fumonisins are needed to accomplish the necessary animal studies. Two papers presented at the symposium addressed this issue. J. David Miller, Agriculture Canada, discussed the production and purification of ^{14}C radiolabeled FB_1 and FB_2 using ^{14}C acetate in liquid culture. Because ^{14}C is distributed throughout the fumonisin molecule, this isotope is useful for studying the fumonisin residues in animal tissues and products as well as for biosynthesis studies. Using ^{13}C, Miller and others determined that methionine, glutarate, and serine and/or alanine are involved in the biosynthesis of the fumonisin hydrocarbon backbone; data indicate a polyketide biosynthetic pathway for the backbone.

Paul E. Nelson, Pennsylvania State University, and others (paper presented by P. Frank Ross) discussed the laboratory production of fumonisins on solid substrate (yellow corn). They found that moisture, temperature, and aeration are important factors in obtaining maximum yields of fumonisins in corn. They described a novel fermentation container that consists of a 30.5 × 61 cm autoclavable polyethylene bag. The greatest yields of fumonisins in a 4-week incubation period at 20–22°C were FB_1 = 6310 ppm, FB_2 = 2290 ppm, and FB_3 = 430 ppm from *Fusarium proliferatum* M-5991. A solvent system using CH_3CN–H_2O (1:1) for 30 min was found most successful in extracting fumonisins from culture materials.

Joseph Le Bars, Institute National de la Recherche Agronomic, France, corroborated that temperature, oxygen, and water availability are the main abiotic factors involved in fumonisin production on corn. Le Bars and others determined the levels of FB_1 produced by spraying plates with *p*-anisaldehyde and using thin-layer chromatography with spectrophotodensitometric quantitation. They surveyed strains of *F. moniliforme* from France and found that 33% of the isolates produced 800–3200 ppm of FB_1. Notably, FB_1 in dried corn cultures is thermally decomposed; half-life times at 150, 125, and 100°C are 10, 38, and 175 min, respectively.

Peter Scott, Health and Welfare Canada, noted that FB_1 and FB_2 are moderately stable, and about 40% was recovered when corn meal was heated at 190°C. However, if the meal was moist and then heated as before, only about 20–30% was recovered. No recoveries were obtained when corn meal was heated to 220°C. Extraction solvents for fumonisins were tested; 91 and 84% of FB_1 and FB_2 were extracted, respectively, with methanol–borate buffer (pH 9.2) (3 + 1) solvent. Recoveries of 50% were obtained from mixed baby cereal regardless of the extraction solvent used. Except for hydrated ferrous sulfate, additives normally present in commercial mixed cereal had no effect on the recoveries of FB_1 and FB_2 when added to ground corn or corn flakes.

The occurrence of fumonisin-producing fungi in four European countries (France, Italy, Poland, and Spain) was discussed by Angelo Visconti, Instituto Tossine e Micotossine da Parasiti Vegatali, Italy. Visconti reported that all strains of *F. moniliforme* examined from these countries produced from 0.7 to 4100 µg/g fumonisins (in laboratory cultures). The greatest fumonisin-producing isolates came from corn. Much lower levels were produced by isolates from barley, sorghum, and wheat. This work demonstrates the almost universal occurrence of fumonisin-producing *Fusarium* species.

The mechanism of action of fumonisins was discussed by Ronald T. Riley, U.S. Department of Agriculture. He noted the significant advances made in this area of research and provided an example of the recently found relationship between FB_1 and sphingoid bases. Riley and others found that FB_1 is a potent inhibitor of *de novo* sphingolipid biosynthesis and results in the accumulation of sphinganine (an intermediate in the biosynthetic pathway of complex sphingolipids). Results of their studies with both horses and swine fed fumonisin-contaminated corn indicate that the ratio of sphinganine to sphingosine in serum and tissues may be used as a diagnostic aid for fumonisin toxicosis in animals. These changes appear to occur prior to other serum biochemical changes of fumonisin toxicosis in these two species.

Mary Ann Dombrink-Kurtzman, U.S. Department of Agriculture, presented research results that establish the cytotoxicity of fumonisins in avian lymphocytes. The *in vitro* toxicity of FB_1 and FB_2 to turkey lymphocytes was determined by using the tetrazolium salt clevage bioassay. This work demonstrated that toxins caused cytoplasmic vacuolization and inhibited proliferation of cells exposed to 0.4–5 µg/mL toxin.

Analytical methodology for the detection and quantitation of fumonisins has advanced rapidly in the last year. The use of liquid chromatography for the separation and quantitation of FB_1, FB_2, and FB_3 was presented by Glenn A. Bennett, U.S. Department of Agriculture. Factors influencing the method's performance include extraction efficiencies of solvents, performance of SPE cleanup columns, stability of a fluorescent derivative, and efficiency of LC columns to resolve fumonisins from matrix interferences. This presentation described the use of a stable, highly fluorescent fumonisin derivative, naphthalene dicarboxaldehyde, which can be readily resolved with a simple gradient mobile phase in less than 30 min. Also, hydrolyzed fumonisins are separated from the parent compounds in this system. This method has been used to determine fumonisins in corn, culture material, and wet-milled corn products.

Another advance in analytical methodology was reported by Ronald D. Plattner, U.S. Department of Agriculture. Plattner and Bruce Branham developed a method to produce a stable isotope of FB_1 in liquid culture. This label, arising from methyl-d_3 methionine, is incorporated into the fumonisin at branch methyl groups. The isolated labeled compound is used as an internal standard to accurately determine levels of fumonisins in corn, corn products, and culture materials.

A unique screening procedure to test for the presence of fumonisins in foods, feeds, and tissues was described by James J. Pestka, Michigan State University. Pestka and others developed a direct competitive ELISA procedure with a 50 ng/mL detection limit. This system uses antibodies (monoclonal and polyclonal) that react with fumonisins but not with hydrolyzed fumonisins or the tricarbalyllic acid side chains.

The forthcoming publication of the full papers in the *Journal of AOAC International* will provide an up-to-date compilation of information on fumonisins regarding their occurrence, chemistry, production, analysis, mode of action, and toxicity.

–J.L. Richard and G.A. Bennett
U.S. Department of Agriculture

Acceptance by AOAC of Collaborative Studies Conducted by Other Organizations

As part of its harmonization efforts and in support of efficient use of limited resources that can be devoted to methods validation work, AOAC encourages the mutual adoption of methods by various methods and standards organizations.

By using informal and formal relationships with other organizations, AOAC presents AOAC Official Methods to those organizations for their consideration and adoption. AOAC-designated experts serve on technical committees, working groups, and task forces. Such experts participate in the detailed, specific procedures by which consensus is reached on a particular item. This requires identification of the need, submission, review, and comment on existing or proposed AOAC methods that fill the need, and then the process for agreeing and accepting that method.

Such activity enhances the worldwide harmonization and use of voluntary and mandated standards, including terminology, specifications, criteria, methodology, and laboratory practices.

In turn, AOAC encourages submission of methods from other organizations for AOAC consideration and adoption. AOAC has adopted the ISO/IUPAC/AOAC Guidelines for Collaborative Study Procedure to Validate Characteristics of a Method of Analysis. This means that performance of methods submitted to AOAC must have been validated according to these guidelines. For methods validated according to those guidelines, the conditions for AOAC adoption are as follows:

(*1*) Each collaborative study and method submitted will be subjected to peer review, just as a method and study that has been conducted under AOAC auspices. This review includes the appropriate General Referee, Committee Statistician, Committee Safety Advisor, and Methods Committee, before a recommendation for first action adoption can be submitted to the Official Methods Board.

(*2*) The statistical analysis must be adequate to generate the performance parameters required: s_r, RSD_r, s_R, and RSD_R for quantitative methods; sensitivity rate and specificity rate for qualitative methods.

(*3*) Documents must be submitted in English; if the method is not written in AOAC style, the submitter must work with the AOAC staff methods editor to prepare the method in the necessary format.

(*4*) The report of the collaborative study must be published in the scientific literature, either the *Journal of AOAC International* or another journal.

Thus, methods can be studied outside of the AOAC process, and still be adopted as AOAC Official Methods. Such methods are reviewed and must meet the same requirements imposed on methods studied under the AOAC Official Methods Program.

To further ensure that studies and methods will meet the AOAC requirements, AOAC encourages joint collaborative studies. Then, the validated method can move concurrently through the review and adoption process of more than one organization.

For additional information, contact Nancy Palmer at AOAC Technical Services, telephone 703/522-3032, fax 703/522-5468.

1993 Short Course Program

DESIGNED FOR THE ANALYTICAL SCIENCE PROFESSIONAL

SATISFACTION GUARANTEED

AOAC INTERNATIONAL

REGISTER NOW!
CALL: Carol Rouse
+1-703-522-3032

PRESENTED BY
AOAC International & American Association for Laboratory Accreditation (A2LA)

AOAC Short Courses Offered

- QA for Analytical Laboratories – *UPDATED for '93*
- QA for Microbiological Laboratories
- Statistics for Methodology – *REVISED for '93*
- Laboratory Waste Disposal, Environmental Compliance and Safety
- How to Testify as an Effective Witness
- Improving Your Technical Writing Skills

A2LA Courses Offered

- Laboratory Accreditation vs. Registration
- Environmental Laboratory Quality Assurance and Assessment
- Environmental Lead Laboratory Accreditation
- How to Design a Quality Manual
- Achieving Accreditation for Your Laboratory
- Laboratory Equipment Calibration Practices
- National Association for Testing Authorities (NATA) Lead Assessor Quality Training
- Statistical Measurement Control
- Laboratory Information Management Systems
- Laboratory Occupational Safety & Health Standards

Member rates are available to all members of either AOAC or A2LA.

Discounts are available if you register for more than one course or more than one person from the same organization.

Locations for AOAC and A2LA 1993 Short Courses*

Chicago, IL – February 22-27
Jacksonville, FL – May 24-29
Washington, D.C. – July 24-30
(In conjunction with the 107th AOAC International Meeting & Exposition)
Manhattan Beach, CA – October 4-9

* Not all courses are offered at all locations.

Call to request a 1993 Short Course Catalog for a complete listing of other short courses available from AOAC and A2LA.

AOAC Wants to Publish Your Books

Do you have an idea for a book on a subject in the analytical sciences?

Do you have a manuscript but no other publisher committed to publishing it?

Are you preparing a workshop, symposium, or training course and want to publish the proceedings or work with AOAC to develop a manual?

If you have answered "Yes" to any of the questions we've posed, please call or fax:

Krystyna McIver
Director of Publications
AOAC International
+1 (703) 522-3032 or
fax +1 (703) 522-5468

The Publications Department of AOAC International is seeking proposals from authors for books to be published by AOAC.

AOAC offers competitive contract terms, royalties, and comprehensive marketing. Promotional campaign efforts are designed to provide the widest appropriate exposure through the use of space ads, exchange ad programs, conference displays, and targeted mailings. AOAC publications reach a worldwide audience of analytical chemists, microbiologists, and other biologists and administrators in industry, government, and academia.

AOAC INTERNATIONAL

Books in Brief

Environmental Remediation: Removing Organic and Metal Ion Pollutants. Edited by G.F. Vandegift, D.T. Reed, and I.R. Tasker. Published by the American Chemical Society, 1155 16th St, NW, Washington, DC 20036, 1992. 270 pp. Price: U.S. & Export $66.95. ISBN 0-8412-2479-X.

This book discusses current applications and new developments in separation science for the remediation of contaminated groundwater and soil. It examines waste treatment and waste avoidance technologies and the applications of separation science to waste minimization and preconcentration. It presents fundamental research for developing new technologies in the remediation of contaminated aquifers and surface and subsurface media. Insights are offered into measuring the depth of problems and the effectiveness of treatment. An overview chapter examines the extent of environmental abuse, the high cost of cleanup, and the federal regulations that govern cleanup and disposal of present waste.

Practical Techniques for Groundwater and Soil Remediation. By Evan K. Nyer. Published by Water Information Center, Inc., 125 East Bethpage Rd, Plainview, NY 11803, 224 pp. Price: $64.00. ISBN 0-87371-731-7.

The third book in the Geraghty & Miller Environmental Science and Engineering Series, *Practical Techniques for Groundwater and Soil Remediation,* has been published through Water Information Center, Inc., by Lewis Publishers, Inc. It is a compilation of a series of popular articles written by the author for "Groundwater Monitoring Review," the publication of the National Ground Water Association. All the practical aspects of a remediation program are discussed, such as actual remediation programs with technical data on treatment equipment performance and costs and data from treatment system that did not work, a unique feature not found in such presentations. Evan Nyer brings to this collection of papers a wealth of experience. "We can see the actual results and compare them to our original ideas on what would happen as we conducted the remediation program. We now know enough about groundwater and soil remediation so that we can develop a strategy at the beginning of the project."

Detectors for Capillary Chromatography. By Herbert H. Hill and Dennis G. McMinn. Published by John Wiley & Sons, Inc., 1 Wiley Dr., Somerset, NJ 08875-1272, 1992. 444 pp. Price: $95.00. ISBN 0-471-50645-1.

Recently, there has been a great deal written about the benefits capillary chromatography offers in the area of separation and analysis. However, there is a need now to focus on the detection methods commonly used with capillary chromatography. *Detectors for Capillary Chromatography* presents the writings of 20 authorities who describe the unique features of each detector that are required for successful interfacing with capillary columns. To understand the information gained in high resolution chromatography, detector response must be interpreted correctly; this book provides the necessary insights into how this can be accomplished. The contributors to this book are all experts in the field. In many cases, they are the researchers who originally developed the detector or have made major contributions to understanding its operation.

Fluid Sterilization by Filtration: The Filter-Integrity Test and Other Filtration Topics. By Peter Johnston. Published by Interpharm Press, 1358 Busch Parkway, Buffalo Grove, IL 60089, 1992. Price: $82.56.

This book provides practical, hands-on information for formulation scientists, production, and quality control personnel working in the pharmaceutical, biotechnology, and bulk chemical industries. The book explains all aspects of filter integrity testing in detail, providing practical answers to critical industry concerns such as the following: how to ensure that a filter failure will not cause waste of expensive product; what measure of filtration efficiency is sufficient to qualify a filter to be a sterilizing filter; how bubble-point testing, integrity, and stress testing correlate and what the data really mean; similarities and differences in liquid and gas filtration; how to characterize filter media from plots of fluid flow rate vs driving pressure and the key differences between liquid and gas plots; cartridge filter testing vs plate filter testing; and determining optimum fluid flow and increased production efficiency with sterilizing filters.

The Basis of Toxicity Testing. By Donald J. Ecobichon. Published by CRC Press, Inc., 2000 Corporate Blvd, NW, Boca Raton, FL 33431, 1992. 176 pp. Price: U.S. $69.95/Outside U.S. $84.00. ISBN 0-8493-7814-1.

The Basis of Toxicity Testing discusses fundamental principles and concepts behind toxicological tests, their design and implementation, interpretation of results, and significance of individual studies in the overall assessment of toxicants. The book covers the complete spectrum of toxicological investigations required for the registration of a chemical (including in vivo and in vitro studies); describes the design of toxicological studies in simple terms, reinforced with diagrams of their design, possible results, and interpretation; examines the various factors that can affect the outcome of a toxicological study; and discusses the limitations of various toxicological tests and their significant in the overall process of hazard and risk evaluation.

Cumulative Author Index

COMING IN THE NEXT ISSUE

SPECIAL REPORTS

- Methods of Analysis for Toxic Elements in Foods. Part IV. General Method of Ashing for the Determination of Toxic Elements—I. Skurikhin
- New Metrological Characteristics of Analytical Methods of Analysis Used for Safety Control of Food and Environmental Materials—I. Skurikhin

DRUG RESIDUES IN ANIMAL TISSUES

- Simultaneous Determination of Multiple Tetracycline Residues in Milk Using Metal Chelate Affinity Chromatography—M. Carson

TECHNICAL COMMUNICATIONS

- Incidence of Fungi in Shared-Use Cosmetics Available to the Public—P.B. Mislivec, R. Bandler, and G.A. June

KEYNOTE ADDRESS

International Perspectives in Certification and Accreditation

A. SILVA MENDES
Commission of the European Communities, Directorate General III, Internal Market and Industrial Affairs, Certification Unit (III/B/5), Rue de la Loi, 200 B–1049 Brussels, Belgium

On May 7, 1985, the Council of Ministers of the European Communities adopted a new approach to technical harmonization and standards. This resolution laid the foundations for a complete and comprehensive policy on quality, which calls for removal of unnecessary technical barriers to trade; restriction of legislative texts to "essential requirements" for protecting the general interest (particularly health, safety, and consumer and environmental protection); and use of nonmandatory European Standards or National Standards, if needed, as a transitional measure for defining the technical characteristics of products to satisfy the essential requirements.

Following the adoption of the May 7, 1985, resolution, standardization activity increased to satisfy the new needs for standards, particularly at the European level and in the regulated area. In general, standardization is a highly transparent process, in which all interested parties should participate. Standardization combines the advantages of democracy with state-of-the-art technology. The reference to standards in legislation simplifies the legislative process (legislation only refers to the essential requirements). Technical specifications are defined in the standards, and therefore, most of the costs of production of technical specifications are transferred from the public to the private sector.

In a June 18, 1992, resolution, the Council (*1*) reiterated the importance of a cohesive system of European Standards, based on transparency, openness, consensus, independence, efficiency, and decision-making, with national representation; (*2*) considered that European standardization, although organized on a voluntary basis, also serves the public interest; (*3*) confirmed the interest of an international standardization system; (*4*) endorsed the desire to avoid the fragmentation of work on European standardization; (*5*) stressed the urgent need for high-quality European Standards; (*6*) stressed the importance of strengthening the links between research and developmental activities and standardization; (*7*) considered that the use of European Standards should be further encouraged as an instrument of economic and industrial integration within the European market and as a technical basis in support of legislation, particularly in defining technical specifications for products and services or for testing methods to be used in other areas falling within the scope of Community legislation; and (*8*) invited the Commission, where appropriate, to apply the principle of referring to European Standards in future draft Community legislation.

Because standardization is only a first step toward the completion of an Internal Market where the quality of products will play a decisive role, the Council stated in the May 7, 1985, resolution "that the new approach will have to be accompanied by a policy on conformity assessment." In other words, standards specifying the essential requirements are necessary but not sufficient. A transparent framework must be built up to carry out conformity assessment of products with regard to EEC directives. The Community adopted a global approach to conformity assessment as a complete and comprehensive policy on quality. This policy is an indispensable part of any industrial policy and fundamental to the very concept of the Internal Market.

In a December 21, 1989, resolution, the Council adopted the global approach to conformity assessment and created, in the context of the Internal Market, a technical environment in which public authorities, economic operators, and consumers should have confidence. This technical environment is composed of product specifications (expressed in the standards and the technical regulations) and structures for the conformity assessment (certification bodies, inspection and quality audit bodies, testing laboratories, and the manufacturers' quality systems).

To create the conditions that are conducive to confidence in this technical environment, the global approach to conformity

Presented at the 106th Annual AOAC International Meeting, August 31–September 2, 1992, at Cincinnati, OH.

assessment is aimed at making these structures as homogeneous, transparent, and credible as possible throughout the Community.

Compliance Procedures

The Council approved the decision on the modules on December 13, 1990, for the various phases of the conformity assessment procedures to be used in the technical harmonization directives. This decision, which takes into account each developmental stage of the conformity assessment mechanisms (quality management and quality assurance techniques, certification and accreditation procedures, etc.) states that the directives must define the limit of choice of procedures open to the manufacturer for ensuring compliance with essential requirements.

The conformity assessment procedures in Community legislation should ensure that products are in full conformity with the essential requirements stated in the technical harmonization directives and that this level of quality is without unnecessarily onerous conditions. The modular approach provides a means of spreading the burden of conformity assessment, with greater flexibility, over the entire production process, and adapting it to the needs of each individual operation. Also, the desired safety level should be achieved without unnecessarily burdening the economic operators, especially the small- and medium-sized enterprises, with excessively onerous conformity assessment procedures:

Conformity assessment procedures usually come into play at the design and production stages in the manufacturing process. The following modules can be combined to form a complete procedure:

Module A Internal production control,
Module B EEC-type examination,
Module C Conformity to type,
Module D Production quality assurance (EN 29002),
Module E Product quality assurance (EN 29003),
Module F Product verification,
Module G Unit verification, and
Module H Full quality assurance (EN 29001).

In one directive, several alternative modules can be applied to the same function provided that a degree of equivalence between their results is obtained. For example, they all ensure that the products meet the required technical specification or afford a given level of safety.

CE Mark of Conformity

To harmonize the texts of a number of Community directives, based on Article 100a of the EEC Treaty, the Commission proposed a regulation for affixing the CE mark of conformity. The regulation is now being discussed in the Council.

The CE mark affixed to a product indicates that the natural or legal person who affixed the mark, or had it affixed, has determined that the product conforms to all binding Community provisions applicable to it.

Conformity Assessment Bodies

In the past, conformity assessment bodies, particularly testing bodies, were recognized by using administrative procedures. Now, for the purpose of the operating modules, Member State National Authorities will be responsible for notifying bodies under their jurisdiction. Bodies must be technically competent and comply with the requirements of the directives. Notification consists of designating the competent body and recognizing its competence to ensure objectivity, transparency, and conformity with the relevant criteria. Member States must ensure that the notified bodies are permanently and technically qualified as required by the directives. Verification may be done by Member State National Authorities themselves, if they have their own assessors, or they can use their National Accreditation Systems.

Notified bodies, which can prove their conformity with the EN 45000 series of standards, will presumably conform to the technical requirements of the directives.

In addition, subcontracting of some conformity assessment work by the notified bodies will be subject to certain conditions. Areas of concern include the competence of subcontractors on the basis of conformity with the EN 45000 series of standards, the capability of the Member State National Authority to effectively monitor the subcontracted work of a notified body, and the ability of the notified body to exercise effective responsibility for the work carried out under contract.

The responsibility of the subcontracting body should not, in any circumstance, diminish on the basis of private law contracts signed between the 2 bodies, for subcontracted work relating to the verification of the degree of conformity, particularly testing and quality audits. Subcontracting procedures do not have any geographical limitation. They are conceived to define the main issues of internal EEC subcontracting activities, but in conformity with Technical Barriers to Trade (TBT) Code rules, they also apply to subcontracting to bodies in third countries.

Use of the European Standards

European Standards are not only used to improve the quality and acceptability of products, but they also are necessary to enhance confidence in the ability of the manufacturer to supply quality products and to ensure confidence in the operation and competence of the bodies in charge of conformity assessment (certification, inspection and quality audits, and testing). In this way, the standards demonstrate their competence.

The European Committee for Standardization/European Committee for Electrotechnical Standardization (CEN/CENELEC) approval of Standards EN 29000 in 1987 and EN 45000 in 1989 is intended to satisfy the needs expressed by the economic operators and may be seen as one of the most important elements of the new Community strategy.

Conformity Assessment in the Nonregulatory Sphere

To eliminate technical barriers to trade, any product lawfully produced and marketed in a Member State must be admitted to the market of every other Member State. This principle of the Internal Market is designed to ensure the full implementation of mutual recognition. It seeks to create conditions whereby the conformity assessment bodies, which are designated or accredited according to objective criteria of

transparency and competence, are authorized to attest conformity of products under national regulations when the operation of such bodies also meet these criteria. In the nonregulatory sphere, the market is completely free to make its own choices in terms of the conformity assessment procedures. However, because the effective operation of conformity assessment under the directives relies on a strong private sector, the Community promoted the European Organization for Testing and Certification (EOTC) as a means of ensuring a greater degree of coherence in the market's choices.

To provide the environment in which the modules will work, the Community needs a competent and transparent private market in conformity assessment, and EOTC is a stimulant to the development of such a market.

The different levels of development in the Community and in industrial sectors with regard to institutional quality infrastructures may influence the successful completion of the Internal Market. The Community launched the Preparation of Regional Industry for the Single Market Programme to support the development of conformity assessment infrastructures in lesser developed regions of the Community (Objective 1 regions). Similarly, the Commission plans to identify the entities working in the quality area; to evaluate the capacity, the relative efficiency, and the competitiveness of the sector; and to estimate further needs in infrastructure in this field.

External Aspects of the Global Approach

The Community's adoption of the global approach to conformity assessment, which provides a means of assessing the competence and responsibilities of the conformity assessment bodies, should facilitate the relationship between the Community and its international partners. The strengthening of confidence throughout Community structures will, in turn, generate greater confidence in third countries, either through the relevant international organizations or on a bilateral basis.

Such Community actions in third countries are intended to promote international trade through the provision of information on European Standards and conformity assessment procedures. This action, which conforms with and goes beyond the TBT/General Agreement on Tariffs and Trade Code obligations, is being developed through the external commitment of the Community and the specific needs of the third countries' economies (for developing countries). Also, the Community's economic interest in the use by third countries of equivalent conformity assessment means and procedures will facilitate bilateral trade.

Mutual Recognition Agreements

The Council's global approach to conformity assessment clearly stated that the Community is prepared to negotiate Mutual Recognition Agreements (MRAs) with third countries and determine those issues that must be negotiated. Assuming that the levels of safety required for the products have to be assured in such agreements, the Council underlines 3 conditions: (*1*) The bodies to be recognized in the third countries should demonstrate the same level of technical competence as the Community's notified bodies; (*2*) the MRAs are confined to reports, certificates, and marks drawn up and issued directly by the bodies designated in the agreements; and (*3*) in cases where the Community wishes to have its own bodies recognized, the agreements establish a balanced situation with regard to the advantages derived by the parties in all matters relating to conformity assessment for the products concerned.

MRAs, the mutual acceptance of the conformity assessment work carried out by the other party, are signed by the governmental authorities of the 2 parties and concern all bodies listed in the annex to the agreement.

The link between the MRAs and Subcontracting is made to clarify and make transparent the relations between these 2 types of activities.

MRA activities are related to the conformity evaluation tasks while subcontracting is related to the verification tasks. Although subcontracting operates until the moment where it is necessary to delegate the issue of a certificate, MRA is necessary.

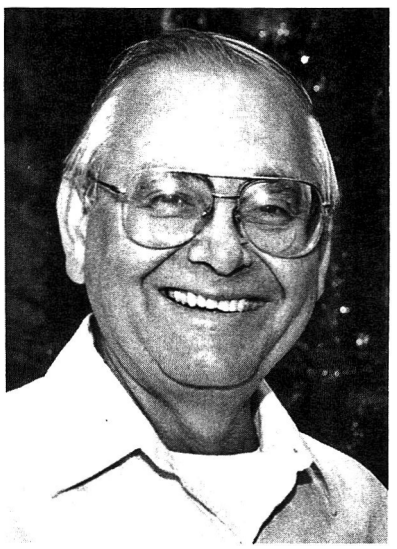
PRESIDENT'S ADDRESS

AOAC International — 1991–1992: The Year in Review

EDGAR R. ELKINS
National Food Processors Association, 1401 New York Ave, NW, Suite 400, Washington, DC 20005

This has, without doubt, been a busy and exciting year for me. In my wildest dreams, I never thought I would become president of this Association. It has truly been a high point in my career.

I thought it would be worthwhile and informative to review with you our accomplishments this past year. It has been a good year, and we have got a lot done. When I say we, I mean the fine, hardworking, and dedicated AOAC staff in Arlington, VA; the hardworking and also dedicated Board of Directors; and you—the volunteers—who make this Association what it is. At the annual business meeting last year when I assumed the presidency, I said that H. Michael Wehr had left us with a full plate. A large portion of that plate was the result of initiatives that were developed at the Board of Directors retreat in March 1991. So, let us begin with implementation of those initiatives.



Implementation of Retreat Initiatives

Making AOAC a Truly International Organization

AOAC has operated for many years on an international basis in the technical area. That is because volunteers in the AOAC official methods program are drawn from analytical scientists worldwide. These scientists include the methods committee members, the General Referees, the Associate Referees, and the collaborators.

AOAC works toward harmonization on an ongoing basis through its liaison officers and representatives who participate in both AOAC and other organizations involved in the pursuit of quality analytical science. An excellent example of that is the ISO/IDF/AOAC Tripartite group of experts who oversee the development and validation of methods in dairy chemistry so that methods in that discipline will be acceptable to all 3 organizations and Codex. AOAC is also a partner in the IUPAC/ISO/AOAC Interdivisional working party on the harmonization of quality assurance for analytical laboratories, with a project completed this year on harmonization of proficiency testing schemes.

As part of their harmonization efforts, AOAC committees are looking seriously for the first time at the possibility of adopting the internationally accepted ISO format for analytical method presentation.

The greatest effort this year has been focused on activity in Europe and the impact of decisions of the European Community on accreditation and analysis. AOAC requested and was granted liaison status in the technical committee, "Food Analysis, Horizontal Methods," in CEN, the European standards organization, and it is seeking status in additional agricultural and environmental committees. It is important to have AOAC methods, which have been thoroughly validated according to international standards, become the methods of choice for international regulations and trade. AOAC has organized, cooperated in, or sponsored several more international symposia this year and, as a result, has become more visible and has brought additional international participants into all AOAC program activities. These symposia have included the AOAC Europe section symposium in November 1991, in Maastricht, The Netherlands, on "Protection of Public Health—A Challenge for Food and Environmental Analysts" (AOAC Board Member Alan R. Hanks and I had the pleasure of attending that meeting); the Fifth International Symposium on Biological and Environmental Reference Materials held in May 1992, in Aachen, Germany; and the international seminar on Analytical Quality Assurance and Good Laboratory Practice in Dairy Laboratories sponsored by the International Dairy Federation, the Commission of European Communities, the German National Committee of IDF, and AOAC.

The AOAC Meetings, Symposia, and Educational Programs Committee has made a commitment to continue the increasing international emphasis of the annual meeting. International representation on the committee has been expanded, and efforts have been focused on increasing international participa-

Presented at the 106th Annual AOAC International Meeting, August 31–September 2, 1992, at Cincinnati, OH.

tion in the 1992 meeting. It begins with this year's theme, "Highlighting Chemical and Biological Analysis in the International Context," and continues with the keynote address by António Silva Mendes of the European Communities on "International Perspectives in Certification and Accreditation." This year, the number of symposia chairs from outside the United States has tripled, the number of presenters from outside the United States has doubled, and 3 of our poster session coordinators are from outside the United States. In addition, the regulatory roundtable has expanded to a full day and features 6 speakers from outside the United States.

Test Kit Testing Program and the AOAC Research Institute

After a year of preparation and planning, a new corporation was launched by AOAC International, the AOAC Research Institute. The Institute was formed to administer the third party review of proprietary, commercial test kits to confirm manufacturer's claims for performance.

The general principles by which the test kit testing program will operate were developed by an AOAC task force. Those principles include submission by the manufacturer of a defined data package, review of the submitted data and development of a testing protocol by Institute-recruited expert reviewers, testing according to the protocol by an Institute-designated independent laboratory, evaluation of the test results by the reviewers, and licensing of the AOAC performance tested seal for successful kits.

As the program evolved, a cooperative relationship was sought between the Institute and the U.S. Food and Drug Administration as a mechanism to evaluate kits intended to detect drug residues in milk under the pasteurized milk ordinance. Under a memorandum of understanding, FDA and AOAC will cooperate in setting the protocol requirements and in evaluating the application data and independent testing data so that Institute-validated test kits will be acceptable in the FDA milk monitoring regulations.

Similar cooperative arrangements are underway so that mycotoxin kits will be acceptable to the U.S. Department of Agriculture, Federal Grain Inspection Service, and environmental immunoassay kits will be acceptable to the U.S. Environmental Protection Agency.

New Peer-Verified Methods Process

The planning retreat pointed out the need for new methods products from AOAC to meet user's requests for more methods and more state-of-the-art methods than the AOAC official methods program is able to generate. An AOAC task force has been working this year on designing a program for a class of methods that have not been subjected to full collaborative study. The program is intended to be less resource-intensive than the AOAC official methods program and, thus, will provide a rapid entry point for useful, validated methods into AOAC and distribution to the user community. As developed so far, the method sponsor will generate in-house information and data about the method and method performance, the sponsor will arrange for a second, independent laboratory testing of performance (hence, "peer-verified"), and AOAC will provide review and approval of the submitted information. Successfully verified methods will be quickly published in a format that will also permit users to submit comments.

It is intended that the requirements for in-house data for the peer-verified method program will harmonize with the entry requirements for the AOAC official methods program. This will facilitate movement of methods into the collaborative study process, if desired.

Streamlining of Full Collaborative Study Methods Validation Process

Although a common criticism of the traditional AOAC methods process is its slow-moving, nonresponsive pace, the AOAC program is in fact one of the more responsive among the various standard methods organizations. One standards organization admits to an average of 84 months for finalization of a standard! At AOAC in the past year, 25% of submitted protocols were approved within 6 months, while 80% overall were approved within 1 year. Of the methods submitted, 35% were approved within 6 months; 65% were approved within 1 year. These intervals involve several review levels in AOAC as well as time in the hands of the method author, which is out of AOAC's control.

The New AOAC Look

After the new name and tagline were developed, it soon became apparent that new logo and letterhead designs were needed, as well. A crisp new logo was designed. It incorporates the new name, retains the symbols that serve to both clearly identify the Association as scientific and provide continuity with earlier logos, and fulfills the universally held criteria for a logo to be memorable and easily recognized.

The *Journal of AOAC International* was also redesigned and now has a metallic gold cover with the world globe at the top reflecting worldwide contributors and leadership.

Nominating and Election Process

In December 1991, the AOAC Board of Directors approved terms of reference for a task force to review the nominating and election process. The task force members have reviewed the bylaws of 10 other societies and have identified the pros and cons of the current AOAC International process and of proposed changes. The task force is scheduled to meet here in Cincinnati, OH, this week and plans to finalize its recommendations, including any proposed changes in the AOAC Bylaws, and present them to the AOAC Board of Directors at its December 1992 meeting.

Task Force on Nutrient Labeling Analyses

At the December 1991 Board of Directors meeting, a task force on nutrient methodology was formed. This was necessary because FDA has proposed new mandatory nutrition labeling regulations, and there are foreseeable methods problems for some of the nutrients for which labeling will be required.

The task force, chaired by Jonathan W. DeVries, has done its work well. This is a classic case of AOAC volunteers responding to the call for quick action. The July 1992 issue of *The Referee* contained a report listing those methods judged by the task force to be adequate for nutrition labeling purposes.

Also, the August 1992 issue of *The Referee* contained information on available standard reference materials that can be used with these analyses.

Journal of AOAC International

The number of articles submitted to the *Journal of AOAC International* has increased in 1992 due partly to the efforts of the publications department to increase the quality of the articles published and to improve publication time. They have also been encouraging authors who publish in competing journals to submit research to the *Journal of AOAC International*. If you are presenting a poster here at the meeting, you probably received a letter from the publications department encouraging you to submit your finished research paper to the managing editor of the Journal. Not everyone's paper will be accepted, however. The *Journal of AOAC International* is a fine journal, and articles published undergo a rigorous peer review. You should feel proud and privileged to have your article published in the *Journal of AOAC International*, and I encourage you to submit your research to the Journal.

New Books

While I am on the subject of publications, AOAC has published 2 books this year: *Ecology and Management of Food Industry Pests*, Richard Gorham, editor, and the *U.S. EPA Manual of Chemical Methods for Pesticides and Devices*. The Youden and Steiner *Statistical Manual of the AOAC* is now being revised by John G. Phillips, chair of the Statistics Committee.

AOAC is expanding its book publishing program and is offering competitive royalty contracts to authors. AOAC also has an extensive marketing program. The 1992 marketing budget for publications is in excess of $300,000. A thorough, customized marketing plan is developed for each new publication prior to release. So, if you have a book idea and an outline, consider AOAC as your publisher.

Regional Section Program

Surprisingly, the regional section program was in existence for 11 years before a section was formed to include the Washington, DC, metropolitan area, so long the home of AOAC. The new section, which covers Maryland, Delaware, Northern Virginia, and Eastern Pennsylvania, as well as the District of Columbia, was chartered in February 1992, and held its first meeting in March of this year. I was privileged to have attended this meeting.

In the meetings area, many of you have already experienced one of our customer service initiatives. To reduce registration waiting lines, badges and tickets were mailed to all registrants who sent their forms by the August 3 deadline. All you had to do was pick up your badge holder and program.

Along these same lines, the membership department has made adjustments in the way it processes membership applications to enable a 1-week turnaround time between receipt of application and notification of acceptance. We have also instituted a policy of asking members when they call what they like and dislike about the Association and to make suggestions.

Which brings me to my last point—for an Association to meet the needs of its members and other constituents, it needs to ask its members what their needs are. AOAC has been doing just that. Members were surveyed on long-range planning questions in 1989, which led to the more in-depth focus group survey accomplished in 1990, which, in turn, led to the many important initiatives about which this talk began. This spring, a membership survey was undertaken jointly with many of the other associations belonging to the Council of Engineering and Scientific Society Executives, and we fully expect to implement measures that will respond to the needs brought to light by this survey.

So, there you have it, a review of what AOAC International did this year. I hope it has been informative. Again, it has been a pleasure serving as your president, and I want to thank the staff for all their help and commend and thank you for the excellent accomplishments and progress made this year. Have a good meeting.

SPECIAL REPORT

Quantitative Planar Chromatography as a Tool in Pharmaceutical Analysis

BERND RENGER
Byk Gulden Pharmazeutika, Konstanz, Germany

Contemporary Planar Chromatography—Scope and Limitations

Modern quality assurance systems in the pharmaceutical industry try to reduce final product analysis in favor of in-process controls and process-parametric assessments. Because of the stringent requirements for testing and monitoring of drugs for approval, the number of analyses to be performed has increased rather than decreased. Also, demands on quality, validation data, and performance of analytical methods have risen dramatically. The main task of a pharmaceutical analysis laboratory, therefore, is to provide reliable analytical data rapidly, as accurately as required, day-by-day, at low costs, and on a wide range of different samples and materials (1).

To cope with these demands, a variety of very different analytical methods and techniques are used, ranging from simple, compendial limit tests to sophisticated combination techniques such as gas chromatography/mass spectrometry (GC/MS). Because unspecific assay methods are no longer accepted by registration authorities, chromatography is the premier method, with liquid chromatography (LC) being the most significant variation.

In looking at the spectrum of compendial analytical methods, there is an "analytical gap" between the pharmacopoeially accepted chromatographic methods (LC and GC) and all the other unspecific methods, such as photometry or gravimetry. Contemporary planar chromatography seems to be a convenient, reliable, and specific chromatographic method that can be used to close this gap (2–12).

In contrast to the traditional compendial, semiquantitative thin-layer chromatographic (TLC) procedures, where spots of diluted test solutions are visually matched against the impurity spots in the undiluted test solution, contemporary quantitative planar chromatographic methods (3–17) use the following features: (prewashed) TLC-grade stationary phases, automated or semiautomated sample application devices, controlled development environment, defined or automated developing chambers or forced-flow techniques, semiautomated derivatization procedures, computer controlled densitometry and quantitation, and fully validated procedures.

Received September 25, 1991. Accepted September 15, 1992.
Adapted from a plenary lecture given at the National Symposium on Planar Chromatography, September 23–25, 1991, Somerset, NJ.

These features result in methods that are convenient, fast, robust, and cost efficient; require minimal sample cleanup; allow high simultaneous sample throughput; offer a wide range of mobile and stationary phases and, therefore, a high separation selectivity and specific pre- and postchromatographic detection possibilities; allow an "in-system" calibration; and require minimal quantities of solvents, thus reducing solvent waste.

"Cost efficient" means that costs per test are relatively low because of high sample throughput and the time-sharing basis on which spotting, development, visualization, scanning, and evaluation can be done. "In-system calibration," achieved by running several standard tracks on one plate, avoids difficulties that may arise from retention time instability in LC. Of course, LC is and will continue to be indispensable in pharmaceutical analysis. But very often, planar chromatography offers a complementary approach to solve problems.

We have found planar chromatography to be the method of choice for complex and dirty samples with poor detection characteristics or impurities that remain adsorbed to the stationary phase. Also, planar chromatography works well for the analysis of large numbers of samples not suitable for automated LC systems because of content uniformity and stability testing.

The lower sensitivity and accuracy of planar chromatography compared with LC can often be accepted if the method's reliability was validated (18–23) according to the U.S. Food and Drug Administration (24) and Committee for Proprietary Medicinal Products guidelines (25, 26). Also, the analytical methods should be "as accurate as required" and not "as accurate as possible," and "the degree of validation depends, to a large extent, on the problem posed" (25). Planar chromatography can be performed in full accordance with good laboratory practices (GLP) and good manufacturing practices (GMP).

The specificity (or resolving power) of planar chromatography is limited by the limited migration distance in the nonforced-flow planar techniques. This disadvantage may be compensated for by the wider variety of sorbents and solvent systems that can be used and by the observation and evaluation of the whole sample track containing the complete chromatogram. A lack of linearity can easily be overcome by specifying working ranges with linear response.

Normally, precision, in terms of reproducibility or repeatability, is lower for planar chromatography than for LC. Reported relative standard deviations for repetitive scans of a sin-

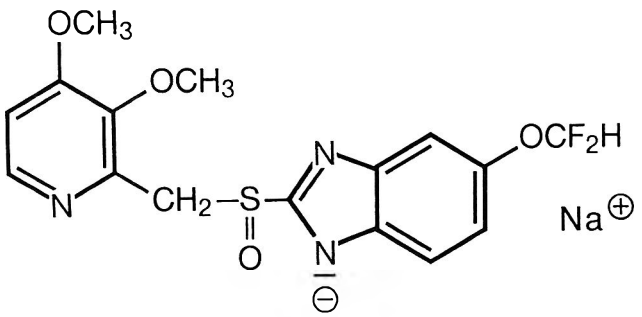


Figure 1. Nonoxynol(9).

gle sample track are ca 0.2% and for multiple applications of the same sample, 0.8–2.0%. Realistic relative standard deviations for the entire analytical procedure are 1.5–3.0%. This precision may be inadequate for some applications but is quite acceptable for many others, considering that "the objective of validation of a test procedure is to demonstrate that it is adequate for its intended use" (25).

Applications

Poly(ethylene glycol) Ethers

Certain classes of chemical compounds, e.g., the widely used poly(ethylene glycol) ether surfactants (27), are well-suited for analysis via TLC.

The pharmacopoeial monograph for nonoxynol(9) (Figure 1), a poly(ethylene glycol) ether surfactant (28), contains an LC assay that shows the several oligomers in one distorted ("bumps and shoulders") peak with poor repeatability (2% for repetitive injections), giving no information about the toxicologically significant chain-length distribution that may be determined by other LC methods (29). Quantitation is done via the UV absorption for the aromatic moiety of the molecule. The fact that nonylphenol ethoxylate surfactants may absorb onto metal surfaces and this absorption may lead to quantitation problems in LC (30) is not taken into consideration; specification limits are 90–110%.

The usefulness of planar chromatographic techniques in solving problems such as this one may be demonstrated by the several convenient TLC assay procedures reported for polidocanol (Thesit®, laureth 9 (CTFA), $CH_3(CH_2)_{11}$-$OCH_2CH_2)_n$ OH, $n \approx 9$), a local anaesthetic (31) with a similar structure.

All 3 methods assay polidocanol in complex matrixes with excellent to moderate repeatabilities relative to those of the repeated injections in the USP XXII LC method for nonoxynol(9) (32–34). The last 2 methods additionally provide information about chain-length distribution (Table 1).

The proposed compendial gravimetric purity test on poly(ethylene glycol) compounds in nonoxynol(9) includes several extraction and filtration steps using chloroform and ethyl acetate. One of them requires warming a separatory funnel filled with ethyl acetate to an internal temperature of 45°C in a water bath.

Table 1. Assay of polidocanol in pharmaceuticals by TLC

Matrix	Repeatability, %	*n*	Ref.
Acrylic polymer gel	2.9	9	32
Poly(ethylene glycol) ointment	1.8	6	32
Hard fat suppositories	3.4	10	33
Hard fat suppositories	1.4	6	34

Our convenient, alternative TLC procedure uses silica gel as stationary phase and toluene–methanol–ethanol–concentrated aqueous ammonia (60 + 30 + 20 + 8, v/v) as mobile phase. Sample preparation and application is very convenient: ca 3 g sample is dissolved in 10 mL dichloromethane, and 1 µL is applied to the plate. PEG 1000 is used as a reference standard. After development, the plate is dipped into Dragendorff reagent, and the red spots on a yellow background are scanned in the absorption mode.

This procedure clearly separates the poly(ethylene glycol) impurities as one unresolved peak from the nonoxynol(9) peak (Figure 2).

The reliability of the TLC method is proven by the following validation data, which are more than acceptable and well-suited to stand any official examination, especially when compared with the compendial method: recovery, 96.6% (RSD = 3.2%, $n = 6$); repeatability, 3.6% ($n = 7$).

Very similar simple TLC methods were suitable for testing the purity and stability of poly(ethylene glycol) methyl ether phosphoric acid esters and the purity of poloxamers.

Escin

Another classical field of application for planar chromatographic methods are assays and purity tests of plant extracts or phytopharmaceuticals (35, 36). In these cases, an advantage offered by planar chromatography is its ability to cope with dirty samples that contain material that might remain adsorbed on the stationary phase. Another advantage is the variety of possible post- and prechromatographic derivatization procedures. Numerous applications were reported (37), but only one will be presented here.

Escin, a common phytotherapeutic principle used in the prevention or treatment of peripheral vascular disorders (38), consists of a mixture of triterpenic saponins similar in structure (39) to those occurring in the seeds of the horse chestnut tree (Figure 3).

The compendial assay, as described in the German Pharmacopoeia, is a complicated and time-consuming sequence of extraction steps followed by a color reaction that requires painstakingly precise observance of the reaction conditions (40). Hardly more than 2 tests can be run simultaneously by one skilled technician per day.

One proposed alternative TLC method (41) uses silica gel as the stationary phase and a mobile phase consisting of *n*-propanol–ethyl acetate–water (4 + 3 + 3, v/v). For sample preparation, a sample containing ca 20 mg escin is sonicated in

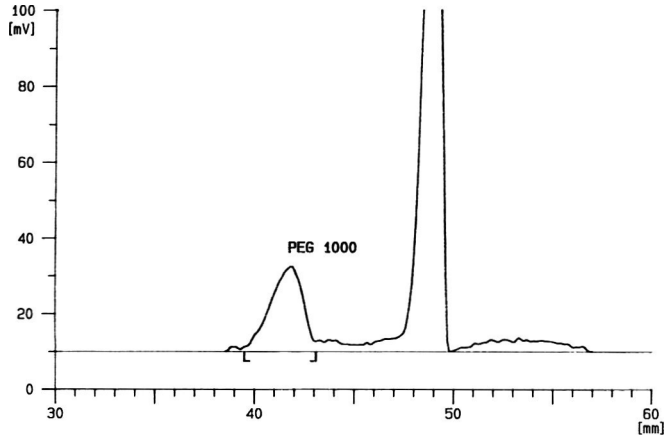


Figure 2. Original densitogram of poly(ethylene glycol) compounds in nonoxynol(9).

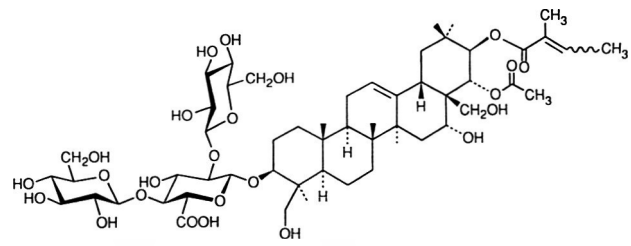


Figure 3. Principal saponins of escin; the C-21 position is acylated by either angelic acid or tiglic acid (indicated by the wavy line).

50 mL 50% ethanol, and 2 µL is applied to the plate. β-Escin reference standard is used for quantitation. Postchromatographic derivatization is done by dipping the plate into a diluted 20% methanolic iron(III) chloride detection reagent. The resulting red spots are scanned at 540 nm, followed by quantitation and evaluation via peak area. The escin components are quantified as a compact, unresolved spot well separated from all other possibly interfering constituents in the sample matrixes (e.g., horse chestnut extract).

Because this method was included in a document for approval, it had to be validated according to the German (42) and European (25) guidelines. In this case, accuracy of the proposed method has to be proven by comparison of the results with those derived with the compendial assay. Our data (Tables 2 and 3), supported by standard addition experiments, clearly demonstrate the suitability of this time-saving TLC alternative, which allows a large increase of sample throughput compared with the compendial method. The most impressive feature of this proposed TLC method is its ruggedness, that is, its ability to provide good results for different analysts in separate locations and even with changes in the chromatographic or evaluation steps.

Parallel to our method, another TLC procedure was proposed (43). The ruggedness mentioned above is one of the main advantages of TLC and was proven in interlaboratory tests (44). It may be explained by the simultaneous multisample analysis on one plate that allows a real in-system calibration in contrast tc other on-line chromatographic procedures, leading to precise and accurate results.

Pantoprazole, Stability Testing

As already mentioned, LC is a more sensitive analytical method and is virtually indispensable, especially in the analysis of new chemical entities, where even traces of undesirable components have to be quantified. There are moments in the development of new drugs, however, where alternative methods are to be considered. Fixed, validated, and synthetic production schemes, known impurity profiles, and degradation pathways of the product allow the replacement of LC by other methods.

A realistic monitoring of the stability of a new drug upon storage and under stress for registration purposes (45–47) covered 3 formulations or strengths with 3 batches each in 3 types of containers, which were stored at 4 different storage conditions. Samples were analyzed after 3, 6, 9, 12, 18, 24, 36, 48, and 60 months, which adds up to nearly 1000 analyses to be performed and does not cover additional "exotic" stress data demanded by several registration authorities. When analyzing tablets of pantoprazole sodium, a new antiulcerative (48) (Figure 4), by LC, slurries had to be filtered to get clear injection solutions. This made it very difficult to install automated, continuous systems and required a higher frequency of standard runs in the nonautomated LC procedure.

Duplicate weighings and injections and the required system suitability tests resulted in a total number of ca 6000 LC runs.

The overall working time of this proposed stability protocol caused us to consider the replacement of the time-consuming LC procedure by a TLC method. The precursor molecules (Fig-

Table 2. Validation data for escin assay

Sample	Assay, %		Standard deviation
	Compendial method	TLC	
Fluid extract	1.7	1.56	−0.04 (−2.4%)
Fluid extract	2.9	3.24	+0.34 (+4.7%)
Spray-dried extract	22.2	21.47	−0.73 (−3.3%)
Repeatability (TLC)			CV = 1.6% (n = 10)
Repeatability (compendial method)			CV = 2.8% (n = 5)

Table 3. Assay of escin by TLC with method variations

Sample	TLC assay, %				
	A[a]	B[b]	C[c]	D[d]	Mean (RSD)[e]
Fluid extract	1.66	1.64	1.70	1.81	1.70 (4.46%)
Fluid extract	3.24	3.22	3.47	3.14	3.28 (4.76%)
Spray-dried extract	21.47	21.22	23.19	22.9	22.20 (4.50%)

[a] Proposed method, 3 point calibration, linear regression (Camag TLC Scanner II, Cats software).
[b] Proposed method, 3 point calibration, polynomial regression (Camag TLC Scanner II, Cats software).
[c] Proposed method, chamber saturation, manual application, 1 point calibration (Desaga DS 60).
[d] Proposed method, 1 point calibration (Zeiss KM3, Merck D 2000 integrator).
[e] RSD = relative standard deviation.

ure 5, peaks 6 and 7) and the relevant degradation products (peaks 1, 3, and 4/5 [a mixture of isomers]) are well-resolved and separated from pantoprazole sodium (peak 2), demonstrating the efficient separation power of the proposed silica gel TLC procedure method.

The differences in polarity of the compounds requires a more complex mobile phase, consisting of ethyl acetate–cyclohexane–methanol–concentrated ammonia (48 + 35 + 15 + 2, v/v). Sample preparation involves no filtration or cleanup step; a sample containing ca 0.3 mg pantoprazole is simply sonicated in 10 mL acetonitrile, and 10 µL supernatant is applied in a band to the plate. Evaluation is done by absorbance scanning at 295 nm.

The following validation data for one of the main impurities clearly prove suitability and conformity with approval requirements: linearity, 10–50 µg/mL (n = 6, r = 0.99996, coefficient of variation [CV] = 0.6%); repeatability, CV for chromatography, 0.5% (n = 6); CV for analytical procedure, 1.8% (n = 6); accuracy (recovery), 100.6% (n = 6, CV = 1.6%).

Replacement of the LC procedures by the proposed TLC method in the stability protocol results in a marked increase of sample throughput.

Phospholipids

One of the most outstanding features of planar chromatography is its ability to handle numerous, sometimes exotic, prechromatographic derivatization procedures (49, 50). By official requirements, we were forced to individually assay 1,2-dipalmitoylphosphatidyl choline (DPPC) and 1-palmitoyl-2-oleylphosphatidyl choline (POPC) (Figure 6) in a lyophilizate by using a stability-indicating analytical procedure.

TLC and high-performance TLC are still the most accepted techniques in phospholipid analysis because of the limited progress of LC in this area (51). Usually, planar chromatography only separates the phospholipid classes. To distinguish between individual phospholipid species, this separation has to be followed by enzymatic splitting of the phospholipid fractions and subsequent analysis of the corresponding diglycerides by capillary GC (52). To avoid this complicated and time-consuming analytical sequence, we developed a prechromatographic derivatization step based on unsaturated phospholipid complexes formed with mercury salts (53, 54).

The contents of one vial are dissolved in chloroform, centrifuged, and diluted with methanol, and an aliquot is reacted with 6% mercury(II) acetate solution in the dark. After complete reaction, 5 µL is applied to the plate. Only the unsaturated POPC reacts to yield a mercury complex, which is easily separated from DPPC by chromatography on silica gel with chloroform–methanol–66% acetic acid (13 + 5 + 2.4, v/v) as mobile phase. Chromatography has to be performed under strictly controlled conditions at 22–23°C, ≤ 60% relative humidity, and 30 min chamber saturation. Visualization uses the known copper(II) sulfate reagent (55). Evaluation is done via absorbance scan-

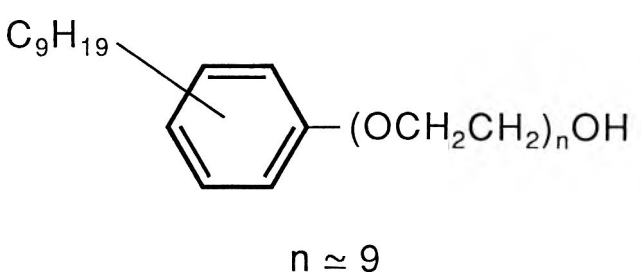


Figure 4. Pantoprazole sodium.

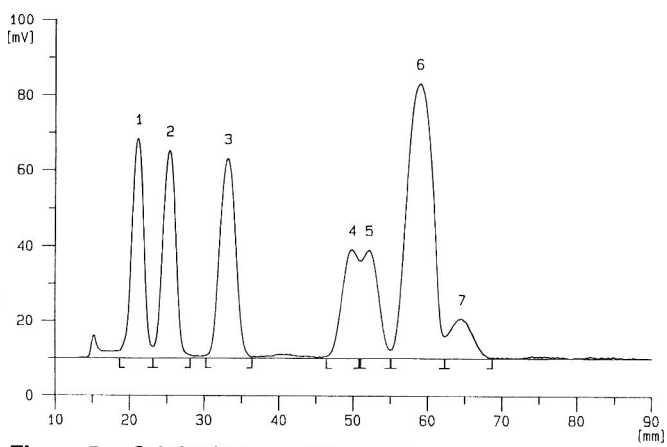
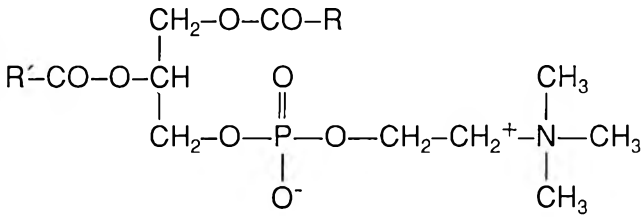


Figure 5. Original densitogram of equal amounts of pantoprazole sodium (peak 2), its precursor molecules (peaks 6 and 7), and its degradation products (peaks 1, 3–5).



DPPC : R = R′ = $C_{16}H_{33}$
POPC : R = $C_{16}H_{33}$
R′ = cis–9–$C_{18}H_{35}$

Figure 6. 1-2-Dipalmitoylphosphatidyl choline and 1-palmitoyl-2-oleylphosphatidyl choline (DPPC and POPC).

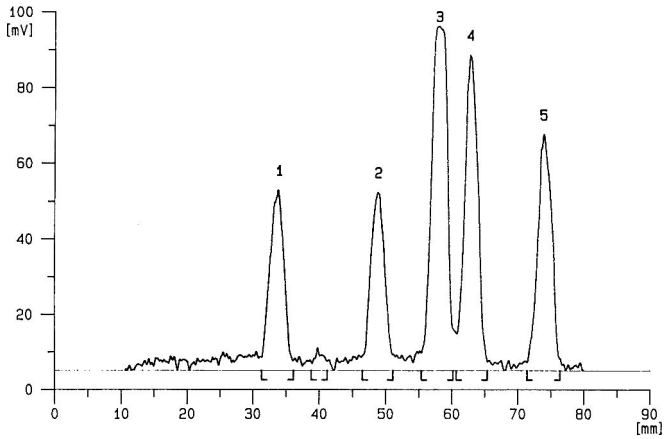


Figure 7. Original densitogram of a mixture of DPPC (peak 3), POPC (peak 4), and 1-2-dipalmitoylphosphatidyl glycerol (DPPG) (peak 5) and the corresponding lysophospholipids lyso-PPC (peak 1) and lyso-PPG (peak 2).

ning at 365 nm and quantitation via peak height with 3 point calibration. The chromatographic characteristics of added dipalmitoylphosphatidyl glycerol (DPPG) and the corresponding lysophospholipids are unaffected by the derivatization steps (Figure 7).

The validation data (Table 4) of this proposed procedure are impressive. They stand any comparison with other chromatographic methods, proving planar chromatography's value in pharmaceutical analysis.

Bacitracin

Bacitracin is a complex peptide antibiotic (56) whose present compendial bioassay (57) by agar diffusion test has a number of inherent disadvantages: Production, suitability tests, and quality control of agar plates and storage, maintenance, and quality control of test organism are very time consuming and laborious; and the bioassay is characterized by large sources of potential error and bias in its experimental design (preparation of inoculum, standards, samples, and inactivation of interferences) and an undesirable incubation time. This results in extremely poor reproducibility or repeatability of the analytical results.

The weakest point of the bioassay, however, is its lack of any information about composition changes and/or possible degradation of the products.

Our TLC approach, based on an already reported method (58), uses RP8 reversed-phase stationary phase and a mobile phase of methanol–0.1M aqueous sodium sulfate solution–triethylamine–85% phosphoric acid (70 + 29 + 1 + ca 3.8, v/v), with pH adjusted to 2.3. Chamber saturation and development are run at 6°C. Postchromatographic derivatization uses fluorescamine (0.04% in acetone), and the resulting spots are scanned in the fluorescence mode with evaluation done via peak height ($\lambda_{ex = 366\ nm}$, $\lambda_{fl} \geq 420$ nm [filter]).

Sample preparation is still under development. From simple formulations, bacitracin is extracted with Bond-Elut SCX/C18 columns. Microbiologically active bacitracin components are separated from inactive or less active components and fermentation by-products (Figure 8).

In the next step, this proposed TLC method has to be validated (59) by using isolated bacitracin subspecies with known antimicrobiological potency to correlate microbiological activity with the chromatographic profile. Although the TLC method does not separate the bacitracin compounds as well as the most recent LC method (60), the fractions representing more than 95% of the microbiological potency are well concentrated and separated from degradation products.

Table 4. Validation data for phospholipid assay via TLC

Parameter	Phospholipid	Result	Statistical data
Accuracy (Rec.)	DPPC	100.1%	CV = 1.9%, *n* = 8
	POPC	98.9%	CV = 1.4%, *n* = 8
	DPPG	101.7%	CV = 1.4%, *n* = 8
Repeatability	DPPC	1.4%	*n* = 9
	POPC	1.4%	*n* = 9
	DPPG	2.2%	*n* = 9
Linearity	DPPC	0.24–0.35 μg/spot	R = 0.995, CV = 1.2%, *n* = 6
	POPC	0.18–0.27 μg/spot	R = 0.997, CV = 0.8%, *n* = 6
	DPPG	0.18–0.27 μg/spot	R = 0.993, CV = 1.3%, *n* = 6

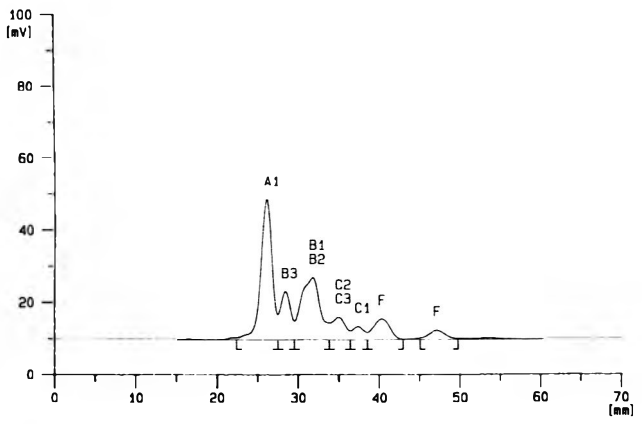


Figure 8. Original densitogram of bacitracin. Peaks were identified by spiking with samples of isolated bacitracin fractions (courtesy of Dr. Frøyshov, Apothekernes Lab. A.S., Oslo, Sweden). Peak areas A, B1, B2, and B3 represent 96% of the mixture's biological activity.

Conclusions

Whereas GC and LC have gained wide acceptance as fully instrumentalized, highly efficient methods, planar chromatography has long been considered a simple, cheap technique that only provides raw qualitative and quantitative data. The progress and advances in instrumentation, layers, and methodology (61–65), however, have enhanced the status of contemporary planar chromatography and allowed steady improvement in reliability, accuracy, and reproducibility. Planar chromatographic analytical methods offer a simple, economical, and realistic alternative to all the other chromatographic techniques in pharmaceutical analysis. They can be performed in full accordance with GLP and GMP principles and validated, and therefore, they are able to withstand all legal challenges in pharmaceutical analysis.

An increasing number of publications in this field of application indicates the usefulness and increasing popularity (9, 10) of planar chromatography. Contemporary planar chromatography deserves a place within the spectrum of chromatographic techniques used in pharmaceutical analysis and acceptance as a complementary technique.

References

(1) Kaiser, R.E. (1989) *Labo Analytik* **89**, 7–15

(2) Jork, H. (1987) *Proc. 4th. Int. Symp. Instrum. HPTLC (Planar Chromatogr.)* H. Traitler, A. Studer, & R.E. Kaiser (Eds), Selvino, IfC, Bad Dürkheim, Germany, pp. 193–239

(3) Renger, B., Ziegenbalg, W., & Wenz, K. (1987) *Proc. 4th. Int. Symp. Instrum. HPTLC (Planar Chromatogr.)* H. Traitler, A. Studer, & R.E. Kaiser (Eds), Selvino, IfC, Bad Dürkheim, Germany, pp. 331–339

(4) Renger, B., Brahm, R., Wenz, K., & Ziegenbalg, W. (1989) *Proc. Int. Symp. Instr. Thin-Layer Chromatogr./Planar Chromatogr.*, R.E. Kaiser (Ed.), Brighton, IfC, Bad Dürkheim Germany, pp. 185–192

(5) B. Renger (1991) *Proc. 6th. Int. Symp. Instrum. Planar Chromatogr.* H. Traitler, O.I. Voroshilova, & R.E. Kaiser (Eds), Interlaken, IfC, Bad Dürkheim, Germany, pp. 291–299

(6) Ng, L.L. (1991) *Chromatogr. Sci.* **55**, 717–754

(7) Siouffi, A.M., Mincsovics, E., & Tyihak, E. (1989) *J. Chromatogr. Biomed. Appl.* **492**, 471–538

(8) Langhorst, M.L. (1989) *J. Planar Chromatogr.* **2**, 346–354

(9) Jork, H. (1992) *Labor Praxis*, 110–119

(10) Jork, H. (1992) *J. Planar Chromatogr.* **5**, 4–5

(11) Sherma, J. (1990) *Anal. Chem.* **62**, 371A–381A

(12) Grinberg, N., Bicker, G., Tway, P., & Baiano, J.A. (1988) *J. Liq. Chromatogr.* **11**, 3184–3204

(13) Sherma, J. (1991) *Chromatogr. Sci.* **55**, 3–41

(14) Poole, C.F., & Poole, S.K. (1989) *Anal. Chem.* **61**, 1257A–1269A

(15) Jaenchen, D.E. (1991) *Chromatogr. Sci.* **55**, 113–134

(16) *Quantitative Thin-Layer Chromatography and Its Industrial Applications* (1987) L.R. Treiber (Ed.), Marcel Dekker, Inc., New York, NY

(17) Wilson, I.D. (1988) *Recent Advances in Thin-Layer Chromatography*, F.A.A. Dallas, H. Read, R.J. Ruane, & I.D. Wilson (Eds), Plenum Press, New York, N.Y., pp. 1–8

(18) Kirschbaum, J.J. (1988) *Trends Anal. Chem.* **7**, 16–20

(19) Evans, M.B., Haywood, P.A., Johnson, D., Martin-Smith, M., Munro, G., & Wahlich, J.C. (1989) *J. Pharm. Biomed. Anal.* **7**, 1–22

(20) Inman, E.L., Frischmann, J.K., Jiminez, P.J., Winkel, G.D., Persinger, M.L., & Rutherford, B.S. (1987) *J. Chromatogr. Sci.* **25**, 252–256

(21) Carr, G.P., & Wahlich, J.C. (1990) *J. Pharm. Biomed. Anal.* **8**, 613–618

(22) *Statistische Methoden in der Wasseranalytik* (1985) W. Funk, V. Dammann, C. Vonderheid, & G. Oehlmann (Eds), VCH Publishers, Inc., New York, NY

(23) Ebel, S. (1987) *Proc. 4th. Int. Symp. Instrum. HPTLC (Planar Chromatogr.)* H. Traitler, A. Studer, & R.E. Kaiser (Eds), Selvino, IfC, Bad Dürkheim, Germany, pp. 133–144

(24) *Guideline for Submitting Samples and Analytical Data for Methods Validation* (1987) U.S. Food and Drug Administration, Department of Health and Human Services, Beltsville, MD

(25) *Note for Guidance on Analytical Validation, Final Draft* (1989) Committee for Proprietary Medicinal Products, Document No. III/844/87-EN, EEC

(26) *Note for Guidance on Specifications and Control Tests on the Finished Product, Draft 7* (1990) Committee for Proprietary Medicinal Products, Document No. III/3324/89-EN

(27) *Handbook of Pharmaceutical Excipients* (1986) American Pharmaceutical Association, Washington, DC, and The Pharmaceutical Society of Great Britain, London, UK, pp. 217–220

(28) *U.S. Pharmacopeia XXII/National Formulary XVII* (1990) U.S. Pharmacopeial Convention, Inc., Rockville, MD, pp. 955–956

(29) Walter, B.A., & Digenis, G.A. (1990) *Pharm. Res.* **9**, 409–411

(30) Sithole, B.B., Zvilichovsky, B., Lapointe, C., & Allen, L.H. (1990) *J. Assoc. Off. Anal. Chem.* **73**, 322–324

(31) *Martindale, The Extra Pharmacopoeia* (1989) 29th Ed., J.E.F. Reynolds (Ed.), The Pharmaceutical Press, London, UK, p. 373

(32) Brahm, R., Ziegenbalg, W., & Renger, B. (1990) *J. Planar Chromatogr.* **3**, 77–78

(33) Krumholz, B., & Wenz, K. (1991) *J. Planar Chromatogr.* **5**, 370–372

(34) Hahn-Deinstrup, E., Heumann Pharma, Nürnberg, Germany, private communication

(35) Wagner, H., Bladt, S., & Zgainski, E.-M. (1983) *Drogenanalyse, Dünnschicht-chromatographische Analyse von Arzneidrogen*, Springer-Verlag, Berlin, Germany

(36) Eilert, U. (1989) *Dtsch. Apoth. Ztg.* **129**, 2159–2168

(37) Vanhaelen, M., & Vanhaelen-Fastre, R. (1983) *J. Chromatogr.* **261**, 263–271; (1988) *Recent Advances in Thin-Layer Chromatography*, F.A.A. Dallas, H. Read, R.J. Ruane, & I.D. Wilson (Eds), Plenum Press, New York, NY, pp. 187–200

(38) *Martindale, The Extra Pharmacopeia* (1989) 29th Ed., J.E.F. Reynolds (Ed.), The Pharmaceutical Press, London, UK, 1539–1540

(39) Tschesche, R., & Wulff, G. (1973) *Fortschr. Chem. Org. Naturst.* **30**, 461–606

(40) *Deutsches Arzneibuch* (1991) 10th Ed., Monographie "Roßkastaniensamen," Deutscher Apotheker Verlag, Stuttgart, Germany

(41) Renger, B. (1990) *J. Planar Chromatogr.* **3**, 160–162

(42) *Erläuterungen des BGA zum Antrag auf Zulassung eines Arzneimittels* (1988) 2nd Ed., Bundesanzeiger Verlagsgesellschaft, Köln, Germany

(43) Lumberti, E., Martinelli, E.M., & Pitteri, G. (1989) *Fitoterapia* **61**, 57–60

(44) Funk, W., Dammann, V., & Donnevert, G. (1988) *J. Planar Chromatogr.* **1**, 78–80

(45) Joint QC-PDS Stability Committee (1991) *Pharm. Technol.* **15**, 92–96

(46) Gamlen, M.J. (1991) *Pharm. Ind.* **53**, 950–954

(47) von Akker, C.R. (1991) *Pharm. Ind.* **53**, 483–489

(48) Kohl, B., Sturm, E., Senn-Bilfinger, J., Simon, W.A., Krüger, U., Schaefer, H., Rainer, G., Figala, V., Klemm, K., Ife, R.J., & Leach, C.A. (1992) *J. Med. Chem.* **35**, 1049–1057

(49) Funk, W. (1984) *Fresenius Z. Anal. Chem.* **318**, 206–219

(50) Jork, H., Funk, W., Fischer, W., & Wimmer, H. (1988) *J. Planar Chromatogr.* **1**, 180–292

(51) Lendrath, G., Bonekamp-Nasner, A., & Kraus, L. (1991) *Fat Sci. Technol.* **93**, 53–61

(52) Traitler, H., Ducret, P., Perrenoud, M.C., Fay, L., & Richli, U. (1991) *Proc. 6th Int. Symp. Instrum. Planar Chromatogr.*. H. Traitler, O.I. Voroshilova, & R.E. Kaiser (Eds), Interlaken IfC, Bad Dürkheim, Germany, pp. 357–365

(53) Rao, Y.N., Prasad, R.B.N., & Rao, S.V (1989) *Fat Sci. Technol.* **91**, 482–484

(54) Mangold, H.K. (1961) *J. Am. Oil Chem. Soc.* **38**, 708–727

(55) Rustenbeck, I., & Lenzen, S. (1989) *J. Planar Chromatogr.* **2**, 470–471

(56) *Analytical Profiles of Drug Substances* (1980) G.A. Brewer, & U.K. Florey (Eds), Academic Press, New York, NY, **9**, 1–70

(57) *U.S. Pharmacopeia XXII/National Formulary XVII* (1990) U.S. Pharmacopeial Convention, Inc., Rockville, MD, pp. 132, 1488–1493

(58) Oka, H., Ikai, Y., Kawamura, N., Yamada, N., Harada, K.-I., Yamazaki, Y., & Suzuki, M. (1988) *J. Chromatogr.* **449**, 448–454

(59) Thomas, A.H. (1987) *J. Pharm. Biomed. Anal.* **5**, 319–324

(60) Bell, R.G. (1992) *J. Chromatogr.* **590**, 163–168

(61) Touchstone, J.C. (1990) *Modern Thin-Layer Chromatography*, N. Grinberg (Ed.), Marcel Dekker, Inc., New York, NY, pp. 465–480

(62) Jaenchen, D.E., & Isaaq, H.I. (1988) *J. Liq. Chromatogr.* **11**, 1941–1965

(63) Wilson, I.D. (1988) *Methodol. Surv. Biochem. Anal.* **18**, 313–320

(64) Jork, H., Funk, W., Fischer, W., & Wimmer, H. (1990) *Thin-Layer Chromatography, Vol. 1a, Reagents and Detection Methods*, VCH Publishers, Inc., New York, NY

(65) Kaiser, R.E. (1989) *Proc. Int. Symp. Instr. Thin-Layer Chromatogr./Planar Chromatogr.*, R.E. Kaiser (Ed.), Brighton, IfC, Bad Dürkheim Germany, pp. 251–262

CHEMICAL CONTAMINANTS MONITORING

Survey of Arsenic in Total Diet Food Composites and Estimation of the Dietary Intake of Arsenic by Canadian Adults and Children

ROBERT W. DABEKA, ARTHUR D. MCKENZIE, GLADYS M.A. LACROIX, CHANTAL CLEROUX, SUSAN BOWE, RUSSELL A. GRAHAM, and HENRY B.S. CONACHER
Health and Welfare Canada, Health Protection Branch, Food Directorate, Bureau of Chemical Safety, Food Research Division, Ottawa, ON, K1A 0L2, Canada
PAM VERDIER
Health and Welfare Canada, Health Protection Branch, Food Directorate, Bureau of Nutritional Sciences, Nutrition Research Division, Ottawa, ON, K1A 0L2, Canada

During a comprehensive total diet study extending from 1985 to 1988, foods were collected in 6 Canadian cities (in one of them, a pilot study was conducted twice). For each of the 7 collections, foods were processed into 112 composites (105 in the initial pilot trial). Total arsenic was determined in all samples. The mean, median, and range of arsenic concentrations in all samples were 73.2, 5.1, and <0.1–4830 ng/g, respectively. Food groups containing the highest mean arsenic levels were fish (1662 ng/g), meat and poultry (24.3 ng/g), bakery goods and cereals (24.5 ng/g), and fats and oils (19.0 ng/g). The estimated daily dietary ingestion of total arsenic by the average Canadian was 38.1 μg and varied from 14.9 μg for the 1- to 4-year-old group to 59.2 μg for 20- to 39-year-old males.

Arsenic is a naturally occurring element, and levels of arsenic in foods generally reflect normal accumulation from the environment. The chronic and acute toxicities of arsenic and its compounds are well known (1), and knowledge of the level of human exposure to arsenic is considered an integral part of the health protection programs in Canada and the world community (2).

In 1985, the Canadian Health Protection Branch (HPB), as part of an ongoing Total Diet Program, initiated a new survey in which 112 food composites were chosen to represent foods consumed in Canada (3, 4). Arsenic was one of the elements chosen for determination when the survey was designed. This paper describes the analytical results obtained for total arsenic in both the preliminary trial and the completed national phases of the survey and presents estimates of the dietary intake of arsenic by Canadian children and adults. The food intakes used to calculate the dietary intakes are also included for different age and sex groups.

Received January 13, 1992. Accepted May 5, 1992.

Experimental

Sample Choice

The study was started in 1985, when foods were collected at the retail level in Ottawa. Foods were taken into the laboratory and prepared for consumption as they would be prepared in a home setting. The individual, prepared foods were then combined into 105 composites, homogenized, and frozen until analysis. This process was repeated for Ottawa; then, the number of composites was increased to 112, and the process was repeated for 5 other Canadian cities.

In some cases, constituents of the food composite that were either unprocessed or prepared in a specific manner were determined to obtain information about the effects of processing, cooking, etc. These are designated in Table 1 as A, B, C, etc. For example, because of the high arsenic levels in fish (2, 5), both raw and cooked fish were analyzed, although the actual composites included only the cooked fish.

Analytical Reagents

Deionized water (ASTM Type III) was used wherever water is specified. All other chemicals were reagent grade.

Analytical Instrumentation

A Varian Model 875-ABQ atomic absorption spectrometer was used in conjunction with a GTA-95 graphite-furnace atomizer to determine arsenic. Plateau-type, pyrolytically coated graphite tubes with pyrolytic graphite platforms were purchased from the manufacturer. Arsenic was determined at the 193.7 nm wavelength using an electrodeless discharge lamp line source and instrumental conditions discussed in the method (6).

Arsenic Methodology

Arsenic was determined by graphite-furnace atomic absorption spectrometry after nitric acid digestion, dry ashing with magnesium oxide ashing aid, coprecipitation of the ammonium pyrrolidine dithiocarbamate complex with copper and iron carriers, and dissolution of the complex in nitric acid containing

Table 1. Arsenic levels (ng/g) found in individual composites from different cities

No.	Category and composite	City[a] 1	2	3	4	5	6	7	n	$\bar{X}$	Median	Range Min.	Max.
	Milk and dairy products												
1	Milk, whole	<0.5	<0.6	0.4	<2.5	<2.9	<1.6	1.2	7	1.4	1.2	0.4	<2.9
2	Milk, 2% B.F.	<0.5	<0.6	0.4	<2.5	<2.9	<1.6	<0.9	7	1.3	<0.9	0.4	<2.9
3	Milk, skim	<0.5	<0.6	0.6	<2.5	<2.9	<1.6	<2.6	7	1.6	<1.6	<0.5	<2.9
4	Evaporated milk, canned	—	—	3.5	<2.5	<2.9	2.6	1.6	5	2.6	2.6	1.6	3.5
4A	Instant breakfast, prepared	1.8	<0.6	—	—	—	—	—	2	1.2	1.2	<0.6	1.8
5	Cream	<0.5	<1.0	2.0	<2.5	<2.9	4.8	2.8	7	2.4	<2.5	<0.5	4.8
6	Ice cream, mixed	5.5	2.0	5.5	—	<2.9	8.5	3.9	6	4.7	4.7	2.0	8.5
6A	Ice cream (ice milk)	—	0.7	3.7	10.0	5.7	7.6	<3.1	6	5.2	4.7	0.7	10.0
7	Yogurt, mixed	0.9	<0.6	1.2	<2.5	3.1	<1.6	<1.7	7	1.7	<1.6	<0.6	3.1
7A	Yogurt, plain	1.1	<0.6	0.8	<2.5	<2.9	2.0	<1.7	7	1.6	<1.7	<0.6	2.9
8	Cheese	2.6	2.3	2.6	6.9	3.4	11.0	<7.6	7	5.2	3.4	2.3	11.0
9	Cottage cheese	<1.0	<1.0	2.2	<2.5	<2.9	5.4	<3.4	7	2.6	<2.5	<1.0	5.4
10	Cheese, processed	19.0	4.1	9.6	26.0	7.1	15.0	<6.8	7	12.4	9.6	4.1	26.0
11	Butter	5.5	4.7	11.0	<2.5	7.2	3.7	14.0	7	6.9	5.5	<2.5	15.0
	Meat and poultry												
12	Beef steak, cooked	6.1	15.0	14.0	4.6	11.0	11.0	<6.5	7	9.8	11.0	4.6	15.0
12A	Beef steak, raw	3.8	15.0	9.7	3.3	6.0	11.0	<5.3	7	7.8	6.0	3.3	15.0
13	Roast beef	12.0	15.0	7.2	—	8.7	15.0	<6.1	6	10.7	8.7	6.1	16.0
14	Ground beef, cooked	<2.0	2.4	6.2	<2.3	<2.9	23.0	<5.3	7	6.3	<2.9	<2.0	24.0
14A	Ground beef, raw	<2.0	19.0	2.7	<2.3	5.8	15.0	<7.3	7	7.8	5.8	<2.0	19.0
15	Pork, cooked	25.0	5.1	8.9	7.3	7.8	19.0	<5.6	7	11.3	7.8	5.1	25.0
15A	Pork, raw	8.9	7.3	8.9	<2.3	4.7	21.0	<5.1	7	8.2	7.3	<2.3	22.0
16	Pork, cured	13.0	8.1	17.0	28.0	18.0	28.0	15.0	7	17.9	17.0	8.1	28.0
17	Veal, cooked	2.4	<1.3	10.0	<2.3	10.0	19.0	<4.9	7	7.2	<4.9	<1.3	19.0
17A	Veal, raw	2.4	3.6	7.1	<2.3	31.0	16.0	<4.6	7	9.6	<4.6	<2.3	31.0
18	Lamb, cooked	3.9	<1.3	7.1	<2.3	1.6	2.1	<5.7	7	3.4	<2.3	<1.3	7.1
18A	Lamb, raw	<1.8	<1.3	19.0	<2.3	4.0	16.0	<7.7	7	7.5	4.0	<1.3	19.0
19	Poultry, cooked	62.0	—	100.0	18.0	25.0	57.0	22.0	6	47.1	41.0	18.0	100.0
19A	Poultry, raw	16.8	36.0	60.0	14.0	28.0	39.0	16.0	7	29.9	28.0	14.0	60.0
20	Eggs	5.1	<1.4	5.0	7.1	3.9	17.0	<4.1	7	6.2	5.0	<1.4	17.0
21	Meat organs	388.0	536.0	179.0	284.0	19.0	29.0	104.0	7	219.8	179.0	19.0	536.0
22	Cold cuts, luncheon meats	6.2	8.9	8.9	15.0	15.0	37.0	<9.4	7	14.4	<9.4	6.2	37.0
23	Luncheon meat, canned	8.3	4.6	8.4	8.9	14.0	31.0	<8.6	7	12.0	<8.6	4.6	31.0
	Fish												
24	Marine fish, cooked	3270	3140	2920	4830	1850	2360	2970	7	3048	2970	1850	4830
24A	Marine fish, raw	2570	2360	2490	—	3030	1500	2850	6	2466	2530	1500	3030
25	Freshwater fish, cooked	181	275	294	77	341	1350	603	7	446	294	77	1350
25A	Freshwater fish, raw	214	257	285	—	431	1060	411	6	443	348	214	1060
26	Fish, canned	1170	913	1370	1340	1370	996	1250	7	1201	1250	913	1370
27	Shellfish	1670	2300	1320	4200	1820	1950	1010	7	2041	1820	1010	4200
	Soups												
28	Soups, meat, canned	3.2	2.7	6.2	3.5	5.1	6.7	4.5	7	4.6	4.5	2.7	6.7
29	Soups, pea, canned	<0.3	2.3	5.1	2.3	3.0	7.5	11.0	7	4.5	3.0	<0.3	11.0
30	Tomato soup, canned	<0.3	3.3	<0.2	0.7	5.7	7.2	4.4	7	3.1	3.3	<0.2	7.2
31	Soups, dehydrated	<0.3	1.6	0.2	3.8	6.5	8.4	11.0	7	4.6	3.8	0.2	11.0

Table 1. (*Continued*)

No.	Category and composite	City[a] 1	2	3	4	5	6	7	*n*	$\bar{X}$	Median	Range Min.	Max.
	Bakery goods and cereals												
32	White bread, all	11.0	5.5	8.3	3.9	25.0	23.0	14.0	7	12.9	11.0	3.9	25.0
32A	White bread no raisins	15.0	7.1	—	—	—	—	—	2	11.1	11.0	7.1	15.0
33	Bread, whole wheat and rye	16.0	6.3	11.0	9.2	10.0	7.9	7.1	7	9.6	9.2	6.3	16.0
34	Bread rolls and biscuits	5.5	6.0	8.0	5.5	11.0	13.0	14.0	7	9.0	8.0	5.5	14.0
34A	Rolls and buns only	—	3.2	—	—	—	—	—	1	3.2	3.2	3.2	3.2
35	Wheat flour	5.0	<3.0	7.0	<0.1	6.5	5.0	12.0	7	5.5	5.0	<0.1	12.0
36	Cake[b,f]	9.2	6.4	6.4	57.0	8.6	4.3	<4.6	7	13.8	6.4	4.3	57.0
36A	Cake, yellow and white[b]	8.8	<2.1	6.1	142.0	3.6	3.8	12.0	7	25.6	6.1	<2.1	142.0
36B	Cake, chocolate	—	—	4.2	8.9	8.4	4.5	<6.1	5	6.4	<6.1	4.2	8.9
37	Cookies, chocolate chip	6.3	2.6	13.0	2.4	8.2	10.0	21.0	7	9.1	8.2	2.4	21.0
37A	Cookies, oatmeal, arrowroot	10.0	2.8	—	—	—	—	—	2	4.0	4.0	2.6	5.5
37B	Cookies, all	5.5	2.6	—	—	—	—	—	2	6.6	6.6	2.8	10.0
38	Danish and donuts	6.0	3.8	8.1	5.1	2.6	13.0	9.1	7	6.8	6.0	2.6	13.0
39	Crackers	11.0	9.5	12.0	25.0	5.3	25.0	27.0	7	16.4	12.0	5.3	27.0
40	Waffles and pancakes	8.9	3.8	5.9	15.0	6.0	11.0	20.0	7	10.0	8.9	3.8	20.0
41	Cooked wheat cereal	<0.8	3.0	12.0	8.0	2.8	5.9	12.0	7	6.4	5.9	<0.8	12.0
41A	Cream of wheat, dry	19.0	4.8	3.3	8.3	5.6	13.0	<9.0	7	9.0	8.3	3.3	19.0
42	Oatmeal cereal	3.2	2.5	13.0	12.0	9.5	8.8	17.0	7	9.3	9.5	2.5	17.0
42A	Oatmeal cereal, dry	8.6	2.6	4.4	12.0	0.4	7.9	<12.0	7	6.8	7.9	0.4	12.0
43	Corn cereal	<1.8	8.5	14.0	12.0	19.0	17.0	23.0	7	13.4	14.0	<1.8	23.0
44	Wheat and bran cereals	16.0	17.0	7.3	9.8	7.9	1.1	<9.9	7	9.8	9.8	1.1	17.0
45	Rice cereal, cooked	114.0	86.0	75.0	121.0	78.0	114.0	89.0	7	96.7	89.0	75.0	121.0
45A	Rice cereal, dry	237.0	277.0	230.0	287.0	286.0	307.0	365.0	7	284.1	286.0	230.0	365.0
46	Apple pie	6.0	2.8	4.2	14.0	<1.6	15.0	11.0	7	7.8	6.0	<1.6	15.0
47	Pie, others	7.2	4.9	5.4	22.0	10.0	9.0	<7.3	7	9.4	<7.3	4.9	22.0
47A	Pie, no raisins	9.1	<2.7	—	—	—	—	—	2	5.9	5.9	<2.7	9.1
47B	Raisin pie	4.6	8.7	—	—	—	—	—	2	6.6	6.6	4.6	8.7
48	Pizza, combination	9.3	3.6	14.0	21.0	16.0	15.0	<10.0	7	12.6	14.0	3.6	21.0
49	Pasta, canned	4.5	3.0	3.1	21.0	9.7	9.0	16.0	7	9.5	9.0	3.0	21.0
50	Pasta, plain, cooked	3.5	3.2	0.7	13.0	4.5	3.9	2.9	7	4.6	3.5	0.7	13.0
	Vegetables												
51	Corn, raw and canned, cooked	2.9	2.2	11.0	<0.1	4.3	4.9	12.0	7	5.3	4.3	<0.1	12.0
51A	Corn, raw	4.4	1.1	13.0	<0.1	—	9.9	8.9	6	6.3	6.7	<0.1	13.0
51B	Corn, kernel, canned	3.4	2.7	5.3	<0.1	6.8	9.6	11.0	7	5.5	5.3	<0.1	11.0
51C	Corn, creamed, canned	—	—	6.7	<0.1	7.5	4.1	10.0	5	5.7	6.7	<0.1	10.0
52	Potatoes, raw	4.2	0.8	11.0	<0.1	2.3	11.0	9.1	7	5.5	4.2	<0.1	11.0
53	Potatoes, baked	6.2	4.7	13.0	<0.1	6.8	11.0	11.0	7	7.5	6.8	<0.1	13.0
54	Potatoes, boiled, skins	2.8	3.0	7.0	<0.1	5.2	13.0	9.8	7	5.8	5.2	<0.1	13.0
55	Potatoes, peeled, boiled	1.9	<2.7	6.5	<0.1	6.3	8.9	10.0	7	5.2	6.3	<0.1	10.0
56	French fries	4.0	<2.7	14.0	<0.1	20.0	30.0	11.0	7	11.7	11.0	<0.1	30.0
57	Potato chips	15.0	8.5	44.0	12.4	32.0	20.0	28.0	7	22.8	20.0	8.5	44.0
58	Cabbage, cooked and coleslaw	1.3	<2.7	2.5	<0.1	5.1	12.0	8.6	7	4.6	<2.7	<0.1	12.0
59	Celery	3.2	3.2	7.0	9.6	5.2	10.0	<5.9	7	6.4	<5.9	3.2	10.0
60	Peppers, green and red	<0.4	<2.7	2.3	<0.1	4.2	8.9	7.6	7	3.7	<2.7	<0.1	8.9
61	Lettuce	3.3	<0.7	0.3	<0.1	1.6	5.4	<5.4	7	2.4	1.6	<0.1	5.4
62	Cauliflower, raw and cooked	<0.4	1.7	0.2	<0.1	<0.1	11.0	0.9	7	2.0	<0.4	<0.1	11.0

Table 1. (*Continued*)

No.	Category and composite	City[a] 1	2	3	4	5	6	7	*n*	$\bar{X}$	Median	Range Min.	Max.
62A	Cauliflower, raw	—	2.9	5.8	<0.1	<0.1	9.6	2.4	6	3.5	2.7	<0.1	9.6
63	Broccoli, raw and cooked	2.6	3.7	5.4	<0.1	<0.1	11.0	2.0	7	3.5	2.6	<0.1	11.0
64	Beans, raw and canned, cooked	<0.4	4.0	3.5	<0.1	<0.1	14.0	1.7	7	3.3	1.7	<0.1	14.0
64A	Beans, raw	<0.4	<1.0	5.7	<0.1	<0.1	12.0	2.4	7	3.1	<1.0	<0.1	12.0
64B	Beans, canned	<0.4	1.3	5.7	<0.1	<0.1	11.0	1.8	7	2.9	1.3	<0.1	11.0
65	Peas, raw and canned, cooked	<1.1	3.0	4.0	<0.1	<0.1	2.1	3.3	7	2.0	2.1	<0.1	4.0
65A	Peas, raw	<1.1	1.8	3.9	<0.1	<0.1	2.1	0.8	7	1.4	<1.1	<0.1	3.9
65B	Peas, canned	4.6	<1.0	6.8	<0.1	<0.1	0.4	5.2	7	2.6	<1.0	<0.1	6.8
66	Carrots, raw & canned, cooked	3.5	2.8	5.3	<0.1	12.0	6.0	11.0	7	5.8	5.3	<0.1	12.0
66A	Carrots, raw	3.9	5.8	5.0	<0.1	4.2	3.0	9.5	7	4.5	4.2	<0.1	9.5
66B	Carrots, raw and canned	5.6	—	—	—	—	—	—	1	5.6	5.6	5.6	5.6
66C	Carrots, canned	<1.1	5.1	—	—	—	—	—	2	3.1	3.1	<1.1	5.1
67	Onions, cooked	—	—	3.9	<0.1	<0.1	14.0	3.3	5	4.2	3.3	<0.1	14.0
67A	Onions, raw	3.4	3.6	2.8	<0.1	6.4	20.0	2.9	7	5.6	3.4	<0.1	20.0
68	Turnips, rutabagas	—	<0.4	3.6	<0.1	0.7	7.9	1.9	6	2.4	1.3	<0.1	7.9
69	Tomatoes, raw and cooked	3.7	<1.0	3.1	<0.1	2.4	7.7	3.4	7	3.1	3.1	<0.1	7.7
69A	Tomatoes, cooked	2.6	<1.0	—	—	—	—	—	7	2.9	1.7	<0.1	11.0
69B	Tomatoes, raw	2.3	<1.0	2.6	<0.1	1.6	11.0	1.7	2	1.8	1.8	<1.0	2.6
70	Tomato juice, canned	3.4	<0.4	<0.9	2.1	9.5	8.9	2.8	7	4.0	2.8	<0.4	9.5
71	Tomatoes, canned, ketchup, sauce	—	—	7.0	10.4	7.0	19.0	11.0	5	10.8	10.0	7.0	19.0
71A	Tomatoes, canned	2.3	1.0	0.3	3.4	2.4	8.8	15.0	7	4.7	2.4	0.3	15.0
71B	Ketchup	—	—	4.7	12.9	5.0	15.0	2.9	5	8.1	5.0	2.9	15.0
72	Mushrooms, canned & raw, cooked[c]	36.0	12.0	<1.5	10.4	27.0	44.0	24.0	7	22.1	24.0	<1.5	44.0
72A	Mushrooms, raw	58.0	52.0	20.0	27.9	23.0	84.0	61.0	7	46.4	52.0	20.0	84.0
72B	Mushrooms, canned	2.3	38.0	—	—	—	—	—	2	20.2	20.0	2.3	38.0
73	Cucumber, raw, pickled	12.0	9.9	<0.6	2.7	5.3	16.0	7.6	7	7.7	7.6	<0.6	16.0
73A	Cucumber, raw	8.3	14.0	0.3	6.0	4.8	33.0	9.3	7	10.8	8.3	0.3	33.0
	Fruit and fruit juices												
74	Citrus fruit, raw	2.0	2.5	0.9	<1.1	2.7	14.0	3.5	7	3.8	2.5	0.9	14.0
75	Citrus fruit, canned	<0.8	<0.4	<0.4	<1.1	<0.1	9.8	<2.1	7	2.1	<0.8	<0.1	9.8
76	Citrus juice	2.5	2.5	1.7	<1.1	4.8	17.0	2.2	7	4.6	2.5	<1.1	17.0
77	Citrus juice, canned	<0.8	<0.4	0.1	<1.1	0.2	0.5	<1.2	7	0.6	0.5	<0.1	1.2
78	Apples	2.4	1.7	0.3	<1.1	8.1	4.4	8.5	7	3.8	2.4	0.3	8.5
79	Apple juice, canned	4.4	3.5	—	5.4	11.0	11.0	30.0	6	11.0	8.2	3.5	30.0
79A	Apple juice, bottled	8.4	6.3	—	—	—	—	—	2	7.4	7.4	6.3	8.4
79B	Apple juice, canned and bottled	6.0	4.5	9.4	—	—	—	—	3	6.6	6.0	4.5	9.4
80	Apple sauce, canned	<0.9	0.5	0.2	<1.1	3.0	<1.5	7.9	7	2.2	1.1	0.2	7.9
80A	Apple sauce, canned and bottled	<0.4	<0.3	—	—	—	—	—	2	0.4	0.4	0.3	0.4
80B	Apple sauce, bottled	<0.9	1.9	—	—	—	—	—	2	1.4	1.4	0.9	1.9
81	Bananas	1.4	0.7	<0.5	<1.1	11.0	<1.5	<7.6	7	3.3	1.4	<0.5	11.0
82	Grapes	5.1	—	3.6	5.6	5.9	5.5	<3.3	6	4.8	5.3	<3.3	5.9
83	Grape juice, bottled	8.9	5.4	3.7	5.4	11.0	7.2	16.0	7	8.2	7.2	3.7	16.0
84	Peaches, canned and raw	4.0	<1.8	0.3	<1.3	2.1	<1.5	<3.4	7	2.1	<1.8	0.3	4.0
84A	Peaches, raw	2.2	—	<0.5	7.6	2.0	2.3	<3.2	6	3.0	2.3	<0.5	7.6

Table 1. (*Continued*)

No.	Category and composite	City[a] 1	2	3	4	5	6	7	*n*	$\bar{X}$	Median	Range Min.	Max.
84B	Peaches, canned	—	—	<0.4	<1.3	3.4	0.2	<3.4	5	1.7	<1.3	0.2	<3.4
85	Pears, raw and canned	<0.9	0.7	5.3	6.2	4.3	<1.5	<3.3	7	3.2	<3.3	0.7	6.2
85A	Pears, canned	<0.9	1.6	<0.8	<1.3	<1.2	<1.5	<3.4	7	1.5	<1.3	<0.8	<3.4
85B	Pears, raw	3.1	—	10.0	5.7	3.4	<1.5	<3.0	6	4.5	3.2	<1.5	10.0
86	Plums, prunes, dry and canned	8.2	1.2	3.3	5.0	10.0	0.7	<6.3	7	5.0	10.0	0.7	10.0
86A	Plums, raw	—	—	1.5	—	11.0	4.2	<3.4	4	5.0	3.8	1.5	11.0
86B	Prunes, dry	—	—	0.9	—	25.0	4.9	<4.9	4	8.9	4.9	0.9	25.0
87	Cherries, raw and canned	11.0	4.4	4.6	—	—	—	<3.3	4	5.8	4.5	<3.3	11.0
87A	Cherries, canned	6.7	—	11.0	5.0	5.1	3.5	<4.4	6	5.9	5.1	3.5	11.0
88	Mellons	14.0	6.1	8.8	4.5	11.0	6.5	10.0	7	8.7	10.0	4.5	14.0
89	Strawberries	3.9	3.7	1.3	9.5	6.1	5.8	37.0	7	9.7	5.8	1.3	37.0
90	Blueberries	7.3	6.7	4.6	8.6	10.0	7.4	<3.9	7	6.9	9.4	<3.9	10.0
91	Pineapple, canned	—	<0.4	1.0	2.0	0.7	1.7	<3.6	6	1.6	1.3	<0.4	<3.6
91A	Pineapple, raw and canned	0.8	1.0	—	—	—	—	—	2	0.9	0.9	0.8	1.0
91B	Pineapple, raw	1.2	0.6	1.5	4.8	8.8	2.4	<3.3	7	3.2	2.4	0.6	8.8
	Fats and oils												
92	Cooking fats and salad oils	<4.0	2.1	5.7	57.0	41.0	24.0	12.0	7	20.8	12.0	2.1	57.0
93	Margarine	<4.0	<1.0	14.0	29.0	21.0	33.0	<38.0	7	19.9	21.0	<1.0	<38.0
94	Peanut butter and peanuts[d]	26.0	15.0	22.0	6.6	9.7	13.0	<22.0	7	16.3	15.0	6.6	26.0
	Sugar and candies												
95	Sugar	<4.0	<2.5	<2.6	<2.8	6.3	1.4	<18.0	7	5.3	<2.8	1.4	<18.0
96	Syrup	5.9	8.0	2.2	<2.8	6.7	16.0	<13.0	7	7.8	6.7	2.2	16.0
97	Jams	8.0	7.6	1.9	<2.8	3.3	7.7	<11.0	7	6.0	9.4	1.9	<11.0
98	Honey	<4.0	2.7	1.7	<2.8	—	5.5	<11.0	6	4.6	3.4	1.7	<11.0
99	Pudding, chocolate from powder	4.4	3.8	1.4	4.3	22.0	28.0	6.8	7	10.0	4.3	1.4	29.0
99A	Pudding, canned and from powder	<4.0	2.6	—	—	—	—	—	2	4.1	4.1	3.8	4.4
100	Candy, chocolate bars	8.9	4.4	<3.6	15.0	32.0	39.0	105.0	7	29.8	15.0	<3.6	105.0
101	Candy, other	7.3	<2.5	3.3	8.9	16.0	17.0	—	6	9.3	8.1	<2.5	17.0
	Beverages												
102	Coffee	1.1	0.8	1.5	2.1	4.5	3.6	<2.4	7	2.3	2.4	0.8	4.5
103	Tea	<0.8	0.4	1.5	2.1	5.1	2.8	<2.3	7	2.1	2.1	0.4	5.1
104	Soft drinks	<0.8	0.6	<2.0	<2.8	3.1	2.8	<2.2	7	2.0	<2.2	0.6	3.1
105	Wines	5.6	4.2	4.8	6.5	9.0	6.7	3.4	7	5.8	5.6	3.4	9.0
105A	Wine and beer, cans & bottles[e]	4.1	3.2	—	—	—	—	—	2	3.7	3.7	3.2	4.1
106	Beer, bottles and cans[e]	—	—	3.1	<2.8	1.1	5.9	<1.9	5	3.0	<2.8	1.1	5.9
106A	Beer, bottles	—	—	3.0	4.6	5.1	2.9	<2.5	5	3.6	3.0	<2.5	5.1
106B	Beer, cans	—	—	2.7	3.1	2.9	4.0	<2.5	5	3.0	2.9	2.5	4.0
	Miscellaneous												
107	Muffins with raisins	—	—	24.0	11.0	22.0	16.0	19.0	5	18.4	19.0	11.0	24.0
107A	Muffins without raisins	—	—	33.0	—	11.0	—	—	2	21.7	22.0	11.0	33.0
107B	Bran muffins, plain	—	—	—	—	24.0	—	—	1	24.4	24.0	24.0	24.0

Table 1. (*Continued*)

No.	Category and composite	City[a] 1	2	3	4	5	6	7	*n*	$\bar{X}$	Median	Range Min.	Max.
108	Baked beans	—	—	<0.8	3.5	16.0	2.0	<4.3	5	5.2	3.5	<0.8	16.0
109	Raisins	—	—	8.5	41.0	24.0	28.0	18.0	5	23.6	24.0	8.5	41.0
110	Wieners	—	—	10.0	14.0	27.0	12.0	13.0	5	15.3	13.0	10.0	27.0
111	Gelatin, dessert	—	—	1.9	3.4	1.8	3.7	<3.9	5	2.9	3.4	1.8	3.9
112	Beets, raw and canned, cooked	—	—	4.9	4.3	4.3	4.6	<4.5	5	4.5	<4.5	4.3	4.9

[a] 1, 2 = Ottawa; 3 = Halifax; 4 = Winnipeg; 5 = Vancouver; 6 = Montreal; 7 = Toronto.
[b] Cakes included muffins for sets 1 and 2. Muffins were transferred to a separate composite (No. 107) for sets 3–7.
[c] Canned mushrooms were only present in sets 1 and 2.
[d] Peanuts included in only sets 3–7.
[e] Sample No. 105A was the composite for sets 1 and 2, and beer was included in it.
[f] Cake, yellow, white, chocolate, and coffee cake with fruit.

modifier (6). Each sample (excluding those used for quality control) was analyzed once.

Arsenic Quality Control

Quality control measures were performed for each analytical batch. They consisted of calculating the recovery of arsenic from samples spiked with sodium arsenate. Duplicates of the unspiked and spiked samples were analyzed. After the preliminary stage of the study, a laboratory reference material (powdered soya base infant formula) was included in each analytical batch. When this material was depleted, it was replaced with National Institute of Science and Technology Standard Reference Material Pine Needles, NIST SRM No. 1575.

Dietary Intake Estimations

Food intake data from the Nutrition Canada Survey (7) were used as a basis for the 112 final food composites (3) used in this study. A detailed list according to age/sex groups is given in Table 2.

Estimations of total arsenic intake were based on the sum over all composites of the product of each composite intake in grams and the concentration found. To estimate arsenic intakes averaged over all cities, the mean concentrations obtained for each composite were used. To estimate arsenic intakes by city, some of the concentrations for individual composites were missing, because sample availability was limited and the number of composites changed during the study. The missing concentrations were substituted either by those of the individual food (subset A, B, etc.) contributing to the composite, when available, or by those of other composites that most closely approximated the composites with missing concentrations.

If the concentrations were less than the detection limit of the analytical batch (defined as 3 times the standard deviation of a minimum of 2 reagent blanks run within each batch), the detection limit was used for calculation purposes.

Results and Discussion

Arsenic Quality Control Results

All the samples (1044) were analyzed in 71 analytical batches by 4 different analysts. Quality control for each analytical batch included reference samples and recovery studies on samples spiked with 0.5 or 1.0 μg arsenic (approximately 50–1000 ng/g, depending on sample weight) within the food category of interest.

Recoveries of arsenic from spiked samples, presented in Table 3 according to city, averaged 98% and ranged from 71 to 120% for individual analytical batches. The standard deviation of all recoveries was 9.6%.

Duplicates of powdered soya base infant formula, the laboratory reference material used for quality control, were included in each analytical batch. For 39 analytical batches, the mean arsenic concentration in the formula was 19 ng/g, with individual concentrations ranging from 2 to 60 ng/g (Table 3). The standard deviation for the formula concentrations was 11.5 ng/g (61% relative standard deviation). Some of the reasons for this large variance are the proximity of the concentration to the detection limit of the method for solid samples and poorer precision when the method is applied routinely to a large number of samples.

After the supply of the laboratory reference material was depleted, NIST SRM No. 1575, Pine Needles, certified at 210 ± 40 ng/g arsenic, was substituted. A mean of 221 ± 31 ng/g was found for the 6 analytical batches in which this material was included. The relative standard deviation was 14% at this level.

In summary, accuracy was good at arsenic concentrations above 50 ng/g. An increase in the between-batch relative standard deviation for the method with decreasing arsenic concentrations was generally noted: 9.6% for spiked samples (50–1000 ng/g), 14% for NIST SRM No. 1575 (210 ng/g), and 61% for the laboratory reference material (20 ng/g).

Arsenic Survey Results

Determination of arsenic in the individual samples (Table 1) revealed that marine fish contained the highest levels, with a mean of 3048 ng/g for the cooked composites and 2466 ng/g for the raw samples. Canned fish, usually marine species, and shellfish also contained high means (1201 and 2041 ng/g, respectively). Most of the arsenic is present in marine fish and shellfish as organoarsenical compounds, usually arsenobetaine

Table 2. Food intakes (g/person/day) by age (years) and sex: Values compiled from data contributing to reference 7

		Male and female		Male				Female				
No.	Composite	1–4	5–11	12–19	20–39	40–64	65+	12–19	20–39	40–64	65+	Male and female, all ages
1	Milk, whole	389.46	323.16	309.40	218.29	167.36	138.87	205.63	129.04	76.86	123.15	214.33
2	Milk, 2%	186.49	185.61	236.61	99.21	56.77	39.84	155.79	67.00	51.53	47.97	118.65
3	Milk, skim	57.11	55.57	87.91	20.52	35.35	20.39	58.27	35.95	40.94	21.44	44.78
4	Evaporated milk, canned	10.15	6.54	8.59	12.27	13.37	17.75	5.63	9.25	10.00	7.13	10.22
5	Cream, 10–12% BF	1.81	2.83	3.34	10.30	12.21	12.48	2.01	7.02	8.67	12.51	6.75
6	Ice cream	16.47	25.59	29.44	19.39	12.97	12.95	22.37	10.33	10.72	12.14	17.44
7	Yogurt	0.86	0.48	1.50	2.25	1.57	0.44	0.28	0.61	2.98	0.72	1.26
8	Cheese	2.84	3.18	8.10	12.13	9.24	7.47	3.39	8.83	7.04	4.79	6.84
9	Cheese, cottage	1.78	1.33	1.95	1.85	3.16	10.34	1.55	5.18	4.54	9.13	3.27
10	Cheese, processed, cheddar	3.99	4.92	6.15	7.61	3.81	1.16	6.70	4.57	2.72	2.81	4.70
11	Butter	7.68	12.94	20.21	20.83	17.37	11.73	13.38	13.97	9.98	7.31	14.02
12	Beef, steak	3.26	7.37	13.14	33.06	23.57	10.51	8.80	10.58	17.67	7.16	14.51
13	Beef, roast and stewing	7.17	12.21	32.42	54.08	37.41	22.31	14.76	16.23	20.86	12.46	23.78
14	Beef, hamburg	16.16	19.23	41.98	32.07	19.90	20.80	20.47	28.90	15.20	12.01	23.59
15	Pork	8.07	11.98	30.35	41.07	27.83	17.75	15.65	24.93	13.16	11.99	21.01
16	Pork, cured	2.17	3.96	5.02	14.43	9.55	9.34	3.83	5.51	4.99	4.14	6.34
17	Veal	0.56	0.33	1.04	0.63	1.41	3.05	2.48	2.00	1.73	5.21	1.46
18	Lamb	0.03	1.80	0.13	0.60	1.35	0.35	2.19	0.77	0.85	0.54	0.97
19	Poultry, chicken and turkey	14.60	16.72	22.92	28.02	25.19	18.96	17.90	21.82	18.61	12.88	20.20
20	Eggs	25.45	21.05	26.38	43.91	39.08	36.63	16.95	26.23	26.53	23.77	28.31
21	Organ meats	1.01	1.85	3.04	4.58	2.56	1.61	1.57	3.68	2.03	2.29	2.54
22	Cold cuts and luncheon meats	6.39	7.85	11.12	15.42	14.36	8.62	11.41	9.76	4.29	3.18	9.55
23	Luncheon meats, canned	0.97	0.97	2.64	2.70	2.59	2.54	1.80	2.44	0.98	1.66	1.86
24	Fish, marine, fresh or frozen	1.67	4.81	6.81	8.33	5.69	5.87	3.31	4.79	9.74	3.82	5.70
25	Fish, freshwater, fresh or frozen	1.25	1.08	0.56	2.14	0.88	2.80	1.59	0.75	1.08	0.25	1.16
26	Fish, canned	0.48	1.84	3.55	6.59	3.92	2.99	4.67	2.40	6.13	1.40	3.57
27	Shellfish, fresh or frozen	0.31	0.64	0.02	3.05	2.51	0.55	1.92	1.92	1.90	1.31	1.54
28	Soups, meat, canned	41.78	42.77	37.68	67.03	69.36	56.93	34.33	45.32	45.94	47.30	48.60
29	Soups, pea, canned	15.73	19.97	40.60	29.75	40.35	40.30	34.76	27.24	22.99	24.48	28.31
30	Soups, tomato, canned	8.53	11.66	7.93	7.46	9.84	3.23	6.89	7.06	6.12	8.02	8.01
31	Soups, dehydrated	11.00	7.98	6.85	6.43	7.93	6.65	8.92	11.17	7.44	4.50	7.93
32	Bread, white	37.42	76.80	117.32	101.39	81.58	63.96	74.00	58.46	52.49	50.79	72.50
33	Bread, whole wheat and rye	5.96	6.47	8.44	20.76	29.46	22.36	6.49	13.67	12.50	24.52	14.03
34	Rolls and biscuits	4.03	11.63	16.51	19.80	9.82	5.65	15.38	9.61	9.05	5.41	11.37
35	Flour, wheat	4.31	10.38	6.87	7.82	9.96	3.58	3.58	4.84	8.56	5.49	7.07
36	Cake	9.56	25.62	46.94	28.42	25.13	16.75	38.41	20.70	16.81	13.42	24.66
37	Cookies	19.78	26.00	29.90	18.06	20.14	19.66	16.73	12.42	12.21	12.71	18.73
38	Danish and donuts	4.02	5.39	12.38	5.70	9.42	2.02	6.88	6.07	5.36	2.54	6.29
39	Crackers	5.03	5.14	3.77	5.13	3.85	3.34	7.44	3.49	2.52	2.57	4.29

Table 2. (*Continued*)

No.	Composite	Male and female		Male				Female				Male and female, all ages
		1–4	5–11	12–19	20–39	40–64	65+	12–19	20–39	40–64	65+	
40	Pancakes	2.39	2.93	4.33	2.94	1.55	1.34	2.48	0.77	2.30	4.04	2.39
41	Cereals, cooked wheat	9.39	5.72	8.43	0.91	8.13	15.04	1.28	2.67	6.27	8.57	6.09
42	Cereals, cooked oatmeal	18.94	19.95	19.72	12.90	26.14	39.88	5.32	7.48	7.23	12.40	15.77
43	Cereals, dry, corn	3.79	5.37	5.02	1.32	1.92	3.31	1.89	1.27	1.71	1.74	2.70
44	Cereals, dry, wheat and bran	3.69	3.37	5.47	2.09	2.46	3.64	1.38	2.01	1.54	2.80	2.66
45	Rice, cooked	7.45	13.98	16.22	19.29	16.10	11.71	13.01	16.14	14.89	11.19	14.50
46	Pie, apple	2.22	3.87	4.41	14.11	5.02	15.32	3.06	8.33	8.05	6.64	6.72
47	Pie, other	4.07	10.35	12.92	15.49	14.28	12.95	8.76	8.25	10.24	10.18	10.69
48	Pizza	0.13	3.09	5.03	6.38	1.69	0.03	5.15	1.73	0.50	0.23	2.68
49	Pasta, mixed dishes	19.71	36.90	49.64	21.12	27.43	5.59	44.52	19.96	10.94	5.37	25.74
50	Pasta, plain	12.09	26.24	10.70	11.02	10.11	12.73	9.98	25.23	8.39	12.21	14.79
51	Corn	10.61	17.60	14.49	12.18	7.06	3.23	9.73	9.59	10.84	3.09	10.95
52	Potatoes, raw	0.28	0.00	0.00	0.12	0.00	0.00	0.00	0.05	0.05	0.00	0.05
53	Potatoes, baked	2.17	2.95	4.37	8.86	4.17	5.41	1.83	3.24	5.44	2.50	4.18
54	Potatoes, boiled in skin	2.32	1.81	4.89	4.43	5.51	9.66	2.29	5.13	3.57	5.83	4.00
55	Potatoes, boiled without skin	47.78	77.66	125.92	126.42	98.29	96.05	77.76	66.00	55.59	63.45	82.84
56	Potatoes, french fries, frozen	20.37	22.78	39.97	44.63	22.53	13.10	26.56	22.39	13.32	7.54	24.56
57	Potato chips	1.83	5.18	9.59	3.69	0.77	0.22	6.16	2.15	0.65	0.21	3.25
58	Cabbage	3.01	5.05	5.10	7.61	10.79	14.98	7.25	9.26	9.20	11.18	7.70
59	Celery	1.61	2.43	2.90	5.79	7.29	9.06	3.96	5.65	11.20	11.41	5.65
60	Peppers	0.06	0.27	0.12	0.94	2.90	0.44	0.71	1.16	1.38	0.21	0.93
61	Lettuce	2.64	4.49	7.50	15.77	10.71	9.47	8.87	13.26	14.96	10.39	9.86
62	Cauliflower	0.29	0.11	1.29	0.98	1.45	1.39	0.95	2.07	1.88	0.41	1.11
63	Broccoli	0.38	1.34	0.25	6.00	1.71	1.83	0.16	1.18	2.17	0.29	1.74
64	Beans	2.90	4.27	3.84	9.97	6.86	4.27	5.09	8.29	5.96	4.87	5.94
65	Peas	4.87	6.09	9.13	9.92	10.73	9.09	6.29	9.17	7.52	10.42	8.46
66	Carrots	8.49	10.34	10.90	13.44	16.23	15.29	11.25	14.80	12.42	13.13	12.44
67	Onions	0.98	2.45	2.03	5.58	6.17	5.98	4.00	6.35	6.31	6.37	4.53
68	Rutabagas or turnip	2.59	3.51	4.28	5.36	6.35	10.97	2.37	2.75	5.08	5.30	4.36
69	Tomatoes, raw	3.56	7.47	11.14	25.65	15.54	13.91	11.17	19.26	19.83	10.56	14.41
70	Tomato juice, canned	5.84	4.52	2.34	12.55	13.27	7.81	8.71	10.94	9.32	4.36	8.33
71	Tomatoes/sauce canned & ketsup	5.47	7.15	9.05	6.38	6.34	3.24	8.72	10.18	5.57	5.30	7.03
72	Mushrooms, canned	0.52	0.86	2.46	2.90	1.44	0.50	1.79	1.72	2.38	0.05	1.62
73	Cucumbers	3.09	8.27	12.22	19.88	8.41	8.31	10.39	11.64	12.20	6.72	10.70
74	Citrus fruit, raw	12.54	24.70	18.27	21.86	40.60	36.03	26.04	19.54	41.03	41.54	26.92
75	Citrus fruit, canned	0.00	0.17	0.00	0.01	0.18	0.19	0.08	0.02	0.36	0.13	0.12
76	Citrus juice	37.08	22.54	26.81	60.27	21.81	9.26	38.72	47.58	35.86	29.51	34.77
77	Citrus juice, canned	8.56	12.96	12.36	7.45	10.28	11.33	9.83	16.97	20.98	7.81	12.61
78	Apples, raw	29.24	41.38	31.06	30.98	17.62	12.87	36.44	19.98	22.73	17.20	27.26

Table 2. (*Continued*)

No.	Composite	Male and female		Male				Female				Male and female, all ages
		1–4	5–11	12–19	20–39	40–64	65+	12–19	20–39	40–64	65+	
79	Apple juice, canned, unsweetened	44.69	26.66	9.62	18.37	10.92	8.86	9.68	14.41	12.62	14.86	17.69
80	Apple sauce, canned, sweetened	3.90	8.81	4.13	5.60	5.18	12.14	2.25	3.70	4.36	7.50	5.34
81	Bananas	13.04	21.42	9.96	14.87	17.81	10.94	12.33	10.12	11.91	11.09	14.05
82	Grapes	0.92	1.52	2.67	0.36	6.56	2.54	2.67	2.35	3.53	1.06	2.47
83	Grape juice, bottled	4.45	2.52	5.12	2.99	0.97	0.29	4.93	3.10	3.62	0.64	3.12
84	Peaches, fresh	11.67	10.27	5.90	8.63	12.89	11.38	7.17	7.85	10.28	10.33	9.53
85	Pears, fresh	10.23	6.70	3.06	7.91	9.64	5.83	5.00	5.99	8.35	8.45	8.28
86	Plums and dried prunes	2.33	2.72	2.68	3.80	6.54	8.09	2.61	2.57	2.81	6.64	3.57
87	Cherries, fresh	1.01	1.15	1.16	1.82	1.54	1.88	0.62	1.71	0.93	2.46	1.33
88	Melons	1.31	7.39	6.24	3.28	4.64	1.41	1.57	4.40	29.92	4.61	7.46
89	Strawberries, fresh	3.35	7.56	6.51	6.26	6.23	11.19	4.34	2.94	8.54	14.48	6.34
90	Blueberries, fresh	0.74	1.00	2.45	1.22	1.39	3.67	0.64	1.94	1.29	3.35	1.49
91	Pineapple, fresh	0.78	1.68	1.03	3.23	2.58	2.26	2.25	2.85	0.75	2.02	1.96
92	Vegetable fats and oils	1.37	2.21	4.36	7.77	4.70	3.34	3.61	5.44	4.87	2.99	4.25
93	Margarine	2.94	6.13	10.38	5.44	8.29	9.58	6.44	3.22	5.49	6.80	6.03
94	Peanuts	3.32	6.08	9.63	8.01	5.08	2.57	3.78	1.67	2.66	1.37	4.70
95	Sugar	7.56	11.66	18.29	26.82	25.02	21.45	10.89	18.52	12.83	11.54	16.57
96	Syrup	3.20	6.45	5.04	5.85	6.81	6.80	6.10	3.04	3.22	5.25	5.04
97	Jams	3.94	6.76	13.30	6.05	7.61	7.99	6.21	4.37	5.38	6.32	6.50
98	Honey	0.95	2.02	2.92	1.76	2.82	4.46	0.91	1.29	1.76	1.38	1.89
99	Puddings	12.75	8.85	11.99	12.72	9.19	11.42	9.29	7.27	6.17	7.72	9.62
100	Candy, chocolate bars	3.47	5.45	8.27	8.11	4.06	2.04	7.94	3.35	2.26	1.75	4.89
101	Candy, others	5.96	8.47	10.44	6.06	3.45	3.54	10.34	5.97	4.71	3.05	6.39
102	Coffee	7.17	11.99	94.26	398.49	392.64	294.86	74.36	345.02	369.11	243.48	226.12
103	Tea	9.31	22.20	78.33	279.77	419.66	380.59	84.73	287.07	383.82	375.30	218.19
104	Soft drinks	111.02	193.57	285.79	237.30	94.81	48.49	198.73	153.11	76.89	32.76	154.89
105	Alcoholic drinks, wine	0.02	0.73	2.07	33.20	52.55	17.36	1.63	9.54	15.68	12.82	15.11
106	Alcoholic drinks, beer	1.18	1.93	41.23	375.84	222.17	63.09	3.03	41.13	27.98	23.17	89.43
107	Muffins	0.43	0.53	1.51	1.03	2.08	1.95	2.69	1.56	1.46	1.20	1.36
108	Baked beans	3.08	7.27	11.13	11.37	8.23	7.66	13.08	7.32	8.94	4.29	8.43
109	Raisins	0.56	0.53	1.94	0.93	0.35	0.84	0.27	0.45	0.78	0.43	0.68
110	Wieners, fresh	2.50	6.35	5.60	4.32	3.26	1.39	5.31	2.04	2.32	0.72	3.69
111	Gelatin dessert	9.66	7.49	4.89	7.81	4.87	9.86	7.00	7.04	8.02	10.71	7.31
112	Beets, raw	0.48	1.26	0.67	2.59	2.51	2.48	1.30	0.71	1.28	1.75	1.44

Table 3. Quality control results for arsenic

Set No.	City	No. of batches	Rec., %			Reference sample, ng/g[a]		
			Mean	Minimum	Maximum	Mean	Minimum	Maximum
1	Ottawa	10	105	97	112	—	—	—
2	Ottawa	10	97	91	101	—	—	—
3	Halifax	12	97	87	109	14	3	22
4	Winnipeg	11	99	89	108	8	2	9
5	Vancouver	8	98	85	112	18	17	24
6	Montreal	10	98	90	120	28	14	60
7	Toronto	10	91	71	112	28	9	38
						221[b]	174[b]	253[b]

[a] Soya infant formula. No reference material was included for the Ottawa samples.
[b] NIST SRM 1575 Pine Needles, certified at 210 ± 40 ng/g.

(8). Seventy-six percent of this arsenic is excreted unaltered from humans within several days (9), and it is considered to be relatively nontoxic (2). Of the remaining composites, only freshwater fish, meat organs, and rice cereal contained arsenic levels that approached or exceeded 100 ng/g. Higher arsenic levels were found in individual samples of cooked poultry (100 ng/g), raw mushrooms (84 ng/g), and chocolate bars (105 ng/g) (Table 1).

Evaluation of the effect of cooking on arsenic level for the community 6 samples (Montreal) showed that for ground beef, poultry, marine fish, and freshwater fish, the increase in arsenic level with cooking generally agreed closely with the decrease in weight (loss of water and other volatiles) of the food (Table 4). The precision of the analytical method at the concentrations present in the other meat products appeared too low to obtain meaningful correlations for this study.

Summary of the arsenic levels according to city and food category (Table 5) showed no significant difference between cities where the foods were collected. The levels in the fish category were highest, and there was a direct correlation between the mean arsenic level for the city and the mean level for the fish category. Meat and poultry, bakery goods and cereals, and fats and oils contained the next highest levels, averaging 24.3, 24.5, and 19.0 ng/g, respectively. The mean, median, and range of arsenic levels for all 1044 samples were 73.2, 5.1, and <0.1–4830 ng/g, respectively.

Table 4. Weight loss for meat and fish and relationship with arsenic in cooked and raw samples for set 6 from Montreal

Food	Cooking method	Concn ratio[a]	Wt. gain ratio[b]
Beef steak	Broiled	1.04	1.81
Ground beef	Broiled	1.53	1.57
Pork	Roasted	0.94	1.31
Veal	Fried	1.18	1.72
Lamb	Broiled	0.13	1.63
Poultry	Roasted	1.48	1.43
Marine fish	Baked	1.57	1.57
Freshwater fish	Baked	1.28	1.57

[a] Ratio of arsenic concentrations for cooked and uncooked product.
[b] Ratio of raw weight to cooked weight.

Dietary Intake of Arsenic

By using the food intake estimations in Table 2 and the arsenic concentrations in Table 1, the estimated dietary intakes of arsenic were calculated according to city, age, and sex (Table 6). Averaged over all cities, the daily ingestion of arsenic varied from 14.9 μg for the 1- to 4- year-old group to 59.2 μg for 20- to 39-year-old males. Winnipeg samples contributed to the highest daily intake of arsenic, 50.9 μg for all ages, but this resulted from the higher arsenic level in the fish samples obtained from Winnipeg. The average estimated ingestion by 20- to 65-year-old adults was 47.3 μg/day. This is roughly 3 times the average intake of 15.6 μg/day found in a Canadian 24 h duplicate diet survey (10). Individuals in that study had intakes varying from 2.6 to 101 μg/day. A more detailed comparison of the 2 studies (Table 7) shows agreement only in the case of adult males from Montreal.

Examination of the data from both studies indicated that the major factor contributing to the higher arsenic intakes in this study was the fish category, which accounted for an average of 64% (range, 46–78%) of the dietary arsenic, depending on age and sex. In the previous 24 h duplicate diet study, the category containing fish, meat, and poultry contributed only 32.1% of the ingested arsenic (10). These data suggest that a discrepancy exists between either the amount of fish actually consumed in the 2 studies or the arsenic levels actually present in the composites containing fish.

The average dietary intake of arsenic by adults (48.5 μg/day from Table 7) is generally similar to or higher than intakes estimated previously in Canada and other countries: 16.7 μg/day (10) and 30 μg/day (11) in Canada; 7 μg/day (12), 45 μg/day (13), 62 μg/day (14), and 13–16 μg/day (15) in the United States; 89 μg/day in the United Kingdom (16); 12 μg/day in Belgium (17); 27 μg/day in Austria (18); and 55 μg/day in New Zealand (19).

The Food and Agriculture Organization/World Health Organization (FAO/WHO) provisional tolerable weekly intake (PTWI) of arsenic (15 μg inorganic arsenic/kg body weight), translated to a daily basis, is 2.1 μg/kg/day (2). As a worst-case

Table 5. Summary of arsenic levels (ng/g) in food categories by city

Food category		Mean arsenic concentrations (ng/g) by city[a]						
		1	2	3	4	5	6	7
Milk and dairy products		3.2	1.5	3.3	5.5	3.8	5.2	3.9
Meat and poultry		31.6	40.1	26.6	23.9	12.0	22.6	13.4
Fish and shellfish		1514.2	1543.5	1446.4	2608.8	1473.0	1535.5	1515.3
Soups		1.0	2.5	2.9	2.6	5.1	7.4	7.9
Bakery goods and cereals		20.1	17.0	19.8	34.8	22.8	26.9	30.4
Vegetables		5.9	5.6	6.5	2.6	5.9	13.9	8.8
Fruit and fruit juices		4.0	2.4	2.9	3.8	6.5	4.7	7.0
Fats and oils		11.4	5.9	13.9	30.9	23.8	23.3	23.9
Sugar and candies		5.8	4.3	2.4	5.6	14.6	16.4	27.4
Beverages		2.5	1.8	2.7	3.4	4.4	4.1	2.5
Miscellaneous		—	—	11.9	12.8	16.3	10.9	10.3
All categories	Mean	72.6	73.9	66.5	84.0	68.7	74.8	72.7
	Median	4.0	2.9	5.0	3.5	6.0	9.6	7.3
	Minimum	<0.3	<0.3	<0.1	<0.1	<0.1	<0.2	<0.8
	Maximum	3270.0	3140.0	2920.0	4830.0	3030.0	2360.0	2970.0
	Count	147	146	153	145	151	151	151

Food category		All cities			
		n	Mean	Minimum	Maximum
Milk and dairy products		89	3.8	<0.4	26.0
Meat and poultry		124	24.3	<1.3	536.0
Fish and shellfish		40	1662.4	77.0	4830.0
Soups		28	4.2	<0.2	11.0
Bakery goods and cereals		177	24.5	<0.1	365.0
Vegetables		262	7.0	<0.1	84.0
Fruti and fruit juices		176	4.5	<0.1	37.0
Fats and oils		21	19.0	<1.0	57.0
Sugar and candies		49	10.9	1.4	105.0
Beverages		45	3.0	0.4	9.0
Miscellaneous		33	12.5	<0.8	41.0
All categories	Mean	1044	73.2	<0.1	4830

[a] 1, 2 = Ottawa; 3 = Halifax; 4 = Winnipeg; 5 = Vancouver; 6 = Montreal; 7 = Toronto.

scenario for a 60 kg adult, the maximum average dietary intake of total inorganic and organic arsenic, according to Table 6 data for a 20- to 39-year-old male from Winnipeg, would be 1.3 µg/kg/day, about 60% of the PTWI of inorganic arsenic. For a 1-year-old child from Montreal with an intake of 18.1 µg arsenic per day and a body weight of 10 kg, the average dietary intake of total arsenic would be 1.8 µg/kg/day. However, the FAO/WHO PTWI refers to inorganic arsenic, whereas a substantial portion of the arsenic present in food, and particularly in fish, is in the less-toxic organic form (8).

References

(1) WHO Task Group on Environmental Health Criteria for Arsenic: *Arsenic* (1981) Environmental Health Criteria 18, World Health Organization, Geneva, Switzerland, pp. 1–174

(2) Joint FAO/WHO Expert Committee on Food Additives: *Evaluation of Certain Food Additives and Contaminants* (1989) 33rd Report, Tech. Rep. Ser. 776, World Health Organization, Geneva, Switzerland, pp. 27–38

(3) Conacher, H.B.S., Graham, R.A., Newsome, W.H., Graham, G.F., & Verdier, P. (1989) *Can. Inst. Food Sci. Technol. J.* **22**, 322–326

(4) Dabeka, R.W., & McKenzie, A.D. (1992) *J. AOAC Int.* **75**, 954–963

(5) Steering Group on Food Surveillance (1982) *Survey of Arsenic in Food*, 8th Report of the Steering Group on Food Surveillance, Ministry of Agriculture, Fisheries and Food, Her Majesty's Stationery Office, London, UK

(6) Dabeka, R.W., & Lacroix, G.M.A. (1987) *J. Assoc. Off. Anal. Chem.* **70**, 866–870

(7) Bureau of Nutritional Sciences (1977) *Nutrition Canada Food Consumption Patterns Report*, Bureau of Nutritional

Table 6. Estimated intake of arsenic (μg/person/day) depending on age, sex, and city

Age, years	Sex	City[a] 1	2	3	4	5	6	7	All cities
1–4	Male and female	12.8	11.4	13.2	17.7	14.1	18.1	16.2	14.9
5–11	Male and female	28.2	25.5	27.2	39.7	25.8	31.1	31.0	29.9
12–19	Male	39.4	34.6	38.3	53.1	35.1	41.9	42.0	40.9
20–39	Male	57.2	52.9	54.7	78.7	51.1	62.4	56.4	59.2
40–65	Male	40.7	37.5	39.3	57.4	38.4	45.4	41.1	43.0
>65	Male	33.6	30.3	33.4	45.4	30.6	38.7	36.7	35.7
12–19	Female	28.8	26.3	28.5	42.0	29.6	34.4	31.2	31.7
20–39	Female	32.5	30.2	30.8	46.2	29.8	36.0	32.4	34.1
40–65	Female	51.9	48.1	49.5	73.5	43.7	50.3	51.3	52.8
>65	Female	24.3	22.5	23.0	34.7	22.8	26.9	25.3	25.8
All ages	Male and female	36.3	33.3	35.0	50.9	33.2	39.7	37.5	38.1

[a] 1, 2 = Ottawa; 3 = Halifax; 4 = Winnipeg; 5 = Vancouver; 6 = Montreal; 7 = Toronto.

Table 7. Comparison of current dietary intakes (μg/day) of arsenic with those obtained in a duplicate diet study for the same cities

City	Males		Females	
	This study[a]	Previous[b]	This study[a]	Previous[b]
Halifax	47.0	5.5	40.2	7.7
Winnipeg	68.1	16.7	59.9	9.1
Vancouver	44.8	14.0	36.8	4.2
Montreal	53.9	55.7	43.2	15.0
Toronto	48.8	8.6	41.9	19.3

[a] The 20–39- and 40–65-year age groups were averaged.
[b] The mean ages for males and females were 37 and 34 years, respectively.

Sciences, Health Protection Branch, Health and Welfare Canada, Ottawa, ON, pp. 1–26

(8) Lawrence, J.F., Michalik, P., Tam, G.K.H., & Conacher, H.B.S. (1986) *J. Agric. Food Chem.* **34**, 315–319

(9) Tam, G.K.H., Charbonneau, S.M., Bryce, F., & Sandi, E. (1982) *Bull. Environ. Contam. Toxicol.* **28**, 669–673

(10) Dabeka, R.W., McKenzie, A.D., & Lacroix, G.M.A. (1987) *Food Addit. Contam.* **4**, 89–101

(11) Smith, D.C., Leduc, R., & Charbonneau, C. (1973) *Pestic. Sci.* **4**, 211–214

(12) Mahaffey, K.R., Corneliussen, P.E., Jelinek, C.F., & Fiorino, J.A. (1975) *Environ. Health Perspect.* **12**, 63–69

(13) Podrebarac, D.S. (1984) *J. Assoc. Off. Anal. Chem.* **67**, 176–185

(14) Gartrell, M.J., Craun, J.C., Podrebarac, D.S., & Gunderson, E.L. (1985) *J. Assoc. Off. Anal. Chem.* **68**, 862–875

(15) Kowal, N.E., Johnson, D.E., Kraemer, D.F., & Pahren, H.R. (1979) *J. Toxicol. Environ. Health* **5**, 995–1014

(16) Food Additives and Contaminants Committee (1984) *Report on the Review of the Arsenic in Food Regulations*, Ministry of Agriculture, Fisheries and Food, FAC/REP/39, Her Majesty's Stationery Office, London, UK

(17) Buchet, J.P., Lauwerys, R., Vandevoorde, A., & Pycke, J.M. (1983) *Food Chem. Toxicol.* **21**, 19–24

(18) Pfannhauser, W., & Pechanek, U. (1977) *Lebensm. Ernaehr.* **30**, 88–92

(19) Dick, G.L., Hughes, J.T., Mitchell, J.W., & Davidson, F. (1978) *N. Z. J. Sci.* **21**, 57–69

CHEMICAL CONTAMINANTS MONITORING

Survey of Bottled Drinking Water Sold in Canada. Part 2. Selected Volatile Organic Compounds

B. DENIS PAGE[1], HENRY B.S. CONACHER[1], and JOHN SALMINEN[2]
Health and Welfare Canada, Health Protection Branch, Food Directorate, Bureau of Chemical Safety, Ottawa, ON, K1A 0L2, Canada
GERALD R. NIXON and GUNTHER RIEDEL
Health and Welfare Canada, Health Protection Branch, Field Operations Directorate, Bureau of Field Operations, Ottawa, ON, K1A 0L2, Canada
BRIAN MORI
Health and Welfare Canada, Health Protection Branch, Field Operations Directorate, Bureau of Field Operations, Burnaby, BC, V5G 4P2, Canada
JACQUES GAGNON and ROBERT BROUSSEAU
Health and Welfare Canada, Health Protection Branch, Field Operations Directorate, Bureau of Field Operations, Longueuil, PQ, J4K 1C1, Canada

Selected volatile organic compound (VOC) contaminants were determined in 182 samples of retail bottled waters purchased in Canada. Samples included spring water (86) packaged in containers of polyethylene or in smaller containers of transparent plastic or glass, mineral water (61) packaged only in transparent plastic or glass, and miscellaneous bottled waters (35). Analyses were performed by 3 laboratories, each using headspace sampling and capillary gas chromatography with either mass spectrometric (1 laboratory) or flame ionization detection with mass spectrometric confirmation, if required (2 laboratories). Benzene, the contaminant of primary interest, was detected in only 1 of the 182 samples at 2 μg/kg. Other VOC contaminants detected (number of positive samples, average, and range of positives in μg/kg) included toluene (20, 6.92, 0.5–63), cyclohexane (23, 39.2, 3–108), chloroform (12, 25.8, 3.7–70), and dichloromethane (4, 59, 22–97). Cyclohexane was found in the plastic and as a migrant from the plastic in 20 samples of spring water, but it was found in only 1 of 61 mineral water samples analyzed at only 3 μg/kg. Chloroform was found almost exclusively in samples that could have been obtained from public water supplies. It was not found in mineral water samples, but it was found in 1 spring water sample at 3.7 μg/kg. The source of the toluene contamination was not known. Other VOCs detected include ethanol and limonene, associated with added flavoring; pentane, as a migrant from a foamed polystyrene cap liner; and 1,1,2,2-tetrachloroethylene in a sample of demineralized water.

Received December 6, 1991. Accepted March 19, 1992.
[1] Food Research Division.
[2] Chemical Evaluation Division.

In early 1990, considerable public and analytical interest was directed toward the occurrence of benzene in bottled mineral waters (1, 2) at levels exceeding the Canadian (3) and U.S. (4) maximum acceptable concentration (MAC) guideline of 0.005 mg/L for drinking water. This finding generated public concern about the quality of bottled water. Therefore, a small survey for benzene and other volatile organic compounds (VOCs) in a number of locally purchased bottled waters was initiated. This sampling then became part of a more extensive survey that included other VOC contaminants and samples from across Canada. Several trace elements, including lead, cadmium, arsenic, aluminum, and fluoride, also were included in the survey. Results for these trace elements were already reported in Part 1 of this survey (5).

Most samples were analyzed for both VOCs and trace elements; however, 21 of the 182 samples were analyzed only for VOCs.

Purge and trap sample concentration followed by capillary gas chromatography (GC) with mass spectrometric (MS) detection is probably the most sensitive analytical technique available to determine benzene and other VOCs in bottled waters. In our laboratories, however, this configuration was not available. Instead, the headspace sampling technique was used with capillary GC and either MS or flame ionization detection (FID).

In our preliminary analysis of several bottled water samples for benzene, several significant peaks in the FID chromatogram were noted. These peaks were identified by MS and subsequently included in our analytical standards. Analyses of the

Table 1. Method performance data for some volatile organic compounds (VOCs) in water

VOC	Spike, µg/kg[a]	CV, %[a]	Detection limit, µg/kg		
			Laboratory 1 (FID)	Laboratory 2 (MS)	Laboratory 3 (FID)
Benzene	4.1	7.41	0.2	1.0	0.5
Toluene	4.2	9.31	0.1	1.0	0.5
Cyclohexane	3.8	7.52	1.0	1.0	0.5
Chloroform	1.5	9.28[b]	0.5	2.0	3.0
Dichloromethane	1.3	24.6[b]	1.0	2.0	>5.0[c]

[a] Data from laboratory 3.
[b] Repeatability CV determined by MS.
[c] General laboratory contamination increased reliable detection limit.

182 samples for VOC contaminants were performed in 3 laboratories. Each laboratory used essentially the same headspace GC procedure, using either MS or FID, but with a few minor differences, as noted in *Experimental*.

Experimental

Samples

Samples were obtained as described in Part 1 of this survey (5). Samples for determination of VOC contaminants were taken from bottles different from those sampled for trace elements. An additional 21 samples obtained from the Ottawa area were analyzed only for VOC contaminants.

Apparatus and Instrumentation

(**a**) *Gas chromatograph.*—Model Vista 6000 gas chromatograph with MS or FID system (Varian Analytical Instruments, Sunnyvale, CA 94034) and Hewlett-Packard 5988 GC/MS system (Hewlett-Packard Ltd., Mississauga, ON, L4V 1M8, Canada). Chromatographs were equipped with cryogenic oven cooling; on-column or splitless injector; and a DB-624 (1.8 µm film thickness) column, a DB-5 (1.0 µm film thickness; J&W Scientific Inc., Folsom, CA 95630) column, or an Ultra 2 (0.52 µm film thickness; Hewlett-Packard Ltd.) 30 m × 0.32 mm fused silica capillary column. Typical operating conditions were as follows: He carrier gas, 1.5 mL/min (0.31 m/s); oven temperature program, –20°C (hold 1 min) to 200°C at 10°C/min; and injector, 100°C. Other programs and temperatures that provided suitable chromatographic performance were also used.

(**b**) *Mass spectrometer.*—Model 5988 GC/MS system (Hewlett-Packard Ltd.) operating in the electron impact mode (70 eV) was used for specific GC detection by one laboratory and for confirmation of all FID-detected VOCs by another. A VG Analytical Model 7070EQ tandem mass spectrometer using the conventional double-focusing configuration with direct interface to the Model Vista 6000 GC system (Varian) was used for confirmation by the third laboratory. The mass spectrometer was operated in the electron impact mode (70 eV) at mass resolution of 1000. The reconstructed ion chromatogram at the particular analyte *m/z* was examined, and the peak height or area was used for quantitation. Analyte identity was confirmed by a full mass spectrum (after background subtraction) at concentrations as low as 0.03 µg/kg (e.g., benzene).

(**c**) *Headspace sampling syringe and needles.*—1 mL gastight locking syringe series A-2 (Dynatech Precision Sampling Corp., Baton Rouge, LA 70895) fitted with a 2 in. sideport needle. If necessary, this needle was replaced by a modified on-column injection needle, as described previously (6).

(**d**) *Headspace sample vials.*—30 mL Hypovials (actual capacity, ca 37 mL) were sealed after sample addition with Teflon-laminated silicone rubber disks (discarded after single use) and aluminum seals. Vials were tightly crimped so that aluminum seals could not be turned. Teflon-coated magnetic stirring bars (25 × 7.5 mm) were used in the vials.

Standards and Quantitation

Stock solutions were prepared by injection of 10–50 µL quantities of each VOC under the surface of ca 99 mL of cold (<4°C) liquid chromatographic (LC)-grade water (Milli-Q system, Millipore Corp., Bedford, MA 01730) in a 100 mL volumetric flask. Added weights were calculated from appropriate densities. The flask was then shaken to ensure dissolution of the added analytes, and the contents were diluted to volume with LC-grade water. The LC-grade water was boiled 15 min in an electric kettle before use and then analyzed to ensure that interference from any of the VOCs included in our analyses was minimal. The VOCs and concentration levels studied are given in Table 1. Stock solutions also were prepared by adding 10 µL aliquots of the particular VOC to a tared 10 mL volumetric flask containing methanol, reweighing to accurately determine the weight of each added VOC, and finally diluting to volume (7). By using cold pipets or syringes, milliliter or microliter serial dilution of the above standards at 4°C into the LC-grade water then gave the required secondary standards. These standards were prepared at concentrations dependent on the volatility of the analyte. To reduce VOC losses, all standards were stored and used at 4°C. Calibration standards and curves were prepared for quantitation by microliter additions of the aqueous standards to 20 mL of the boiled water and the salt in the headspace vial at 4°C. These responses were monitored daily, and the appropriate standards were prepared fresh if any reduction in response was noted. Quantitation standards at other concen-

Table 2. Summary of survey results for some volatile organic compounds (VOCs) in 182 samples of bottled water

VOC	No. positive samples (%)	Av. of positives., µg/kg	Range, µg/kg
Benzene[a]	1 (0.55)	2	—
Toluene[b]	20 (11.0)	6.92	0.5–63
Cyclohexane[c]	23 (13.2)	39.2	3–108
Chloroform[d]	12 (6.59)	25.8	3.7–70
Dichloromethane[e]	4 (2.20)	59.0	22–97

[a] Levels greater than 0.2, 1.0, and 0.5 µg/kg reported by laboratories 1, 2, and 3, respectively.
[b] Levels greater than 0.1, 1.0, and 0.5 µg/kg reported by laboratories 1, 2, and 3, respectively.
[c] Levels greater than 1.0 and 0.5 µg/kg reported by laboratories 2 and 3, respectively. Data from laboratory 1 combined and reported with that of laboratory 2.
[d] Levels greater than 0.5, 2.0, and 3.0 µg/kg reported by laboratories 1, 2, and 3, respectively.
[e] Levels greater than 1.0, 2.0, and 5.0 µg/kg reported by laboratories 1, 2, and 3, respectively.

trations and for other VOCs were also prepared by microliter additions to provide comparable sample peak areas, as required for quantitation. Other VOCs found in the bottled water samples, but not included in Table 1, were pentane, ethanol, 1,1,2,2-tetrachloroethylene, and limonene.

Sampling of Bottled Waters

Bottled water samples (20 g) stored at 4°C (refrigerator) were decanted into cold, tared 30 mL headspace vials and weighed on a top-loading balance. Before sampling, vials were kept at 0°C in a water–ice bath and loosely covered with the silicone disk and aluminum cap. They contained a magnetic stirring bar and 4 g anhydrous sodium sulfate or sodium chloride, which was added to increase the headspace partition. All vials were immediately sealed and weighed to determine sample weight. They were brought to room temperature over 15 min with stirring in a 250 mL beaker containing ca 75 mL of 45–50°C water. One laboratory equilibrated the vials for 30 min (with stirring) at room temperature before headspace sampling using the locking 1 mL gas-tight syringe. Two other laboratories used an equilibration temperature of 35°C. All time and temperature conditions used were tested to ensure that equilibrium was attained.

Headspace Sampling

With the lock open, the barrel of the A-2 locking syringe and attached sideport needle were flushed through the needle with nitrogen. The plunger was replaced, trapping 1 mL nitrogen; the needle was inserted 1–2 cm through the septum of the headspace vial, and the 1 mL nitrogen was injected. The plunger was withdrawn slowly (5 s) to beyond the 1 mL mark. The barrel was allowed to fill (10 s), the plunger tip was aligned with the 1 mL mark, the syringe was locked, and the needle was withdrawn. If necessary for injection, the sideport needle was removed and replaced with the modified on-column injection assembly. The needle was inserted into the injector, the syringe was unlocked, and the headspace sample was injected onto the column. The temperature program was started, and the particular VOC was identified by its retention time and confirmed, if required, by its mass spectrum. Only one injection was made from each headspace vial.

Evaluation of Method Performance

To determine the coefficient of variation (CV) of the spiking of standards in water, 6 separate vials containing 20 g water were individually spiked with 10 µL aliquots of a mixed standard. VOCs included in this study and the concentration levels are given in Table 1. In another experiment, the overall CV for benzene was determined from the individual preparation of 6 stock solutions through to the final GC determination.

Water used to prepare the standards was analyzed to ensure that interference from any of the VOCs listed in Table 1 was minimal. A correction was applied to peak areas or heights of

Table 3. Results of survey for volatile organic compounds (VOCs) in bottled spring waters

	Laboratory 1 (n = 44)			Laboratory 2 (n = 30)			Laboratory 3 (n = 12)			Total (n = 86)		
VOC	No. positive	Av., µg/kg	Range, µg/kg	No. positive	Av., µg/kg	Range, µg/kg	No. positive	Av., µg/kg	Range, µg/kg	No. positive	Av., µg/kg	Range, µg/kg
Benzene[a]	0	—	—	1	2	—	0	—	—	1	2	—
Toluene[b]	5	2.5	1.6–4	3	12.2	2.1–32	1	0.5	0.5–0.52	9	5.5	0.5–32
Cyclohexane[c]	—	—	—	16	48.6	3–108	4	16.3	6.3–24.7	20	42.2	3–108
Chloroform[d]	0	—	—	1	3.7	—	0	—	—	1	3.7	—
Dichloromethane[e]	0	—	—	4	59.0	22–97	0	—	—	4	59.0	22–97

[a–e] See footnotes of Table 2.

Table 4. Results of survey for some volatile organic compounds (VOCs) in bottled mineral waters

VOC	Laboratory 1 (n = 38)			Laboratory 2 (n = 16)			Laboratory 3 (n = 7)			Total (n = 61)		
	No. positive	Av., μg/kg	Range, μg/kg	No. positive	Av., μg/kg	Range, μg/kg	No. positive	Av., μg/kg	Range, μg/kg	No. positive	Av., μg/kg	Range, μg/kg
Benzene[a]	0	—	—	0	—	—	0	—	—	0	—	—
Toluene[b]	5	2.3	1.4–4	1	1.7	—	0	—	—	6	2.2	1.4–4
Cyclohexane[c]	—	—	—	1	3	—	0	—	—	1	3	—
Chloroform[d]	0	—	—	0	—	—	0	—	—	0	—	—
Dichloromethane[e]	0	—	—	0	—	—	0	—	—	0	—	—

[a–e] See footnotes of Table 2.

the standard for any interference found. A 20 g blank water sample was also prepared and analyzed concurrently with the standards and each set of 5–10 samples. This was done to determine any contribution from the laboratory environment as well as to assess the possibility of any carryover from one sample to the next.

The linearity of response for the VOCs listed in Table 1 was studied using FID over a 50-fold range of concentrations from ca 10 to 500 times the detection limits shown in Table 1. In another laboratory, the linearity of the benzene response was evaluated from 1 to 53 μg/kg.

Identification of Plastic Containers

Plastic containers were identified by a combination of appearance and several test procedures for distinguishing plastics, including ignition with a small flame and a copper wire test for chlorinated polymers (8).

Results and Discussion

Sampling Problems

In every analysis for VOCs, there is the problem of analyte loss by volatility when transferring the standard or sample from one container to another. Steps taken to minimize this loss include minimizing manipulative steps and cooling syringes, other equipment contacting the sample, samples themselves, and standards to reduce analyte volatility. Pouring the sample into the headspace vial may result in VOC losses not only from the sample in the headspace vial but also from the sample remaining in the bottle. In the analysis of the survey samples, therefore, replicate sampling from the same container was performed only for confirmation of identity by GC/MS.

With the sampling of carbonated water, however, VOC losses can occur as soon as the container is opened; pressure over the drink falls to atmospheric level, and degassing, with possible purging of the analyte, can occur. Furthermore, degassing, and possible VOC loss, can also occur during any subsequent sample manipulation (pouring, pipetting, or other sample transfer). With headspace analysis, degassing is especially difficult to avoid when the sample is added to the headspace vial containing sodium sulfate or sodium chloride. Cold aqueous standards in water decanted into the headspace vial, however, give only a slightly lower (<5%) analytical response for the more volatile analytes when compared with equivalent standards prepared by syringe spiking through 20 mL water to the bottom of the vial, which was quickly sealed. Thus, with noncarbonated samples, losses on sample decanting should be minimal, but for pressurized products, reported VOC levels are probably lower than actual VOC levels in unopened products.

Method Performance

CVs obtained for 6 individually spiked vials and the detection limits are given in Table 1 for 5 of the VOCs studied. The repeatability CVs, determined in 1 laboratory, were acceptable for benzene, toluene, cyclohexane, and chloroform and are expected to be indicative of the precision of the method in the 2 other laboratories. The poor repeatability for dichloromethane

Table 5. Results of survey for some volatile organic compounds (VOCs) in miscellaneous bottled waters

VOC	Laboratory 1 (n = 9)			Laboratory 2 (n = 24)			Laboratory 3 (n = 2)			Total (n = 35)		
	No. positive	Av., μg/kg	Range, μg/kg	No. positive	Av., μg/kg	Range, μg/kg	No. positive	Av., μg/kg	Range, μg/kg	No. positive	Av., μg/kg	Range, μg/kg
Benzene[a]	0	—	—	0	—	—	0	—	—	0	—	—
Toluene[b]	1	1.7	—	4	18.4	1.3–63	0	—	—	5	15.1	1.3–63
Cyclohexane[c]	—	—	—	2	27.6	8.1–47	0	—	—	2	27.6	8.1–47
Chloroform[d]	0	—	—	11	27.8	7.3–70	0	—	—	11	27.8	7.3–70
Dichloromethane[e]	0	—	—	0	—	—	0	—	—	0	—	—

[a–e] See footnotes of Table 2.

Table 6. Packaging materials identified for different types of bottled water

	No. of bottled water containers									
	Spring water			Mineral water			Miscellaneous water			
Container material[a]	Lab. 1	Lab. 2	Lab. 3	Lab. 1	Lab. 2	Lab. 3	Lab. 1	Lab. 2	Lab. 3	All waters, all laboratories
PE	22	9	6	0	0	0	3	16	0	56
PET	—	10	3	—	0	0	—	5	0	18
PVC	—	4	2	—	3	2	—	0	0	11
PET, PVC, or PC[b]	16	—	—	18	—	—	5	—	—	39
Glass	6	4	1	20	13	5	1	1	2	53
Metal	0	0	0	0	0	0	0	1	0	1
Not identified[c]	0	3	0	0	0	0	0	1	0	4

[a] PE, polyethylene; PET, polyethylene terephthalate; PVC, poly(vinyl chloride); and PC, polycarbonate.
[b] Not differentiated by laboratory 1.
[c] Containers not retained for analysis.

was attributed to a general contamination of the laboratory environment arising from the use of dichloromethane as an extraction solvent. Repeatability CVs were 9.87, 8.26, 12.7, and 10.4%, respectively, for 1,1,2,2-tetrachloroethylene, pentane, limonene, and hexane. One laboratory participating in the study reported a general contamination problem with 1,1,2,2-tetrachloroethylene of unknown origin. Detection limits for VOCs listed in Table 1 are dependent on the mode of detection and the sophistication of the instrumentation used in each particular laboratory. Detection limits are generally defined at a signal-to-noise ratio of about 3:1. With the mass spectrometer operated in the full-scan mode, one laboratory was able to use headspace GC/MS to confirm the presence of benzene at levels as low as 0.03 μg/kg in bottled water.

Overall CV for benzene from the individually prepared stock solutions through the final GC determination was 14%. Linearity studies showed the headspace technique to give adequate linear response for all VOCs studied. No carryover of, or residual contamination from, VOCs was observed when the headspace from unspiked LC-grade water controls was injected after that of LC-grade water spiked with high VOC levels.

Volatile Organic Compounds

In total, 182 samples were analyzed for VOC contaminants. Results are summarized in Table 2 and separated into the categories of spring water in Table 3 (86 samples), mineral water in Table 4 (61 samples), and other miscellaneous bottled waters in Table 5 (35 samples). The last group includes waters labeled as carbonated, demineralized, deionized, treated (by reverse osmosis), or distilled. This categorization results in a differentiation in which only Table 5 includes samples that could have been obtained from a public water supply. Some of these waters are expected to be chlorinated. Chloroform, a by-product of this chlorination process, was found in 11 of 35 (31.4%) samples; average level in positive samples was 27.8 μg/kg. By definition, spring or mineral waters are not to be obtained from public water supplies and thus are not be expected to be chlorinated. Only 1 of the 147 samples of these waters contained detectable chloroform (3.7 μg/kg). The other trihalomethanes associated with the chlorination of water were not detected. In the above cases, the total trihalomethane levels would reflect only the chloroform contaminant and would be well below the Canadian MAC of 350 μg/kg for the combined trihalomethanes (3).

Bottled water can also be differentiated on the basis of packaging (Table 6). Of the 182 containers, 4 were not available for analysis, and another 39 transparent plastic containers were classified only as poly(ethylene terephthalate) (PET), poly(vinyl chloride) (PVC), or polycarbonate (PC).

All mineral waters were bottled in transparent packages of glass, PVC, PET, or possibly PC, typically 0.2–1.5 L in capacity. Over half the spring waters, 46 of the 83 samples (55%) for which the container was identified were also in transparent packages. The other 37 (45%) were packaged in larger (1–10 L) containers identified as polyethylene (PE). This material is associated with the average 42 μg/kg cyclohexane contamination of spring water in 20 of these 37 samples, as noted in Table 3. Cyclohexane is used as a polymerization solvent in some manufacturing processes of high-density polyethylene (HDPE) (9). Its presence in the PE containers was verified by headspace analysis of container shavings dispersed in water. Furthermore, in all water samples containing cyclohexane, including the 2 samples in Table 5, the same VOC was found in the packaging material. This clearly identifies the package as the source of the cyclohexane. Only 1 of 61 samples (1.6%) of mineral water contained cyclohexane, at a low level of 3 μg/kg.

Pentane, another VOC associated with packaging, was noted at 18.7 μg/kg in one sample of spring water. Pentane is used as a blowing agent for polystyrene foam, as a component of cap liners, and as a polymerization solvent in the production of HDPE (10). In this instance, pentane was found only in the cap liner.

VOCs associated with the flavoring of some carbonated waters were also noted but were not included in the tables. These included limonene (187 μg/kg) in one sample and ethanol (0.7 g/kg).

VOC contaminants such as benzene, toluene, and dichloromethane from unknown sources were noted in one or

more samples. These results are included in Tables 2–5. Benzene, the VOC of primary interest, was found in only one sample (spring water) at 2 μg/kg, a level below the Canadian MAC of 5 μg/kg. Toluene was found in 11% of samples (average, 6.9 μg/kg), and dichloromethane was found in 2.2% of samples (average, 59 μg/kg). 1,1,2,2-Tetrachloroethylene was noted in one sample of the miscellaneous bottled waters (11 μg/kg).

In general, survey results in Tables 3–5 show that mineral waters contain lower levels of VOC contaminants at a lower incidence than the other bottled waters. Packaging and municipal chlorination are probably the major sources of VOCs in the other waters.

Classification of a sample as spring water, mineral water, or miscellaneous bottled water also relates to its status as an imported or a domestic product. Most of the imported samples were mineral waters (ca 70%); the remainder of the imports were spring waters. However, most spring waters were of domestic origin (ca 83%), and all miscellaneous waters were domestic products.

Conclusions

Selected VOC contaminants were determined in 182 samples of retail bottled waters, including 86 samples of spring waters, 61 samples of mineral water, and 35 samples of other bottled waters. In general, results show that the incidence of VOC contamination is lower in bottled mineral waters than in other bottled waters. Packaging and municipal chlorination are proposed as the major sources of VOCs in these other waters. Mineral waters were packaged mainly in glass or transparent plastic, whereas spring waters were packaged mainly in PE. Cyclohexane was found in the plastic and as a migrant in the spring waters. Chloroform was found almost exclusively in samples that could have been obtained from public water supplies. The low incidence and level of the VOC contaminants encountered in this survey are not considered to represent a health concern to the public.

Acknowledgments

The authors wish to thank G. Lacroix and D. Weber for their technical assistance in this survey.

References

(1) Anon. (1990) *Food Chem. News* **31**(50), 60–61

(2) Borman, S. (1990) *Chem. Eng. News* **68**(8), 5–6

(3) *Guidelines for Canadian Drinking Water Quality* (1989) 4th Ed., Canadian Government Publishing Centre, Ottawa, ON, Canada

(4) National Primary Drinking Water Regulations: Synthetic Organic Chemicals; Monitoring for Unregulated Contaminants (1987) *Fed. Regist.* **52**(130), 25690–25734

(5) Dabeka, R.W., Conacher, H.B.S., Salminen, J., Nixon, G.R., Riedel, G., Crocker, R., & Dubé, G. (1992) *J. AOAC Int.* **75,** 949–953

(6) Page, B.D., & Avon, R.J. (1989) *J. Assoc. Off. Anal. Chem.* **72,** 815–822

(7) Methods for Determination of Organic Compounds in Drinking Water (1988) Method 524.2 (Rev. 3) U.S. Environmental Protection Agency Publication No. EPA-600/4-88/039, Cincinnati, OH

(8) Braun, D. (1982) *Simple Methods for Identification of Plastics,* Macmillan Publishing Co., New York, NY

(9) Hogan, J.P. (1980) in *Kirk-Othmer Encyclopedia of Chemical Technology,* Vol. 16, M. Grayson (Ed.), John Wiley & Sons, New York, NY, p. 430

(10) Dale, G.H., & Drehman, L.E. (1980) in *Kirk-Othmer Encyclopedia of Chemical Technology,* Vol. 12, M. Grayson (Ed.), John Wiley & Sons, New York, NY, p. 924

Aflatoxin Contamination in Foods and Foodstuffs in Tokyo: 1986–1990

SETSUKO TABATA, HISASHI KAMIMURA, AKIHIRO IBE, HIDEKI HASHIMOTO, MAMI IIDA, YUKIHIRO TAMURA, and TAICHIRO NISHIMA
Tokyo Metropolitan Research Laboratory of Public Health, 3-24-1 Hyakunin-cho, Shinjuku-ku, Tokyo 169, Japan

Aflatoxins were determined in 3054 samples of foods or foodstuffs, including cereals, nuts, beans, spices, dairy products, dry fruits, and edible oil. Samples were collected in Tokyo from 1986 to 1990. Aflatoxins were found in rice products, adlay, corn, crude sugar, peanut products, pistachio nuts, brazil nuts, sesame products, butter beans, white pepper, red pepper, paprika, nutmeg, and mixed spices. The highest incidence of aflatoxin contamination was observed in nutmeg (80%), and the highest level of aflatoxin B_1 was observed in pistachio nuts (1382 ppb).

Contamination of foods and foodstuffs with aflatoxins, which are highly carcinogenic compounds produced by *Aspergillus flavus* and *A. parasiticus*, is one of the most serious problems of food hygiene. To reduce the risk, many countries regulated the maximum permissible levels of aflatoxins in foods; examples are as follows: 20 ppb in the United States, 15 ppb in Canada, 10 ppb in France, 10 ppb in the United Kingdom, 5 ppb in Australia, and 10 ppb in Japan (1).

Analytical results of aflatoxin contamination were reported by many researchers (2–6), but most of them examined only a few types of samples collected in a short period.

In our laboratory, about 600 samples, collected in Tokyo and mainly consisting of cereals, nuts, beans, spices, and dairy foods, were assayed for aflatoxins every year, beginning in 1982. In a previous study, we reported that aflatoxins were found in buckwheat, adlay, corn, peanut butter, butter beans, bean jam, nutmeg, white pepper, peanut oil, and natural cheese in the period from 1982 to 1985 (7).

This report summarizes analytical data on aflatoxin contamination in 3054 samples covering a wide variety of products collected in Tokyo from 1986 to 1990 and compares the results with our previous report (7) and other reports.

Materials and Methods

Samples

Commercially available market basket samples of foods and foodstuffs were collected in Tokyo during the years 1986—1990. Aflatoxins were determined in 3054 samples, which were made up of 803 samples of cereals, 540 samples of nuts, 540 samples of beans, 564 samples of spices, and 607 samples of other foods.

Received January 2, 1992. Accepted April 6, 1992.

Aflatoxin Determination

Aflatoxins were determined and confirmed by the method reported earlier (8), as follows: 200–1000 g samples were ground; aflatoxins were extracted with chloroform–water from 20 g ground sample, purified by Florisil column chromatography, and determined by high-performance thin-layer chromatography. Detection limits (ppb) were 0.2 for B_1, 0.1 for B_2, 0.2 for G_1, 0.1 for G_2, and 0.1 for M_1. Aflatoxin recoveries were 91% for B_1, 89% for B_2, 91% for G_1, 78% for G_2, and 92% for M_1.

Results and Discussion

Cereals, Sugars, and Their Products

Measurable levels of aflatoxins were found in rice products, coix seed, corn, muscovado (crude sugar), and fiber snack (Table 1).

Two samples of rice products were positive for aflatoxins: "siratamako" (a kind of rice powder) and a rice bran product used for Japanese pickles. In our survey, this was the first detection of aflatoxins in a rice product. It seemed likely that aflatoxin contamination of rice products resulted from imported rice, considering the temperature and humidity in Japan.

Of the coix seeds (*Coix lacryma-jobi* L. var. *ma-yuen*) examined, 10% contained aflatoxin. This incidence was lower than the 25% result in the 1982–1985 period (7).

Aflatoxin B_1 was found at levels of 0.1–0.4 ppb in 4 of 181 corn products examined. Of the positive samples, 2 were corn grits and 2 were corn snacks. Because aflatoxin B_1 was found in the corn grits used for the corn snack, we reasoned that the source of aflatoxin might be corn grits.

Of 9 sugars examined, 3 were contaminated with aflatoxins. All of the positive samples were muscovado (crude sugar) from the extreme south of Japan. No aflatoxin was detected in refined sugar.

The positive sample under "Others" in Table 1 was fiber snack. In recent years, vegetable fibers have drawn so much public attention as healthy food components that fibers are added to some snacks or drinks available in Japanese markets.

Table 1. Incidence and level of aflatoxins in cereals, sugar, and their products

Sample	No. of samples	No. of positives	Range (av. of positives), ppb			
			B_1	B_2	G_1	G_2
Rice	74	2	0.3–2.7 (1.5)	0–tr[a] (<0.1)	0–0.9 (0.5)	—
Wheat	168	0	—	—	—	—
Barley	104	0	—	—	—	—
Oat	14	0	—	—	—	—
Rye	6	0	—	—	—	—
Coix seed	49	5	0.1–0.6 (0.4)	—	—	—
Corn	181	4	0.1–0.4 (0.2)	—	—	—
Buckwheat	84	0	—	—	—	—
Sugar	9	3	1.0–1.5 (1.2)	0.1–0.2 (0.1)	—	—
Mixed flour	66	0	—	—	—	—
Others	48	1	0.4	—	—	—
Total	803	15	—	—	—	—

[a] tr = <0.1 ppb.

Fiber content of the positive sample was 30%, and the fiber originated from corn and beets. Because the risk of aflatoxin contamination of other materials (wheat flour, dry vegetables, and oil) was negligible, the source of aflatoxin appeared to be aflatoxin-contaminated corn that was carried through to the final product after processing.

No aflatoxin was detected in the buckwheat samples examined, although 20% of those examined were contaminated with aflatoxins during the period 1982–1985 (7). The reason for this difference was probably that buckwheat from Brazil was not imported after 1986.

Nuts and Their Products

Measurable levels of aflatoxins were found in peanut products, pistachio nuts, brazil nuts, and sesame products (Table 2).

Of 11 samples of aflatoxin-positive peanut products, 1 was crushed peanuts for sweets, 1 was peanut sweet, 4 were powdered peanuts for cooking, and 5 were peanut butter. No aflatoxin was detected in whole peanuts, but a high incidence of aflatoxins was detected in crushed peanuts (Table 3). The reason was probably that whole peanuts were higher grade, and crushed peanuts, whose grade the consumer cannot estimate, were lower grade.

Of 165 samples of pistachio nuts examined, 5 samples contained aflatoxins. The levels were high: 1 sample contained >1000 ppb, 2 contained >100 ppb, and the other 2 contained >10 ppb. All of the positive samples were from Iran. From 1982 to 1985, no aflatoxin was detected in any of the 95 samples of pistachio nuts examined (7). The difference seemed to be the country of origin. In the previous period, most of the samples were from the United States, whereas in this period, most samples were from Iran, because pistachio nut importation from Iran was increased.

One of 4 Brazil nut samples contained over 10 ppb aflatoxin B_1. The sample was from Brazil.

Table 2. Incidence and level of aflatoxins in nuts and their products

Sample	No. of samples	No. of positives	Range (av. of positives), ppb			
			B_1	B_2	G_1	G_2
Peanut	149	11	0.4–21.7 (4.1)	0.1–5.3 (0.9)	0–22.1 (2.8)	0–6.7 (0.8)
Cashew nut	56	0	—	—	—	—
Almond	43	0	—	—	—	—
Pistachio nut	165	5	11.5–1382 (323)	0.5–260 (58.0)	0–306 (61.3)	0–48.3 (9.7)
Brazil nut	4	1	10.2	0.8	3.2	0.3
Walnut	25	0	—	—	—	—
Macadamia nut	13	0	—	—	—	—
Sesame	19	2	0.6–2.4 (1.5)	0.2–0.5 (0.4)	—	—
Pumpkin seed	12	0	—	—	—	—
Pine seed	12	0	—	—	—	—
Mixed nut	15	0	—	—	—	—
Others	27	0	—	—	—	—
Total	540	19	—	—	—	—

Table 3. Incidence and level of aflatoxins in peanut and their products

Sample	No. of samples	No. of positives	Level of aflatoxin B_1, ppb	
			Av.	Range
Whole	125	0	—	—
Crushed	7	2	7.3	0.7–13.9
Powdered	9	4	5.9	0.5–21.7
Paste	8	5	1.4	0.4–2.7

Of 19 samples of sesame examined, 2 contained aflatoxins; 1 sample was washed sesame, and the other was topping for rice.

The level and incidence of aflatoxins in nuts in Tokyo were lower than those reported from other countries (3). Apparently, the check of imported nuts for aflatoxins in Japan is effective in removing aflatoxin-contaminated nuts.

Beans and Their Products

Measurable levels of aflatoxins were found in beans for bean-jam and white bean-jam (Table 4). All of the positive beans for bean-jam were butter beans from Myanma. No aflatoxins were detected in soybean. These results for beans were similar to those during the period 1982–1985 (7).

Spices

Measurable levels of aflatoxins were found in white pepper, red pepper, paprika, nutmeg, and mixed spice (Table 5).

Aflatoxins were found in 13% of white pepper samples. The incidence was lower than that in the 1982–1985 period (7), but the level was similar. Scott and Kennedy (2) and Wood (3) reported that no aflatoxins were detected in white pepper. We reasoned that the difference was due to the detection limit of the analytical methods: the limit for the method of Scott and Kennedy was 2.5 ppb for aflatoxin B_1 (2), whereas the limit of our method was 0.2 ppb (8).

Aflatoxins were detected in 2 of 10 red peppers examined. The aflatoxin B_1 level of one sample was 9.1 ppb, which is close to the regulatory level in Japan. Scott and Kennedy (2) reported that 10 of 33 red peppers (cayenne peppers) contained aflatoxins, a result that is in agreement with our results.

The level of aflatoxin in paprika was low, but the incidence was high (42%). This result was contrary to the data of Scott and Kennedy (2) and Wood (3): they reported the absence of aflatoxins in paprika. The difference in the country of origin of the paprika might be the reason.

The incidence of nutmeg samples contaminated with aflatoxins was extremely high (80%), but the level was lower than that in the 1982–1985 period (7). Nutmeg was imported from Indonesia or Malaysia. High incidences of aflatoxins in nutmeg were reported by Wood (3) and Beljaars et al. (4).

Other Foods

No aflatoxin was detected in the following foods (number of samples in parentheses): edible oil (27), cheese (37, tested for aflatoxin M_1 and M_2 as well as aflatoxin B_1, B_2, G_1, and G_2), butter (4), beef (14), pork (23), chicken (12), liver of hog (10), neat's liver (4), egg (13), hamburger steak (10), dry fruit (14), tea (27), coffee bean (8), cacao (5), cookie (48), fruit sauce (51), herb (27), and others (273).

Although 16% of natural cheese contained aflatoxin M_1 during the 1982–1985 period, no aflatoxin was detected in 37 samples assayed in the 1986–1990 period. In the previous period, the incidence and level of aflatoxin M_1 decreased year by year (2), and the tendency continued in this period. Aflatoxin M_1 in dairy foods is a metabolite of aflatoxin B_1 in dairy cattle; therefore, this result indicates that the aflatoxin contamination in feed for dairy cattle decreased during the past 10 years. The reason seems to be that the number of countries with legislation controlling aflatoxin in feedstuffs increased from 22 in 1981 to 35 in 1986. Also, the European Community directive was tight-

Table 4. Incidence and level of aflatoxins in beans and their products

Sample	No. of samples	No. of positives	Range (av. of positives), ppb			
			B_1	B_2	G_1	G_2
Soy bean	35	0	—	—	—	—
Soy bean paste	19	0	—	—	—	—
Azuki-bean	32	0	—	—	—	—
Tebo-bean	13	0	—	—	—	—
Beans for bean-jam	293	4	4.7–52.3 (17.9)	0.4–1.5 (0.7)	—	—
Bean-jam	68	1	0.1	—	—	—
Others	80	0	—	—	—	—
Total	540	5	—	—	—	—

Table 5. Incidence and level of aflatoxins in spices

Sample	No. of samples	No. of positives	Range (av. of positives), ppb			
			B_1	B_2	G_1	G_2
Black pepper	46	0	—	—	—	—
White pepper	84	11	0.1–1.8 (0.8)	0–0.3 (<0.1)	0–0.9 (0.2)	—
Red pepper	10	2	2.6–9.1 (5.9)	0–0.4 (0.2)	0–1.9 (1.0)	—
Paprika	12	5	0.2–1.3 (0.6)	—	0–0.2 (<0.1)	—
Nutmeg	108	84	0.2–13.4 (3.6)	0–2.5 (0.5)	0–2.8 (<0.1)	0–0.4 (<0.1)
Mace	22	0	—	—	—	—
Caraway	15	0	—	—	—	—
Thyme	14	0	—	—	—	—
Horseradish	9	0	—	—	—	—
Cinnamon	9	0	—	—	—	—
Coriander	6	0	—	—	—	—
Laurel	12	0	—	—	—	—
Clove	7	0	—	—	—	—
Hop	27	0	—	—	—	—
Ginger	13	0	—	—	—	—
Sage	14	0	—	—	—	—
Curry powder	11	0	—	—	—	—
Mixed spice	78	16	0–1.9 (0.8)	0–0.4 (<0.1)	0–0.5 (<0.1)	—
Others	67	0	—	—	—	—
Total	564	118	—	—	—	—

ened in 1984 when the tolerance for aflatoxin B_1 in feedstuffs for dairy cattle was reduced from 20 to 10 ppb (1).

References

(1) Egmond, H.P. (1989) *Food Addit. Contam.* **6**, 139–188

(2) Scott, P.M., & Kennedy, B.P.C. (1973) *J. Assoc. Off. Anal. Chem.* **56**, 1452–1456

(3) Wood, G.E. (1989) *J. Assoc. Off. Anal. Chem.* **72**, 543–548

(4) Beljaars, P.R., Schumans, J.C.H.M., & Konen, P.J. (1975) *J. Assoc. Off. Anal. Chem.* **58**, 263–271

(5) Davis, N.D., Currier, C.G., & Diener, U.L. (1986) *Cereal Chem.* **6**, 467–470

(6) Gagliardi, S.J., Cheatle, T.F., Mooney, R.L., Llewellyn, G.C., O'Rear, C.E., & Llewellyn, G.C. (1991) *J. Food Prot.* **54**, 627–631

(7) Tabata, S., Kamimura, H., Tamura, Y., Yasuda, K., Ushiyama, H., Hashimoto, H., Nishijima, M., & Nishima, T. (1987) *J. Food Hyg. Soc. Jpn.* **28**, 395–401

(8) Kamimura, H., Nishijima, M., Yasuda, K., Ushiyama, H., Tabata, S., Matsumoto, S., & Nishima, T. (1985) *J. Assoc. Off. Anal. Chem.* **68**, 458–461

CHEMICAL CONTAMINANTS MONITORING

U.S. Food and Drug Administration Survey of Methyl Mercury in Canned Tuna

NORMA J. YESS
U.S. Food and Drug Administration, Division of Contaminants Chemistry, Washington, DC 20204

Methyl mercury was determined by the U.S. Food and Drug Administration (FDA) in 220 samples of canned tuna collected in 1991. Samples were chosen to represent different styles, colors, and packs as available. Emphasis was placed on water-packed tuna, small can size, and the highest-volume brand names. The average methyl mercury (expressed as Hg) found for the 220 samples was 0.17 ppm; the range was <0.10–0.75 ppm. Statistically, a significantly higher level of methyl mercury was found in solid white and chunk white tuna than was found in chunk light and chunk tuna. Methyl mercury level was not related to can size. None of the 220 samples had methyl mercury levels that exceeded the 1 ppm FDA action level.

As a public health agency, the U.S. Food and Drug Administration (FDA) is interested in determining the presence and amount of potentially toxic elements in the food supply. One of the elements that has received great attention recently is mercury.

Mercury can enter the aquatic environment either as a result of human activity or from natural geologic sources, and mercury may be converted to methyl mercury (1). Fish can become a depository for methyl mercury directly through the water or by bioconcentration, the latter being especially important in large predatory fish such as tuna, halibut, shark, and swordfish (1). Because methyl mercury has a biological half-life of 2 years, it can be a persistent contaminant in fish (2).

Fish consumption increased considerably in the United States over the past 2 decades. Canned tuna, because of its relatively low cost, its convenience, its perception as a low-fat food (when water-packed), and its versatility as a recipe ingredient, is the most popular form of seafood consumed in the United States. According to the National Oceanic and Atmospheric Administration (3), 3.6 lb (1.6 kg) of canned tuna was consumed per capita in the United States in 1988, compared with 2.4 lb (1.1 kg) in 1968. Canned tuna accounted for 71% of the canned fish consumed in 1988 (3).

In view of increasing consumption of canned tuna and because of the potential toxic effects of methyl mercury (4), FDA has monitored this food/chemical combination for a number of years. An action level of 0.5 ppm total mercury was set in 1969. In 1978, an action level of 1 ppm methyl mercury was set (5).

In 1991, FDA conducted a survey of canned tuna for methyl mercury to provide information on the current levels of methyl mercury and to assist in the agency's ongoing evaluation of potential dietary exposure to methyl mercury through consumption of canned tuna.

Survey

Samples

FDA districts were instructed to collect 12 cans in duplicate for each sample (total of 24 cans per sample) from 12 randomly selected cases in a lot from large grocery store warehouses or wholesale distribution centers. U.S. consumption patterns show that 85% of the canned tuna consumed is water-packed and 15% is oil-packed (6); therefore, emphasis was placed on the former category.

Units consisted of the smallest can sizes available, and they included as many different brand names as possible, with emphasis on the highest-volume brand names. Samples included as many different styles of tuna (chunk, solid, etc.), colors (white, light, etc.), and pack (white chunk, light chunk, etc.) as available. The choice of samples was not based on any "statistical" sampling plan.

Because the standard of identity for canned tuna does not require that the species of tuna be listed on the label (7), specific species of tuna in cans could not be collected. In fact, canned tuna may represent a blend of different species; and the fish constituting the blend may vary widely in size, age, and region of catch (8).

Analysis

Samples were collected by inspectors from 18 FDA Districts throughout the United States. They were analyzed by 10 FDA laboratories.

Water-packed tuna was drained, and 12 individual cans of tuna from each sample were composited (12 cans were held in reserve). Because oil from oil-packed tuna might typically be mixed with the tuna before consumption, oil-packed tuna was not drained. Methyl mercury was determined by a liquid chromatographic/atomic absorption spectrophotometric (LC/AAS) method (9, 10). Briefly, the procedure consisted of the follow-

Received April 3, 1992. Accepted June 18, 1992.

ing steps: Each composite was blended in a food processor. Methyl mercury was isolated from the blended test sample by chloroform elution from a diatomaceous earth–hydrochloric acid column. The organomercury compound was then extracted into a small volume of sodium thiosulfate solution. An aliquot of this solution was injected into an LC column, and the methyl mercury was eluted with methanol–ammonium acetate solution (3 + 2). Mercury was determined by flameless AAS. The limit of quantitation of the method is approximately 0.1 ppm mercury (methyl-bound) (9, 10). Appropriate reagent blanks, standards, and fortified and unfortified test portions were analyzed.

Results and Discussion

A total of 220 samples of canned tuna were collected and examined for methyl mercury. Of those, 50 test samples contained <0.10 ppm methyl mercury (expressed as Hg). Statistical values of methyl mercury (expressed as Hg) found in the 220 samples were as follows: average, 0.17 ppm; median, 0.14 ppm; range, <0.10–0.75 ppm; and 90th percentile value, 0.42 ppm.

Descriptive information was obtained for many of the 220 samples from the collection reports filed by the FDA inspectors. This information was matched with the analytical data (Table 1). There was a statistically significant (analysis of variance) difference between the levels of methyl mercury in water-packed and spring water-packed tuna and in oil-packed and vegetable oil-packed tuna, with the former group being higher (0.18 and 0.21 ppm vs 0.07 and 0.10 ppm, respectively). (The probability that differences as large as those observed would occur if the group averages were really the same is, at most, 5%.) This difference is at least partially attributable to the dilution effect of the oil, which was blended with the tuna meat before analysis. Cappon and Smith (8) also found a higher level of methyl mercury in water-packed canned tuna than in the oil-packed type. However, the authors stated that because of the small number of samples (3 water-packed and 10 oil-packed), a statistical assessment was not possible.

In the FDA survey, levels of methyl mercury in solid white and chunk white tuna were significantly higher (analysis of variance) than in those products categorized as chunk light or chunk (0.26 and 0.31 ppm vs 0.10 and 0.10 ppm, respectively). The influence of oil or water on these methyl mercury levels cannot be assessed, because there were relatively few oil-packed products (5 solid white, 5 chunk, and no chunk white). There were 26 oil-packed chunk light tuna samples, with an average methyl mercury level of 0.06 ppm, compared with 0.11 ppm for 80 samples of water-packed tuna. There was no significant difference in methyl mercury levels for the different size cans in which the tuna was packed.

As part of the analytical process, mercury was determined in 93 reagent blanks by the participating laboratories. All of these blanks had values <0.10 ppm, the limit of quantitation of the method. In addition, 29 canned tuna samples fortified with 0.55–1.90 ppm methyl mercury were examined. The recoveries ranged from 70.0 to 107.4% (average 97.4%). Also, standard solutions of reagent methyl mercuric chloride were carried through the analytical procedure (9, 10) a total of 38 times by 1 of the participating laboratories to verify its recoverability. The recoveries ranged from 96.8 to 136.8% (average 105.2%).

Table 1. Analytical data for methyl mercury in 220 canned tuna samples matched with descriptive information

Variable	No. samples	Methyl mercury (expressed as Hg), av., ppm[a]	SD
Packing medium indicated on can[b]			
Water	117	0.18	0.16
Spring water	65	0.21	0.17
Vegetable oil	26	0.07	0.10
Oil	11	0.10	0.11
Style indicated on can[c,d]			
Chunk light	106	0.10	0.11
Solid white	71	0.26	0.16
Chunk white	19	0.31	0.17
Chunk	14	0.10	0.12
Can size, oz.[e,f]			
6 1/8	105	0.17	0.17
6 1/2	62	0.17	0.14
3 1/4	17	0.26	0.16

[a] Values recorded as <0.10 ppm were treated as 0.00 for calculations.
[b] No information, 1 sample.
[c] No information, 4 samples.
[d] Three samples of smoke-flavored and 3 samples of solid light tuna were also collected.
[e] No information, 21 samples.
[f] Fifteen samples were packed in other size cans.

Between 1978 and 1990, FDA determined methyl mercury in a total of 42 samples of canned tuna. The findings ranged from <0.10 to 0.67 ppm methyl mercury expressed as Hg with an average of 0.14 ppm (FDA, unpublished data, 1992); these results are similar to those of the 1991 survey. In 1973, FDA conducted a survey of canned tuna for total mercury. The average level of total mercury was 0.24 ppm for 253 samples (FDA, unpublished data, 1992). The ratio of total mercury to methyl mercury may vary among species (11). Hall (11) found that 89% (range, 64–119%) of the mercury in canned tuna was methyl mercury. If that relationship is assumed, then the average value for the 1973 survey analyses would be 0.21 ppm methyl mercury, somewhat higher than the results from the 1991 FDA survey.

Only 8 of the 220 samples of canned tuna in this survey contained >0.50 ppm methyl mercury (expressed as Hg). None of the 220 samples in the survey contained methyl mercury levels that exceeded FDA's 1 ppm action level.

Acknowledgments

The author acknowledges the FDA District inspectors and scientists who collected and analyzed the canned tuna. Thomas A. Allison and Ronald L. Lyles, Division of Program Operations, assisted with the design of the survey and furnished the descriptive information. Richard H. Albert, Division of Mathematics, provided the statistical evaluation.

References

(1) Wallace, R.A., Fulkerson, W., Shults, W.D., & Lyon, W.S. (1971) *Mercury in the Environment—The Human Element*, Oak Ridge National Laboratory, Oak Ridge, TN, pp. 4–6, 15–19

(2) "Hazards of Mercury" (1971) *Environ. Res.* **4**, 1–69

(3) Fisheries of the United States 1988 (May 1989) Current Fishery Statistics No. 8800, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Washington, DC, p. 80

(4) Tollefson, L., & Cordle, F. (1986) *Environ. Health Perspect.* **68**, 203–208

(5) U.S. Food and Drug Administration (1979) Action level for mercury in fish, shellfish, crustaceans, and other aquatic animals. *Fed. Regist.* **44**, 4012

(6) *Competitive Conditions in the U.S. Tuna Industry* (Oct. 1986) Publication 1912, U.S. International Trade Commission, Washington, DC

(7) *Code Fed. Regulat.* (1991) Title 21, Part 161.190

(8) Cappon, C.J., & Smith, J.C. (1982) *J. Appl. Toxicol.* **2**, 181–189

(9) Holak, W. (1989) *J. Assoc. Off. Anal. Chem.* **72**, 926–930

(10) *Official Methods of Analysis* (1990) 15th Ed., 1st Supplement, AOAC, Arlington, VA, sec. **990.04**

(11) Hall, E.T. (1974) *J. Assoc. Off. Anal. Chem.* **57**, 1068–1073

DRUGS IN FEEDS

Assay of Oxytetracycline in Animal Feed by Liquid Chromatography and Microbiological Plate Assay

MARY LEE HASSELBERGER
Nebraska Department of Agriculture Laboratory, 3703 S 14th St, Lincoln, NE 68502

In a proposed method for determining oxytetracycline (OTC) in animal feed, OTC is extracted with acidic methanol, and the extract is centrifuged and assayed by microbiological plate assay and by reversed-phase liquid chromatography (LC). Riboflavin and furazolidone elute at retention times similar to that of OTC. Successful separations are made by using both dimethylformamide and acetonitrile mobile phases. Variation between microbiological and LC results is comparable with variation within the microbiological assay. The relative standard deviation for 6 replicates of feed at the 100 ppm level is 3.6% for the LC assay and 3.1% for the microbiological assay.

Oxytetracycline (OTC), an antibiotic, is added to feeds for a variety of animal species. The official AOAC method (**968.50**), a microbiological plate assay, not only is time-consuming but also is nonspecific. Chlortetracycline (CTC), another antibiotic commonly added to feed, is a positive interference in the microbiological method. This paper compares the OTC results from microbiological and liquid chromatographic (LC) testing of extracts of animal feed.

Experimental

Scope

OTC guarantees range from 100 g/lb in premixes to 10 g/ton in finished feeds. The proposed LC method gives baseline separation, peak symmetry, and correlation with microbiological methods for the most common finished feed level, 100 g/ton, and for those feeds guaranteed at higher levels. Quality of chromatograms for feeds labeled <100 g/ton is matrix-dependent.

Apparatus

(a) *Liquid chromatograph.*—Waters Lambda Max Model 480 detector, Model 6000A pump, WISP Model 712 automatic sampler, and Model 730 data module (Millipore Corp., Waters Chromatography Division, Milford, MA 01757). Operating parameters: mobile phase flow rate, 1.3 mL/min; chart speed, 0.4 cm/min; wavelength, 365 nm; attenuation, 0.2 (premixes and concentrates) and 0.02 (finished feeds) AUFS; column temperature, ambient; injection volume, 10 μL.

(b) *LC columns.*—*(1)* Regis Octadecyl Workhorse, octadecylsilyl, 10 μm, 30 × 0.46 cm (Regis Chemical Co., Morton Grove, IL 60053-9975). Guard column, Waters Bondapak Corisil C_{18}. *(2)* Zorbax RX-C8 5 μm, 15 × 0.46 cm, and guard cartridge (MacMod Analytical Inc., Chadds Ford, PA 19137).

(c) *Sample filters.*—5 mL syringe barrels (No. 1603, Becton Dickinson and Co., Rutherford, NJ 07070) with 13 mm glass fiber filters (Gelman 66073, Gelman Sciences, Ann Arbor, MI 48106).

Reagents

(a) *OTC extractant, 957.23B(i).*—Concentrated HCl–methanol (1 + 50).

(b) *Mobile phases.—(1) Dimethylformamide (DMF).*—To be used with Regis column. Add 44.6 g sodium pyrophosphate (SX 0740, EM Science) to mixture of 1620 mL LC grade water and 20 mL phosphoric acid. Dissolve, add 360 mL DMF (DX 1726-1, EM Science, or equivalent), and adjust pH to 2.5 with additional phosphoric acid. Filter and degas. Adjust mobile phase to pH 2.5 daily. (See caution regarding column stability in section on columns and mobile phases.) *(2) Acetonitrile mobile phase.*—To be used with Zorbax column. Add 42.5 g potassium phosphate tribasic to mixture of 1680 mL LC grade water and 20 mL phosphoric acid. Dissolve, add 300 mL CH_3CN, and adjust pH to 2.5 with additional phosphoric acid. Add 1 mL triethylamine, mix, filter, and degas.

(c) *OTC stock solution.*—Accurately weigh ca 25 mg (to nearest 0.01 mg) United States Pharmacopeia Oxytetracycline Reference Standard into 50 mL volumetric flask, and dilute to volume with OTC extractant. Make appropriate dilutions in OTC extractant to generate 3-point standard line encompassing expected concentrations of samples to be tested. Correlation coefficient for 3-point line, based on either peak height or peak area, is usually 0.9999.

Extraction

Both extractions follow **968.50**, except that a 30 min shake is used instead of the specified 5 min shake. Aliquots with-

Received February 26, 1992. Accepted May 15, 1992.

Table 1. Sample weight and dilution table

Guarantee, g/ton	Sample weight, g	Extraction volume, mL	Dilution	Theoretical concn, µg/mL
1000	10	200	25/100	14
425	10	100	7/25	13
150	10	100	20/25	13
100	12	100	None	13

drawn from a series of extractions at 5, 15, 30, and 45 min show that maximum recovery is achieved between 15 and 30 min. The 30 min extraction time is commonly used in laboratories testing OTC in animal feed.

Sample weights are determined on the basis of the guarantee level and the sample homogeneity. Generally, 2–5 g weights are used for concentrates guaranteed at >1000 g/ton, and 10–20 g weights are used for lower guarantees. The dilution chart in Table 1 can be used for some common guarantee levels. These samples are analyzed at a detector setting of 0.020 AUFS against a 3-point standard line encompassing a concentration range of 9–15 µg/mL. The 15 µg/mL standard is prepared by diluting 3.0 mL stock standard, 25 mg (weighed accurately to 4 significant figures)/50 mL, to 100.0 mL. Then, 20.0 mL of the 15 µg/mL standard is diluted to 25.0 mL for a 12 µg/mL concentration, and 15.0 mL is diluted to 25.0 mL for a 9 µg/mL concentration. All standards are calculated to nearest 0.01 µg based on the actual weight used. Note that in these calculations, the actual potency of the current standard (indicated on the standard vial) and the factor (1.08) for converting base to OTC HCl should be used.

For guarantees higher than those listed in Table 1, samples can be either analyzed at 0.200 AUFS against a standard line ranging from 300 to 500 µg/mL or diluted to the range cited in Table 1.

The microbiological assay involves diluting to volume and filtering the pH-adjusted aliquot rather than filtering the subsequent dilution(s). Also, 0.15 µg/mL is used as the reference concentration instead of the specified 0.2 µg/mL. These minor modifications of the method were studied in the Associate Referee's laboratory and will be discussed in another paper after interlaboratory study has been completed. In many cases, more than one dilution of the sample was plated. If only one result is cited, it represents the zone that is most similar in diameter to the reference zone of the standard line.

For LC, the acidic methanol extract is clarified by centrifugation; diluted, if necessary, with extractant; filtered; and injected into the LC system. Standards are also prepared in extractant. The quantitative response is different if standards are prepared or diluted in mobile phase or 0.1N HCl.

Interferences

OTC and CTC are separated by LC. However, other interferences can be a problem with the LC method. Nonmedicated vitamin premixes, feed concentrates, and base mixes were extracted and chromatographed to determine possible interferences from other common feed ingredients. The only interferences noted were at retention times corresponding to riboflavin and furazolidone.

Adequate baseline separation of OTC, riboflavin, and furazolidone should be confirmed initially and as the column ages. Retention times of interest for the Regis Workhorse column used with the DMF mobile phase were as follows: OTC, 12.6 min; riboflavin, 11.3 min; furazolidone, 15.3 min; and CTC, 27.6 min. For the Zorbax RX C_8 15 cm column used with the CH_3CN mobile phase, retention times were as follows: OTC, 4.4 min; riboflavin, 6.2 min; furazolidone, 8.9 min; and CTC, 19.30 min.

Comparisons

During the past 8 years, several hundred sample extracts were tested by LC and microbiological methods. Good correlation between the 2 techniques was noted over this period. To obtain precision data, one sample labeled at 100 g/ton was tested in replicate by LC using the DMF mobile phase and by the microbiological plate assay. Sample weights of 10 g were extracted in 100 mL acidic methanol and processed as described above. The average for 6 replicates was 81.6 g/ton by the microbiological method and 78.4 g/ton by the LC method (relative standard deviations were 3.6 and 3.1%, respectively). Throughout this paper, all microbiological/LC comparisons are based on results from a single extract. Unless otherwise indicated, values represent single results, not averages of multiple determinations.

For a more structured comparison, I decided to test laboratory-fortified blank feeds and a series of feeds having different matrixes. Because few finished feeds are nonmedicated, a pig concentrate was diluted 1:1 with corn, as stated in the feeding instructions, and used as a blank feed. A premix was extracted, and the extract was used to wet spike corn and a 1:1 mixture of corn and pig concentrate to a level comparable with 100 g/ton. Extracts were tested by microbiological assay and by LC. Several dilutions of the pH-adjusted extract were plated. Results are shown for those dilutions giving zone diameters similar to that of the reference concentration, expressed as percent theoretical potency recovered (Table 2). The difference between the LC and microbiological results is comparable with the difference between the 2 sets of microbiological results.

Low-level cross-contamination of feeds can occur. If CTC precedes OTC in the manufacturing cycle, the feed may contain CTC at a low level. When feeds are tested for OTC by the

Table 2. Recovery (%) of OTC from wet spikes

Matrix	Microbiological		LC
	Dilution 1	Dilution 2	
Premix alone	102.3	100.0	98.9
Spiked corn	94.5	99.0	98.9
Spiked feed	94.9	91.2	96.8

Table 3. Recovery (g/ton) from feed spiked with OTC and with OTC and CTC

Matrix	Microbiological	LC
Feed spiked with OTC	92.0	91.3
Feed spiked with OTC and CTC	111.9	92.8

microbiological method, the result will be erroneously high because the test organism, *Bacillus cereus*, responds to both CTC and OTC. The microbiological response of CTC is 5 times greater than that of OTC, as evidenced by the reference concentration for the respective standard lines. To illustrate the microbiological and LC responses to CTC contamination, separate portions of a corn/pig concentrate mix were fortified with OTC standard alone and with a mixture of OTC and CTC standards. This was done to simulate the difference between a feed containing OTC alone and one contaminated with CTC. The feed fortification in Table 3 corresponded to levels of 100 g OTC/ton and 4 g CTC/ton. The CTC spike of 4 g/ton increased the apparent OTC potency from 92 g/ton to 112 g/ton, as expected.

The LC responses for OTC are identical for both spikes, because CTC elutes much later and does not interfere with quantitation of OTC. Chromatography using the OTC LC system is poor for the CTC peak; late-eluting peaks are broad. Levels of 10 g/ton would have to be present for a discernible peak to be obtained. If an analyst wishes to document CTC contamination below 10 g/ton, bioautography or a residue method for CTC should be considered.

The first set of feed samples used in this comparison consisted of the most common guarantee level (100 g/ton) from various major manufacturers. Portions of the same acidic methanol extract were tested both by the microbiological method and by LC. DMF mobile phase was used with the Regis column, and acetonitrile was used with the Zorbax column. These samples were chromatographed at 365 nm (see Table 4).

Results for samples 1–4 in Table 4 show little difference between microbiological and LC responses, despite the use of 2 different mobile phases and 2 different columns. LC results for sample 5 are in agreement but are lower than the microbiological result. Subsequent retesting gave parallel results. The baseline in the chromatogram for sample 5 shows a small blip at the retention time for CTC. As seen in the spiking study above,

Table 4. Comparison of microbiological result (g/ton) and LC results (g/ton) using Regis and Zorbax columns from the same sample extract

Sample No.	Microbiological	Regis	Zorbax
1	96	96	97
2	82	81	83
3	108	105	107
4	89	91	93
5	103	87	93

Table 5. Comparison of LC results (g/ton) using Regis and Zorbax columns and 2 different wavelengths

	365 nm		280 nm	
Sample No.	Regis	Zorbax	Regis	Zorbax
1	99	97	99	97
2	90	87	89	93
3	110	111	110	110
4	97	94	101	97
5	85	88	86	88

CTC at 3 g/ton or less can cause a 15 g/ton OTC response. Because the retention time for CTC with either column and mobile phase system is quite long, the low level of analyte and peak broadening make contaminant quantitation implausible within this method.

This same series of samples was re-extracted and tested again by LC using the analytical wavelength, 365 nm, and a secondary wavelength, 280 nm. Results are shown in Table 5. Although the chromatography at 280 nm is inferior to that at 365 nm, the correspondence of results at the 2 wavelengths supports the lack of interfering peaks in all samples (see Table 5).

In addition to the comparison study involving the 100 g/ton level, another comparison, shown in Table 6, involved a wider range of guarantee levels and swine and beef feed matrixes. Samples selected included the following: (*1*) a swine premix (8000 g/ton); (*2*) a beef concentrate (800 g/ton); (*3*) a sow concentrate (400 g/ton); and (*4*) a finished swine feed (100 g/ton), which also contained furazolidone and arsanilic acid. Figure 1 shows chromatograms obtained for the standard, sample 1, and sample 4 using the Regis column with the DMF mobile phase; Figure 2 shows the chromatograms obtained using the Zorbax column with acetonitrile mobile phase.

For the data in Table 6 and the previous data cited, the microbiological result and the LC results were determined from the same extract. In the microbiological method, different dilutions of the same pH-adjusted extract were made, and the average of the results from these dilutions appears in Table 6. Individual results expressed in g/ton are as follows: 5480, 5680, and 5860 for sample 1; 538, 559, and 540 for sample 2; 256, 261, and 283 for sample 3; and 73, 73, and 79 for sample 4. Thus, the difference between the average microbiological result and either of the LC results is no greater than the difference

Table 6. Comparison of microbiological result (g/ton) and LC results (g/ton) using Regis and Zorbax columns

Sample	Microbiological	Regis	Zorbax
1	5670	6000	5680
2	546	546	529
3	267	263	259
4	75	75	75

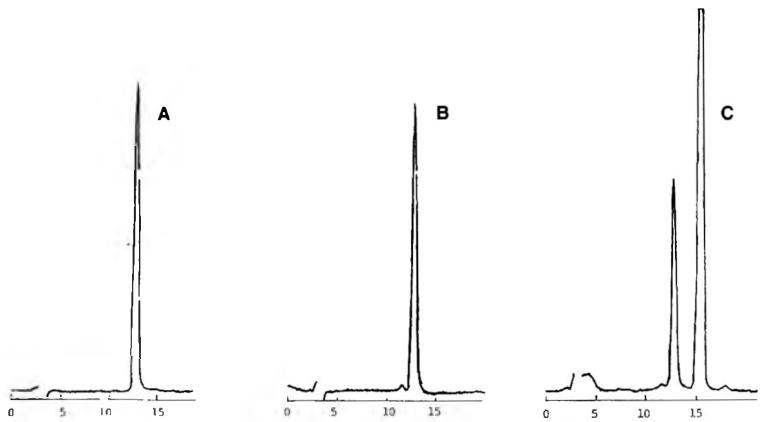


Figure 1. Chromatograms of OTC standard (A), sample 1 (B), and sample 4 (C) using Regis column with DMF mobile phase.

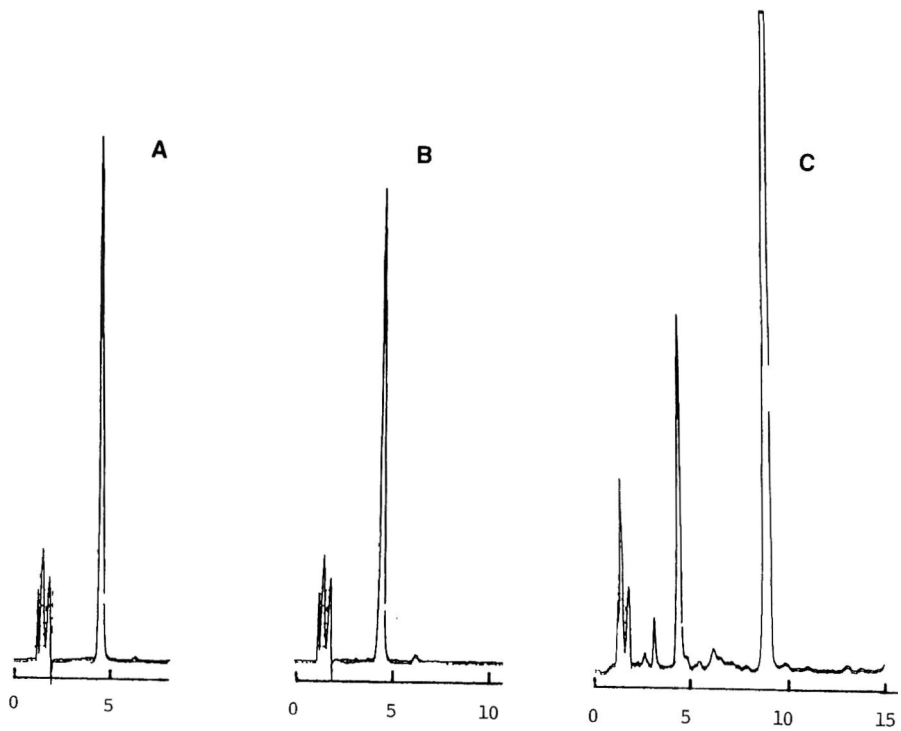


Figure 2. Chromatograms of OTC standard (A), sample 1 (B), and sample 4 (C) using Zorbax column with acetonitrile mobile phase.

between the microbiological results derived from different dilutions of the same pH-adjusted extract.

Comparison of Columns and Mobile Phases

The acetonitrile mobile phase not only is less toxic but also does not require that the column be dedicated. The DMF mobile phase has consistently given cleaner chromatograms; however, for long column life, the column should be stored in the buffered mobile phase. Dedicating a column can be a problem for small laboratories, although in practice the same column can be used for OTC, CTC, and carbadox–pyrantel tartrate analysis. The analysis was attempted on other C_8 and C_{18} columns, but the chromatograms were inferior with respect to baseline separation and peak shape.

Before a new column is used for analytical work, it should be equilibrated overnight with the DMF mobile phase at a low flow rate. Responses on our equilibrated columns show less than 2% drift in standard peak height or peak area, and responses vary less than 1% in retention time during a 12 h run. Results from multiple injections of the same test solution were well within 1%. If this stability is not achieved, more standards should be interspersed with the samples, and results should be calculated by bracketing.

The column and precolumn are stored in the DMF mobile phase. This procedure, presented by Barbatschi and Leese at the 97th AOAC International Meeting, Oct. 3–6, 1983, Washington, DC, was followed with all reversed-phase columns used with the DMF mobile phase. Column life is approximately 1 year. The LC system, after removal of the column, is thoroughly rinsed with DMF and water, then with water, and then with methanol–water. In addition, the pump seals are carefully flushed with water.

We have had over 10 years of experience with the DMF mobile phase. Use of the acetonitrile mobile phase was initiated last year, because some laboratories did not want to use DMF. Our experience with the acetonitrile system is limited. During a 4 h run, we noted approximately 1% change in retention time and 1–2% drift in peak area and peak height of standards.

Conclusion

For over 10 years, experience within our laboratory has shown good correlation between microbiological and LC results using the LC mobile phase suggested by Barbatschi and Leese for CTC separations. During the past year, good correlation was also obtained by using the Zorbax column with the acetonitrile mobile phase. The separation of furazolidone and riboflavin from OTC is column-dependent. As we become aware of more columns that will achieve adequate separation, we will make this information available.

Acknowledgments

The author thanks R. Beekley and L. Wetzler for the analytical work, C. Focht for assistance in preparation of the manuscript, and Robert Moody of MacMod for technical assistance.

EXTRANEOUS MATERIALS

Extraction of Light Filth from Oriental Fish Products Containing Spice: Collaborative Study

LARRY E. GLAZE
U.S. Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Collaborators: W. Davis, Jr; D.M. Floyd; C.R. Graham; J.K. Nagy; R.E. Olson; J. Palau; L.F. Spurlock; W.A. Sumner; W.T. VanVelzen

A collaborative study was conducted to validate a new method for the extraction of light filth from oriental fish products containing spice. A 100 g test portion is digested by boiling in a mixture of HCl, Igepal DM-710, and CO-730. Light filth is isolated by wet-sieving on a No. 230 plain-weave sieve with Tergitol, deaeration boiling in 40% isopropanol, and extracting with mineral oil–heptane (85 + 15) and 40% isopropanol in a Wildman trap flask. Three spiking levels for rat hairs and insect fragments were used in the study. For rat hairs, recoveries at the low, medium, and high levels averaged 80.0, 71.6, and 88.0%, respectively. Recoveries of insect fragments for low, medium, and high levels averaged 87.8, 83.7, and 89.4%, respectively. The method was adopted first action by AOAC International.

The increasing demand for oriental fish products in the United States is indicated by the large number of imported brands found in the retail markets. These products are prepared from several types of salt water and fresh water fishes, such as anchovy, carp, dace, eel, filefish, lungfish, mackerel, milkfish, sardine, saury, shark, tuna, and mud-skipper. They are imported from several Asian countries, including Thailand, the Philippines, the People's Republic of China, and Taiwan.

There is no AOAC official method for determination of light filth in oriental fish products. A new method was developed, which involves a digestion step that uses a combination of emulsifying agents and acid, wet-sieving with Tergitol, extraction of light filth with flotation liquid from a 40% isopropanol solution, and trapping in a Wildman trap flask. The present report describes a collaborative study of the method.

Submitted for publication November 1, 1991.
The recommendation was approved by the General Referee, Committee Statistician, Committee Safety Advisor, and by the Committee on Environmental Quality. The method was adopted first action by the Official Methods Board at their January 1992 meeting. Association actions will be published in "Changes in Official Methods of Analysis" (1993) *J. AOAC Int.* **76**, January/February issue.

Collaborative Study

The collaborative study was performed as a new method for the extraction of light filth from oriental fish products containing spice. The product was spiked at 3 different levels. The low spike level consisted of 5 insect fragments (elytral squares of *Tribolium confusum*, approximately 0.5 mm sq.) and 5 rat hairs (1–3 mm long). The middle spike level consisted of 15 insect fragments and 10 rat hairs. The high spike level consisted of 30 insect fragments and 15 rat hairs. Six test samples of product in metal containers and 2 vials of each spike level were sent to each of 9 collaborators. Test samples were numbered 1–6; spike vials were coded to correspond to the appropriate test sample. Two spiked samples (P1 and P2) were provided for use as "practice" portions. Collaborators were instructed to report results and analysis times and to return the extraction papers so that their results could be checked by the Associate Referee.

992.10 Light Filth from Fish Products Containing Spice—Flotation Method

First Action 1992

Method Performance
See Table 992.10 for method performance data.

A. Principle

The product is digested without affecting insect exoskeleton or mammalian hair filth contaminants. These oleophilic filth elements are separated from non-oleophilic food product by attraction to oil phase of oil/aqueous mixture. Oil phase is trapped off, filtered, and examined microscopically.

B. Pretreatment

Weigh 100 g test portion into 2 L beaker. Add 800 mL 5% HCl (40 mL HCl + 760 mL H_2O) and 15 mL Igepal (5 mL DM-710 and 10 mL CO-730), **945.75C(j)**. Cover beaker with watch glass, and bring contents to full boil while stirring on magnetic stirrer-hot plate, **945.75B(n)**. Remove watch glass, and boil gently with magnetic stirring for 60–90 min or until homoge-

Table 992.10. Method Performance for 992.10

Spike added		$\bar{X}$	s_r	s_R	RSD_r	RSD_R
Rat hairs	5	3.9 (4.0)[a]	1.2 (1.2)	1.3 (1.2)	31.1 (28.9)	33.2 (31.0)
	10	7.0 (7.2)	2.3 (2.3)	3.0 (2.9)	32.3 (32.6)	42.4 (40.0)
	15	11.9 (13.2)	2.7 (2.1)	2.7 (2.1)	22.2 (16.1)	22.3 (16.1)
Insect fragments	5	4.4 (4.4)	1.0 (1.0)	1.0 (1.0)	22.0 (22.0)	22.0 (22.0)
	15	12.5 (12.6)	1.7 (1.6)	2.8 (2.8)	13.7 (13.0)	22.1 (21.9)
	30	26.3 (26.8)	3.1 (3.4)	3.3 (3.5)	11.9 (12.6)	12.7 (13.0)

[a] Third party counts in parentheses.

neous slurry is obtained. (*Note*: Do not let product boil over during digestion procedure.)

C. Isolation

Transfer slurry portionwise onto No. 230 plain weave sieve and wet sieve, **970.66B(a)**, with forceful stream of hot tap water (55–70°) until rinse is clear. Use rubber policeman or spatula to remove residue adhering to sides of beaker. Add 25 mL Tergitol, **945.75C(bb)**, to residue on sieve, and let stand 2–3 min. (*Note*: Soaking residue in Tergitol on sieve for 2–3 min aids in dispersing clumped material.)

Wet sieve until all foam washes through and rinse is clear. Control excessive foaming with 40% isopropanol from wash bottle. Repeat Tergitol procedure. Wet residue on sieve with 40% isopropanol, and quantitatively transfer residue to 2 L Wildman trap flask, **945.75B(h)(4)**, using 40% isopropanol. (*Note*: Insert No. 10 rubber stopper into flask neck. If stopper bottom extends 1.5–2.0 cm into neck, flask has proper neck diam.; if <1.5 cm, flask may allow spillage during trapping procedure.) Dilute to 800 mL with 40% isopropanol, and boil gently 10 min with magnetic stirring. Remove from heat, wash walls of flask with 40% isopropanol, and cool in water bath to 28–30°. Remove from water bath, add 75 mL flotation liquid, **945.75C(k)**, to flask, and stir magnetically 10 min, **970.66B(c)**. Fill flask with 40% isopropanol. Stir bottom layer every 4–5 min during first 20 min, then let stand 10 min. Spin wafer disc or stopper to remove sediment and trap off while rinsing neck of flask with 40% isopropanol. Add 50 mL flotation liquid. Hand stir bottom contents with gentle up-and-down motion for 30 s. Fill flask with 40% isopropanol. Let stand 20 min. Trap off as before while rinsing neck with 40% isopropanol.

Filter onto ruled paper, **945.75B(i)**, and examine microscopically at ca 30×.

Ref.: JAOAC **76**, January/February issue (1993)

Results and Discussion

Statistical parameters calculated were mean recovery, $\bar{X}$; percent recovery, $\bar{X}$%; standard deviation and relative standard deviation for repeatability of the method, s_r and RSD_r, respectively; and standard deviation and relative standard deviation for reproducibility of the method, s_R and RSD_R, respectively.

Most collaborators obtained good recoveries of rat hairs (Table 1). The mean recoveries of rat hairs at the low, middle, and high spike levels were 80.0, 71.6, and 88.0%, respectively. Relative standard deviations for the spike levels were 28.9, 32.6, and 16.1% for repeatability and 31.0, 40.0, and 16.1% for reproducibility (Table 992.10). Collaborator G obtained low recoveries in both replicates of each spike level; the 2 replicates of the high spike level (7 and 9) qualified as outliers by the Grubbs test. No background striated hairs were reported.

Table 1. Collaborative results for recovery of rat hairs (blind duplicates) added to oriental fish product containing spice

	Spike level					
Coll.	5		10		15	
A	5	5	7	10	16 (15)[a]	10 (11)
B	3 (4)	6 (5)	8	6	12	14
C	5	2	6 (7)	9	11 (12)	13 (15)
D	4	4	10	9	12	14
E	4	4	8	8	7	15
F	5	4	7	3	10	11 (15)
G	1	3	1 (2)	3	7[b]	9[b]
H	2	4 (5)	9 (10)	2	14	11 (12)
I	5	5	10	10	15	14

[a] Third party counts are in parentheses if different from those of collaborator.
[b] Outlier by Grubbs test; not included in calculations.

Table 2. Collaborative results for recovery of insect fragments (blind duplicates) added to oriental fish products containing spice

	Spike level							
Coll.	5		15			30		
A	5	5	15		15	30	29	
B	5	5	14		13	26	27	(30)[a]
C	4	3	10		10	25	(29)	25
D	4	5	14		14	26	30	
E	3	5	12	(13)	15	27	26	(28)
F	4	5	12		7	19	29	
G	2	5	7		10	18	25	
H	5	4	15		12	26	28	
I	5	5	15		15	28	30	

[a] Third party counts are in parentheses if different from those of collaborator.

As shown in Table 2, recoveries of insect fragments were good. The mean recoveries for insect fragments at the low, middle, and high spike levels were 87.8, 83.7, and 89.4%, respectively. Relative standard deviations for the spike levels were 22.0, 13.0, and 12.6% for repeatability and 22.0, 21.9, and 13.0% for reproducibility (Table 992.10). Collaborator F reported low recoveries in 1 replicate of the middle spike level (47%) and in 1 replicate of the high spike level (63%). Collaborator G reported low recoveries in 1 replicate of the low spike level (40%) and in 1 replicate of the middle spike level (47%). No replicates qualified as outliers by the Cochran and Grubbs tests.

Collaborators' Comments

Six collaborators reported either no problem with the method or that the method was easy to perform, with easy-to-read extraction papers. Three collaborators reported that using 75 mL flotation liquid as specified in the method resulted in spillage during trapping. This spillage may account for some of the low recoveries reported. A variation in the manufacturer's design of the trap flasks used by these collaborators narrowed the neck in the flask. Therefore, all 2 L flasks to be used in this method must be checked for proper neck diameter. To do so, insert a No. 10 rubber stopper into the neck of the flask. If the bottom of the stopper extends into the neck a distance of 1.5–2.0 cm, the flask has the proper neck diameter. If the distance is less than this, spillage may result during the trapping procedure.

Several collaborators reported that some test samples weighed slightly less than the 100 g amount listed on the original container label; however, this discrepancy posed no problem with the method. Filter papers returned to the Associate Referee had light-to-medium product debris. The collaborators reported an average of 1.8 h (range 0.7–3.0 h) per test sample to perform the method.

Recommendation

I recommend that the proposed method for the extraction of light filth from oriental fish products containing spice be adopted first action. Recoveries were generally satisfactory, and the filter papers obtained were usually free of interfering debris.

Acknowledgments

I thank the following collaborators for their participation in the study:

Willie Davis, Jr, Florida Department of Agriculture, Tallahassee, FL

Deborah M. Floyd, U.S. Food and Drug Administration (FDA), Dallas, TX

C. Robert Graham, Lancaster Laboratories, Hershey, PA

Joseph K. Nagy, FDA, Philadelphia, PA

Richard Olson, FDA, Kansas City, MO

Joaquin Palau, FDA, San Juan, PR

Lisa F. Spurlock, FDA, New Orleans, LA

Wilfred A. Sumner, FDA, San Francisco, CA

W.T. VanVelzen, FDA, Seattle, WA

I also thank Foster D. McClure, Division of Mathematics, FDA, for statistical analysis of the data and Jack Boese, Division of Microbiology, FDA, for editing assistance.

EXTRANEOUS MATERIALS

Extraction of Light Filth from Oriental Sauces Containing Soy Sauce, Thickeners, and Spices: Collaborative Study

MARVIN J. NAKASHIMA
U.S. Food and Drug Administration, Division of Microbiology, HFF-237, Washington, DC 20204

Collaborators: J.A. Gallman; R.R. Haynos; C.E. Highfield; G.P. Kelly; H.K. Loechelt; P.A. Millward; W. Ocus; A. Rodriguez; M.D. Wirth

Results are reported for a collaborative study of a method for the extraction of light filth from oriental sauces containing soy sauce, thickeners, and spices. A 100 g test portion is pretreated in a 2% solution of Tergitol Anionic 4 over a steam bath, and oils are removed by wet-sieving on No. 230 sieve. Filth is isolated from 40% isopropanol by using Na_4EDTA and mineral oil. Average recoveries by 9 collaborators for 3 spike levels of rat hairs (5, 10, and 15) were 84, 78, and 79%, respectively; for insect fragments (5, 15, and 30), recoveries were 92, 95, and 96%, respectively. The method was adopted first action by AOAC International.

Oriental sauces encompassed by this method typically contain soy sauce (made from water, soybeans, wheat, and salt) as the main ingredient, thickener (xanthan gum, guar gum, starch, or flour), garlic, spices, onions, sesame seeds, and chilies. Other thickeners that are also important ingredients in sauces are black beans and pastes of sesame seeds, tomatoes, and soybeans. Other spices in the sauces are black and white peppers and ginger. Types of oriental sauces tested with the method, in descending order of frequency encountered, were hoisin, barbecue, shabu-shabu, steak, teriyaki, sweet and sour, stir-fry, seasoning, black beans, spare-rib, szechuan, chap kam, char siu, hunan, Cantonese, bulgogi, goma syabu, tonkatsu, kung pao, plum, peking, oriental salad dressing, and oriental marinade. Sauces are generally packed in jars and are not refrigerated.

A principal source of contamination of oriental sauces is rodent and insect-defiled spices. Because of the variety of sauce types, a method for the extraction of light filth from oriental sauces was developed to cover as many types of sauces as possible under one generalized description. The method consists of removing oils from sauces by treatment with Tergitol Anionic 4 and wet-sieving on a No. 230 screen. The residue is transferred to a 2 L Wildman trap flask with 40% isopropanol, and light filth is extracted with Na_4EDTA and mineral oil. The mineral oil is trapped and filtered, and the filth is counted.

Submitted for publication October 28, 1991.
This report was presented at the 105th Annual AOAC International Meeting, August 12–15, 1991, at Phoenix, AZ.
The recommendation was approved by the General Referee and the Committee on Microbiology and Extraneous Materials and was adopted by the Official Methods Board of the Association. See "Changes in Official Methods of Analysis" (1993) *J. AOAC Int.* **76**, January/February issue.

Collaborative Study

Each collaborator received 2 jars of hoisin sauce and 6 separate vials randomly numbered from 1 through 6. Two vials each of the following 3 analyte levels were provided: 5 rat fur hairs (2.5–3.5 mm long) and 5 insect fragments (elytral squares of *Tribolium confusum* (ca 0.5 mm sq.); 10 rat fur hairs and 15 insect fragments; and 15 rat fur hairs and 30 insect fragments. The spike was mixed with granules of sucrose to prevent collaborators from seeing the enclosed spikes. Collaborators were to weigh six 100 g test portions from the 2 jars of hoisin sauce and add to each portion the contents from a numbered vial. Collaborators were instructed to report their analytical times and to return the filter papers so that the Associate Referee could check their results.

992.12 Light Filth in Sauces Containing Soy Sauce, Thickeners, and Spices—Flotation Method

First Action 1992

(Applicable to determination of light filth in sauces containing soy sauce, thickeners, and spices.)

Method Performance

See Table 992.12 for method performance data.

A. Principle

Oils are removed from the sauce sample by pretreatment with wetting agent over steam and then by wet-sieving. Residue is transferred to Wildman trap flask with 40% isopropanol, and light filth is extracted with Na_4EDTA and mineral oil. The flotation liquid is trapped and filtered, and the filth is counted.

Table 992.12. Method performance for 992.12, light filth in sauces containing soy sauce, thickeners, and spices[a]

Spike added		$\bar{X}$	s_r	s_R	RSD_r	RSD_R
Rat hairs	5	4.5 (4.2)	0.7 (0.8)	0.8 (1.0)	15.6 (19.0)	17.8 (23.8)
	10	7.9 (7.8)	1.5	2.0 (2.1)	19.0 (19.2)	25.3 (26.9)
	15	11.9 (11.8)	1.2 (1.3)	2.4 (2.5)	10.1 (11.0)	20.2 (21.2)
Insect fragments	5	4.9 (4.6)	0.8 (0.6)	1.0 (0.7)	16.3 (13.0)	20.4 (15.2)
	15	13.6 (14.2)	1.0	1.5 (1.0)	7.4 (7.0)	11.0 (7.0)
	30	28.8	1.6 (1.0)	1.6 (1.0)	5.6 (3.5)	5.6 (3.5)

[a] Third party's counts in parentheses.

B. Reagents

(a) *Wetting agent.*—Sodium tetradecyl sulfate (Tergitol Anionic 4, Sigma Chemical Co, or equivalent).

(b) *Tetrasodium EDTA solution.*—Na_4EDTA in 40% isopropanol, **945.75C(z)**.

C. Pretreatment

Add 100 g well-mixed sample to 1.5 L beaker. Add 980 mL H_2O and 20 mL Tergitol Anionic 4, **(a)**, to beaker. Heat over steam bath 30 min. Stir beaker contents occasionally with glass rod.

D. Isolation

Rinse rod with water, and wet-sieve beaker contents portionwise, **970.66B(a)**, onto No. 230 plain weave sieve, **945.75B(r)**, with a forceful stream of hot tap H_2O, if necessary. Wet-sieve until foaming subsides, excess oil is removed, and no further reduction of residue is noted.

Work residue to side of sieve with gentle spray of hot tap H_2O. Wet residue with 40% isopropanol and quantitatively transfer residue to 2 L trap flask, **945.75B(h)***(4)*, with 40% isopropanol. Fill flask to 400 mL by rinsing sides of flask with 40% isopropanol. Boil flask contents *gently* 10 min with magnetic stirring. Remove flask from heat, and immediately add 50 mL Na_4EDTA solution, **(b)**, slowly down-stirring rod. Hand-stir contents 1 min with gentle rotary motion, ensuring that residue does not adhere to sides of flask. Let stand 5 min. Fill flask to 800 mL by rinsing sides of flask with 40% isopropanol. Add 50 mL mineral oil, **945.75C(p)**, down-stirring rod. Stir contents magnetically, **970.66B(c)**, 3 min. Fill flask with 40% isopropanol down-stirring rod. Stir contents intermittently every 5 min for 20 min. Bring stirring rod to midpoint of flask, rinse rod with 40% isopropanol and clamp, and let stand 10 min. Trap off into 400 mL beaker and rinse neck, wafer, and rod with 40% isopropanol. Add 35 mL mineral oil and hand-stir contents 1 min to disperse oil. Repeat procedures from "Fill flask with 40% isopropanol . . ." as for first trapping.

Filter beaker contents onto ruled paper, **945.75B(i)**, washing 400 mL beaker, and filter washings with 40% isopropanol. Examine paper microscopically at ca 30×.

Ref.: JAOAC **76**, January/February issue (1993)

Results and Discussion

Collaborators' counts of recoveries of added rat hairs and insect fragments (Tables 1 and 2, respectively) were checked by the Associate Referee and verified by an expert microanalyst when the Associate Referee's counts differed from the collaborator's. Before an analysis of variance of the data, Grubbs and Cochran tests were done for outliers (Youden, W.J., & Steiner, E.H. (1975) *Statistical Manual of the AOAC*, AOAC International, Arlington, VA). Average recoveries for 3 spike

Table 1. Collaborative results for recovery of rat hair spikes (blind duplicates) from oriental sauces containing soy sauce, thickeners, and spices

	Spike level					
Coll.	5		10		15	
1	5 (4)[a]	3	4	8 (7)	12	11 (9)
2	5	6 (5)	10	10	11	13
3	5	5	9	8	13	11
4	4	5	7 (6)	9	15 (14)	12
5	4	5	8	8	14	14
6	4	4	7	4	10	13
7	3	4 (3)	7 (6)	6 (7)	11 (12)	11
8	5	5	11	10	15	15
9	4 (2)	5	7	10	7	7

[a] Third party counts are in parentheses if different from those of collaborator.

Table 2. Collaborative results for recovery of insect fragment spikes (blind duplicates) from oriental sauces containing soy sauce, thickeners, and spices

Coll.	Spike level											
	5			15				30				
1	6	(5)[a]	7	(5)	14		14		30	(29)	30	
2	5		5		14		13	(14)	25	(28)	29	(30)
3	4		3		11	(11)[b]	11	(11)[b]	28		30	
4	5		5		14		13		29	(30)	30	
5	3		5		12	(12)[b]	12	(12)[b]	28		29	
6	4		5		15		14		28		28	
7	6	(5)	5		16	(15)	14		29		29	(28)
8	5		5		14		15		29		28	
9	6	(5)	4		16		13		27		32	(29)

[a] Third party counts are in parentheses if different from those of collaborator or if either duplicate value is an outlier.
[b] Outlier by Grubbs test; not included in calculations.

levels were 84, 78, and 79% for rat hairs and 92, 95, and 96% for insect fragments (Table 992.12), which are comparable to in-house studies in which recoveries were 82 and 91%, respectively, for 99 replicates representing 20 brands of oriental sauces. None of the data for rat hairs or insect fragments indicates any recovery problems with the method. Variability measures (standard deviation, s, and relative standard deviation RSD) were acceptable for rat hairs (s, 0.8–2.5; RSD, 11–27%) (Table 992.12), with more variability in measures of reproducibility than repeatability. Variability measures were low for insect fragments (s, 0.6–1; RSD, 3–15%) (Table 992.12).

Collaborators took an average of 1.8 h (range 0.5–3 h) to isolate the filth and 0.33 h (range 0.1–0.5 h) to count the plates. All collaborators needed only 1 paper per test portion. Collaborators 2, 5, and 9 had 3–6 test portions for which the amount of trapped product was acceptable but was heavier than expected. Papers from collaborator 2 had many more pieces of garlic tissue than those of other collaborators. Discussions with collaborator 2 revealed no reason for this difference. Collaborator 5 indicated that the water used for sieving the first 3 test portions was not hot, as required by the method. Although the sauce does not contain large amounts of oil, it is still necessary to remove the oil; otherwise, residual oil may remain on the product and be trapped along with the filth. The collaborator's final 3 test portions sieved with hot water had less product debris. A talk with collaborator 9 disclosed that the individual vigorously stirred the mineral oil for the second trapping. The collaborator observed that considerably more product was trapped in the second trapping compared with the first.

Recommendation

On the basis of satisfactory to excellent analyte recoveries and acceptable to excellent precision measurements, I recommend that the proposed method for extraction of light filth from oriental sauces containing soy sauce, thickeners, and spices be adopted first action.

Acknowledgments

I express my appreciation to the following analysts for participating in this study:

James A. Gallman, U.S. Food and Drug Administration (FDA), Atlanta, GA

Richard R. Haynos, Certified Analytical Group, Corona, NY

Charles E. Highfield, Health Protection Branch, Scarborough, ON, Canada

Gerald P. Kelly, Herbert V. Shuster, Inc., Quincy, MA

Hans K. Loechelt, FDA, Bothell, WA

Patricia A. Millward, FDA, Winchester, MA

Warren Ocus, McCormick and Co., Hunt Valley, MD

Alfredo Rodriguez, Anresco, Inc., San Francisco, CA

Michael D. Wirth, FDA, Buffalo, NY

I also wish to thank the following individuals from FDA, Washington, DC, for their assistance: Linda K. Bullock and Foster D. McClure, Division of Mathematics, for statistical analyses, and John R. Bryce, Division of Microbiology, for check counting of collaborative extraction plates.

EXTRANEOUS MATERIALS

Extraction of Light Filth from Tofu: Collaborative Study

MARVIN J. NAKASHIMA
U.S. Food and Drug Administration, Division of Microbiology, HFF-237, Washington, DC 20204

Collaborators: J.M. Hajoway; H. Keizer; E.D. McGary; W.A. Senff; M.L. Silva; L.F. Spurlock; M.A. Stenske; A. Torn; M. Wheeler

Results are reported for a collaborative study of a method for the extraction of light filth from tofu. A 100 g test portion is digested in HCl solution with Igepal CO-730 and Igepal DM-710. Hairs and insect fragments are isolated by wet-sieving on a No. 230 sieve, dispersing remaining residual product with Aerosol OT 75%, and filtering. Average recoveries by 9 collaborators for 3 spike levels of rat hairs (5, 10, 15) were 80, 78, and 84%, respectively; for 3 spike levels of insect fragments (5, 15, 30), recoveries were 97, 99, and 99%, respectively. The method was adopted first action by AOAC International.

Tofu is a precipitated soybean curd product prepared by adding calcium sulfate to heated soybean milk. Tofu has been an important source of protein for centuries in the Orient. As a result of the great influx of Asian immigrants into the United States over the past few years, demand for oriental food products, including tofu, has greatly increased.

Tofu is generally stored under refrigeration, except when the product is canned. The degree of product firmness (soft, regular, or firm) and other product names, such as bean curd, soybean curd, or soybean cake, may be stated on product labeling in addition to the name tofu.

Because the name tofu is found on related tofu products, which should not be confused with the off-white, gelatinous product of this method, a brief explanation of these products that were altered to extend the shelf life of tofu is presented. Tofu is fried (fried tofu, plain and flavored), dried (freeze-dried), and fermented (preserved). Because of tofu's bland nature, products containing tofu as a primary ingredient are flavored either by soaking the tofu in seasoning solution or by adding sauce to the tofu cubes. Flavored, fried tofu is usually canned, whereas preserved tofu is packed in jars. Neither form is refrigerated. Plain, fried tofu and flavored tofu are usually packed in cellophane or vacuum packages and refrigerated.

The tofu method reported here represents the first collaborative study of a method for soybean curd products. This method entails boiling for 30 min in 5% HCl solution with Igepal CO-730 and Igepal DM-710 to digest the curd, wet-sieving (No. 230), further dispersing of any undigested product with Aerosol OT 75%, and filtering. The method cannot be applied to other forms of tofu, because the original gelatinous composition has been changed by frying and drying, or condiments have been added that are not digested by HCl and Igepal.

Collaborative Study

Each collaborator received 3 cans of tofu and 6 gelatin capsules of spike material in separate vials randomly numbered from 1 through 6. Two capsules each of the following 3 analyte levels were provided: 5 rat fur hairs (2.5–3.5 mm long) and 5 insect fragments (elytral squares of *Tribolium confusum* about 0.5 mm sq.), 10 rat fur hairs and 15 insect fragments, and 15 rat fur hairs and 30 insect fragments. Capsules were filled with filter-paper clippings to prevent collaborators from seeing the enclosed spikes. A container of Aerosol OT 75% was sent to each collaborator, because small quantities of this surfactant are not currently available. Collaborators were instructed to weigh six 100 g test portions from the 3 cans of tofu and to add a spike capsule from a numbered vial to each portion. Collaborators were instructed to report their analytical times and to return the filter papers so that the Associate Referee could check their counts.

992.13 Light Filth in Tofu—Sieving Method

First Action 1992

(Applicable to determination of light filth in tofu.)
Method Performance
See Table 992.13 for method performance data.

A. Principle

Tofu is acid-digested with emulsifiers and wet-sieved. Residual product is dispersed with surfactant solution. Residue is filtered, and filth is counted.

Submitted for publication November 1, 1991.
This report was presented at the 105th Annual AOAC International Meeting, August 12–15, 1991, at Phoenix, AZ.
The recommendation was approved by the General Referee and the Committee on Microbiology and Extraneous Materials and was adopted by the Official Methods Board of the Association. See "Changes in Official Methods of Analysis" (1993) *J. AOAC Int.* **76**, January/February issue.

Table 992.13. Method performance for light filth in tofu[a]

Spike added		$\bar{X}$	s_r	s_R	RSD_r	RSD_R
Rat hairs	5	4.3 (4.0)	1.1 (0.7)	1.2 (1.1)	25.6 (17.5)	27.9 (27.5)
	10	7.9 (7.8)	1.5 (1.6)	2.2 (2.0)	19.0 (20.5)	27.8 (25.6)
	15	12.7 (12.6)	2.1 (2.4)	2.2 (2.4)	16.5 (19.0)	17.3 (19.0)
Insect fragments	5	5.0 (4.8)	0.6 (0)	0.6 (0.4)	12.0 (0)	12.0 (8.3)
	15	14.6 (14.9)	1.2 (0.3)	1.2 (0.3)	8.2 (2.0)	8.2 (2.0)
	30	29.9 (29.8)	1.2 (0.6)	1.2 (0.9)	4.0 (2.0)	4.0 (3.0)

[a] Third party's counts in parentheses.

B. Reagents

(**a**) *Emulsifiers.*—Igepal CO-730, **945.75C(j)***(1)*, and Igepal DM-710, **945.75C(j)***(2)*.

(**b**) *Surfactant solution.*—Sulfobutanedioic acid 1,4-bis(2-ethylhexyl) ester sodium salt (docusate sodium) in ethanol–H_2O (Aerosol OT 75%, American Cyanamid is suitable).

(**c**) *Hydrochloric acid solution.*—37% HCl, v/v.

C. Isolation

Weigh 100 g sample into 2 L beaker. Add 950 mL H_2O and 50 mL HCl (37%) to sample. Add 5 mL Igepal CO-730, **945.75C(j)***(1)*, and 10 mL Igepal DM-710, **945.75C(j)***(2)*. Cover beaker with watch glass, and bring mixture to full boil with magnetic stirring, **945.75B(n)**. Remove watch glass, and boil mixture 30 min, magnetically stirring so top of stirring bar is visible at bottom of vortex. Wet-sieve digestate, **970.66B(a)**, on No. 230 plain weave sieve, **945.75B(r)**, with hot tap water until material no longer washes through. Add ca 10 mL Aerosol OT 75%, and gently wet-sieve until suds disappear. Repeat Aerosol treatments until residue is reduced to filterable amounts. At most, 4 washes should be needed. Quantitatively transfer residue to 400 mL beaker with H_2O. Filter onto ruled paper, **945.75B(i)**. Wash beaker with H_2O; filter washings. Examine microscopically at ca 30×.

Ref.: JAOAC **76**, January/February issue (1993)

Results and Discussion

Collaborators' counts of recoveries of added rat hairs and insect fragments (Tables 1 and 2, respectively) were checked by the Associate Referee and verified by an expert microanalyst when the Associate Referee's counts differed from the collaborator's. Before an analysis of variance of the data, Grubbs and Cochran tests were done for outliers (Youden, W.J., & Steiner, E.H. (1975) *Statistical Manual of the AOAC*, AOAC International, Arlington, VA). Table 2 shows that a number of outliers were found, and up to 2 laboratories were excluded per column (collaborator and third party) of data. Average recoveries for 3 spike levels were 80, 78, and 84% for rat hairs and 97, 99, and 99% for insect fragments (Table 992.13), which are comparable to in-house studies in which recoveries were 84 and 98%, respectively, for 22 replicates. Only collaborator A had consistently low recoveries (rat hairs). A discussion with the collaborator revealed that the sieve used in the study had a 4 × 1/230 in. tear, which could have trapped hairs and prevented their transfer to the beaker. Variability measures (standard deviation, s, and relative standard deviation, RSD) were acceptable for rat hairs (s, 0.7–2.4; RSD, 18–28%) and very low for insect fragments (s, 0–0.9; RSD, 0–8%) (Table 992.13).

Collaborators took an average of 1.6 h (range, 0.8–2.5 h) to isolate the filth and 0.4 h (range, 0.2–1.0 h) to count plates. An

Table 1. Collaborative results for recovery of rat hair spikes (blind duplicates) from tofu

	Spike level					
Coll.	5		10		15	
A	3 (2)[a]	5 (3)	5	3	7 (6)	12
B	7 (5)	5	9	8	12 (13)	14
C	5	5	10	7	14 (12)	15
D	4	2 (3)	8	9	13	15
E	2	5 (4)	10 (8)	12 (11)	13 (12)	13 (15)
F	4	3	9	5	13	15 (14)
G	5	5	7	9	9	13
H	4	5	9	9	14	14
I	4	4	7	7	14	9

[a] Third party counts are in parentheses if different from those of collaborator.

Table 2. Collaborative results for recovery of insect fragment spikes (blind duplicates) from tofu

Coll.	Spike level											
	5				15				30			
A	5[a]		5[a]		15	(15)[a,b]	15	(15)[a,b]	32		29	(30)
B	5		5		15		15		30	(29)	28	
C	5		5		15		15		29	(30)	30	
D	4	(4)[c]	6	(5)[c]	15		15		30		30	
E	6	(6)[c]	4	(4)[c]	15		15		30		30	
F	4[d]	(4)[a]	4[d]	(4)[a]	16	(16)[c]	16	(13)[c]	28[d]	(28)[c]	21[d]	(22)[c]
G	5		5		14	(14)[a]	15	(15)[a]	30		29	
H	5[d]		6[d]	(5)	14	(15)	14	(15)	30		33	(30)
I	5		5		11	(12)[d]	15	(15)[d]	29		29	

[a] Outlier by Grubbs test, but included in calculations because its removal would result in more than a 2/9 reduction in the original number of laboratories.
[b] Third party counts are in parentheses if different from those of collaborator or if either duplicate value is an outlier.
[c] Outlier by Cochran test.
[d] Outlier by Grubbs test.

average of 2.3 (mode and median of 2) Aerosol OT 75% washes were used. With the exception of collaborator H, who used 2 papers for a test portion, all collaborators used only 1 paper per test portion. Collaborators C and E had 4 and 3 test portions, respectively, which had greater than expected, but acceptable, amounts of undigested tofu on their papers. Because Aerosol OT 75% is most effective when the tofu is finely digested, the collaborators may have experienced some problems in the digestion. Conversations with both collaborators disclosed no discernible differences in the performance of the method for test portions that were effectively dispersed by Aerosol OT 75% from those that were dispersed less effectively. Collaborators C and E used tofu from different lots. Four other collaborators used the same lots of tofu as collaborators C and E and had less residual product on their papers. An explanation for this could be product variation. In-house studies showed some differences in the degree of digestion for canned tofu, which resulted in noticeably more residue on the paper, although not to the same degree as experienced by collaborators C and E.

Recommendation

On the basis of excellent analyte recoveries and acceptable to excellent precision measurements, I recommend that the proposed method for extraction of light filth from tofu be adopted first action.

Acknowledgments

I express my appreciation to the following analysts for participating in this study:

Joanne M. Hajoway, U.S. Food and Drug Administration (FDA), Philadelphia, PA

Henry Keizer, Analytical Entomology, Elk River, MN

Eddie D. McGary, FDA, Kansas City, MO

Wynn A. Senff, FDA, Cincinnati, OH

Mary Lou Silva, Rhode Island Dept of Health, Providence, RI

Lisa F. Spurlock, FDA, New Orleans, LA

Mark A. Stenske, Michigan Dept of Agriculture, East Lansing, MI

Alena Torn, Health Protection Branch, Burnaby, British Columbia, Canada

Mark Wheeler, U.S. Department of Agriculture, Beltsville, MD

I also would like to thank Linda K. Bullock and Foster D. McClure, FDA, Division of Mathematics, Washington, DC, for statistical analyses and John R. Bryce, FDA, Division of Microbiology, Washington, DC, for check counting of collaborative extraction plates.

FOOD ADDITIVES

Enzymatic Determination of Sulfite in Foods: NMKL[1] Interlaboratory Study

ULLA EDBERG
Swedish National Food Administration, PO Box 622, S-751 26 Uppsala, Sweden

Collaborators: Maija-Liisa Anttonen; Karen Berg-Nilsen; Karin Blomberg; Karl Olav Gjerstad; Tomas Gustavsson; Per Joner; Jukka Loimaranta; Antti Mustranta; Mikko Oksanen; Leena Pero; Björn Sönsterud

An enzymatic method for the determination of sulfite in foods was collaboratively studied in Nordic industry and government laboratories. The sulfite in liquid foods or extracts of solid foods is analyzed according to the following principle: Sulfite ions are oxidized to sulfate ions by oxygen in the presence of sulfite oxidase, thereby forming hydrogen peroxide. Hydrogen peroxide is transformed to water by reduced nicotinamide adenine dinucleotide (NADH) in the presence of NADH peroxidase. In this reaction, NAD^+ is formed (and NADH is consumed) in amounts proportional to the sulfite concentration. Consumption of NADH can be measured spectrophotometrically at 340 nm. The method was collaboratively tested in 2 separate studies with high and low levels of sulfite tested. Results of both studies are reported here. The study samples consisted of potato flakes, wine, juice, and dried apples containing between 0 and about 960 mg SO_2/kg. Eleven laboratories participated in the full study and analyzed 12 samples. Six laboratories analyzed 8 samples in the complementary study. Before statistical evaluation of the collaborative study data, results were adjusted for the time-dependent decrease of sulfite in the case of materials with high sulfite content (dried apples and wine). For 2 blind duplicate samples of wine containing 75 mg SO_2/kg, the relative standard deviation for repeatability (RSD_r, within-laboratory variation) was 3.9%. Relative standard deviation for reproducibility (RSD_R, between-laboratory variation) was 7.6%. For 2 samples of dried apples containing 800 and 960 mg SO_2/kg, an RSD_r value of 13.3% and an RSD_R value of 13.9% were calculated. The corresponding parameters for 2 juice samples containing about 270 mg SO_2/kg were 4.8 and 10.4% for repeatability and reproducibility, respectively.

Sulfites have been used as preservatives in foods for many years (1). The addition of sulfite to food is regulated in the Nordic countries. Regulatory levels vary between 10 mg/kg and 3 g/kg. In this study, foods that are common in the market were chosen as matrixes. The concentration range of sulfite in the samples were chosen according to the legal limits in most Nordic countries. The limits are as follows: wine, 200–400 mg/L; fruit juice, 10–300 mg/L; dried apples, 1500–2000 mg/kg; and potato flakes, 500 mg/kg. Recently, some people, especially asthmatics (2), were found to exhibit adverse reactions to sulfite in food. Consequently, simple and inexpensive methods for the determination of sulfite are needed. The method of choice for sulfite estimation has been the Monier-Williams method, or some modification of this method (3). This method is based on a distillation procedure that can be difficult to run for some laboratories. The Nordic Committee on Food Analysis (NMKL), therefore, suggested that an enzymatic method (4) for the determination of sulfite in food should be collaboratively studied. Enzymatic methods need no heavy equipment and are very specific, inexpensive, and simple.

Collaborative Study

Four food matrixes were selected for the collaborative study: wine, fruit juice, dried apples, and potato flakes. Study materials for each matrix were prepared at 3 sulfite levels, including the regulatory level. Each material was carefully mixed, and the homogeneity and stability of the material were tested by analyzing the samples at least 10 times during a 3-week period. At the time of the collaborative study, samples were analyzed regularly in the author's laboratory.

A check sample, the concentration of which only I knew, and familiarization samples consisting of solid Na_2SO_3 with

Received December 11, 1991. Accepted April 24, 1992.
This method was accepted as an official NMKL method at the 44th Annual Meeting of the Nordic Committee on Food Analysis, August 29–31, 1990, Gentotle, Denmark.

[1] Nordic Committee on Food Analysis (Secretariat General, c/o Technical Research Centre of Finland, Food Research Laboratory, P O Box 203, SF-02151 Espoo, Finland).

EDTA were sent, with the collaborative study samples, to the participating laboratories.

Determinations of blanks and analyses of blind duplicates were included in the study. Laboratories were asked to perform single determinations on all samples. When the result of the complete study was available, the limit of detection of the method was questioned. To answer this question, a complementary study was performed with 6 collaborators, all of whom had participated in the main study. Matrixes for this study were wine and juice, and 8 samples were analyzed.

Two commercially available, sulfite-treated potato flake products were analyzed both with the Monier-Williams method and the enzymatic method under investigation. Products were then mixed to achieve the desired concentration.

White wine, with a suitable content of sulfite (220 mg/L), was purchased after discussion with The Swedish Wine and Spirit Cooperation, which regularly checks the sulfite content of wine in Sweden. Sulfite-treated dried apple rings were bought from 2 different producers and homogenized with a Moulinette Kitchen homogenizer. Frozen lemon juice was thawed and diluted according to the producer's recommendation. The sulfite concentration of the juice material was analyzed and found to be under 2 mg/L. Half of the juice batch was used as blank samples, and the other half was spiked with Na_2SO_3 stock solution to obtain the desired sulfite concentration. The spiked-sample batch was divided, and the test samples were presented to the participants as blind duplicates.

Sulfite levels of the 12 samples were chosen to meet the regulatory needs in the Nordic countries for the different matrixes. Samples were weighed into capped, plastic containers, except dried apples, which were packed into plastic bags with as little air in the bags as possible. The 15 samples and storage instructions for the samples were mailed by express delivery to each of the 11 participants. Samples reached all the collaborators the next day. Method instructions were sent to the laboratories well in advance to allow them to practice the method.

METHOD

Principle

Sulfite in liquid foods or extracts of solid foods is analyzed according to the following principle, where NADH is reduced nicotinamide adenine dinucleotide:

$$SO_3^{2-} + O_2 + H_2O \xrightarrow{\text{sulfite oxidase}} SO_4^{2-} + H_2O_2$$

$$H_2O_2 + NADH + H^+ \xrightarrow{\text{NADH peroxidase}} 2H_2O + NAD^+$$

Decrease in NADH is measured spectrophotometrically and is proportional to the concentration of sulfite.

Apparatus

(**a**) *Water bath.*—Temperature range, 20–90°C.

(**b**) *Homogenizer*—Ultraturrax, or equivalent.

(**c**) *Graduated micropipets.*—10, 20, 50, and 100 µL. If mechanical pipets with disposable ends/capillaries are used, they must be properly calibrated.

Table 1. Solutions added to sample and blank

Solution	Sample cuvette	Blank
Triethanolamine buffer, mL	1.00	1.00
NADH, µL	100	100
NADH peroxidase, µL	10	10
Sample, µL	100	—
Water, mL	1.90	2.00

(**d**) *pH meter.*

(**e**) *Spectrophotometer.*—With 1 cm cuvette. Use quartz cuvettes or cuvettes of other material suitable for use at 340 nm.

(**f**) *Centrifuge.*

Reagents

All reagents must be analytical grade. Use deionized or distilled water.

(**a**) *Triethanolamine buffer, 0.6 mol/L, pH 8.0.*— Dissolve 5.57 g triethanolamine in 40 mL water in a beaker. Adjust to pH 8.0 with NaOH (0.1 mol/L). Transfer solution to 50 mL volumetric flask, and dilute to mark with water.

(**b**) *Reduced NADH, 0.007 mol/L.*—Dissolve 25 mg disodium NADH and 50 mg $NaHCO_3$ in 5.0 mL water. Solution is stable at least 4 weeks at 4°C.

(**c**) *NADH peroxidase (EC 1.11.1.1) suspension.*—Make a suspension of 15 enzyme units/mL in 2 mol/L ammonium sulfate solution, ca pH 7. Suspension is stable 1 year at 4°C.

(**d**) *Sulfite oxidase (EC 1.8.3.1) suspension.*—Make a suspension of 2.5 enzyme units/mL in 2 mol/L ammonium sulfate solution, ca pH 7. Suspension is stable 1 year at 4°C.

(**e**) *Sodium hydroxide, 0.1 mol/L.*

(**f**) *Sodium hydroxide, 2 mol/L.*

(**g**) *Sodium hydrogen carbonate.*

(**h**) *Reference sample.*—With an accuracy of ±0.1 mg, weigh 600 mg Na_2SO_3 (which contains about 300 mg SO_2) and 37 mg EDTA. Dissolve in water. Transfer the solution quantitatively to a 1 L volumetric flask, dilute to mark with water, and mix. Use 100 µL of this solution as sample, and analyze sulfite content within 30 min. Standard deviation for the reference value must not exceed 0.06.

(**i**) *NADH solutions for spectrophotometer control.*—When using a new spectrophotometer, check the absorption coefficient (ε) of the NADH solutions at 340 nm; the value should be 6.30 ± 0.04 L/(mol · cm). (*1*) *NADH, 0.07×10^{-3} mol/L.*—Dilute 20 µL solution (**b**) with 1980 µL water. (*2*) *NADH, 0.035×10^{-3} mol/L.*—Dilute 20 µL solution (**b**) with 3980 µL water. (*3*) *NADH, 0.0175×10^{-3} mol/L.*—Dilute 10 µL solution (**b**) with 3990 µL water.

Make sure that the wavelength is adjusted to maximum absorption at 340 nm. Read absorbance (A) of solutions *1–3*. Calculate ε of NADH from the Beer–Lambert equation:

$$\varepsilon = A/(d \times c)$$

where A = absorbance value recorded, c = concentration of NADH solution (mol/L), and d = light path (cm).

Calculate ε of NADH from the 3 solutions and their mean value.

Table 2. Collaborative results for enzymatic determination of sulfite (mg/kg)

	Sample No.												
	Potato flakes			Wine			Dried apple			Juice			
Collaborator	1	2	3	4	5	6	7	8	9	10	11	12	13[a]
1	29[b]	17[b]	115	214	108	26	323	782	670	217	3[b]	213	293
2	34[b]	19[b]	106	190	115	27	330	915	765	249	<2[b]	232	308
3	16[b]	5[b]	108	222	116	28	—	—	—	277	3[b]	281	294
4	75[b]	0[b]	150	174	0	25	300[b]	778[b]	550[b]	248	10[b]	245	—
5	16[b]	0[b]	123	231	124	30	394	931	732	270	0	278	317
6	—	—	—	211	105	27	—	—	—	248[c]	3	258[c]	281
7	—	—	—	221	79	33	—	—	—	260	<2[b]	262	321
8	50[b]	0[b]	124	210	107	26	341	863	691	207	0[b]	251	314
9	—	—	—	205	103	50	418	934	828	244	0[b]	258	352
10	<20[b]	<20[b]	81	170	88	21	176	778	601	266	<5[b]	266	295
11	25[b]	<25[b]	115	112	50	12	185	804	572	200	<1[b]	214	306
C[d]	22	10	113	224	121	26	394	938	772	266	<2	266	320

[a] Sodium sulfite, used as check sample.
[b] Absorbance <0.050; suspect values, but included in further calculations.
[c] Result corrected by the author; the collaborator had failed to multiply the result by 2.
[d] Author's values at start of study; not included in evaluation of results.

Sample Preparation and Procedure

Liquid foods.—Analyze white wine and brandy directly. Beer must be filtered. For red wines, adjust 25 mL wine to pH 7.5–8.0 with NaOH (2 mol/L), and dilute with water to 50 mL in a volumetric flask. Usually, red wine must be decolorized. This can be done as described below for fruit juices. Sample volumes are 100–200 µL for wines and 500 µL for brandy and beer.

For fruit juices, centrifuge cloudy juices (ca 4000 rpm). Remove ascorbic acid by adding ca 20 units ascorbate oxidase (EC 1.10.3.3) in solution to 2.0 mL juice previously adjusted to pH 5–6 with NaOH (2 mol/L). Leave sample 10 min, or stir 3 min with an ascorbate oxidase spatula. Adjust pH to 7.5–8.0 with NaOH (2 mol/L). In the case of colored juices, add ca 0.1 g polyvinylpolypyrrolidone, and stir mixture 1 min. Dilute sample to 4.0 mL with water, and filter through filter paper. Sample volume is 200 µL.

Solid foods.—Homogenize sample thoroughly, and extract with water 5 min at 60°C. Shake occasionally, and then cool sample. Vary sample size depending on the amount of sulfite. For matrixes such as potato flakes, add 40 mL water to 5.0 g homogenized material in a 50 mL volumetric flask. Cap flask, and extract in a water bath 5 min at 60°C. Shake occasionally. Cool and dilute with water to volume. If necessary, centrifuge the solution. Sample volume is <500 µL. The following sample quantities of some other foods are suggested: dried fruit, 1.0 g sample/50 mL water; jam, 5.0 g sample/50 mL water; spices, 100 mg sample/50 mL water; and dried potato products, 2.0 g sample/50 mL water.

Perform determination in a 1 cm cuvette by using a sample volume of 100 µL. If sample volume is different from 100 µL, adjust volume of added water so that the final volume of water and sample is 2.00 mL (Table 1).

Mix gently. Measure the absorbance A1 of the sample and of the blank after 5 min. Add 50 µL sulfite oxidase (**d**) to sample and blank cuvettes. Read absorbance A2 after ca 30 min. Make an additional reading after ca 5 min to check that no further change in the absorbance has taken place. If the reaction

Table 3. Collaborative results from complementary study for enzymatic determination of sulfite in wine and juice (mg/kg)

	Sample No.							
	Wine			Juice			Wine	
Laboratory No.	14	15	16	17	18	19	20	21
1	77	4	16	1	2	1	33	69
2	75	4	18	5	4	3	37	76
3	78	5	15	9	10	5	38	79
4	82	4	14	6	5	5	41	83
5	84	4	19	8	6	6	41	82
6	72	3	13	5	4	3	31	66
C[a]	75	4	16	<1	7	2	37	75

[a] Author's values at start of study; not included in evaluation of results.

Table 4. Collaborative results (mg/kg) on which the statistical evaluation was performed[a]

	Sample No.												
	Potato flakes			Wine			Dried apple			Juice			
Collaborator	1	2	3	4	5	6	7	8	9	10	11	12	13[b]
1	29	17	115	214 (4/1)	108 (4/1)	26	323 (4/6)	782	670	217	3	213	293
2	34	19	106	190 (4/7)	115 (4/7)	27	330 (4/7)	915	765	249	<2	232	308
3	16	5	108	222 (4/8)	116 (4/8)	28	—	—	—	277	3	281	294
4	—	—	—	—	—	—	—	—	—	—	—	—	—
5	16	0	123	231 (4/6)	124 (4/6)	30	394 (4/8)	931	732	270	0	278	317
6	—	—	—	211 (4/6)	105 (4/7)	27	—	—	—	248	3	258	281
7	—	—	—	221 (4/11)	79 (4/11)	33	—	—	—	260	<2	262	321
8	50	0	124	210 (4/28)	107 (4/29)	26	341 (4/30)	863	691	207	0	251	314
9	—	—	—	205 (5/6)	103 (5/6)	50	418 (5/14)	934	828	244	0	258	352
10	<20	<20	81	170 (5/8)	88 (5/8)	21	176 (5/13)	778	601	266	<5	266	295
11	25	<25	115	112[c] (5/14)	50 (5/14)	12	185 (5/14)	804	572	200	<1	214	306
C[d]	22	10	113	224	121	26	394	938	772	266	<2	266	320

[a] Dates in parentheses are dates of analysis of samples. Dates after sample 7 are for samples 7, 8, and 9.
[b] Sodium sulfite, used as check sample.
[c] Outlier according to Grubb's test at $P < 0.01$.
[d] Author's values at start of study; not included in evaluation of results.

has not stopped, continue to read the absorbance at intervals of 2 min until the change in absorbance is constant. If the absorbance decreases constantly, extrapolate the absorbance back to the time of addition of the sulfite oxidase to estimate the A2 to be used.

Calculation

Calculate the absorbance of the sample solutions from the following equations:

$$A \text{ sample} = \text{A1 sample} - \text{A2 sample}$$
$$A \text{ blank} = \text{A1 blank} - \text{A2 blank}$$
$$A = A \text{ sample} - A \text{ blank}$$

Calculate the sulfite concentration (g SO_2/L or kg) in the sample from the Beer–Lambert equation, $A = c \times d \times \varepsilon$, rearranged as follows:

$$c \text{ (g/L)} = (V \times MW \times A)/(\varepsilon \times d \times v \times 1000)$$

where c = concentration of sulfite (g SO_2/L); V = final volume (mL), 3.160 mL; v = sample volume (mL), 0.1 mL; MW = mo-

Table 5. Results (mg/kg) for determination of sulfite in samples with high amounts of sulfite analyzed in the author's laboratory over a 6-week period

	Wine sample No.			Dried apple sample No.		
Date	4	5	Date	7	8	9
3/25	230	118	3/31	332	842[a]	796
4/1	217	125	3/31	413	931	772
4/1	217	124	3/31	388	979	803
4/1	219	120	4/7	409	930	734
4/9	224	110	4/7	377	953	698
4/9	224	111	4/7	392	961	690
4/9	222	110	4/22	324	860	690
4/24	207	106	4/22	159	904	772
4/24	206	106	4/22	150	874	695
4/24	205	108	5/6	269	896	645
5/13	190	97	5/6	275	816	613
5/13	185	97	5/6	280	816	603
5/13	185	96				
Concn	224 – 0.829 × day	121 – 0.615 × day		394 – 3.13 × day	961 – 3.31 × day	772 – 3.8 × day
n	12	12		10	11	12
R^2, %	89	96		79	79	89
P	<0.001	<0.001		<0.001	<0.001	<0.001

[a] Outlier.

lecular mass of sulfur dioxide, 64.1; d = light path (cm), 1 cm; and ε = absorption coefficient of NADH at 340 nm, 6.3 L/(mmol · cm).

This procedure is similar in principle to that previously described (5).

Results and Discussion

Results from all 11 participating laboratories are listed in Tables 2 (full study) and 3 (complementary study). Only valid results are listed.

Collaborator 3 deviated from the method for samples 7–9 by treating them with ascorbate oxidase. Therefore, these results were excluded from further calculations.

Half of the results originating from collaborator 4 were calculated from absorbances less than 0.05, which indicated that the collaborator could not handle the method or the spectrophotometer. Collaborator 4 was excluded from the study.

Collaborator 6 deviated from the method (used Millipore filtration) for samples 1–3 and 7–9. These results were excluded.

Table 6. Statistical results from collaborative study for determination of sulfite in individual samples, outliers excluded

Sample No.	Av., mg/kg	s_R, mg/kg	No. of accepted results, n	RSD_R, %
1	28.3	13	6	45
2	13.7	7.6	3	55
3	110	15	7	13
4[a]	220	15	9	6.8
5[a]	110	17	10	15
6	28.0	9.6	10	34
7[a]	372	87	7	23
8[a]	924	81	7	8.8
9[a]	770	9.6	7	13
10	243	27	10	11
11	0	0	6	—
12	251	24	10	10
13	308	20	10	6.4
14	78.0	4.4	6	5.7
15	4.00	0.63	6	16
16	15.8	2.3	6	15
17	5.67	2.8	6	49
18	5.17	2.7	6	52
19	3.83	1.8	6	48
20	36.8	4.1	6	11
21	75.8	7.0	6	9.2
4[b]	208	18	9	8.9
5[b]	100	22	10	22
7[b]	310	95	7	31
8[b]	858	70	7	8.2
9[b]	694	90	7	13

[a] Values corrected for the time-dependent decrease of sulfite.
[b] Values not corrected for the time-dependent decrease of sulfite.

Laboratory 7 reported results only for samples 4–6 and 10–13 because of spectrophotometer breakage.

Collaborator 9 reported spectrophotometer difficulties for samples 1 and 2; therefore, these results were excluded.

Data in Table 2 marked with a footnote *b* were calculated by using absorbances less than 0.05. As stated in the method, the absorbance value A must exceed 0.05. If not, a larger sample volume must be used.

For sample 11, values ≥ 0 are regarded as false positives. For sample 2, values ≤ 0 are regarded as zero.

Statistical evaluation of the collaborative data was carried out according to the International Union of Pure and Applied Chemistry (IUPAC) Harmonized Protocol (6) on the values listed in Tables 3 and 4.

The single Grubbs test identified 1 outlying result (indicated as a footnote), a wine sample analyzed by collaborator 11. The proportion of outlying results is well below the recommendations of IUPAC.

The statistical evaluation indicated that there was a significant, time-dependent decrease in sulfite concentration in 2 matrixes (apples and wine) with a high content of sulfite. Samples were 4, 5, 7, 8, and 9. Because the collaborative samples were analyzed regularly in the author's laboratory at the time of the collaborative study, the time-dependent decrease of sulfite could be estimated. New plastic containers or bags were used every new date. These data are presented in Table 5, together with the dates of analysis.

In Table 5, the equations of the time-dependent decrease for each sample are presented. Corrected values for samples 4, 5, 7, 8, and 9 were used in the evaluation of the results from the study (Table 6).

Sample 13 consisted of pure Na_2SO_3 that was to be dissolved in water and analyzed within 30 min. Analysis of this material gave a very low reproducibility; the relative standard deviation was 6.4% at the 300 mg SO_2/kg level.

Reproducibility relative standard deviations (RSD_R), which reflect the variations between laboratories, and repeatability relative standard deviations (RSD_r), which measure the within-laboratory variations for levels of sulfites between 75 and 900 mg/kg, are presented in Table 7.

RSD_r for 2 blind duplicates of wine (75 mg SO_2/kg) was 3.9%, and RSD_R was 7.6%. For a matched pair of dried apples containing 800 and 960 mg SO_2/kg, the RSD_r value was 13%, and the RSD_R value was 14%. For 2 juice samples with a concentration of 270 mg SO_2/kg, the corresponding parameters were 4.8 and 10% for repeatability and reproducibility, respectively.

Results of the collaborative studies show that the method is well-suited for quantitative analysis of sulfite at levels below 100 mg SO_2/kg. For very low concentrations of sulfite, the type of food is of great importance. In the analysis of solid samples or when the sulfites may adhere to particles (e.g., in juice), a high standard deviation may be expected, especially if the analyst has little experience with enzymatic methods. This can also be seen for the potato flake samples. Standard deviations are high for the low concentrations of sulfite but acceptable for higher concentrations. No statistically significant time-depen-

Table 7. Statistical results from collaborative study for determination of sulfite in matched pairs/blind duplicates

Parameters	Sample combinations		
	8/9[a]	10/12[b]	14/21[b]
Matrix	Apple	Juice	Wine
No. of pairs	7	10	6
Mean of matched pairs/duplicates, mg/kg	834	248	76.9
s_r, mg/kg	109	12	3.0
RSD_r, %	13	4.8	3.9
Repeatability value ($2.8 \times s_r$), mg/kg	305	33	8.4
s_R, mg/kg	112	26	5.9
RSD_R, %	14	10	7.6
Reproducibility value ($2.8 \times s_R$), mg/kg	314	72	16.5

[a] Split level.
[b] Blind duplicate.

dent decrease of sulfite could be seen for potato flakes. Presumably, this depends on the lower concentrations of sulfite in these samples. Variations in the results depending on the inhomogeneity are higher than the decrease in sulfite concentration during the time period. Concentrations in wine of between 1 and 10 mg/L can be determined with good reliability.

The limit of detection of the method, expressed as absorbance difference, is 0.04. For a sample of 1 mL, the limit of detection, calculated as the mean value of a representative number of blanks ($n > 20$) plus 3 times the standard deviation of the mean value (according to recommendations of the European Community), is 1.2 mg SO_2/kg.

Acknowledgments

The author thanks the following persons and laboratories for their participation in the collaborative study:

Maija-Liisa Anttonen, Miljöoch hälsoskyddsförvaltningen, Göteborg

Karen Berg-Nilsen, Oslo Helseråd, Oslo

Karin Blomberg, Finnish Customs Laboratory, Espoo

Karl Olav Gjerstad, Stavanger off. Kjøtt- og næringsmiddelkontroll, Forus

Tomas Gustavsson, Marabou AB, Sundbyberg

Per Joner, Institutt for Næringsmiddelhygiene, Norges veterinærhøgskole, Oslo

Jukka Loimaranta, Valio Mejeriernas Centrallag, Helsingfors

Antti Mustranta, Technical Research Centre of Finland, Espoo

Mikko Oksanen, Lahden kaupungin elintarvikelaboratorio, Lahti

Leena Pero, Tampereen kaupungin elintarviketutkimuslaitos, Tampere

Björn Sönsterud, Statens kvalitetskontroll for vegetabilske konserver, Oslo

The author also thanks Gunilla Everitt for skillful technical assistance and valuable discussions, Annica Jansson for typing the manuscript, Torbjörn Schröder for statistical evaluation of the collaborative results, and Harriet Wallin for critical examination of the manuscript.

References

(1) Modderman, J.P. (1986) *J. Assoc. Off. Anal. Chem.* **69**, 1–3

(2) Stevenson, D.D., & Simon, R.A. (1984) *J. Allergy Clin. Immunol.* **74**, 469–472

(3) Hillery, B.R., Elkins, E.R., Warner, C.R., Daniels, D., & Fazio, T. (1989) *J. Assoc. Off. Anal. Chem.* **72**, 470–475

(4) Beutler, H.O. (1984) *Food Chem.* **15**, 157–164

(5) *Methods of Biochemical Analysis and Food Analysis using Test-Combinations* (1986) Boehringer Mannheim Corp., New York, NY

(6) Horwitz, W. (1988) *Pure Appl. Chem.* **60**, 855–864

(7) Report of the Scientific Group on Reference Methods of Analysis for Residues (1988) Commission of the European Communities, DG VI

FRUITS AND FRUIT PRODUCTS

Sugars, Alcohols, and Hydroxymethylfurfural in Authentic Varietal and Commercial Grape Juices

TIBOR FULEKI and ESTELA PELAYO
Ontario Ministry of Agriculture and Food, Horticultural Research Institute of Ontario, Vineland Station, ON, L0R 2EO, Canada

The quantity of fructose, glucose, and sucrose present in authentic varietal grape juices produced by cold and/or hot pressing from 56 cultivars was determined by liquid chromatography for the 1988 and 1989 seasons. The concentration of fructose, glucose, and sucrose and the fructose:glucose ratio ranged from 5.97 to 10.92, 4.91 to 9.99, trace to 1.04 g/100 mL, and 0.92 to 1.41, respectively. Sorbitol and hydroxymethylfurfural were not detected in the authentic grape juices. Genotype had no significant effect on the fructose and glucose contents. The labrusca-type cultivar group was significantly higher in the sucrose concentration and fructose:glucose ratio than either the vinifera or the French hybrid groups. The pressing method had no significant effect on the sugar composition. The glucose, fructose, total sugar, and total soluble solids contents were significantly higher in the juices from the warmer and sunnier 1988 season. The total sugar contents correlated highly with total soluble solids, but the latter averaged 1.81 units more than the former. Most commercial grape juices had a composition similar to that of the authentic juices, except that they had only traces of sucrose. Also, most commercial grape juices contained measurable quantities of ethanol, glycerol, and hydroxymethylfurfural.

Sugars contribute to the nutritional and sensory qualities of grapes and grape juice. Fructose and glucose are the predominant sugars in grapes, although sucrose may also be present in small quantities. These sugars are the substrates for alcoholic fermentation, but lactic acid bacteria can also metabolize the hexose sugars and result in spoilage. Nonfermentable sugars such as arabinose, galactose, maltose, manninotriose, melibiose, raffmose, rhamnose, stachyose, and xylose were detected in grapes as minor constituents (1–3). The sugar alcohols erythritol, glycerol, sorbitol, and xylitol were also reported in grapes as trace components (4–8).

Received December 9, 1991. Accepted April 30, 1992.

The concentrations of fructose and glucose in grapes are approximately the same; in apples and pears, fructose predominates (9–10). Sorbitol is present in substantial quantities in pome fruits (9–10). Therefore, the adulteration of grape juice and concentrate with a cheaper juice, such as apple and pear juices, can be detected on the basis of the fructose:glucose ratio (F/G) and sorbitol content.

Considerable literature exists reporting individual sugar composition of grapes grown in the major wine-producing areas of the world (9–12). In contrast, relatively little information is available on the sugar composition of grapes produced in the eastern grape-growing regions of North America (13–14). The present study was undertaken to rectify this situation and provide a data base for authentication of fruit juices.

Experimental

Grape Samples

Grapes, obtained from the experimental vineyard of this Institute, were kept in cold storage at 2°C before pressing. Grape cultivars and selections were classified into the following groups: (*1*) Vinifera, cultivars of the species *Vitis vinifera* L.; (*2*) French hybrid, cultivars from Europe (mostly from France) arising from crosses of *V. vinifera* with wild *Vitis* species or North American cultivars and as cultivars from crosses of this group with each other or with *V. vinifera*; (*3*) Labrusca type, cultivars, mostly from North America, which have a definite labrusca flavor character, as indicated by Vineland Grape Flavor Index of >14 (15); and (*4*) miscellaneous hybrid, interspecific hybrids that do not fit into any of the above groups.

Production of Grape Juice

Authentic varietal grape juice was prepared from ca 25 kg grapes by either cold pressing (CP) or hot pressing (HP). Enzymes, press aids, and SO_2 were not used. For CP, clusters were passed through the roller crusher attached to the press and pressed 5 min on a small rack-and-frame type of hydraulic press (Model TPZ 7, Bucher-Guyer, Niederweningen, Switzerland) at 6895 kPa. For HP, clusters were passed through a DVP-25 stainless steel stemmer-crusher (Mearelli Fraz., Cinquemiglia Citta di Castello, Italy) and heated 30 min in a steam-jacketed stainless steel kettle to 63°C. The pressing op-

Table 1. Retention time, detection limit, and constants of the regression equation for sugars, alcohols, sugar alcohols, and hydroxymethylfurfural (HMF)

Compound	Source[a]	R_t, min	Detection limit, ng[b]	Constants of the regression equation[c]	
				A	B
Stachyose	S	6.12	80	−0.34573	0.7248908
Raffinose	R	6.79	80	—	—
Maltose	F	7.75	40	−0.23420	0.3243000
Melibiose	F	7.97	50	—	—
Sucrose	F	8.10	16	−0.00203	0.5270668
Glucose	F	9.57	16	−0.35674	0.5494438
Xylose	R	10.48	40	−0.17785	0.4962648
Galactose	R	10.66	64	—	—
Mannose	B	10.74	50	—	—
Rhamnose	S	10.92	40	—	—
Fructose	R	11.66	16	−0.11928	0.5300648
Arabinose	R	11.86	60	—	—
Inositol	F	12.00	40	—	—
Erythritol	F	13.44	16	—	—
Glycerol	F	13.94	24	0.01167	0.4005940
Methanol	C	14.25	32	−0.01816	0.0723668
Ethanol	A	14.62	32	−0.02845	0.2110776
Mannitol	F	14.67	16	—	—
Xylitol	F	17.30	30	—	—
Sorbitol	F	17.45	20	−0.15297	0.5225400
Ribose	F	17.63	80	—	—
HMF	F	22.20	2.3	−7.26288	108.01293

[a] A = Consolidated Alcohols Ltd, Toronto, ON, Canada; B = BDH, London, England; C = Caledon Laboratories Ltd, Georgetown, ON, Canada; F = Fluka, Buchs, Switzerland; R = Roth, Karlsruhe, Germany; S = Sigma Chemical Co., St. Louis, MO.

[b] Signal-to-noise ratio = 2. Established with refractometer detector sensitivity set at 256× except for HMF, which was measured with the photodiode array detector at 285 nm.

[c] Established with refractometer detector sensitivity set at 32× except for HMF, which was measured with the photodiode array detector at 285 nm. Calculate for 25 µL injection volume as follows:

$$\frac{\text{area} \times 10^{-6} - A}{B} = \text{g/L}.$$

eration was the same as for CP. Small samples of juice were stored in glass containers at −30°C.

Reagents

(a) *Water.*—Deionized, glass-distilled, and filtered through Norganic charcoal (Millipore Corp., Bedford, MA).

(b) *Standards.*—Highest purity commercially available (Table 1), dried 3 h at 105°C and cooled in a desiccator.

(c) *Liquid chromatographic (LC) mobile phase.*—0.0001M calcium propionate–0.001M calcium disodium EDTA, sparged with He and kept at 60°C.

Apparatus

(a) *Liquid chromatograph.*—6000A pump, set at 0.5 mL/min flow rate; 710 WISP autoinjector; 7011 column heater; 990+ photodiode array detector (DAD), set at 3 nm resolution and 1 s measuring interval; 410 differential refractometer detector (RI), set at sensitivity of 32×, scale factor of 10, and temperature of 45°C; 721 system controller; 730 data module, set at 0.35 cm/min chart speed; 990 plotter (Waters, Milford, MA); and Model 3363 Optical Disk Drive (IBM Corp., Armonk, NY).

(b) *LC column.*—Stainless steel 300 × 6.5 mm Sugar-Pak I fixed-ion resin analytical column protected with Sugar-Pak II guard column (Waters), both kept at 90°C.

(c) *Bio-Rex minicolumn.*—Econo-Column packed with 0.6 g dry Bio-Rex 5 intermediate base resin containing primarily tertiary but also some quaternary amines on a polyalkyleneamine lattice (Bio-Rad Labs., Richmond, CA), prepared as described by McCord et al. (16).

(d) *Microwave oven.*—Model 70–40 commercial microwave oven (Litton Industries, Minneapolis, MN).

(e) *Centrifuge.*—Safety-Head benchtop centrifuge (Clay-Adams Co., Inc., New York, NY).

(f) *Water bath.*—B-52 ultrasonic bath (Branson Cleaning Equipment Co., Shelton, CT).

(g) *Refractometer.*—Model-A Abbé refractometer equipped with flow-through cell (C. Zeiss, Oberkochen, Germany). Temperature in cell was controlled with a Model 2000 Masterline constant-temperature water bath and circulator (Forma Scientific Inc., Marietta, OH).

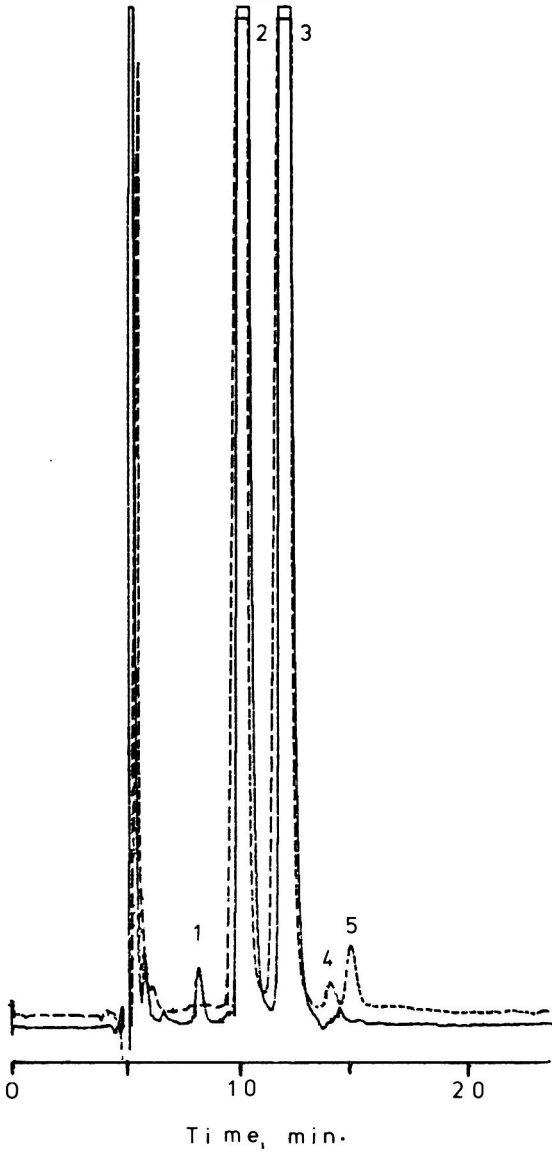


Figure 1. Separation of sugars and alcohols in authentic Niagara juice (—) and in a commercial pure white grape juice (– – –) on Sugar-Pak 1 column. Peak identification: 1 = sucrose, 2 = glucose, 3 = fructose, 4 = ethanol, 5 = glycerol.

(h) *pH meter.*—Model 28 (Radiometer A/S, Copenhagen, Denmark).

Sample Preparation

Frozen sample was thawed in the microwave oven, and pH was adjusted to 8.5 with 28–30% NH_3. Neutralized sample was heated to 90°C in the microwave oven and centrifuged 20 min at 7000 rpm. Exactly 1 mL filtrate was applied on the Bio-Rex 5 minicolumn, washed with water until 25 mL of the neutral fraction was collected, and passed through 0.45 μm Durapore membrane filter (Millipore Corp.).

Sample preparation procedure was designed to minimize enzymatic and acid hydrolysis of sucrose and to bring crystallized tartrate completely into solution. The quick heating was intended to inactivate native enzymes and facilitate dissolving the tartrate. Mild alkaline conditions were required for fractionation on the Bio-Rex 5 resin (16) and prevention of acid hydrolysis of sucrose during heating. To test for inversion of sucrose, a solution of sucrose (5 g/100 mL) and tartaric acid (1.5 g/100 mL) was carried through the sample preparation and fractionation. The test sample was chromatographed untreated, after heat treatment, and after fractionation. Results showed that only 0.87% of the sucrose was hydrolyzed during the heating phase of the sample preparation procedure and no further inversion took place during fractionation on the Bio-Rex 5 resin.

Because simple sugars can enolize under alkaline conditions (17), the sample preparation procedure had to be tested for the occurrence of this reaction. A test solution of glucose (8 g/100 mL) and tartaric acid (1.5 g/100 mL) was put through the test procedure described above. Only glucose appeared on the chromatograms, indicating that enolization did not occur.

The average recoveries of sucrose, fructose, and glucose added to 10 different grape juices were 96.6, 97.2, and 96.6%, respectively, after the sample preparation and ion exchange treatments.

Determination

A 30 μL portion of the filtered neutral fraction was injected on the column. Chromatographic peaks were identified by comparing their retention times with those of authentic standards (Table 1). Sample was also co-chromatographed with standards to verify peak identity. The external standard method was used for quantitation (Table 1). Detector response was linear in the concentration ranges at which the determinations were performed. Determinations were performed on 2 or 3 juice samples from the same pressing.

To determine the total soluble solids (TSS), frozen juice samples were heated to 65°C and held at this temperature in an ultrasonic bath until the tartrate crystals were dissolved. The juice was centrifuged 20 min at 7000 rpm; the supernatant was filtered through a 0.65 μm membrane filter and then used for the determination of TSS.

Statistical Methods

Data were evaluated by the Statistical Analysis System (PC-SAS, SAS Institute, Cary, NC) program package. Analysis of variance (ANOVA) and the General Linear Model (GLM) procedures were used. Means were separated by Duncan's multiple range test. Linear Correlation Analysis (CORR) was also performed by using the above program package.

Results and Discussion

Chromatograms of the neutral fraction of an authentic and a commercial white grape juice are shown in Figure 1. Sucrose, glucose, fructose, ethanol, glycerol, and hydroxymethylfurfural (HMF) were identified. No HMF was found in the authentic juices, and only trace quantities of ethanol and glycerol were found; measurable quantities were present in most of the commercial juices. None of the trace sugars and sugar-alcohols re-

Table 2. Sugar composition of authentic grape juices produced by cold (CP) and hot (HP) pressing

Cultivar	Type[a]	Pressing	Year	Sucrose, g/100 mL	Glucose, g/100 mL	Fructose, g/100 mL	Total, g/100 mL	TSS, %	Fructose/glucose ratio
Agawam	L	CP	88	0.09	7.48	9.00	16.57	18.2	1.20
Agawam	L	CP	89	0.41	7.65	7.72	15.78	17.8	1.01
Aligoté	V	CP	88	0.05	6.84	7.51	14.40	17.4	1.10
Aurore	F	CP	89	0.16	8.68	9.67	18.51	20.0	1.11
Auxerrois	V	CP	89	0.05	5.88	7.06	12.99	15.5	1.20
Bacchus	V	CP	89	0.17	6.60	7.00	13.77	15.2	1.06
Baco noir	F	CP	89	tr[b]	8.42	8.63	17.06	19.3	1.03
Baco noir	F	HP	89	0.06	8.51	8.76	17.33	20.3	1.03
Cabernet Franc	V	CP	89	0.04	8.20	9.18	17.42	19.0	1.12
Cabernet Franc	V	HP	89	0.06	7.57	8.44	16.07	18.0	1.11
Cab. Sauvignon	V	HP	88	tr	9.99	10.49	20.49	22.9	1.05
Cab. Sauvignon	V	HP	89	0.03	8.38	8.55	16.96	19.0	1.02
Canada Muscat	L	CP	88	0.04	6.02	6.78	12.84	14.8	1.13
Canada Muscat	L	CP	89	0.15	6.05	6.64	12.84	14.6	1.10
Cascade	F	CP	89	0.17	8.77	9.00	17.94	19.4	1.03
Catawba	L	CP	89	0.21	6.55	8.20	14.96	15.8	1.25
Cayuga White	M	CP	89	0.04	7.92	9.43	17.39	18.6	1.19
Chambourcin	F	CP	89	0.12	8.01	9.12	17.25	18.2	1.14
Chambourcin	F	HP	89	0.02	7.94	9.01	16.97	17.7	1.13
Chancellor	F	CP	89	0.11	8.67	9.19	17.97	19.6	1.06
Chancellor	F	HP	89	0.03	9.19	9.68	18.90	21.6	1.05
Chardonnay	V	CP	88	tr	7.61	8.21	15.83	18.8	1.08
Chardonnay	V	CP	89	0.06	8.50	8.79	17.35	20.1	1.03
Chelois	F	CP	89	0.07	7.92	8.46	16.45	18.4	1.07
Chelois	F	HP	89	0.03	8.43	8.89	17.35	19.6	1.05
Chenin blanc	V	CP	89	0.53	7.44	7.66	15.63	17.2	1.03
Colobel	F	HP	89	0.18	6.30	6.97	13.45	17.4	1.11
Concord	L	CP	89	0.16	6.44	8.22	14.82	15.6	1.28
Concord	L	HP	88	tr	5.47	6.46	11.94	15.5	1.18
Concord	L	HP	89	tr	6.26	7.96	14.23	17.8	1.27
De Chaunac	F	CP	89	0.04	7.79	8.46	16.29	18.2	1.09
De Chaunac	F	HP	88	0.06	8.38	9.06	17.98	20.2	1.08
De Chaunac	F	HP	89	0.09	8.49	9.40	17.89	20.0	1.11
Delaware	L	CP	88	0.04	8.45	9.82	18.31	21.3	1.16
Delaware	L	CP	89	0.04	8.99	10.84	19.87	21.3	1.21
Dutchess	M	CP	88	0.04	7.98	8.93	16.95	18.0	1.12
Dutchess	M	CP	89	tr	6.75	7.31	14.07	15.0	1.08
Ehrenfelser	V	CP	88	0.05	8.33	8.49	16.87	18.1	1.02
Ehrenfelser	V	CP	89	tr	8.20	8.59	16.80	18.8	1.05
Elvira	L	CP	88	tr	6.83	8.50	15.34	16.7	1.24
Elvira	L	CP	89	0.19	4.91	5.97	11.07	13.4	1.22
Gamay	V	CP	88	0.04	7.27	7.75	15.06	17.4	1.07
Gamay	V	CP	89	tr	5.86	6.21	12.08	14.5	1.06
Gamay	V	HP	88	0.03	7.94	8.37	16.34	19.3	1.05
Gamay	V	HP	89	0.09	6.41	6.52	13.02	15.2	1.02
Gewürztraminer	V	CP	89	0.03	7.95	8.14	16.12	17.5	1.02
Le Commandant	F	CP	89	0.03	6.83	7.72	14.58	16.4	1.13
Le Commandant	F	HP	88	tr	7.90	8.82	16.73	19.2	1.12
Le Commandant	F	HP	89	tr	6.82	7.89	14.72	18.0	1.16
Maréchal Foch	F	CP	89	0.02	7.64	8.22	15.88	18.0	1.08
Maréchal Foch	F	HP	89	0.02	8.20	8.76	16.98	20.1	1.07
Merlot	V	CP	88	0.04	9.02	9.20	18.26	19.6	1.02
Merlot	V	CP	89	0.03	8.91	9.27	18.21	19.8	1.04
Merlot	V	HP	88	0.21	8.93	9.48	18.62	20.8	1.06
Merlot	V	HP	89	0.15	9.03	9.33	18.51	20.4	1.03

Table 2. (*Continued*)

Cultivar	Type[a]	Pressing	Year	Sucrose, g/100 mL	Glucose, g/100 mL	Fructose, g/100 mL	Total, g/100 mL	TSS, %	Fructose/glucose ratio
Müller-Thurgau	V	CP	88	0.05	7.21	7.61	14.87	17.4	1.06
Müller-Thurgau	V	CP	89	0.13	6.67	7.44	14.24	15.6	1.12
NY Muscat	M	CP	88	0.18	8.07	9.29	17.54	18.6	1.15
NY Muscat	M	CP	89	0.09	7.87	8.95	16.91	18.4	1.14
Niagara	L	CP	88	0.39	5.09	6.41	11.89	13.2	1.26
Niagara	L	CP	89	1.04	6.70	7.79	15.53	16.5	1.16
Optima	V	CP	89	0.17	7.45	7.72	15.34	16.6	1.04
Oraniensteiner	V	CP	88	0.04	7.11	7.44	14.59	17.2	1.05
Oraniensteiner	V	CP	89	0.10	7.20	7.34	14.64	16.9	1.02
Pinot noir	V	CP	88	tr	7.32	7.63	14.96	17.0	1.04
Pinot noir	V	CP	89	tr	7.71	8.11	15.83	19.5	1.05
Pinot noir	V	HP	89	0.04	8.63	9.21	17.88	20.0	1.07
Pollux	F	CP	89	0.02	7.17	7.39	14.58	16.8	1.03
Reichensteiner	V	CP	89	0.09	5.45	6.86	12.40	14.0	1.26
Riesling	V	CP	89	0.09	7.38	7.76	15.23	17.1	1.05
Rosette	F	CP	89	0.04	7.70	8.74	16.47	17.8	1.13
Rosette	F	HP	88	0.14	8.60	9.72	18.46	21.5	1.13
Rosette	F	HP	89	0.08	7.71	8.93	16.72	21.6	1.16
Rougeon	F	CP	89	tr	6.44	6.97	13.42	15.7	1.08
Scheu-rebe	V	CP	89	0.14	7.19	7.89	15.22	16.6	1.10
Seyval blanc	F	CP	88	tr	8.85	10.35	19.21	20.3	1.17
Seyval blanc	F	CP	89	0.10	7.31	8.54	15.95	17.5	1.17
Siegfriedrebe	F	CP	88	0.15	7.78	7.59	15.52	17.6	0.98
Siegfriedrebe	F	CP	89	0.03	8.45	7.75	16.23	18.4	0.92
SV-23-512	F	CP	88	tr	8.73	9.97	18.71	19.6	1.14
Valerian	F	CP	89	0.17	7.07	8.30	15.54	16.1	1.17
Veeblanc	F	CP	88	0.22	8.96	9.92	19.10	20.2	1.11
Veeblanc	F	CP	89	0.13	6.27	7.05	13.45	14.2	1.12
Veeport	L	CP	89	0.10	5.36	7.53	12.99	15.8	1.41
Veeport	L	HP	88	0.17	6.88	8.50	15.55	18.6	1.24
Veeport	L	HP	89	tr	6.57	8.08	14.66	17.0	1.23
Ventura	M	CP	88	0.03	7.60	9.65	17.28	19.3	1.27
Ventura	M	CP	89	0.04	6.82	8.64	15.50	17.9	1.27
Verdelet	F	CP	89	0.11	8.32	9.07	17.50	18.4	1.09
Vidal blanc	F	CP	88	0.11	9.43	10.43	19.97	21.5	1.11
Vidal blanc	F	CP	89	0.04	7.17	7.95	15.16	16.5	1.11
Vignoles	F	CP	89	0.36	8.59	9.64	18.59	21.7	1.12
Villard blanc	F	CP	89	0.02	5.99	6.62	12.63	15.4	1.11
Villard noir	F	CP	89	0.06	5.92	7.15	13.13	14.5	1.21
Villard noir	F	HP	88	tr	6.82	7.88	14.71	16.8	1.16
Villard noir	F	HP	89	tr	6.67	7.98	14.66	16.6	1.20
Vincent	M	HP	88	0.03	6.10	7.40	13.53	16.3	1.21
Vinered	L	CP	88	0.02	7.96	8.65	16.63	18.1	1.09
Vinered	L	CP	89	tr	7.09	7.93	15.03	16.5	1.12
Vivant	M	CP	88	0.03	9.30	10.92	20.25	21.6	1.17
Vivant	M	CP	89	0.15	7.83	9.20	17.18	18.8	1.17
Mean				0.09	7.52	8.35	15.97	18.0	1.12
SD				0.13	1.06	1.06	2.05	2.1	0.08
Minimum				tr	4.91	5.97	11.07	13.2	0.92
Maximum				1.04	9.99	10.92	20.49	22.9	1.41
Literature mininum (ref.)				0 (13)	3.32 (18)	3.70 (19)	—	—	0.71 (12)
Literature maximum (ref.)				9.95 (20)	13.0 (12)	12.0 (12)	—	—	1.73 (21)

[a] Genotype: F = French hybrid, L = labrusca type, M = miscellaneous hybrid, V = *V. vinifera*.
[b] For statistical calculations, "trace" (tr) was assumed to be 0.01 g/100 mL.

ported in the literature for grapes and grape juice were detected in the juices, even when the injection volume was raised to 100 μL, although the method used was sufficiently sensitive (Table 1). It is noteworthy that sorbitol, which was reported in grapes (4–5), was not detected in any of the grape juice samples.

Authentic Grape Juice

Results of the determination of sugars in authentic juices produced by CP and HP from 56 grape cultivars are presented in Table 2. F/G values were above 1.00 in all but one cultivar (Siegfriedrebe). Low fructose and glucose concentrations are not unusual in grapes from cool viticultural regions (18, 19). A comparison of results with those in the literature (Table 2) shows that values reported here were in mid-ranges for every sugar measurement except sucrose. Great care was exercised in this study to avoid both enzymatic and acid hydrolysis of sucrose. However, the high sucrose concentrations (>3.5%) reported by some authors (2, 13, 20–25) were not found. It should be noted that the high sucrose values reported in the literature were detected in older labrusca-type cultivars not covered in this study.

The sugar composition of the 4 genotypes was compared with the 1989 data from the cold-pressed juice by applying Duncan's multiple range test (Table 3). Results showed that there were no significant differences in glucose, fructose, total sugar, and TSS. However, the sucrose content of labrusca-type cultivars was significantly higher than that of the other groups. F/G values were also significantly higher in labrusca-type grapes than in either the vinifera or French hybrid cultivars. Lott and Barrett (21) also found higher sucrose (2.14%) and F/G (1.34) in the 18 labrusca-type cultivars they analyzed. Because most of the labrusca-type grapes were developed for use as table and juice grapes, the selection process might have favored those seedlings that contained a higher proportion of the sweeter sugars, fructose and sucrose.

The sugar compositions of authentic juices produced from the same lot of grapes by both CP and HP in 1989 were compared by using ANOVA. With HP, the glucose, fructose, total sugar, and TSS contents were higher, and the sucrose contents and F/G values were lower. Inversion of sucrose upon heating might explain the lower sucrose content in HP juice. However, differences in sugar composition between the 2 pressing methods were not significant at the 5% level. Rice (14) and Gore (26) also found higher sugar content in HP juice; others (27–29) obtained variable results.

Data on the influence of vintage on the sugar composition of the juice from 21 grape cultivars that were cold-pressed in both years are presented in Table 4. The average value for each sugar measurement except sucrose and F/G was significantly higher in 1988. The warmer and sunnier season, as indicated by the increased growing-degree days and total solar radiation for the year, could explain the higher sugar content in 1988.

The total sugar determined by LC and the TSS measurements for all analyzed authentic varietal grape juice samples showed good correlation ($r = 0.92482$). The total sugar values were, on the average, 1.81 units lower than the TSS values; the difference ranged from 0.56 (Valerian, 1989) to 4.88 (Rosette HP, 1989). Because TSS measures not only the sugars but all dissolved substances that have a different refractive index from that of water (acids, salts, etc.), this was to be expected. Lott and Barrett (21) also found that the total sugar was always lower than the TSS, but the 2 measurements showed good correlation ($r = 0.9213$). The following regression equation was established by using all relevant data in Table 2 for the calculation of the total sugar content in grape juice from the TSS measurement:

$$\text{Total sugar g/100 mL} = \text{TSS} \times 0.91 - 0.37$$

Commercial Grape Juice

To get an indication of the changes in sugar content as a result of commercial processing and the authenticity of grape juices available in Ontario, we examined the sugar composition of commercial pure grape juices purchased locally (Table 5). Fructose and glucose contents of domestic commercial juices (A–D) were similar to those of authentic grape juices produced for this study. Sucrose was present only in trace quantities in most commercial juices. Hartmann and Tolman (27) failed to detect any sucrose in commercial Concord juice. These results indicate that sucrose is inverted during processing. One brand (E) contained trace quantities of maltose.

Only trace amounts of ethanol and glycerol were present in the authentic samples, but measurable quantities were detected in most of the commercial grape juices. Ethanol is produced in grapes by anaerobic respiration (29), by fermentative yeasts during transport (30), and by cold detartaration of the juice (27). Hartmann and Tolman (27) found up to 1.07% (v/v) alcohol in Concord juice after 4 months of cold storage. Because glucose usually ferments more rapidly than fructose (12), high alcohol content in the juice could be associated with increased F/G, as in brand A. The presence of alcohol in commercial

Table 3. Comparison of the sugar content of cold-pressed grape juice from different genotypes in 1989[a]

Genotype	No.	Sucrose, g/100 mL	Glucose, g/100 mL	Fructose, g/100 mL	Total, g/100 mL	TSS, %	Fructose/glucose ratio
Vinifera	16	0.10[a]	7.29[a]	7.81[a]	15.20[a]	17.12[a]	1.08[a]
French hybrid	21	0.09[a]	7.58[a]	8.27[a]	15.93[a]	17.64[a]	1.10[a]
Labrusca	9	0.26[b]	6.64[a]	7.87[a]	14.77[a]	16.37[a]	1.20[b]
Miscellaneous	5	0.07[a]	7.44[a]	8.71[a]	16.21[a]	17.74[a]	1.17[b]

[a] Means within each column are not significantly different using Duncan's multiple range test ($p = 0.05$).
[b] Means within each column are not significantly different using Duncan's multiple range test ($p = 0.05$).

Table 4. Effect of vintage on sugar content of cold-pressed grape juice

Vintage	GDD[a]	TSR[a] MJ/m^2	No.	Sucrose, g/100 mL	Glucose, g/100 mL	Fructose, g/100 mL	Total, g/100 mL	TSS, %	Fructose/glucose ratio
1988	2272	2945	21	0.076	7.79[b]	8.69[b]	16.56[b]	18.33[b]	1.12
1989	2015	2668	21	0.132	7.28[b]	8.00[b]	15.41[b]	17.24[b]	1.10

[a] Growing degree days (GDD, base 5°C) and total solar radiation (TSR) at Vineland Station from May 1 to September 30 (E.M. Lauro, personal communication).
[b] Significant at the $p = 0.05$ confidence level.

grape juice is recognized in the food regulations of the European Economic Community by allowing up to 1% (v/v) ethanol in grape juice (31).

Glycerol is a major by-product of molds infesting grapes and is also produced by yeast during fermentation (12). Kupina (32) found 1032 mg glycerol/L in juice produced from grapes with 3% mold defects. He suggested glycerol content as an objective measure of grape spoilage by molds. High ethanol concentration was always accompanied by high glycerol content in the examined commercial juices (A, G, K). Because low temperature favors glycerol formation by yeast (12), fermentation probably occurred during cold detartaration.

Although HMF is not present in fresh grapes, it is produced in the juice and concentrate as a result of thermal stress during heat processing, concentration, and/or storage (33). Kern (34) found that grapes had greater capacity to form HMF than other fruits; he detected 110 and 94 mg HMF/L in freshly bottled red and white grape juice, respectively, held 2 h at 95°C. Wucherpfennig and Burkhardt (35) found 26 mg HMF/L in freshly bottled red grape juice. The HMF content increased to 40 mg/L after the juice was stored at 35°C for 7 weeks. HMF was not detected in the authentic juices, but it was present in all except one of the commercial products. An excessively high concentration of HMF was detected in some of the commercial white juices (E, K). Severe browning was observed in these products, which may have been stored for too long at high temperatures.

In contrast to commercial processing, juices in our laboratory were produced from hand-harvested grapes that were free

Table 5. Composition of commercial grape juice

Brand	No.[a]	Type[b]	Sucrose, g/100 mL	Glucose, g/100 mL	Fructose, g/100 mL	Total, g/100 mL	TSS, %	Fructose/ glucose ratio	Ethanol, mg/L	Glycerol, mg/L	HMF, mg/L
						Red					
A	2	P	tr[c]	5.46	7.01	12.47	14.5	1.28	4688	582	43
B	1	P	0.06	6.63	7.25	13.94	15.9	1.09	735	656	0
C	3	C	tr	6.62	8.08	14.70	16.4	1.22	tr	tr	41
D	4	C	0.07	7.28	7.87	15.15	16.2	1.08	tr	995	38
E	1	P	0.04	7.64	7.99	15.84	17.1	1.05	1030	1129	51
F	1	P–S	0.03	6.67	7.15	13.85	15.5	1.07	1107	642	39
G	1	P–S	0.02	6.64	7.23	13.89	15.9	1.09	6364	908	36
H	1	P–S	0.02	6.63	7.52	14.17	15.5	1.13	tr	112	70
I	1	P	0.09	6.43	7.39	13.91	15.6	1.15	tr	801	41
J	1	P	tr	6.06	5.99	12.05	13.2	0.99	tr	30	45
						Rose					
G	1	P–S	0.01	7.69	8.16	15.86	17.1	1.06	1117	509	37
						White					
A	3	P	tr	6.85	8.48	15.33	16.9	1.24	2012	413	40
C	3	C	0.02	7.10	8.18	15.28	16.3	1.15	tr	tr	42
E	1	P	0.05	8.00	7.98	16.03	16.2	1.00	tr	608	120
G	1	P–S	0.01	7.73	8.20	15.94	17.1	1.06	486	451	38
K	1	P	0.11	6.56	6.91	13.58	15.3	1.05	2285	659	157
L	1	P–S	tr	5.83	6.40	12.24	13.8	1.10	tr	4	59

[a] No. of container sizes analyzed.
[b] P = pure, C = from concentrate, and S = sparkling.
[c] tr = trace.

of spoilage and were stored in the frozen state. These factors could explain the differences in sucrose, ethanol, glycerol, and HMF contents of authentic and commercial grape juices.

Sorbitol was not present in the authentic and commercial grape juices examined. This allowed the detection of apple or pear juice/concentrate in grape juice on the basis of sorbitol content. The sorbitol content of apple juice ranges from 0.16 to 1.67 g/100 mL (36). The detection limit for sorbitol was 20 ng in this study (Table 1). A trial carried out with apple juice containing 0.2 g sorbitol/100 mL showed that addition of 2 and 3% apple juice to grape juice could be detected at the 256× and 32× sensitivity settings of the RI detector, respectively.

Acknowledgments

We thank K.H. Fisher for supplying the grapes, C.L. Chu for advice on statistical analyses, and R.B. Palabay for his able technical assistance. We are also grateful for the financial support of the Agriculture and Food Research Fund of the Ontario Ministry of Agriculture and Food.

References

(1) Kliewer, W.M. (1966) *Plant Physiol.* **41**, 923–931

(2) Richmond, M.L., Brandao, S.C.C., Gray, J.I., Markakis, P., & Stine, C.M. (1981) *J. Agric. Food Chem.* **29**, 4–7

(3) Jurcevic, A., Dzamic, M., Kapor, S., & Jankovic, J. (1983) *Acta Hort.* **138**, 147–153

(4) Kline, D.A., Fernandez-Flores, E., & Johnson, A.R. (1970) *J. Assoc. Off. Anal. Chem.* **53**, 1198–1202

(5) Washüttl, J., Riederer, P., & Bancher, E. (1973) *J. Food Sci.* **38**, 1262–1263

(6) Schwarzenbach, R. (1977) *Mitt. Geb. Lebensm. Hyg.* **68**, 64–71

(7) Mäkinen, K.K., & Söderling, E. (1980) *J. Food Sci.* **45**, 367–371, 374

(8) Shindou, T., Sasaki, Y., Miki, H., Eguchi, T., Hagiwara, K., & Ichikawa, T. (1989) *J. Agric. Food Chem.* **37**, 1474–1476

(9) Wrolstad, R.E., & Shallenberger, R.S. (1981) *J. Assoc. Off. Anal. Chem.* **64**, 91–103

(10) Herrmann, K. (1987) in *Frucht-und Gemüsesäfte*, U. Schobinger (Ed.), Ulmer, Stuttgart, Germany, pp. 39–88

(11) Ribéreau-Gayon, J., Peynaud, E., Ribéreau-Gayon, P., & Sudraud, P. (1975) *Sciences et Techniques du Vin*, Tome 2, Dunod, Paris, France, pp. 117–175

(12) Amerine, M.A., Berg, H.W., Kunkee, R.E., Ough, C.S., Singleton, V.L., & Webb, A.D. (1980) *The Technology of Wine Making* 4th Ed., AVI Publishing Co., Westport, CT

(13) Gallander, J.F. (1974) in *Chemistry of Winemaking*, A.D. Webb (Ed.), American Chemical Society, Washington, DC, pp. 11–49

(14) Rice, A.C. (1974) in *Chemistry of Winemaking*, A.D. Webb, (Ed.), American Chemical Society, Washington, DC, pp. 88–115

(15) Fuleki, T. (1982) *Vitis* **21**, 111–120

(16) McCord, J.D., Trousdale, E., & Ryu, D.D.Y. (1984) *Am. J. Enol. Vitic.* **35**, 28–29

(17) Brewster, R.Q., & McEwen, W.E. (1961) *Organic Chemistry* 3rd Ed., Prentice-Hall, Englewood Cliffs, NJ, pp. 446–448

(18) Dako, D.Y., Trautner, K., & Somogyi, J.C. (1970) *Schweiz. Med. Wochenschr.* **100**, 897–903

(19) Lee, C.Y., Shallenberger, R.S., & Vittum, M.T. (1970) *New York's Food and Life Sciences Bull.*, No. 1, New York State Agricultural Experiment Station, Geneva, NY, pp. 1–12

(20) Alwood, W.B., & Eoff, J.R. (1916) *J. Ind. Eng. Chem.* **8**, 334–335

(21) Lott, R.V., & Barrett, H.C. (1967) *Vitis* **6**, 257–268

(22) Alwood, W.B. (1910) *J. Ind. Eng. Chem.* **2**, 481–482

(23) Alwood, W.B. (1911) *Enological Studies*, U.S. Department of Agriculture, Bureau of Chemistry Bulletin No. **140**

(24) Gore, H.C. (1916) *J. Ind. Eng. Chem.* **8**, 333–334

(25) Caldwell, J.S. (1925) *J. Agric. Res.* **30**, 1133–1176

(26) Gore, H.C. (1909) *J. Ind. Eng. Chem.* **1**, 436–441

(27) Hartmann, B.G., & Tolman, L.M. (1918) *Concord Grape Juice: Manufacture and Chemical Composition*, USDA Bulletin **656**

(28) Noyes, H.A., King, H.T., & Martsolf, J.H. (1922) *J. Assoc. Off. Agric. Chem.* **6**, 197–205

(29) Flanzy, C., & Buret, M. (1970) *C.R. Acad. Agric.* **56**, 422–428

(30) Morris, J.R., Cawthon, D.L., & Fleming, J.W. (1979) *J. Am. Soc. Hort. Sci.* **104**, 166–169

(31) Bielig, H.J., Faethe, W., Fuchs, G., Koch, J., Wallrauch, S., & Wucherpfennig, K. (1987) *RSK-Values: The Complete Manual*, Verlag Flüssiges Obst., Schönborn, Germany

(32) Kupina, S.A. (1984) *Am. J. Enol. Vitic.* **35**, 59–62

(33) Pollard, A., & Timberlake, C.F. (1971) in *The Biochemistry of Fruits and Their Products*, Vol. 2, A.C Hulme (Ed.), Academic Press, London, England, pp. 573–621

(34) Kern, A. (1964) *Intern. Fruchtsaft-Union Ber. Wiss-Tech. Komm.* **5**, 203–218

(35) Wucherpfennig, K., & Burkhardt, D. (1983) *Flüss. Obst.* **50**, 416–422, 424–426

(36) Lee, H.S., & Wrolstad, R.E. (1988) in *Adulteration of Fruit Juice Beverages*, S. Nagy, J.A. Attaway, & M.E. Rhodes (Eds), Marcel Dekker, New York, NY, pp. 343–376

PESTICIDE AND INDUSTRIAL CHEMICAL RESIDUES

Matrix Solid-Phase Dispersion Extraction and Gas Chromatographic Screening of 14 Chlorinated Pesticides in Oysters (*Crassostrea virginica*)

HEIDI M. LOTT and STEVEN A. BARKER
Louisiana State University, School of Veterinary Medicine, Department of Veterinary Physiology, Pharmacology, and Toxicology, The Laboratory for Residue Studies, Baton Rouge, LA 70803

A multiresidue isolation technique known as matrix solid-phase dispersion (MSPD) is presented for the extraction and subsequent gas chromatographic/electron capture detection (GC/ECD) determination of 14 chlorinated pesticides (α-BHC, β-BHC, lindane, heptachlor, aldrin, heptachlor epoxide, *p,p'*-DDE, dieldrin, endrin, 4,4'-DDD, endrin aldehyde, *p,p'*-DDT, endosulfan sulfate, and methoxychlor) from oysters (Crassostrea virginica). Pureed whole oysters (0.5 g aliquots) are fortified with the 14 pesticides and δ-BHC, as an internal standard, and blended with 2 g C_{18} (octadecylsilyl)-derivatized silica. Pesticides are eluted from an extraction column composed of C_{18}/oyster matrix blend and 2 g activated Florisil by addition of 8 mL acetonitrile–methanol (90 + 10). Then, 2 μL of the eluate is analyzed by GC/ECD. Unfortified blank controls are treated similarly. The eluate contained all the pesticide analytes and was free of interfering coextractants. Correlation coefficients for the standard curves of the 14 extracted pesticides (linear regression analysis) ranged from 0.9849 to 0.9980. Average relative percent recoveries over the range of concentrations examined (66 ± 12.7% to 84 ± 25.3%, n = 25 for each pesticide), interassay variability (13.6 ± 8.8% to 30.2 ± 9.1%, n = 25 for each pesticide), and intra-assay variability (5.8–11.8%, *n* = 5 for each pesticide) indicated that the MSPD methodology successfully extracted and determined the 14 chlorinated pesticides in oyster at levels of 31.3–500 ng/g.

The Food Safety and Inspection Service (FSIS) 1990 Annual Residue Plan (1), a residue-monitoring program for all domestic and imported animal food in the United States, includes many of the chlorinated hydrocarbon pesticides currently and previously used in agriculture. These pesticides are of concern, because they persist in the environment and in animal tissues (2) and their significance to human health is not known. Some of the pesticides are still classified as suspected carcinogens (3). Statistical associations between high pesticide concentrations in human tissue and certain diseases were reported (4). One study reports an association between premature births and DDE levels in fetal whole blood (5). On the other hand, other studies indicate no associations between human pesticide residues and disease (6–9). Because the human health effects have not been satisfactorily evaluated, programs such as the Annual Residue Plan are essential in monitoring the presence of pesticides in the human food supply.

Although oysters are a human food, they are not presently included in the Annual Residue Plan. However, to better protect the human food supply, government agencies such as the National Marine Fisheries Service (NMFS) and the U.S. Food and Drug Administration may soon be requested to include oysters in mandatory programs to determine oyster bed contamination (10, 11).

In addition, oysters are commonly used as bioindicators of pollution (12–16). For example, scientists from the Benthic Surveillance and Mussel Watch Projects (of the National Oceanic and Atmospheric Administration within its National Status and Trends Program) use bivalve mollusks to detect pesticide contamination of U.S. coastal waters and estuarine sites (12).

Agencies that monitor the human food supply and the environment could benefit from a quick, simple, and inexpensive screening protocol for detection of chlorinated hydrocarbon pesticides in oysters and related mollusks. The purpose of this study was to develop such a screening protocol for detection of 14 chlorinated pesticides in oysters.

A new screening protocol is needed, because many screening protocols are limited by the techniques available for separating the pesticides from various tissues. These techniques often involve extensive tissue preparation and several extractions, extract purification and concentration, and analysis by gas chromatography (GC) (17–19). Therefore, the extraction process makes many screening protocols time-consuming and complicated. Also, because of the requisite large volumes of solvent, these protocols become expensive to perform, especially for a large number of samples.

Recently, a multiresidue extraction technique called matrix solid-phase dispersion (MSPD) was developed (20–22), which

Received February 4, 1992. Accepted May 19, 1992.

eliminates many of the difficulties associated with extracting various residues from animal tissue. In this process, a small amount of tissue is homogenized and dispersed in a solid support in one step; pesticides (or other compounds) are eluted from a single, potentially multiphasic column in a different step. The tissue actually becomes part of the column. Only 8 mL of extracting solvent is needed. In many cases, samples can then be injected onto the GC apparatus without further cleanup. Because these methods generate less solvent waste, they support general efforts to protect the environment. In this study, the MSPD methods were optimized for and applied to the isolation of 14 chlorinated pesticides from oysters for subsequent determination by GC with electron capture detection (ECD).

Experimental

The multiresidue MSPD pesticide extraction method reported by Long et al. (20) was used in this study. However, the following modifications were made to include more pesticides (i.e., 14 vs 9) and to optimize the assay for oyster tissue.

Reagents

(a) *Pesticides and internal standards.*—Pesticide standards (α-BHC [319-84-6]; β-BHC [319-85-7]; lindane [58-89-9]; heptachlor [76-44-8]; aldrin [309-00-2]; heptachlor epoxide [1024-57-3]; *p,p'*-DDE [72-55-9]; dieldrin [60-57-1]; endrin [72-20-8]; 4,4'-DDD [72-54-8]; endrin aldehyde [7421-93-4]; *p,p'*-DDT [50-29-3]; endosulfan sulfate [1031-07-8]; and methoxychlor [72-43-5]); and internal standards (δ-BHC [319-86-8]; decachlorobiphenyl; and 2,4,5,6-tetrachloro-*m*-xylene) were purchased from Supelco Inc. (Bellefonte, PA). Another internal standard, dibutyl chlorendate, was purchased from Chemical Research Supplies (Addison, IL).

(b) Oysters.—Whole oysters (*Crassostrea virginica*) were obtained from a local market. Tissues from 5–10 oysters were pureed in a blender to a homogeneous, smooth liquid and stored (ca 3.0 g aliquots) in glass vials at –29°C until needed.

Preparation of Stock Pesticide Solutions and Internal Standards

Lindane, heptachlor, aldrin, *p,p'*-DDE, dieldrin, endrin, *p,p'*-DDT, and methoxychlor (group A) were purchased dissolved in isooctane at 200 μg/mL. Stock solutions were admixed and then serially diluted with isooctane to make mixed pesticide solutions containing 1.3, 2.5, 5.0, 10.0, and 20.0 μg of each pesticide/mL.

α-BHC, β-BHC, heptachlor epoxide, 4,4'-DDD, endrin aldehyde, and endosulfan sulfate (group B) were purchased dissolved in methanol and methanol–methylene chloride (98 + 2) at 20 μg/mL. Stock solutions were admixed and then serially diluted with methanol to make mixed pesticide solutions containing 0.2, 0.4, 0.8, 1.7, and 3.3 μg of each pesticide/mL.

δ-BHC was obtained in methanol–methylene chloride (98 + 2) at 20 μg/mL. No further preparation was required. However, the other 3 internal standards (dibutyl chlorendate in methanol, 200 μg/mL; decachlorobiphenyl in acetone, 200 μg/mL; and 2,4,5,6-tetrachloro-*m*-xylene in methanol, 200 μg/mL) were diluted with their respective solvents to make 3 internal standard solutions at 25 μg/mL.

Preparation of Sample Extracts

Pesticide fortification levels in tissue were as follows: (*1*) *5 μL*.—δ-BHC, 100 ng/0.5 g tissue; dibutyl chlorendate, 125 ng/0.5 g tissue; decachlorobiphenyl, 125 ng/0.5 g tissue; or 2,4,5,6-tetrachloro-*m*-xylene, 125 ng/0.5 g tissue. (*2*) *12.5 μL*.—The various pesticide stock solution mixtures of 8 pesticides, group A. (*3*) *75 μL*.—The various pesticide stock solution mixtures of 6 pesticides, group B.

Fortification levels resulted in final concentrations of 15.6, 31.3, 62.5, 125, and 250 ng of each pesticide/0.5 g oyster tissue.

Fortified samples were allowed to stand for 5 min after the last pesticide was added. Samples were then blended with the C_{18} silica. As previously described (20), this material was transferred to a 10 mL syringe barrel column that contained 2 g activated Florisil. Two filter paper discs (Whatman No. 1, 1.5 cm diameter) were placed on the column head, and the column was compressed to 7.5 mL with a syringe plunger that had the rubber end and pointed plastic portion removed. A plastic pipet tip (100 μL) was placed on the column outlet to increase the residence time of the eluting solvent on the column.

Pesticides were eluted by gravity flow with 8 mL acetonitrile–methanol (90 + 10) into a 5 mL conical, screw-threaded, disposable glass centrifuge tube. Flow usually ceased after 25 min. After 27 min, positive pressure was applied to all the column heads to collect the remaining solvent. Final recovered extract volumes varied between 4.7 and 4.8 mL. The entire procedure took ca 40 min to complete.

Apparatus

Gas chromatograph.—Varian Vista 6000 equipped with DB-5 column, 30 m × 0.25 mm id, 0.2 μm coating (J & W Scientific, Folsom, CA). Column temperature program: 120°C for 2 min, increased at 10°C/min to 290°C, and held 4 min. Splitless injection with purge function activated at 0.75 min postinjection. Injection port temperature: 200°C. Detector: ECD, 300°C, –0.10 mV at 32 attenuation and 10 mV full-scale range sensitivity. Carrier gas: ultra high-purity nitrogen at a calculated linear flow rate of 15 cm/s.

Data Analysis

The peak area ratio (PAR) for each extracted pesticide at each concentration was determined by dividing the peak area of each pesticide standard by the peak area of the internal standard. Percent recoveries were then determined by comparing the PARs of the extracted pesticides with the PARs of non-extracted pesticides run under identical conditions on the GC apparatus. To determine interassay variability, the PARs for 5 replicates at each concentration (3.1, 6.3, 12.5, 25, and 50 ng/mL, 2 μL injection) were calculated. These 5 values were averaged to give means ± standard deviations (SDs) and coefficients of variation (CVs). The CVs determined for each concentration were then averaged to give a mean ± SD for each

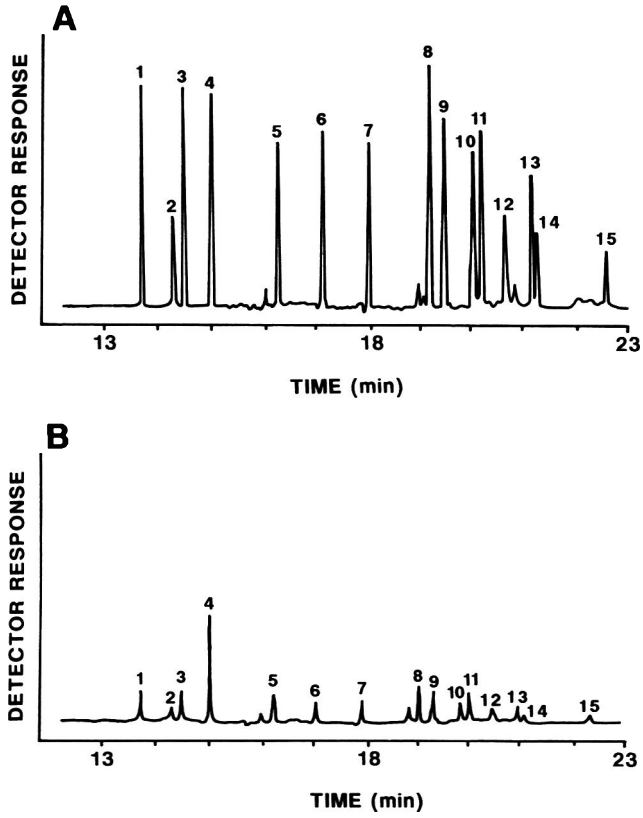


Figure 1. Representative gas chromatograms obtained from the electron capture detector analysis of the acetonitrile–methanol (90 + 10) extract of oyster homogenate fortified with pesticides at (A) 250 ng/g and (B) 31.3 ng/g (2 μL injection). Peak identities are α-BHC (1), β-BHC (2), lindane (3), δ-BHC (4), heptachlor (5), aldrin (6), heptachlor epoxide (7), *p,p'*-DDE (8), dieldrin (9), endrin (10), 4,4'-DDD (11), endrin aldehyde (12), *p,p'*-DDT (13), endosulfan sulfate (14), and methoxychlor (15).

pesticide. These values were the interassay variability. (Interassay variability represents the inherent variability that exists among extraction procedures and the variability that results from any lack of uniformity in the physical conduct of the extraction procedures.) The values for intra-assay variability were the CVs for the means ± SDs of 5 replicates of the same sample. (Intra-assay variability represents the variability associated with the analytical instrumentation used.) Finally, standard curves and correlation coefficients from linear regression analysis were generated by plotting the average PARs ($n = 5$) of extracted pesticide standards at each concentration.

Results and Discussion

The 14 pesticides at levels of 31.3, 62.5, 125, 250, and 500 ng/g in oyster tissue were extracted by MSPD methods adapted from previously reported methods (20–22) applied to catfish, beef muscle, and beef fat. Pesticides were readily detected by GC/ECD (Figures 1A and 1B). Representative gas chromatograms of blank samples (Figures 2A, 2B, and 2C) showed minimal interferences in the region of the pesticide elution times. Correlation coefficients for the standard curves of the 14 extracted pesticides (linear regression analysis, $n = 5$) ranged from 0.9849 to 0.9980. Average relative percent recoveries (66 ± 12.7% to 84 ± 25.3%, $n = 25$ for each pesticide), interassay variability (13.6 ± 8.8% to 30.2 ± 9.1%, $n = 25$ for each pesticide), and intra-assay variability (5.8–11.8%, $n = 5$ for each pesticide) indicated that the MSPD methodology allowed for the successful extraction and determination of the 14 chlorinated pesticides in oyster tissues (Table 1).

The following adaptations were required for oyster tissue: (*1*) changing the eluant from acetonitrile to a mixture of acetonitrile and methanol (90 + 10) and (*2*) using δ-BHC as the internal standard instead of dibutyl chlorendate (20). As previously mentioned, a unique feature of the MSPD method is that the complex biological matrix being analyzed becomes part of the extraction column. Therefore, the various components of this matrix can influence recovery efficiency of analytes. Also, different matrixes will result in different coextracting compounds that may produce interference. The distribution of muscle and fat in oyster differs from that in beef and catfish, which were the matrixes in the studies of Long et al. (20–22). Mollusks are also known to contain a wide variety of atypical sterols (23), unlike marine crustacea and vertebrates, in which cholesterol predominates. Although the anatomical distribution of the many different types of sterols in the American oyster, *Crassostrea virginica*, has not been clearly established, the presence of unusual nonmethylene-interrupted dienoic (NMID) fatty acids was reported (24). The NMID fatty acids were previously observed in only a few plant species. In addition, the sulfated heteropolysaccharides, other structural components isolated from oyster viscera (25), are thought to be a new type. Because of these unique structural and biochemical properties of oysters, the need for a new solvent system was anticipated and found to be necessary for the best recovery of the determined pesticides.

Three elution profiles were examined. Pure acetonitrile gave the clearest extracts (Figure 2A). However, percent recovery fell at the lower pesticide concentrations. For example, at the 31.3 ng/g fortification level, recoveries ranged from 0% for methoxychlor up to only 45% for endosulfan sulfate. A mixture of acetonitrile–methanol (75 + 25) extracted more nonpesticide components (Figure 2B). The mixture of acetonitrile–methanol (90 + 10) produced the cleanest extracts (Figure 2C) and highest consistent pesticide recoveries.

In addition, several internal standards were evaluated, because dibutyl chlorendate, which was previously used (18, 19), was unobtainable in pure form. Decachlorobiphenyl, which is recommended by the U.S. Environmental Protection Agency for pesticide analysis methods, gave an inconsistent extraction recovery and variable peak areas. However, some of the variability in peak area may have resulted from the compound's long retention time on the capillary column (29.89 min). Another compound that was examined, 2,4,5,6-tetrachloro-*m*-xylene, eluted early from the capillary column (12.72 min) and was frequently subject to interference by nonpesticide com-

Table 1. Relative percent recoveries, average percent recoveries, and intra- and interassay variability percents for the 14 chlorinated hydrocarbon pesticides isolated from fortified whole oyster homogenate

	Rec. (n = 5), %						
Concn, ng/g	α-BHC	β-BHC	Lindane	Heptachlor	Aldrin	Heptachlor epoxide	*p,p'*-DDE
31.3	66 ± 16	65 ± 40	73 ± 23	65 ± 15	50 ± 12	71 ± 17	63 ± 11
62.5	84 ± 10	95 ± 30	78 ± 10	83 ± 19	69 ± 8	88 ± 17	78 ± 16
125	79 ± 8	84 ± 17	83 ± 6	81 ± 10	73 ± 7	82 ± 6	87 ± 16
250	86 ± 14	87 ± 12	82 ± 17	78 ± 17	74 ± 16	92 ± 15	86 ± 17
500	72 ± 9	86 ± 17	75 ± 6	69 ± 3	65 ± 2	74 ± 9	91 ± 14
Av. rec., %[a]	77 ± 13	84 ± 25	79 ± 13	75 ± 15	66 ± 13	82 ± 15	81 ± 17
IAV, %[b]	5.8	11.8	6.1	7.9	7.4	9	9
IRV, %[c]	15 ± 6	29 ± 18	16 ± 10	16 ± 8	14 ± 9	16 ± 6	18 ± 2
r[d]	0.9970	0.9980	0.9915	0.9864	0.9930	0.9950	0.9879
Concn, ng/g	Dieldrin	Endrin	4,4'-DDD	Endrin aldehyde	*p,p'*-DDT	Endosulfan sulfate	Methoxychlor
31.3	57 ± 13	65 ± 10	63 ± 18	59 ± 18	53 ± 7	78 ± 25	53 ± 11
62.5	67 ± 15	66 ± 14	79 ± 16	65 ± 12	59 ± 15	75 ± 30	72 ± 31
125	83 ± 14	76 ± 7	78 ± 10	70 ± 10	72 ± 13	73 ± 27	79 ± 33
250	82 ± 19	80 ± 17	91 ± 13	75 ± 13	76 ± 14	88 ± 14	69 ± 10
500	79 ± 6	80 ± 4	90 ± 17	69 ± 18	88 ± 16	70 ± 20	72 ± 8
Av. rec., %[a]	74 ± 16	73 ± 13	80 ± 17	68 ± 14	70 ± 18	77 ± 22	69 ± 22
IAV, %[b]	9.6	7.7	8.1	7.5	8	9.4	10.2
IRV, %[c]	18 ± 7	14 ± 7	19 ± 7	21 ± 7	19 ± 5	30 ± 9	26 ± 16
r[d]	0.9849	0.9854	0.9950	0.9980	0.9899	0.9950	0.9980

[a] $n = 25$.
[b] Intra-assay variability, $n = 5$.
[c] Interassay variability, $n = 25$.
[d] Correlation coefficient from linear regression analysis ($n = 5$).

pounds. Only δ-BHC, one of the less important isomers of hexachlorocyclohexane (benzene hexachloride), extracted consistently with the other pesticides and had an appropriate retention time on the capillary column. In screening for organochlorine pesticides, its use as an internal standard would consequently make it difficult to quantitate if it were present in the biological matrix. However, the gamma-isomer, lindane, is the only effective pesticide among the 8 stereoisomers of hexachlorocyclohexane; it is from 50 to several thousand times as toxic as the alpha- or delta-isomers (26). Early technical mixtures of hexachlorocyclohexane contained mostly the gamma- and beta-isomers, with some of the alpha-isomer also present (27). The delta-isomer was usually not present in the preparations. Furthermore, because the strength of this assay is its speed and simplicity as a screening procedure, the use of δ-BHC as an internal standard is not an absolute hindrance.

The combined effect of the C_{18} and Florisil makes this extraction column truly efficient for working with the large number of pesticides and a very complex biological matrix, such as oyster homogenate. In this column, the tissue being analyzed is combined with C_{18} to make a reversed-phase component for retaining neutral compounds. Florisil, the second component of the column, has historically been used for chromatographic cleanup of pesticide extracts (2, 17–19, 28, 29) and removes compounds too polar to be eluted by all but the most polar solvents. For example, in the mixture of acetonitrile–methanol (75 + 25), the high concentration of methanol caused removal of more nonpesticide compounds from the Florisil. However, 10% methanol enhanced the recovery of the pesticides without apparently removing other interferences from the Florisil.

This column can be adapted to extract many different types of compounds from many different types of tissues by simply changing the solvent system (20–22). Other methods are not as adaptable. For example, methods for some tissues require additional cleanup steps or chemical agents to further separate the pesticide residues from interfering biological substances before chromatographic analysis of an extract; this requirement can severely complicate and lengthen the time for analysis. The cleanup agent (H_2SO_4) of one method (19) actually destroys one of the pesticides being determined (dieldrin). However, the MSPD methods did not require these additional cleanup steps, because very little of the oyster homogenate incorporated into the column was extracted with the pesticides. The average amount of tissue and pesticide residue present in the eluate was 3.73 mg ($n = 5$), which was only 0.75% (on a wet weight basis) of the original amount of tissue and pesticide mixed with C_{18}.

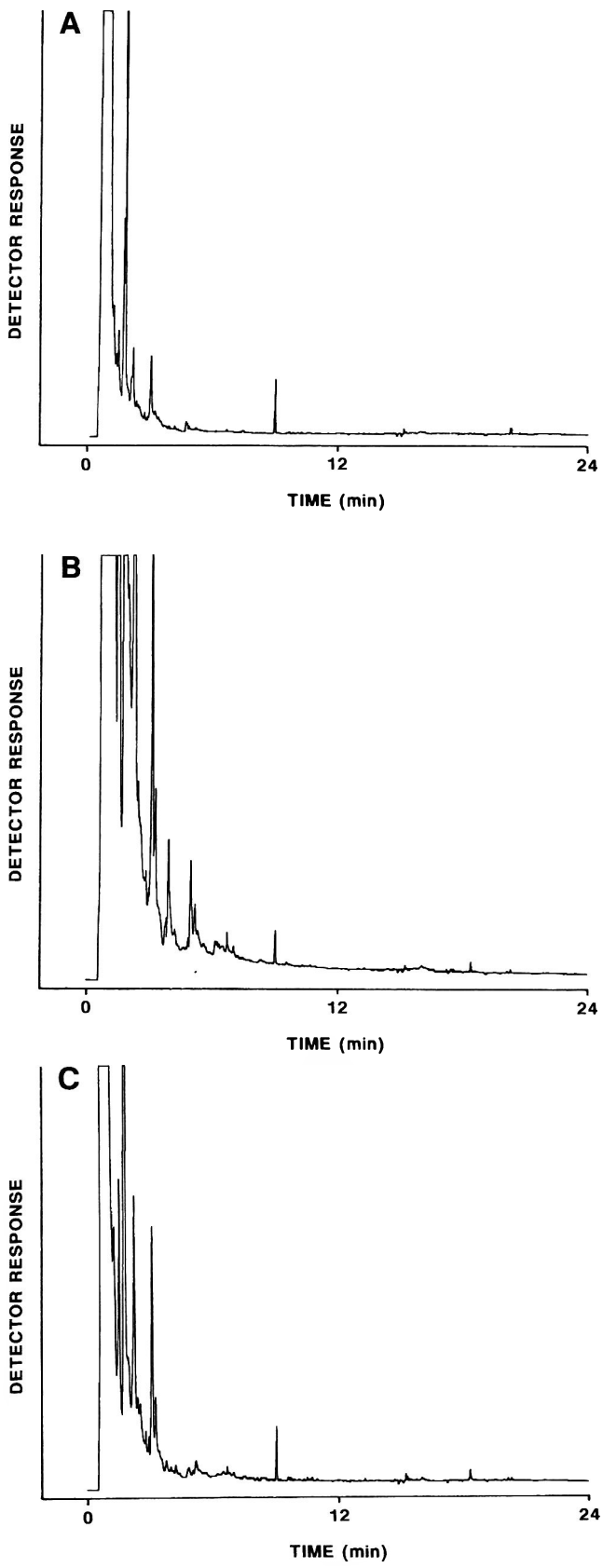


Figure 2. Gas chromatograms obtained from the electron capture detector analysis of the acetonitrile (A), acetonitrile–methanol (75 + 25) (B), and acetonitrile–methanol (90 + 10) (C) extract of blank controls.

Therefore, the 2 µL injections of eluate contained only 1.5 µg of material, a portion of which was pesticides.

Results of this study are based on fortified samples. Incurred samples would be ideal but were not available and were outside the scope of this research.

In conclusion, the MSPD methods were easily applied to the extraction of the 14 chlorinated pesticides from oyster tissue. The procedure is simple and rapid and requires only small samples sizes and volumes of solvent. The outlined methods may serve as rapid screening protocols for pesticides in oysters to detect food supply contamination. In addition, they may be applied to environmental-monitoring programs to screen for pesticides in oysters and other mollusks in the organisms' natural habitats or where they are placed as biomonitors.

Acknowledgments

This research was supported by Cooperative Agreement FDU-000235 with the U.S. Food and Drug Administration. The assistance of Tom Mahier is greatly appreciated.

References

(1) U.S. Department of Agriculture, Food Safety and Inspection Service (1990) *Compound Evaluation and Analytical Capability National Residue Program Plan*, J. Brown (Ed.), USDA, Washington, DC

(2) Nimmo, D.R. (1985) in *Fundamentals of Aquatic Toxicology: Methods and Applications*, G.M. Rand & S.R. Petrocelli (Eds), Hemisphere Publishing Corp., Washington, DC, p. 340

(3) U.S. Department of Health and Human Services, U.S. Public Health Service (1985) *Fourth Annual Report on Carcinogens: Summary 1985*, National Toxicology Program, Research Triangle Park, NC

(4) Casarett, L.J., Fryer, G.C., Yauger, W.L., & Klemmer, H.W. (1968) *Arch. Environ. Health* **17**, 306–311

(5) O'Leary, J.A., Davies, J.E., Edmundson, W.F., & Feldman, M. (1970) *Am. J. Obstet. Gynecol.* **106**, 939

(6) Spindler, M. (1983) *Residue Rev.* **90**, 1–34

(7) Hayes, W.J., Dale, W.E., & Pirkle, C.I. (1971) *Arch. Environ. Health* **22**, 119–135

(8) Hunter, C.G., Robinson, J., & Roberts, M. (1969) *Arch. Environ. Health* **18**, 12–21

(9) Hoffman, W.S., Adler, H., Fishbein, W.I., & Bauer, F.C. (1967) *Arch. Environ. Health* **15**, 758–765

(10) Nightingale, S.L. (1990) *Am. Fam. Physician* **42**, 1657–1658

(11) *J. Am. Vet. Med. Assoc.* (1990) **197**, 182

(12) O'Connor, T.P. (1991) *Environ. Health Perspect.* **90**, 69–73

(13) Marion, K.R., Barker, S.A., & Settine, R.L., (1987) in *Symposium on the Natural Resources of the Mobile Bay Estuary*, T. Lowery (Ed.), Alabama Sea Grant Extension Service, Auburn University, Auburn, AL, pp. 154–164

(14) Farrington, J.W., Goldberg, E.D., Risebrough, R.W., Martin, J.H., & Bowen, V.T. (1983) *Environ. Sci. Technol.* **17**, 490–496

(15) Butler, P.A., Kennedy, C.D., & Schutzmann, R.L. (1978) *Pestic. Monitor. J.* **12**, 99–101

(16) Butler, P.A. (1973) *Pestic. Monitor. J.* **6**, 238–246

(17) Ray, L.E., Murray, H.E., & Giam, C.S. (1983) *Chemosphere* **12**, 1031–1038
(18) Suzuki, T., Ishikawa, K., Sato, N., & Sakai, K. (1979) *J. Assoc. Off. Anal. Chem.* **62**, 681–684
(19) Murphy, P.G. (1972) *J. Assoc. Off. Anal. Chem.* **55**, 1360–1362
(20) Long, A.R., Soliman, M.M., & Barker, S.A. (1991) *J. Assoc. Off. Anal. Chem.* **74**, 493–496
(21) Long, A.R., Crouch, M.D., & Barker, S.A. (1991) *J. Assoc. Off. Anal. Chem.* **74**, 667–670
(22) Barker, S.A., Long, A.R., & Short, C.R. (1989) *J. Chromatogr.* **475**, 353–361
(23) Gordon, D.T., & Collins, N. (1982) *Lipids* **17**, 811–817
(24) Paradis, M., & Ackman, R.G. (1975) *Lipids* **10**, 12–16
(25) Ogamo, A., Watanabe, H., & Nagasawa, K. (1974) *J. Biochem.* **76**, 573–582
(26) Hassall, K.A. (1982) *The Chemistry of Pesticides*, Verlag Chemie, Weinheim, Germany
(27) *The Merck Index* (1976) 9th Ed., M. Windholz (Ed.), Merck & Co., Inc., Rahway, NJ
(28) Luke, M.A., Froberg, J.E., & Masumoto, H.T. (1975) *J. Assoc. Off. Anal. Chem.* **58**, 1020–1026
(29) Moats, W.A. (1963) *J. Assoc. Off. Anal. Chem.* **46**, 172–176

PESTICIDE AND INDUSTRIAL CHEMICAL RESIDUES

Determination of Organic Pollutants in Reagent Water by Liquid–Solid Extraction Followed by Supercritical Fluid Elution

PETER H. TANG
Technology Applications, Inc., 26 West Martin Luther King Dr, Cincinnati, OH 45268
JAMES S. HO and JAMES W. EICHELBERGER
U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, 26 West Martin Luther King Dr, Cincinnati, OH 45268

A selected group of polynuclear aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), organochlorine pesticides, and phthalate esters in reagent water containing no particulate matter were separated and identified by liquid-solid extraction and supercritical fluid extraction (SFE). The water sample is first passed through a cartridge or disk containing a solid matrix coated with a chemically bonded C_{18} organic phase capable of extracting those organic compounds. The cartridge or the disk is then eluted with supercritical carbon dioxide to remove the compounds from the sorbent. Finally, the extract is injected into a capillary column gas chromatographic/quadruple mass spectrometric system. The precisions, percent recoveries of analytes using solvent elution, and percent recoveries using SFE are compared. The total analysis time is greatly reduced by using disk extraction and SFE in place of cartridge extraction and liquid–solvent elution. In addition, the waste solvent generated was minimized by using SFE.

Received December 12, 1991. Accepted April 16, 1992.

After Congress passed the Pollution Prevention Act of 1990, the U.S. Environmental Protection Agency (EPA) issued its strategy for carrying out pollution prevention activities. A key part of this strategy is the Industrial Toxics Project, or the 33–50 Program. The agency intends to cut environmental emissions of the targeted chemicals 50% below 1988 levels by the end of 1995. That 1995 goal and an interim goal of 33% reduction by the end of 1992 provide the project name: the 33–50 Program. Dichloromethane (methylene chloride), which has been widely used as an extraction solvent in EPA methods (1, 2), is one of 17 listed toxic chemicals. Therefore, the agency is interested in the development of alternatives to extractions using methylene chloride. Analytical scale supercritical fluid extraction (SFE) is of interest to EPA because of its low solvent requirements and its potential to yield good recoveries with short extraction times (<1 h). Moreover, SFE uses a fluid (such as carbon dioxide) that is inexpensive, nontoxic, noncombustible, chemically inert, and easy to discard.

Several applications of SFE were reported and were the subject of recent reviews (3, 4). The majority of reports to date have focused on the extraction of analytes from solid matrixes; only a few applications of SFE for aqueous matrixes were reported. Hedrick and Taylor (5) proposed a direct extraction method similar to conventional purge-and-trap systems for semivolatile and polar analytes in a small volume of water. High levels (>500 ppb) of phosphonate, triprolidine, and phenol were extracted from 3 mL water by passing supercritical

carbon dioxide (CO_2) through a water sample via a recirculating pump. Their strategies were limited to small scales. The obvious difficulty is that the extraction cell must be of a geometry that retains the bulk of the water during the extraction. For trace analyte levels (low ppb), the direct extraction of large volumes (i.e., ≥500 mL) of water sample is more difficult because extracting 1 volume of sample requires 7 volumes of supercritical CO_2. Therefore, large volumes of supercritical CO_2 are required to extract the quantifiable amounts of analytes. Much research is needed in order to apply the technology to large-scale samples.

Some other applications of SFE to biological matrixes include extraction of cholesterol from eggs (6), concentration of eicosapentanoic acid from fish oil (7), and extraction of drugs and drug metabolites from a cell culture (8). Samples were mixed with adsorbents or added to adsorbents already loaded in the extraction vessels. After the analytes in biological samples were concentrated on the solid matrixes, they were successfully desorbed with supercritical fluid. To manipulate large volumes of water samples, liquid–solid extraction (LSE) proved to be an alternative method for trace analysis of semivolatile compounds (2). The preconcentration of analytes on solid sorbents (i.e., C_8 or C_{18} sorbent cartridges or disks), followed by supercritical CO_2 elution, may be a more appropriate approach. To investigate this approach, selected analytes were first fortified on Empore™ disks and eluted with supercritical CO_2 using various conditions to determine optimum elution parameters. Next, semivolatile organic compounds in large volumes of reagent water (500 mL or 1 L) were determined by using SFE after LSE. Results were compared with those published in the EPA Method 525 (2) in which LSE cartridges and liquid–solvent elution are used.

Experimental

Reagents and Materials

(a) *Solvents.*—Acetone and methanol, high purity pesticide quality. Solvents were not further purified before use. Reagent water was purified by using a Millipore Water System.

(b) *Standard materials.*—Organic compounds and internal and surrogate standards were the highest purity materials available from commercial sources and the EPA Repository for Toxic and Hazardous Materials, and they were used without purification.

(c) *Anhydrous sodium sulfate.*—Reagent grade. Heated 8 h at 450°C and stored in a tightly capped bottle until used.

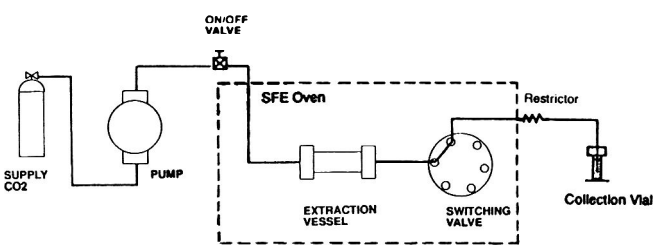


Figure 1. Off-line SFE configuration.

(d) *Carbon dioxide.*—SFC grade CO_2 (Scott, Avon Lake, OH 44012).

(e) *Helium.*—Carrier grade, 99.99%.

(f) *Adsorbents.*—Empore extraction disk (C_{18} bonded, 47 mm diameter) from 3M (St. Paul, MN 55144). LSE cartridge (C_{18} bonded, 500 mg/3 mL) from Baxter (McGraw Park, IL 60085).

Apparatus and Operating Conditions

(a) *SFE system.*—Model SFE/50 (Suprex Co., Pittsburgh, PA 15238) equipped with a 250 mL syringe pump (Figure 1). Pressurized CO_2 was passed through a stainless steel extraction cartridge that was capped at each end with the retaining sleeve and contained a 2 μm stainless steel frit and seal unit. The cartridge was placed inside a stainless steel extraction holder. The extraction cartridge (3.18 × 1.04 cm id) was fabricated in-house at the machine shop. It was designed to facilitate the loading of a LSE cartridge or a disk. An uncoated, deactivated, fused silica capillary column (20 × 50 μm id) was attached to the outlet of the extractor as a restrictor. The restrictor was inserted through a septum of a 15 mL screw cap vial. The vial contained 2 mL acetone. The restrictor was inserted ca 3 mm below the solvent level. Carbon dioxide was vented from the vial through a hypodermic needle inserted through the septum, and the extracted analytes were collected in the solvent.

(b) *Gas chromatography (GC) system.*—Model 5890A GC system equipped with Model 7673A autosampler and mass spectrometric (MS) system (Hewlett-Packard, Palo Alto, CA 94304). Fused silica capillary GC column (30 m × 0.25 mm id; DB-5.625 bonded-phase; 0.25 μm film thickness) obtained from J&W Scientific (Folsom, CA 95630). Carrier gas flow rate was ca 30 cm s^{-1}. Injector temperature, 250°C. Samples were injected (1.0 or 2.0 μL) by autosampler. Multiramp temperature program GC conditions were as follows: initial oven temperature, 60°C; held in splitless mode 1 min. Oven temperature was heated rapidly to 130°C at 35°C/min. At 3 min the following temperature program was started: 130–180°C at 10°C/min; 180–240°C at 6°C/min; 240–320°C at 10°C/min; final hold time 10 min. Data acquisition was started at 4 min.

(c) *Mass spectrometer.*—Model 5970B mass selective detector equipped with 59970C MS ChemStation data system (Hewlett-Packard). Transfer line was maintained at 280°C, and the mass spectrometer was scanned from 45 to 450 amu.

Optimization Study

Elution of compounds from solid sorbents is controlled by many factors, including the affinity of the compounds for the sorbent, the vapor pressures of the compounds, and the solubilities and diffusion coefficients of the compounds in the supercritical fluid. These factors governing analytical-scale SFE were reported for the extraction of polynuclear aromatic hydrocarbons (PAHs) by octadecyl sorbents (9). The above factors are also controlled by a complex relationship between many experimental variables, including pressure, temperature, flow volume, and solvent modifiers. A systematic study was conducted to optimize the elution conditions. In this study, a

Table 1. Effect of flow volume on the elution of analytes from the LSE disk

Compound[a]	Compound No.	Fortified concn, ppb	Rec., %, for 4 flow volumes[b]				ISD ref.[c]
			5 mL	10 mL	20 mL	30 mL	
Internal standards							
Naphthalene-d_8	1	20	—	—	—	—	—
Acenaphthene-d_{10}	2	20	—	—	—	—	—
Phenanthrene-d_{10}	3	20	—	—	—	—	—
Chrysene-d_{12}	4	20	—	—	—	—	—
Surrogate							
Perylene-d_{12}	5	20	—	—	—	—	4
Target analytes							
Naphthalene	6	10	98	97	107	96	1
Acenaphthylene	7	70	117	111	116	117	2
Acenaphthene	8	40	118	114	118	117	2
Fluorene	9	20	112	112	115	115	2
Phenanthrene	10	20	111	111	118	115	3
Anthracene	11	20	107	103	113	107	3
Fluoranthene	12	25	87	95	100	98	3
Pyrene	13	15	72	88	91	88	3
Chrysene	14	20	88	98	97	100	4
Benz[*a*]anthracene	15	25	88	95	98	101	4
Benzo[*b*]fluoranthene	16	15	72	83	92	85	4
Benzo[*a*]pyrene	17	35	83	87	105	99	4
Indeno[1,2,3-*c,d*]pyrene	18	15	65	85	103	112	4
Benzo[*ghi*]perylene	19	15	60	87	92	105	4
Mean	—	—	91	98	105	104	—

[a] With methanol modifier.
[b] Av. of 2 determinations at 400 atm and 50°C.
[c] ISD ref., internal standard reference; an internal standard is chosen to be referenced by other analytes having the closed retention.

single variable was changed while all other variables were kept constant. Three variables were investigated: pressure, temperature, and flow volume (amount of supercritical fluid).

The following selected PAHs, with molecular weights ranging from 128 to 278, were used as model compounds: naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*c,d*]pyrene, and benzo[*g,h,i*]perylene. A fortification standard mixture of all 14 compounds was prepared in acetone by using individual stock standard solutions. A 50 µL aliquot of this standard fortification solution was deposited directly onto the disk, and the acetone was allowed to evaporate. The disk was folded, rolled, and inserted into a 2.7 mL stainless steel SFE cartridge. Clean glass wool was placed at both ends of the cartridge to fill the void volume. Methanol, which was used throughout the systematic study as a modifier, was then added to the top of the sample disk and glass wool. Unless otherwise noted, the methanol modifier added was 400 µL. The extraction cartridge was immediately capped with 2 retaining sleeves at both ends, placed inside an extraction holder, and installed in the supercritical fluid extractor. SFE was then performed in the dynamic mode at selected conditions. Analytes were eluted from the disk, passed through a restrictor, and collected in a 15 mL vial containing 2 mL acetone. The collecting solvent contained 20 µg each of the 4 internal standards naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, and chrysene-d_{12}, and 20 µg of a surrogate, perylene-d_{12}. Eluted analytes in the acetone extract were then transferred to a GC autosampler vial and analyzed by GC/MS.

SFE can be performed in the dynamic mode, static mode, or a combination of the two. The dynamic mode allows for fresh supercritical CO_2 to pass continuously through the extraction vessel. The static mode uses a smaller amount of supercritical CO_2 to access the extractant/matrix by a diffusion-limited process. To determine the optimum elution approach, both the dynamic and static-dynamic modes were investigated. In this study, the following selected PAHs were fortified on the disk: acenaphthylene, fluorene, anthracene, pyrene, chrysene, benz[*a*]anthracene, and benzo[*a*]pyrene.

The dynamic elution was started at an initial pressure of 100 atm. Pressure was then increased rapidly to the optimized

Table 2. Effect of temperature on the elution of analytes from the LSE disk

Compound[a]	Compound No.	Fortified concn, ppb	Rec., %, at 4 temperatures[b]				ISD ref.[c]
			30°C	50°C	80°C	100°C	
Internal standards							
Naphthalene-d_8	1	20	—	—	—	—	—
Acenaphthene-d_{10}	2	20	—	—	—	—	—
Phenanthrene-d_{10}	3	20	—	—	—	—	—
Chrysene-d_{12}	4	20	—	—	—	—	—
Surrogate							
Perylene-d_{12}	5	20	—	—	—	—	4
Target analytes							
Naphthalene	6	10	51	95	101	104	1
Acenaphthylene	7	70	63	114	118	112	2
Acenaphthene	8	40	61	112	121	116	2
Fluorene	9	20	63	113	115	111	2
Phenanthrene	10	20	61	110	113	113	3
Anthracene	11	20	60	110	110	114	3
Fluoranthene	12	25	47	94	99	103	3
Pyrene	13	15	42	91	87	93	3
Chrysene	14	20	45	99	106	105	4
Benz[a]anthracene	15	25	41	96	97	111	4
Benzo[*b*]fluoranthene	16	15	35	78	82	86	4
Benzo[*a*]pyrene	17	35	37	85	91	96	4
Indeno[1,2,3-c,*d*]pyrene	18	15	18	80	88	73	4
Benzo[*g,h,i*]perylene	19	15	6	75	71	70	4
Mean	—	—	45	97	100	101	—

[a] With methanol modifier.
[b] Av. of 2 determinations at 400 atm and 10 mL flow volume.
[c] ISD ref., internal standard reference.

condition at a ramp rate of 100 atm/min and held at the optimized pressure for 20 min. With the static-dynamic mode, supercritical CO_2 was compressed into the extraction vessel and statically held at the optimized condition for 5 min. At 6 min, the dynamic elution was performed for 20 min. Use of methanol as a modifier was evaluated in both the dynamic and static-dynamic modes. Eluted analytes were collected in 2 mL acetone containing an internal standard (phenanthrene-d_{10}, 22.7 µg).

Sample Preparation

Bottles of reagent water, 1 L each, were fortified with PAHs, polychlorinated biphenyls (PCBs), organochlorine pesticides, and phthalates esters at 2 µg/L. Each water sample was then acidified with 2 mL 6N HCl to pH <2 and fortified with 100 µL solution containing internal and surrogate standards at 50 µg/mL each. Internal standards, which included naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, and chrysene-d_{12}, were used to measure the relative analyte responses, thus providing recovery corrections for the target analytes. Perylene-d_{12} surrogate was used to monitor the method performance with each sample.

In Method 525, 5 mL methanol was added to 1 L water sample and mixed well before extraction. Methanol was used to "wet" the support surface and prevent C_{18} ligands from matting down, so that more C_{18} was available for interaction with the sample. Without methanol, C_{18} ligands gradually form a compact mat to escape water during the extraction, leaving little exposure for interaction with the sample and resulting in low recoveries. Also, the number of flow paths are limited, which results in low flow rates. For trace analysis of a large volume of water sample, a low flow rate translates into a long extraction time.

Disk Extraction

Disks were prepared as follows: A 47 mm extraction disk was placed into the 47 mm filter apparatus and washed with 10 mL methanol. A slight vacuum of 13 cm (5 in.) of mercury was used during all operations with the apparatus. The disk was air-dried 1 min; 10 mL methanol was then pulled through the disk, followed by 10 mL reagent water to wash excess metha-

Table 3. Effect of pressure on the elution of analytes from the LSE disk

Compound[a]	Compound No.	Fortified concn, ppb	Rec., %, at 4 pressure values[b]				ISD ref.[c]
			200 atm	300 atm	350 atm	400 atm	
Internal standards							
Naphthalene-d_8	1	20	—	—	—	—	—
Acenaphthene-d_{10}	2	20	—	—	—	—	—
Phenanthrene-d_{10}	3	20	—	—	—	—	—
Chrysene-d_{12}	4	20	—	—	—	—	—
Surrogate							
Perylene-d_{12}	5	20	—	—	—		4
Target analytes							
Naphthalene	6	10	100	95	102	97	1
Acenaphthylene	7	70	116	114	107	111	2
Acenaphthene	8	40	115	112	108	114	2
Fluorene	9	20	115	113	107	112	2
Phenanthrene	10	20	113	110	116	111	3
Anthracene	11	20	111	110	113	103	3
Fluoranthene	12	25	91	94	101	95	3
Pyrene	13	15	75	91	96	88	3
Chrysene	14	20	93	99	97	98	4
Benz[*a*]anthracene	15	25	99	96	106	95	4
Benzo[*b*]fluoranthene	16	15	72	78	84	83	4
Benzo[*a*]pyrene	17	35	83	85	87	87	4
Indeno[1,2,3-*c,d*]pyrene	18	15	68	80	88	85	4
Benzo[*g,h,i*]perylene	19	15	60	75	82	87	4
Mean	—	—	94	97	100	97	—

[a] With methanol modifier.
[b] Av. of 2 determinations at 50°C and 10 mL flow-volume.
[c] ISD ref., internal standard reference.

nol out of the disk. The vacuum was released immediately after the water passed through the disk to prevent the disk from drying. Immediately after this step, 1 L of the water sample containing standards fortified at 2 μg/L was passed through the disk in ca 20 min with vacuum on. The disk was then dried by drawing air through it for 10 min.

Cartridge Extraction

This preparation procedure was based on the recommended steps described in EPA Method 525. An LSE cartridge was inserted through a rubber stopper into the vacuum system. The cartridge was flushed with two 10 mL portions of methanol and allowed to drain dry. Then, 10 mL reagent water was added to the solvent reservoir. Before the reagent water level dropped below the top edge of the packing in the LSE cartridge, the 500 mL water sample containing standards fortified at 4 μg/L was added to the solvent reservoir. The water sample drained into the cartridge and from the exit into the suction flask. More than 1 h was required for the 500 mL water sample to pass through the cartridge. After all of the water sample passed through, the cartridge was washed with 10 mL reagent water and dried by drawing air through the cartridge for 10 min.

SFE

After the extraction step, the flat disk was processed as described in the optimization study. The neck and the other end of the sample cartridge were trimmed to produce a cylinder shape ca 3.4 cm long, so that it could fit into the stainless steel extraction cartridge. Glass wool was then inserted at the open end of the sample cartridge. The cartridge was held upright, and the methanol modifier was added through the glass wool. The sample cartridge was then placed into the extraction cartridge, and 2 retaining sleeves were capped at both ends of the extraction cartridge. After the extraction vessel was installed, SFE was performed at the optimized set of conditions.

Supercritical CO_2 desorbed the analytes from the solid matrix. Desorbed analytes were then passed through a restrictor and collected in a 15 mL vial containing 2 mL acetone. Because supercritical CO_2 underwent expansive cooling upon decompression through the restrictor, the residual water froze at the tip of the restrictor and plugged the tip during the elution.

Table 4. Comparison of dynamic and static-dynamic modes

Compound	Fortified concn, ppb	Rec., %			
		Dynamic		Static-dynamic	
		With methanol modifier	Without methanol modifier	With methanol modifier	Without methanol modifier
		Internal standard			
Phenanthrene-d_{10}	22.7	—	—	—	—
		Target analytes			
Acenaphthylene	17	96	85	88	84
Fluorene	6	103	98	93	90
Anthracene	40	93	86	87	84
Pyrene	34	83	79	73	75
Chrysene	11	74	68	68	66
Benz[*a*]anthracene	11	75	63	72	65
Benzo[*a*]pyrene	6	52	42	48	43

To defrost the frozen tip of the restrictor, air was blown outside the receiving vial during the elution. About 1 mL extract remained in the receiving vial at the end of the elution. The collected analyte solution was then transferred to the GC autosampler vial and analyzed by GC/MS.

Results and Discussion

Elution Optimization

Relative effects of the controllable SFE variables were evaluated and optimized for maximum overall recoveries of sorbed analytes. Fortified PAHs were eluted from the disks under different conditions. The same concentration of fortified PAHs was used throughout the optimization study (Table 1). Results are summarized below.

Effects of flow volume.—Experiments were performed at a constant flow rate of 1.5 mL supercritical CO_2/min to study the effects of flow volume. Table 1 lists the percent recoveries of PAHs with increasing flow volumes of supercritical CO_2. Naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, and anthracene were eluted with 5 mL supercritical CO_2, yielding >95% recoveries. The other compounds required

Table 5. Single-laboratory accuracy and precision from 4 replicate analyses of fortified reagent water[a]

Compound	Concn, µg/L	Group I			Group II		
		R	SD	RSD	R	SD	RSD
Naphthalene	5	101.9	5.6	5.5	94.0	9.4	10.0
Acenaphthylene	25	91.6	4.8	5.3	97.3	8.5	8.8
Acenaphthene	25	109.4	10.2	9.3	107.3	8.0	7.4
Fluorene	25	120.9	15.1	12.5	129.3	25.2	19.5
Phenanthrene	25	106.9	8.3	7.7	113.3	5.8	5.1
Anthracene	5	99.6	18.6	18.7	104.2	5.2	5.0
Fluoranthene	25	107.2	11.5	10.7	99.7	13.1	13.1
Pyrene	5	99.6	15.9	16.0	95.3	14.2	14.9
Chrysene	5	101.9	5.3	5.2	90.2	3.4	3.8
Benz[*a*]anthracene	5	102.4	3.3	3.2	105.2	3.8	3.6
Benzo[*b*]- and benzo[*k*]fluoranthene	17.5	83.8	4.5	5.3	81.7	3.8	4.7
Benzo[*a*]pyrene	5	70.5	3.3	4.7	73.1	1.5	2.0
Perylene-d_{12}	10	61.5	4.4	7.2	65.0	3.4	5.2
Indeno[1,2,3-*c,d*]pyrene	5	50.0	2.2	4.3	46.9	4.4	9.3
Dibenz[a,*h*]anthracene	5	50.0	7.4	14.9	11.5	5.4	47.0
Benzo[*g,h,i*]perylene	5	41.4	4.1	9.8	38.1	4.5	11.8
Mean	—	87.4	7.8	8.8	84.5	7.5	10.7

[a] R, mean rec., %.; SD, standard deviation of mean; RSD, relative standard deviation of mean; Group I disks contained less than 10% (w/w) or 60 µL water; Group II disks contained greater than 10% water.

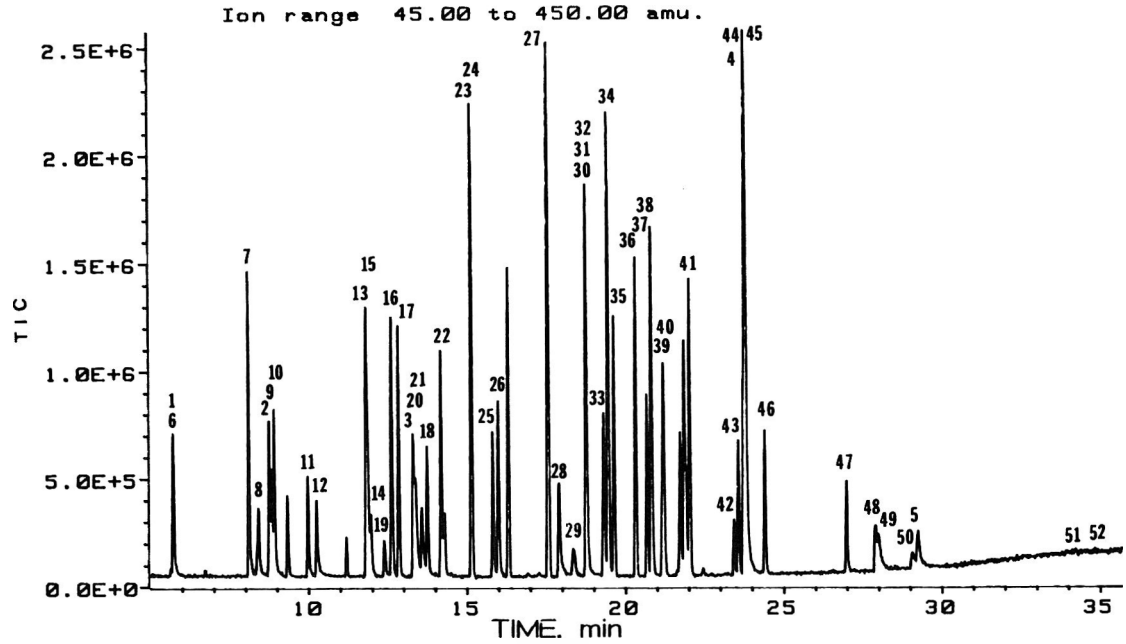


Figure 2. Standard GC/MS chromatogram of the target analytes at 5 ng/μL (1 μL injection). See Table 6 for peak identification and *Experimental* for conditions.

≥20 mL supercritical CO_2 to obtain >90% recoveries. The volume of supercritical CO_2 required is about 7 times the extraction cell volume.

Temperature effects.—Several studies evaluated temperature effects on the desorption of analytes from the disk. Fortified PAHs were eluted from the disks at 4 different temperatures (30, 50, 80, and 100°C) at a constant pressure and flow volume. A 10 mL amount of supercritical CO_2 was purposely chosen to yield less than quantitative recovery of the PAHs so that comparisons could be made between temperatures and pressures. Recoveries increased between 50 and 100°C (Table 2). The lowest temperature, 30°C, was below the critical temperature and produced the lowest results, because the CO_2 was not in the supercritical state. There are no significant differences in the recoveries over the temperature range of 50–100°C.

Pressure effects.—Density and solvent strength of supercritical CO_2 change as the pressure increases. The influence of pressure on the recovery of fortified PAHs from disks was studied at 4 conditions. As Table 3 shows, percent recoveries range from 60 to 116%. Nine compounds showed recoveries >90% over the 200–400 atm pressure range. Increasing the pressure from 200 to 300 atm increased the extraction efficiencies of 4 compounds: pyrene, benzo[*b*]fluoranthene, indeno[1,2,3-*c,d*]pyrene, and benzo[*g,h,i*]perylene. Recoveries of 3 compounds, benzo[*b*]fluoranthene, indeno[1,2,3-*c,d*]pyrene, and benzo[*g,h,i*]perylene, were slightly increased when pressures were varied from 300 to 400 atm. Most PAHs can be extracted with more than 80% efficiency at a pressure of 300 atm or above.

In all the above experiments, internal standards were added to the collecting solvent before elution, and these internal standards were used to measure the relative responses of the target analytes. Because some of the selected PAHs were highly volatile, the internal standards added before elution were intended to minimize the effect of the evaporation losses of the target analytes during the elution. Although some recoveries greater than 100% were obtained, results indicated that these compounds were desorbed from the matrix more efficiently than the other nonvolatile compounds. Results due to losses of highly volatile internal standards were >100%, which resulted in the increased response factors of the target analytes. Nevertheless, the results provided information about the relative effects of flow volume, temperature, and pressure. Conditions of 300 atm at 60°C and 30 mL flow volume produced maximum overall recoveries of analytes.

Comparison of Static-Dynamic and Dynamic Modes

Table 4 lists a comparison of dynamic and static-dynamic elutions. Two sets of duplicates (one with and one without the methanol modifier) were tested in the dynamic and static-dynamic modes. Recovery measurements were based on the relative response of each analyte to an internal standard, phenanthrene-d_{10}. Each percent recovery was obtained by calculating the recovered quantity against the fortified quantity. Under the optimized conditions of SFE, results for all analytes from 4 sets of experiments showed no significant differences. When methanol modifier was not added to the extraction cell, plugging was a problem. The small amount of residual water from the disk caused the restrictor to plug during the elution step. Because supercritical CO_2 underwent expansive (Joule-Thompson) cooling upon decompression, the water froze at the tip of the restrictor. The plugging problem was largely resolved by

Table 6. Accuracy and precision data from the determinations of organic pollutants in reagent water by LSE followed by SFE and GC/MS

Compound	Peak No.	Disk mean rec., %[a]	RSD, %	Cartridge mean rec., %[b]	RSD, %	ISD ref.
			Internal standards			
Naphthalene-d_8	1	—	—	—	—	—
Acenaphthene-d_{10}	2	—	—	—	—	—
Phenanthrene-d_{10}	3	—	—	—	—	—
Chrysene-d_{12}	4	—	—	—	—	—
			Surrogate			
Perylene-d_{12}	5	45	29	36	27	4
			Target analytes			
Naphthalene	6	100	10	120	31	1
Dimethylphthalate	7	96	10	26	19	2
Acenaphthylene	8	54	52	64	70	2
Acenaphthene	9	99	3	97	15	2
2-Chlorobiphenyl	10	92	5	97	15	2
Diethylphthalate	11	124	10	58	53	2
Fluorene	12	113	10	135	27	2
2,3-Dichlorobiphenyl	13	99	20	166	32	2
Hexachlorobenzene	14	70	29	105	30	2
Lindane components						
α-BHC	15	82	14	45	90	3
γ-BHC	16	125	23	19	116	3
β-BHC	17	94	22	45	101	3
δ-BHC	18	75	36	22	118	3
Simazine	19	106	20	0	0	3
Phenanthrene	20	108	5	104	1	3
Anthracene	21	77	20	71	24	3
2,4,5-Trichlorobiphenyl	22	83	15	72	9	3
Heptachlor	23	106	18	57	15	3
Pentachlorophenol	24	97	12	55	18	3
Di-*n*-butylphthalate	25	150	20	172	20	3
2,2′,4,4′-Tetrachlorobiphenyl	26	94	34	84	10	3
Heptachlor epoxide	27	105	12	89	21	3
Fluoranthene	28	106	16	107	5	3
Chlordane components						
α-Chlordane	29	115	21	96	16	3
γ-Chlordane	30	95	32	60	27	3
Endosulfan I	31	144	18	118	7	3
2,3,4,5,6-Pentachlorobiphenyl	32	86	12	116	3	3
Pyrene	33	125	10	145	6	3
4,4′-DDE	34	109	6	121	8	3
Dieldrin	35	154	27	133	5	3
2,2′,4,4′,5,5′-Hexachlorobiphenyl	36	72	19	108	7	3
Endrin	37	136	33	125	11	3
Endosulfan II	38	143	24	71	39	3
4,4′-DDD	39	117	13	123	10	3
Endrin aldehyde	40	152	30	94	53	3
4,4′-DDT	41	106	12	70	20	3
Butylbenzylphthalate	42	117	22	160	5	4
Benz[a]anthracene	43	122	9	64	7	4
Chrysene	44	96	15	156	15	4
2,2′,3,3′,4,4′,6-Heptachlorobiphenyl	45	103	25	106	12	4
Methoxychlor	46	126	14	80	32	4

Table 6. (*Continued*)

Compound	Peak No.	Disk mean rec., %[a]	RSD, %	Cartridge mean rec., %[b]	RSD, %	ISD ref.
Di(2-ethylhexyl)phthalate	47	140	25	101	29	4
Benzo[*b*]fluoranthene	48	77	14	78	22	4
Benzo[*k*]fluoranthene	49	82	10	66	42	4
Benzo[*a*]pyrene	50	56	14	42	33	4
Indeno[1,2,3-*c,d*]pyrene	51	36	75	0	0	4
Benzo[*g,h,i*]perylene	52	20	70	0	0	4

[a] $n = 7$; 1 L reagent water containing target analytes at 2 µg/L was passed through the LSE disk.
[b] $n = 4$; 500 mL reagent water containing the target analytes at 4µg/L was passed through the LSE cartridge.

adding methanol modifier. The dynamic mode with methanol modifier was shown to be the most suitable mode.

Residual Water Effect

To investigate the effect of residual water remaining in the disk on the elution efficiencies of SFE, a 1 L water sample containing 16 semivolatile and 5 deuterated PAHs was passed through the disk. These deuterated PAHs included 4 internal standards, naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, and chrysene-d_{12}, and a surrogate, perylene-d_{12}. The degree of disk drying was controlled by the length of time of the final air-drying step (5–10 min). The content of water was determined by the difference in the weights of the disk before and after the disk extraction process.

Precision and accuracy data were generated under 2 sets of disk conditions (Table 5). Group I disks contained <10% (w/w) water. Group II disks contained >10% water. We found that wet disks, for which the water percentage was >10%, caused the capillary restrictor of the SFE to plug during the elution step. Although the restrictor was not totally plugged, this problem made the elution process inconsistent and lengthy. Passing 30 mL supercritical CO_2 through Group I disks required about 20 min; a longer elution time (>40 min) was required for Group II.

Mean recoveries and relative standard deviations for the 16 analytes under each condition are also shown in Table 5. No significant differences exist between Group I and Group II, except for one analyte, dibenz[*a,h*]anthracene, which has a lower recovery and higher relative standard deviation. The presence

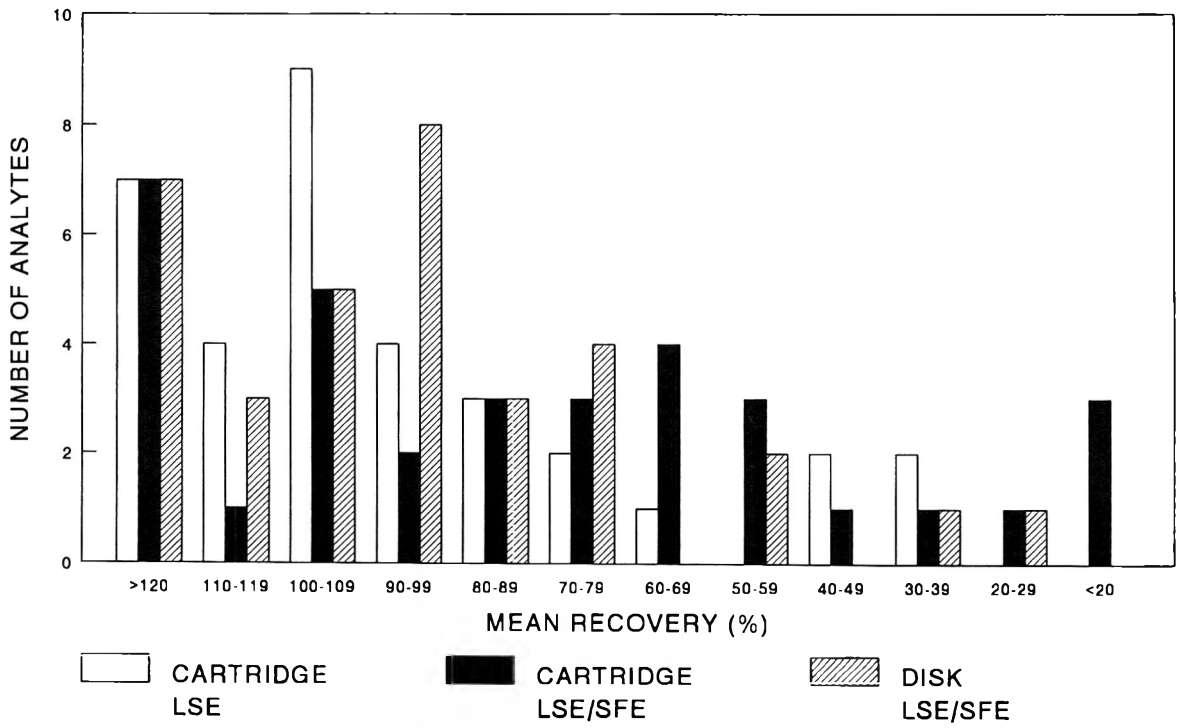


Figure 3. Distribution of mean recovery of 36 analytes from 3 methods: (I) cartridge/LSE, (II) cartridge/LSE/SFE, (III) disk/LSE/SFE.

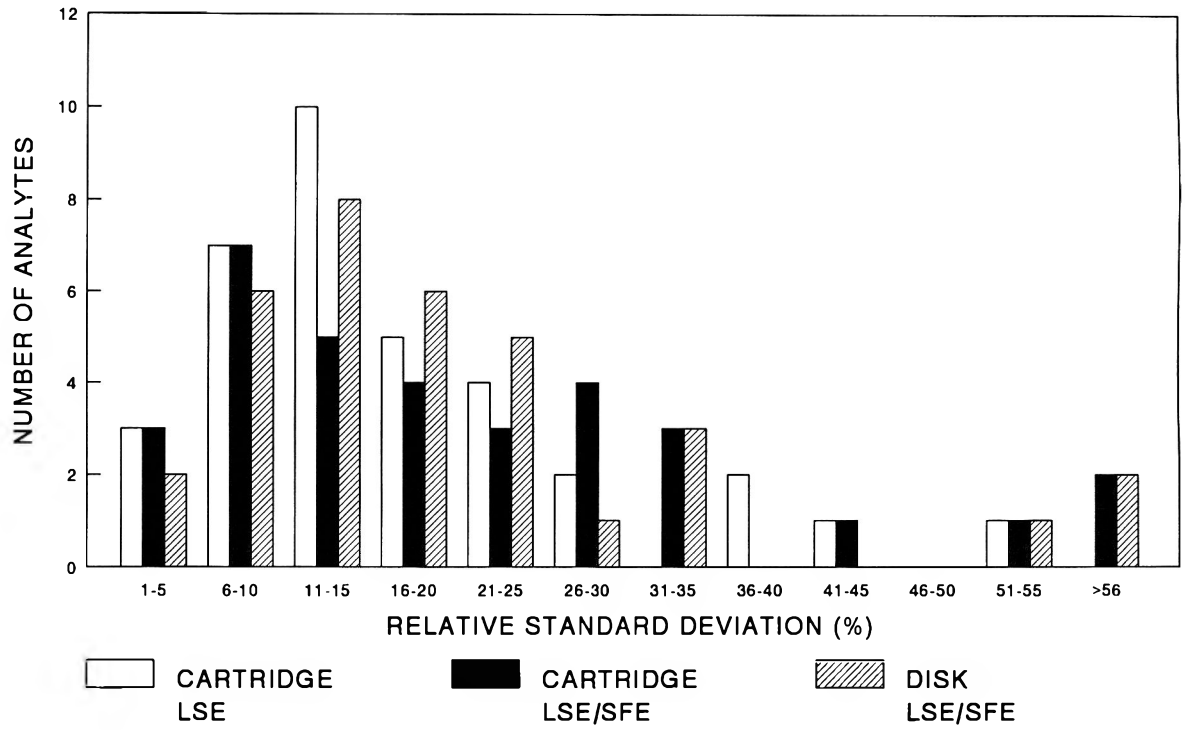


Figure 4. Distribution of relative standard deviations among 36 analytes from 3 methods: (I) cartridge/LSE, (II) cartridge/LSE/SFE, (III) disk/LSE/SFE.

of water (>10% w/w, or >60 μL) appears to affect the recovery of this compound from the disk, but the phenomenon is not understood. A longer drying time (10 min) is recommended to reduce the elution time required.

Comparison Between Disk and Cartridge

The performance of disk extraction was evaluated for a variety of semivolatile organic compounds and compared with the results obtained by using cartridge extraction. Several groups of compounds including PAHs, PCBs, organochlorine pesticides, and phthalate esters were selected for this study. A typical standard chromatogram is shown in Figure 2. Single laboratory accuracy and precision data from the disk extractions are shown in Table 6, along with recovery data from the cartridge extractions. In Method 525, a 1 L water sample is passed through a 6 mL cartridge containing approximately 1 g C_{18} coated solid sorbent. Three mL cartridges containing about 500 mg C_{18} sorbent each were used in this study because of the size limitation of the extraction vessel. To compare the results published in Method 525, in which the final concentration of extract before analysis was 2 μg/mL, 500 mL volumes of water sample at 4 μg standard/L were chosen for the cartridge-SFE study. The cartridge extraction time for 500 mL water samples was 1–2 h, whereas the extraction time using the disk as the sample preparation device was complete within 20 min.

Despite the shorter extraction time required, the general performance of the disk (mean recovery, 100%) is better than that of the cartridge (mean recovery, 86%). With the disk procedure, the relative standard deviations of recovery (RSD) for 3 analytes were >50%; with the cartridge procedure, RSDs for 7 analytes were >50%. With the cartridge, recovery was zero for simazine, indeno[1,2,3-*c,d*] pyrene, and benzo[*g,h,i*]perylene; the disk showed better results for these compounds.

Figures 3 and 4 show the comparison of recoveries and RSDs for 3 approaches: cartridge/LSE, cartridge/LSE/SFE, and disk/LSE/SFE. The mean recoveries of 34 common compounds among 3 methods were chosen to compare the performances of these methods. The mean recovery using disk/LSE/SFE (95%) compares well with the 100% recovery of analytes using cartridge/LSE in Method 525 and is better than cartridge/LSE/SFE recovery (84%) of this study. Average RSDs of the disk and cartridge results in this study were 21% and 22%, respectively. These compare well with the 15% in Method 525.

Figure 3 shows the distribution of mean recoveries among 34 analytes. Recoveries published in Method 525 were 35–175%; recoveries by the disk/LSE/SFE method ranged from 20 to 152%. For both disk/LSE/SFE and Method 525, 30 compounds had mean recoveries of ≥70%, compared with 21 compounds by the cartridge methods. Three analytes were not extracted by the cartridge procedure. Figure 4 shows the distribution of estimated standard deviations of the mean recoveries among 34 analytes. For the disk procedure, 29 recoveries had estimated standard deviations of ≤25%, compared with 25 by the cartridge procedure. Thirty recoveries by Method 525 had estimated standard deviations of ≤25%.

Because use of environmentally unfriendly organic solvents was reduced, the SFE method has the advantage of low solvent

consumption and less waste. Only 2.4 mL organic solvent (2 mL acetone and 400 μL methanol) was used in SFE, whereas at least 15 mL methylene chloride was required for the liquid–solvent elution in Method 525. Although the reduced volume of methylene chloride used to analyze reagent water is not tremendous, the advantage of SFE would be greater for real-world samples. Real-world samples contain particulates onto which many analytes may adsorb rather strongly, and a larger volume of methylene chloride is required to elute the analytes from these particulates. Thus, the extract produced will be of high volume and low concentration and will require extensive concentration before it can be analyzed by GC/MS. In Method 525, about 15 min is needed to concentrate 15 mL extract to 1 mL (2). The total time required for the liquid–solvent elution and concentration steps is about 25 min. In the same time span, SFE combines the elution and concentration into one step, which translates into less sample handling. With trace analysis, less sample handling reduces the possibility of gross experimental error.

Conclusion

The combination of LSE using disks and cartridges and SFE to extract semivolatile and nonvolatile organic compounds from water was demonstrated. Flow volume, temperature, and pressure conditions were evaluated. Higher pressures increase extraction efficiencies for most of the PAHs from the disk. Increased flow volumes result in higher extraction efficiencies. The plateau of flow volume is reached at 20 mL supercritical CO_2, which is about 7 times the extraction cell volume. Temperature had the least effect on the recovery of PAHs in this study. A 50–100°C temperature can be chosen arbitrarily for an extraction. SFE conditions of 300 atm, 60°C, and 30 mL flow volume were chosen to perform extraction experiments in the determination of organic pollutants in reagent water. Analytical time is shortened considerably by using the SFE method and the disk in place of the conventional cartridge–liquid solvent elution method. To concentrate analytes from large volumes of liquid samples, the LSE disk is more efficient than the LSE cartridge.

Although this report focuses only on the use of SFE in reagent water (particulate-free), we believe that SFE should show its elution power for real water samples containing particulates. Because supercritical CO_2 has almost no surface tension, it can penetrate low-porosity particulates that may adsorb some analytes. The application of SFE to real water samples will be explored in the near future. Use of SFE with the disk for lower method detection limits is also under investigation.

Acknowledgment

The authors thank William L. Budde, Director of Chemistry Research Division, Environmental Monitoring Systems Laboratory-Cincinnati, EPA, for his support and suggestions on this research project and for reviewing the manuscript.

References

(1) Eichelberger, J.W., Kerns, E.H.. Olynyk, P.O., & Budde, W.L. (1983) *Anal. Chem.* **55**, 1471–1479

(2) Eichelberger, J.W., Behymer, T.D., & Budde, W.L. (1988) *Revised July, 1991, Method 525: Revision* **2.2**, U.S. Environmental Protection Agency, Environmental Monitoring System Laboratory, Cincinnati, OH

(3) Hawthorne, S.B. (1990) *Anal. Chem.* **62**, 633A–642A

(4) Veuthey, J.L., Caude, M., & Rosset, R. (1990) *Analusis* **18**, 103–111

(5) Hedrick, J.L., & Taylor, L.T. (1990) *J. High Resolut. Chromatogr.* **13**, 312–316

(6) King, J.W., Eisslor, R.L., & Friedlich, J.P. (1987) *ACS Symp. Ser.* **366**, 63–68

(7) Krukonis, V.J. (1987) *ACS Symp. Ser.* **366**, 26–43

(8) McNally, M.E.P., & Wheeler, J.R. (1988) *J. Chromatogr.* **435**, 63–71

(9) Rein, J., Cork, C.M., & Furton, K.G. (1991) *J. Chromatogr.* **545**, 149–160

PESTICIDE FORMULATIONS

Simplified Method to Measure Suspensibility of Water-Dispersible Powder: Use of Microanalytical Techniques to Reduce Wastewater

MASAO OGAWA, TOSHIRO OHTSUBO, SHIGENORI TSUDA, and KOZO TSUJI
Sumitomo Chemical Co., Ltd., Takarazuka Research Center, Agricultural Science Research Laboratory, Takatsukasa, Takarazuka 665, Japan

A method was developed for suspensibility of pesticides in the form of water-dispersible powders (WPs) as an alternative to CIPAC method MT 15.1. After the suspensibility study, the methods, along with several variations, were compared on the basis of reduction of waste, simplicity of the procedures used, and time required to measure suspensibility. WPs in this study were prepared by using fenitrothion and procymidone as representatives of liquid and solid active ingredients. The best method was obtained by modifying CIPAC MT 15.1 as follows: (a) A 100 mL measuring cylinder was used instead of a 250 mL cylinder, (b) the process for preparing a suspension in a beaker was deleted, (c) the content of the active ingredient in the top 90% was analyzed instead of the bottom 10%, and (d) the process for evaporating water from a suspension removed by pipet from the top 90% was deleted. Suspensibility determined by this method showed good consistency with that determined by the original CIPAC method, and results were reproducible. Time required for determination of suspensibility and the amount of waste produced by this method was about 40% of that for the original CIPAC method. Furthermore, this method is suitable for WPs made with both liquid and solid active ingredients.

Water-dispersible powder (WP) is one of the most widely used pesticide formulations (1). To develop an excellent WP, many kinds of physicochemical properties must be considered; the most important is suspensibility, i.e., the uniformity of the suspension after the WP is diluted in water.

Received October 10, 1991. Accepted May 15, 1992.
This report was presented at the 35th CIPAC Symposium, June 20, 1991, at Braunschweig, Germany.

Although many methods of measuring suspensibility have been proposed (2, 3), the CIPAC MT 15.1 method is one of the most widely used (4). However, the CIPAC method is a little complicated, and a long time is required to prepare an analytical sample. Ogawa et al. (5) previously proposed a simplified method to measure the suspensibility of WPs; the method is less complicated and requires less time for the preparation of the samples.

Recently, stricter regulations on waste water containing pesticides have increased the difficulty of treating such waste water, not only at the production sites but also at quality control laboratories. However, in method CIPAC MT 15.1, 250 mL of the suspension is the required volume for measuring WP suspensibility, and a large amount of waste solution is produced after the suspensibility test. In the present study, further modifications of the CIPAC method were studied to reduce the amount of waste solution and to greatly simplify the method. Suspensibility is measured by using WPs containing fenitrothion and procymidone as representative of liquid and solid active ingredients, respectively.

Experimental

Preparation of Powders

(a) *Fenitrothion 40 wt% WPs.*—The following 3 kinds of fenitrothion 40 wt% WPs were prepared by blending fenitrothion, precipitated hydrated silicone dioxide, diatomite, and several surfactants: Recipe A, excellent suspensibility; Recipe B, excessive flocculation during the suspensibility test after dilution at 25 g (a.i.)/L; and Recipe C, poor suspensibility.

(b) *Procymidone 50 wt% WPs.*—Four kinds of procymidone 50 wt% WPs having different particle sizes were prepared by pulverizing procymidone and blending it with surfactants and kaolin clay. Average particle sizes of procymidone were as follows; Recipe D, 4.7 μm; Recipe E, 8.3 μm; Recipe F, 17.5 μm; and Recipe G, 24.5 μm.

Susceptibility Measurement

CIPAC MT 15.1.—As shown in Figure 1, fenitrothion 40 wt% WP (15.625 and 0.5 g) or procymidone 50 wt% WP

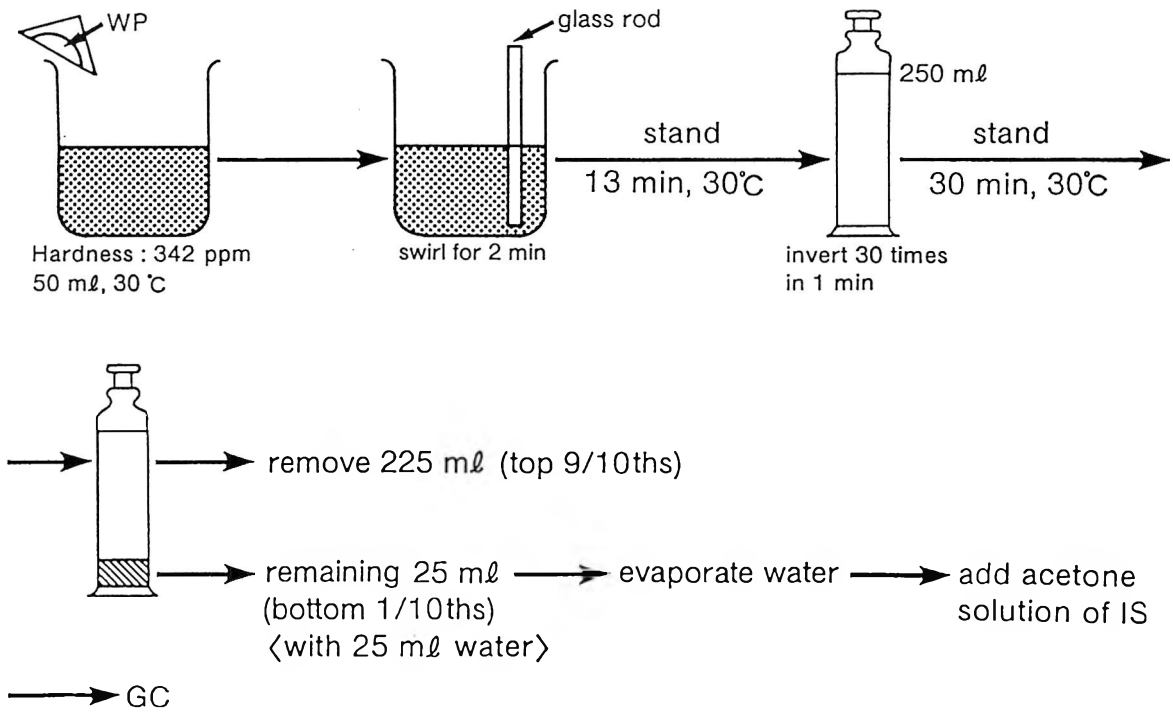


Figure 1. Method for measuring suspensibility by CIPAC MT 15.1.

(5 and 0.5 g) was weighed. The weighed sample was added slowly to a beaker containing 50 mL 342 ppm hard water (at 30°C) and swirled by a glass rod in a circular motion 120 times/min for 2 min. The suspension was left to stand a further 13 min in a water bath at the same temperature.

The suspension was transferred to the measuring cylinder and diluted to 250 mL with 342 ppm hard water at 30°C. The cylinder was inverted 30 times in 1 min in a 180° arc. The cylinder was then placed in the 30°C water bath. After 30 min, 225 mL (top 90%) of the suspension was removed with a suction tube.

The remaining 25 mL in the cylinder (bottom 10%) was transferred to a flask with ca 25 mL distilled water. The water was evaporated by vacuum, and an acetone solution of internal standard (IS) was added to prepare an analytical sample for gas chromatography (GC). With sample solutions of very high concentrations, 9 or 19 mL acetone was added to a 1 mL aliquot of the sample solution. The content of active ingredient in

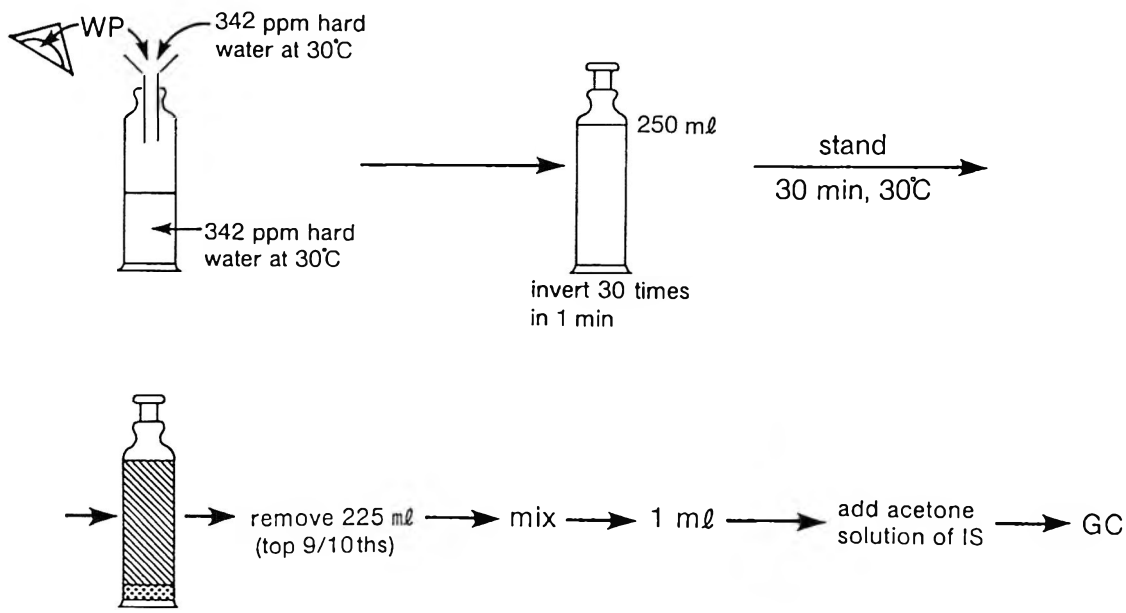


Figure 2. Modified method for measuring suspensibility (method B-7).

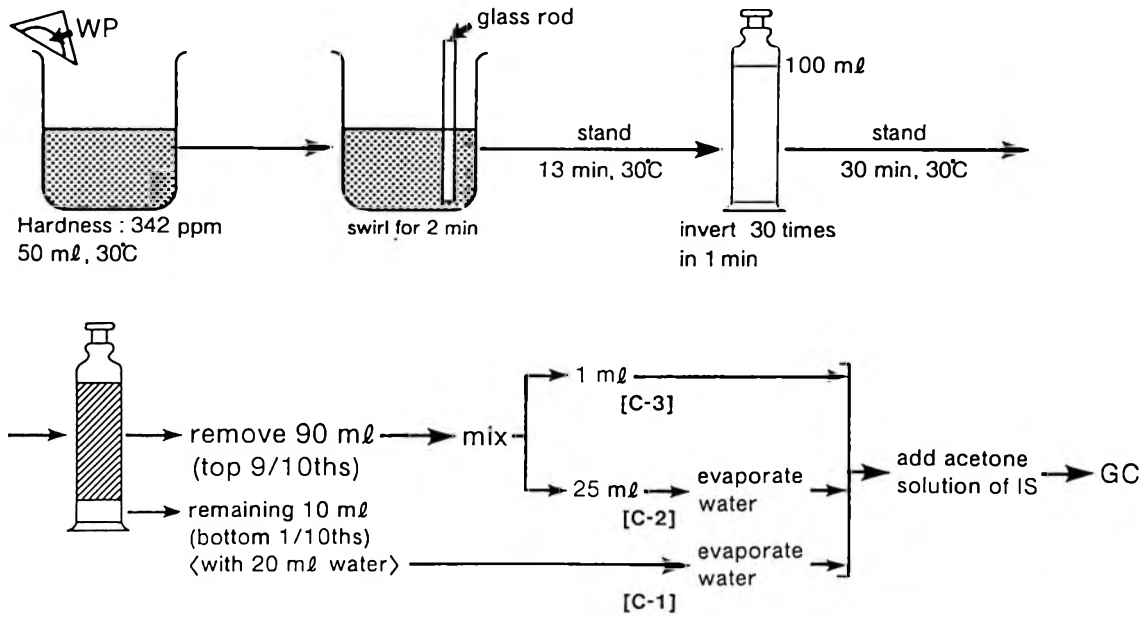


Figure 3. Modified methods for measuring suspensibility (methods C-1 and C-2).

the original sample was determined separately by the same GC method.

For fenitrothion 40 wt% WPs, the IS was fluoranthene, and the conditions of GC were as follows: flame ionization detection (FID) system; column, 2 m × 3 mm id; column packing, Chromosorb W-HP (100–120 mesh) treated with 30 mL PPE-6 R/L; oven temperature, 195°C; injection temperature, 200°C; detector temperature, 250°C; carrier gas, nitrogen (30 mL/min).

For procymidone 50 wt% WPs, the IS was dicyclohexyl phthalate, and the conditions of GC were as follows: FID system; column, 1.1 m × 3 mm id; column packing, Chromosorb W (AW, DMSC, 60–80 mesh) treated with 5% silicone XE-60; oven temperature, 200°C; injection temperature, 250°C; detector temperature, 250°C; carrier gas, nitrogen (50 mL/min).

Suspensibility was calculated by using the following equation (4).

$$\text{Suspensibility} = \frac{10}{9} \times \frac{100\,(c - Q)}{c} = \frac{111\,(c - Q)}{c} \quad \% \qquad (1)$$

where c = the weight (g) of active ingredient in the sample actually taken, and Q = the weight (g) of active ingredient in the bottom 10%.

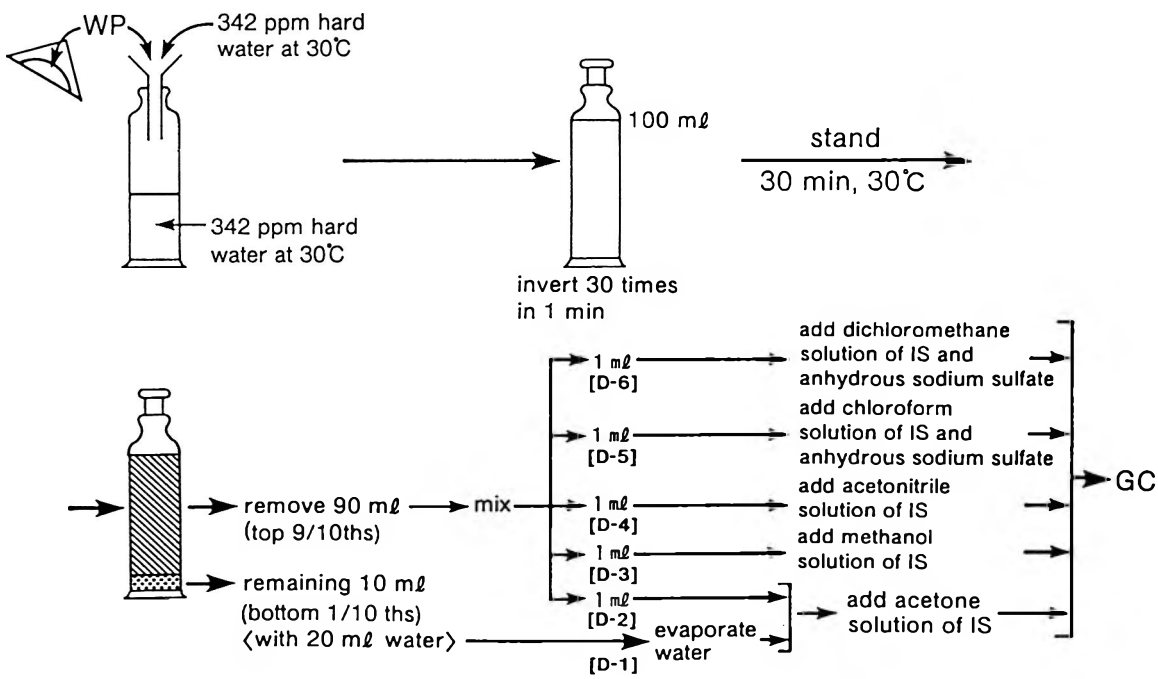


Figure 4. Modified methods for measuring suspensibility (methods D-1 to D-6).

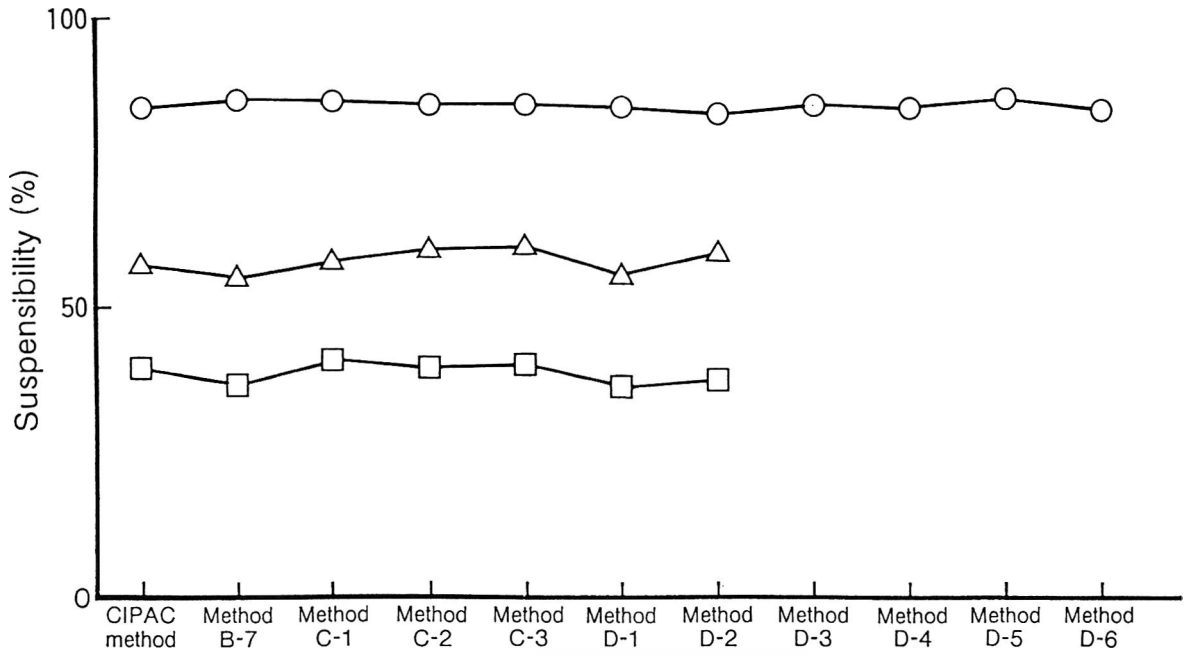


Figure 5. Suspensibility of fenitrothion 40% WP (342 ppm hard water, 30°C, 25 g [a.i.]/L, 30 min after dilution): ○—○, Recipe A; △—△, Recipe B; □—□, Recipe C.

Simplified method (method B-7).—Method B-7, which was the same method described in our previous paper (5), was the best simplification of the CIPAC method.

As shown in Figure 2, the following points were modified: The process for preparing a suspension in a beaker was deleted, the content of active ingredient in the top 90% was analyzed instead of the bottom 10%, and the process for evaporating water from a suspension removed from the top 90% by pipet was deleted.

Suspensibility was calculated by the following equation:

$$\text{Suspensibility} = Q/c \times 250/d \times 100 \quad \% \qquad (2)$$

where c = the weight (g) of active ingredient in the sample actually taken, d = the volume (mL) of the suspension removed by pipet from the top 90%, and Q = the weight (g) of active ingredient in the d mL aliquot removed by pipet from the top 90%.

Simplified method (methods C-1 to C-3).—Suspensibility was measured as in the CIPAC method described above, except

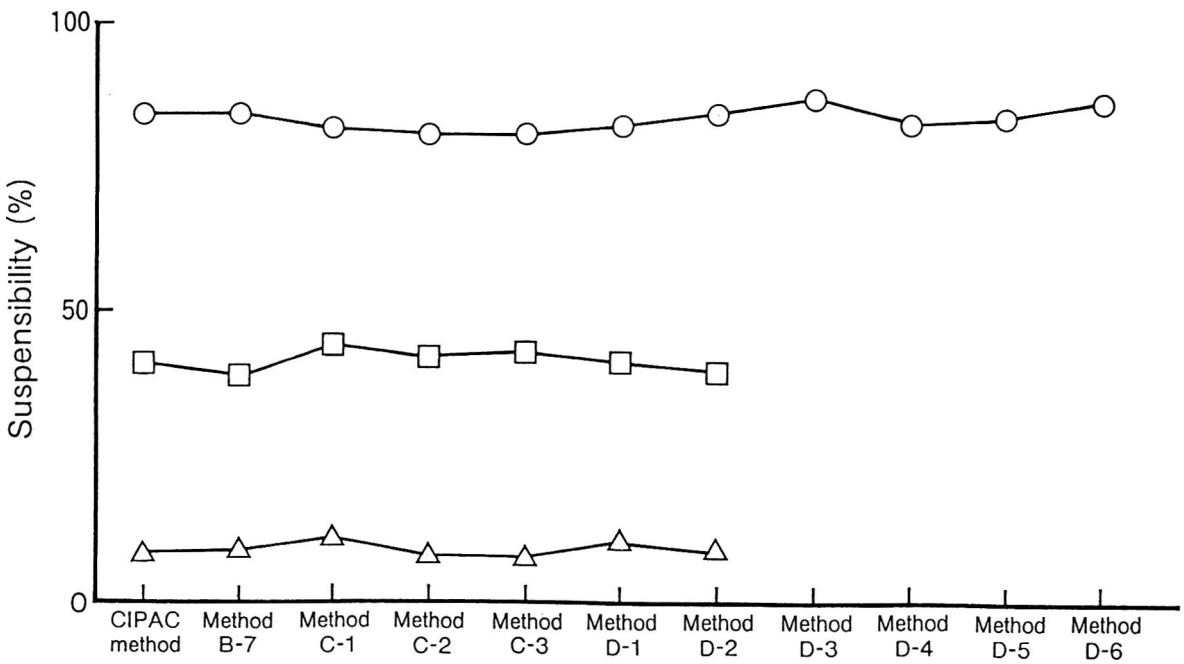


Figure 6. Suspensibility of fenitrothion 40% WP (342 ppm hard water, 30°C, 0.8 g [a.i.]/L, 30 min after dilution): ○—○, Recipe A; △—△, Recipe B; □—□, Recipe C.

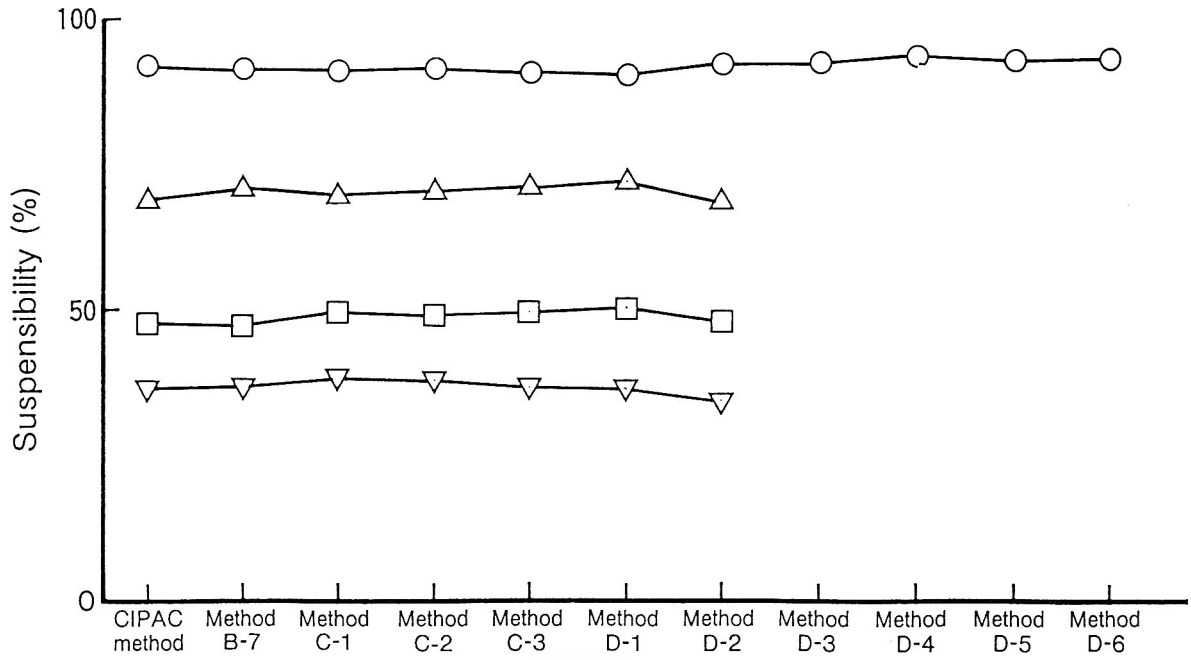


Figure 7. Suspensibility of procymidone 50% WP (342 ppm hard water, 30°C, 10 g [a.i.]/L, 30 min after dilution): ○—○, Recipe D; △—△, Recipe E; □—□, Recipe F; ▽—▽, Recipe G.

a 100 mL measuring cylinder was used instead of a 250 mL cylinder.

As shown in Figure 3, the weight of active ingredient in the remaining 10 mL in the cylinder was obtained as in the CIPAC method described above, and suspensibility was calculated by using Equation 1 in the case of method C-1.

For methods C-2 and C-3, the content of active ingredient in the top 9/10 was analyzed instead of the bottom 1/10. The 90 mL (top 9/10) that was removed was thoroughly mixed, and 25 mL (method C-2) or 1 mL (method C-3) was removed by pipet. Then the analytical sample was prepared and analyzed in the same manner as described above, except the water evaporation procedure was deleted in the case of method C-3.

Suspensibility was calculated by using the following equation:

$$\text{Suspensibility} = Q/c \times 100/d \times 100 \quad \% \qquad (3)$$

where c = weight (g) of active ingredient in the sample actually taken, d = volume (mL) of the suspension removed by pipet

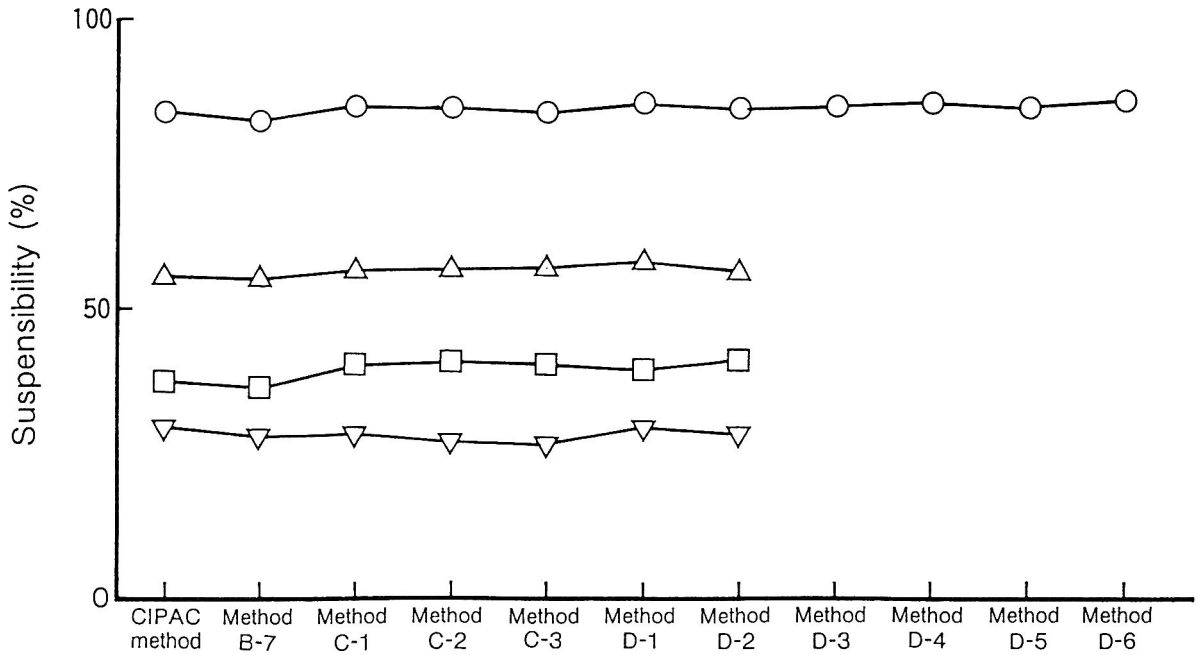


Figure 8. Suspensibility of procymidone 50% WP (342 ppm hard water, 30°C, 1 g [a.i.]/L, 30 min after dilution): ○—○, Recipe D; △—△, Recipe E; □—□, Recipe F; ▽—▽, Recipe G.

Table 1. Reproducibility of suspensibility using procymidone 50% WPs for 4 methods[a]

	Recipe D				Recipe G			
Parameter	CIPAC	B-7	C-1	D-2	CIPAC	B-7	C-1	D-2
Suspensibility, %	91.7	91.4	90.8	92.1	36.4	36.9	38.0	37.5
	92.6	90.0	89.5	91.6	39.9	34.0	36.1	38.8
	93.6	92.6	89.5	92.7	35.6	34.1	35.1	38.9
	90.0	94.0	91.5	93.2	35.8	35.2	34.6	36.1
	90.8	90.1	90.3	92.9	34.5	35.6	37.6	36.4
Av., %	91.7	91.6	90.3	92.5	36.4	35.2	36.3	37.5
SD, %	1.3	1.5	0.8	0.6	1.8	1.1	1.3	1.2
CV, %	1.4	1.6	0.9	0.6	4.9	3.1	3.7	3.1

[a] 342 ppm hard water, 30°C, 10 g (a.i.)/L, 30 min after dilution.

from the top 90%, and Q = weight (g) of active ingredient in the d aliquot removed by pipet from the top 90%.

Simplified method (methods D-1 to D-6).—Suspensibility was measured as in methods C-1 and C-2 described above, except the process for preparing a suspension in a beaker was deleted.

As shown in Figure 4, WP was placed directly in the cylinder and diluted to 100 mL with the hard water, and the cylinder was inverted 30 times in 1 min. The cylinder was kept standing in a water bath (30°C). After 30 min, 90 mL (top 90%) of the suspension was removed. The weight of active ingredient in the remaining 10 mL in the cylinder was obtained as in the CIPAC method described above, and suspensibility was calculated by using Equation 1 (method D-1).

The 90 mL (top 90%) removed was thoroughly mixed, and 1 mL was removed from the mixture by pipet. The analytical sample was prepared and analyzed as described above, except the water evaporation was deleted (method D-2). In methods D-3 to D-6, a methanol IS (method D-3), an acetonitrile IS (method D-4), a chloroform IS and anhydrous sodium sulfate as a desiccant (method D-5), or a dichloromethane IS and anhydrous sodium sulfate (method D-6) were added instead of acetone IS, as in method D-2. Suspensibility was calculated by using Equation 3.

Reproducibility of Data

Reproducibility of data was examined for Recipe D and Recipe G of procymidone 50 wt% WP by replicating the procedure for measurement of suspensibility 5 times. Methods B-7, C-1, and D-2 were used for the reproducibility tests.

Analytical Time

Time required to measure the suspensibility by each method, excluding the GC analysis, was determined.

Amount of Waste Solution

The amount of waste solution produced was determined by measuring the suspensibility, including an analytical GC sample.

Results and Discussion

Suspensibility of fenitrothion 40 wt% WP.—As shown in Figure 5, the suspensibilities of Recipes A, B, and C determined by the CIPAC method were about 85, 55, and 40%, respectively, when each fenitrothion 40 wt% WP was diluted to 25 g (a.i.)/L. The suspensibility of Recipe A determined by method C-1, in which a 100 mL measuring cylinder was used instead of a 250 mL cylinder, was almost the same as that determined by the CIPAC method. The suspensibilities of the formulation determined by the simplified measuring methods using a 100 mL cylinder (methods C-2, C-3, and D-1 to D-6) also were almost the same as those determined by the CIPAC method and method B-7. Similar results were obtained when Recipes B and C were used instead of Recipe A.

As shown in Figure 6, when fenitrothion 40 wt% WP was diluted to 0.8 g (a.i.)/L, the suspensibilities of Recipes A, B, and C determined by the CIPAC method were about 85, 40, and <10%, respectively. The suspensibilities of Recipes A, B, and C determined by the simplified measuring methods using a 100 mL cylinder were almost the same as those of the corresponding formulation determined by the CIPAC method and method B-7.

Data obtained by these simplified methods using a 100 mL cylinder and the CIPAC method show good consistency. Furthermore, hydrophilic solvents, such as acetone, methanol, and acetonitrile, and hydrophobic solvents, such as chloroform and dichloromethane with anhydrous sodium sulfate, probably could be used for preparing an analytical sample.

Suspensibility of procymidone 50 wt% WP.—After procymidone 50 wt% WP was diluted to 10 g (a.i.)/L, the suspensibility was higher when the average diameter of procymidone was smaller, i.e., suspensibilities of Recipes D, E, F,

Table 2. Time required to measure suspensibility excluding GC analysis

Method	Time, min
CIPAC MT 15.1	95
B-7	37
C-1	84
C-2	80
C-3	51
D-1	69
D-2 to D-6	37

Table 3. Amount of waste solution

Method	Amount of waste solution, mL		
	Remaining suspension[a]	Analytical sample	Total
CIPAC MT 15.1	225	20–39	245–264
B-7	249	5–10	254–259
C-1	90	10–29	100–119
C-2	75	10–29	85–104
C-3	99	5–10	104–109
D-1	90	10–29	100–119
D-2 to D-6	99	5–10	104–109

[a] Suspension minus an aliquot sample for analytical use.

and G determined by the CIPAC method were about 90, 70, 50, and 35%, respectively. As shown in Figure 7, suspensibilities of Recipes D, E, F, and G determined by the simplified measuring methods using a 100 mL cylinder were almost the same as those of the corresponding formulation determined by the CIPAC method and method B-7.

As shown in Figure 8, similar results were obtained when procymidone 50 wt% WP was diluted to 1 g (a.i.)/L.

Reproducibility of data.—Table 1 shows the reproducibility of the data obtained with procymidone 50% WP. The CIPAC method and methods B-7, C-1, and D-2 gave almost the same suspensibility values. Coefficients of variation were 0.6–1.6% for Recipe D and 3.1–4.9% for Recipe G. In both cases, the reproducibility of data for the simplified methods using a 100 mL cylinder was comparable with that of the CIPAC method.

We concluded that these simplified measuring methods can be used instead of the current CIPAC method.

Time required to measure suspensibility.—Table 2 shows the analytical time required to measure the suspensibility by every method except GC. Suspensibility determination by the CIPAC method required 95 min. On the other hand, after deletion of the process for preparing a suspension in a beaker or for evaporating water from a suspension removed by pipet from the top 90%, analytical time was greatly reduced.

Analytical times required for method B-7 and methods D-2 to D-6, which are the most simplified methods, were about 40% of the required time of the CIPAC method.

Amount of waste solution.—Table 3 shows the amount of waste solution produced by measuring the suspensibility of the analytical sample. In the CIPAC method, a large amount of IS was needed to prepare the analytical sample for GC because of the large amount of active ingredient in the sample. In contrast, methods D-2 to D-6 required only a small amount of IS. Therefore, the total amount of waste solution in methods D-2 to D-6, where the most simplified methods use a 100 mL cylinder, decreased to about 40% of that in the CIPAC method and method B-7, where the most simplified methods use a 250 mL cylinder.

Conclusion

Methods D-2 to D-6 were obtained by modifying the following points in CIPAC MT 15.1: (a) A 100 mL measuring cylinder was used instead of a 250 mL cylinder, (b) the process for preparing a suspension in a beaker was deleted, (c) the content of the active ingredient in the top 90% was determined instead of the bottom 10%, and (d) the process for evaporating water from a suspension removed by pipet from the top 90% was deleted. These are the best methods for reducing the analytical time and the waste solution, simplifying the CIPAC method, and maintaining the accuracy and reproducibility of the data. Furthermore, these methods are suitable for WPs made with both liquid and solid active ingredients.

Acknowledgment

We are indebted to Sumitomo Chemical Co., Ltd., for its permission to publish this work.

References

(1) Van Valkenburg, W. (1973) in *Pesticide Formulations*, Marcel Dekker, Inc., New York, NY, pp. 174–186

(2) *Specifications for Pesticides Used in Public Health* (1985) 6th Ed., World Health Organization, Geneva, Switzerland, pp. 183–185

(3) Ashworth, R.B., Henriet, J., & Lovett, J.F. (1970) in *CIPAC Handbook*, Vol. 1, G.R. Raw (Ed), Collaborative International Pesticides Analytical Council Limited, Hertfordshire, England, pp. 865–868

(4) Ashworth, R.B., Henriet, J., & Lovett, J.F. (1970) in *CIPAC Handbook*, Vol. 1, G.R. Raw (Ed), Collaborative International Pesticides Analytical Council Limited, Hertfordshire, England, pp. 861–865

(5) Ogawa, M., Ohtsubo, T., Tsuda, S., & Tsuji, K. (1991) *J. Assoc. Off. Anal. Chem.* **74**, 848–852

TECHNICAL COMMUNICATIONS

Determination of Oxyfluorfen in Pesticide Formulations by Liquid Chromatography

RODNEY J. BUSHWAY and LEWIS B. PERKINS
University of Maine, Department of Food Science, 102B Holmes Hall, Orono, ME 04469

A normal-phase liquid chromatographic method was developed to determine oxyfluorfen, a herbicide, in pesticide formulations. Technical and liquid formulations were dissolved in 10% water and 90% tetrahydrofuran. A 5 μL aliquot was injected into a Phenomenex Spherex amino column and monitored at 240 nm. The mobile phase was tetrahydrofuran–hexane (5 + 95). Total analysis time was 80 min. Six different liquid formulations and 1 solid formulation were analyzed. Within-day percent coefficients of variation (%CVs) ranged from 0.5 to 3.5, and between-day %CVs ranged from 1.4 to 3.9. Purity of each oxyfluorfen chromatographic peak was checked by using a photodiode array detector in the spectrum mode. No interferences were observed.

Oxyfluorfen (Goal; 2-chloro-1-[3-ethoxy-4-nitrophenoxy]-4-[trifluoromethyl]benzene) is a pre- and post-emergence herbicide that is used to control broad-leaf weeds. Its use is increasing because of its low mammalian toxicity (acute oral LD_{50} for rats, >5000 mg/kg) and its minimal leaching. It is formulated as a technical solid at approximately 70% and a technical liquid at approximately 19%. It can also be purchased as a liquid formulation containing 0.25% active ingredient.

Gas chromatography was the preferred method of analysis for oxyfluorfen formulations (1), but recently Rohm and Haas developed a reversed-phase liquid chromatographic (LC) method for the analysis of oxyfluorfen in liquid technical formulations (2).

This paper describes a normal-phase LC method for the analysis of a wide variety of technical and other formulations of oxyfluorfen.

METHOD

Apparatus and Reagents

(a) *Liquid chromatograph.*—Waters Associates (Milford, MA 01757) 510 pump, Valco pneumatic injector Model EC6u (Vici Instruments, Houston, TX 77225), and Hewlett-Packard photodiode array detector Model 1040A (Andover, MA 01810). Operating conditions: injection volume, 5 μL; flow rate, 1.5 mL/min; wavelength, 240 nm; absorbance range, 0.04 AUFS.

(b) *Chromatographic column.*—Phenomenex Spherex amino, 5 μm particle size, stainless steel, 25 × 4.6 cm id (Phenomenex, Torrance, CA 90501).

(c) *Mobile phase.*—Tetrahydrofuran–hexane (5 + 95). All solvents were LC grade (VWR Scientific, Boston, MA 02101).

(d) *Sample extraction solvent.*—Tetrahydrofuran containing 10% water. All solvents were reagent grade (VWR).

(e) *Oxyfluorfen standard solution.*—Accurately weigh ca 21 mg oxyfluorfen reference standard, 99% pure (Rohm and Haas, Philadelphia, PA 19477), into 10 mL volumetric flask. Dilute to volume with reagent grade tetrahydrofuran. Transfer 1 mL sample to 10 mL volumetric flask, and add 1 mL water. Dilute to volume with reagent grade tetrahydrofuran (working standard).

Preparation of Sample

Accurately weigh amount of technical liquid or solid oxyfluorfen formulation containing ca 21 mg active ingredient into 10 mL volumetric flask, and dilute to volume with tetrahydrofuran. Transfer 1 mL sample by pipet to 10 mL volumetric flask, and add 1 mL water. Dilute to volume with reagent grade tetrahydrofuran. For water-based liquid formulations guaranteed at 0.25%, place 1 mL in 10 mL volumetric flask by using pipet, and dilute to volume with reagent grade tetrahydrofuran. Filter sample through 0.45 μm filter before injection.

Determination

Inject standard, followed by 2 injections of sample. Finally, inject another standard. Use peak area to calculate content:

$$\text{Compound, \%} = (R/R') \times (W'/W) \times \text{\% purity of std}$$

where R and R' = average peak area of sample and standard, respectively; W' = mg standard; and W = mg sample extracted. Duplicate injections should be within 1%.

Results and Discussion

Figure 1 shows a typical chromatogram of an oxyfluorfen liquid formulation guaranteed at 0.25% active ingredient.

Received January 6, 1992. Accepted March 24, 1992.

Table 1. LC determination of oxyfluorfen in formulations

		Intraassay[a]		Interassay[a]	
Formulation	Concn, %	Found, %	CV, %[b]	Found, %	CV, %[b]
Liquid	0.25	0.25	2.2	0.24	3.1
Liquid	0.25	0.23	3.5	0.22	3.4
Liquid	0.25	0.27	1.9	0.28	3.2
Liquid	0.25	0.25	1.6	0.26	2.1
Liquid	0.25	0.24	1.7	0.24	2.3
Tech. liquid	19.0	18.6	2.0	19.3	3.9
Tech. solid	70.0	70.2	0.5	69.5	1.4

[a] $n = 6$.
[b] CV = coefficient of variation.

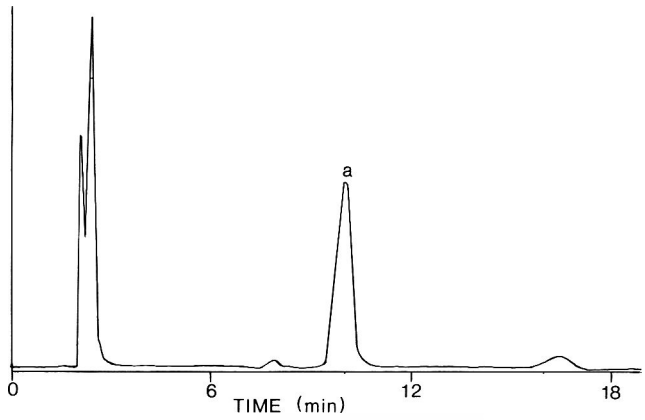


Figure 1. Chromatogram of an oxyfluorfen liquid formulation containing 0.25% active ingredient. Peak a = oxyfluorfen.

Baseline separation from all impurities was accomplished in 10 min, with a late-eluting peak coming off at 16.3 min. Thus, the total analysis time for each sample, including sample preparation, was 80 min.

The purity of each oxyfluorfen peak in all formulations was checked by using a photodiode array detector in the spectrum mode. Ultraviolet spectra from 190 to 350 nm were taken for each oxyfluorfen peak at the upslope, pinnacle, and downslope. All purity checks indicated that no co-eluting compounds existed.

Seven formulations were analyzed to determine within-day (6 analyses per day) and between-day (6 analyses on 6 different days) variations. Results are shown in Table 1. The %CVs for the intraassay ranged from 0.5 to 3.5; the %CVs for the interassay varied from 1.4 to 3.9. All %CVs were excellent; interassay variations were slightly higher than the intraassay %CVs, except 1 formulation for which the %CVs were approximately the same. However, all %CVs were <4; most were below 2.4, indicating an excellent method.

Linearity of the response for oxyfluorfen was checked on the basis of area; at 240 nm, oxyfluorfen produced a linear response to area for the range 0.04–10.3 μg injected. The correlation coefficient was 0.9999. Such linear ranges are more than adequate to cover formulations that are higher or lower than 0.25–70.0%. The detection limit of this method for oxyfluorfen was 0.01 μg injected.

Because of water-based formulations and a normal-phase system, tetrahydrofuran was the solvent of choice for dissolving the formulations. Furthermore, to keep the water content equal for all samples and the standard, dilutions had to be made for some of the samples to which 1 mL of water was added. To maintain detector response consistency and peak symmetry, the water content had to be equal for the samples and standards.

In conclusion, this LC method for oxyfluorfen is more versatile than other methods (because of interference problems with some formulations by the other LC method) and is as fast; reproducibility is excellent both within days and between days.

Acknowledgment

This manuscript is Maine Agricultural Experiment Station No. 1643.

References

(1) Adler, L., & Hofmann, C.K. (1980) "Oxyfluorfen," in *Analytical Methods for Pesticides and Plant Growth Regulators-XI*, G. Zweig and S. Sherma (Eds), Academic Press, New York, NY, p. 331

(2) Rohm and Haas Co. (1991) Technical Report 0672-05, Philadelphia, PA

TECHNICAL COMMUNICATIONS

Determination of Cymiazole Residues in Honey by Liquid Chromatography

PAOLO CABRAS and MARINELLA MELIS
Dipartimento di Tossicologia, Viale Diaz 182, 09126 Cagliari, Italy
LORENZO SPANEDDA
Istituto di Merceologia, Viale Fra' Ignazio 74, 09123 Cagliari, Italy

A liquid chromatographic method is described for the determination of cymiazole residues in honey. This acaricide is determined on a reversed-phase (C_{18}) column, with a CH_3CN–0.001N HCl–NaCl mixture (950 mL + 50 mL + 0.3 g/L) as the mobile phase, and UV detection at 265 nm. Cymiazole is extracted with *n*-hexane from aqueous alkalinized (pH 9) honey solutions. No further cleanup of the honey extract was required before chromatographic analysis. Recoveries on control samples fortified with 0.01, 0.10, and 1.00 ppm cymiazole ranged from 92 to 102%. The limit of determination was 0.01 ppm.

The appearance and rapid diffusion in Europe of *Varroa jacobsonii*, a parasitic mite, has caused significant damage to apiculture. The chemical treatment for the control of this honeybee parasite inside beehives resulted in the contamination of hive products. The most widely used compounds are amitraz, bromopropylate, cymiazole, coumaphos, flumethrin, fluvalinate, formic acid, malathion, and tetradifon. Limits for residues in honey were established in a few countries: 0.05 ppm is the maximum residue limit fixed in the United States for fluvalinate, and 0.10 ppm bromopropylate and 0.01 ppm coumaphos are allowed in Germany.

A number of analytical methods were proposed for the determination of acaricide residues in honey (1–12). Residue values reported in previous investigations were usually low and exceeded 0.05 ppm only in a few samples (13–17). However, no analytical method or residual data for cymiazole, 2-(2,4-dimethylphenylimino)-3′-methylthiazole (Figure 1), has been reported in the current literature on the active ingredients used to control the *Varroa* mite.

In this paper, a simple and rapid method is described for the liquid chromatographic (LC) determination of cymiazole in honey.

Experimental

Apparatus and Reagents

(a) *Liquid chromatograph.*—Spectra-Physics Model SP 8700 (Spectra-Physics, San Jose, CA), fitted with Model 770 UV/Vis variable wavelength detector (Spectra-Physics) and Valco AH 20 injection valve (250 μL loop), connected to HP Model 3390 A reporting integrator (Hewlett-Packard, Avondale, PA).

(b) *Column.*—S_5 ODS2 (250 × 4.6 mm id, 5 μm) (Spherisorb, Weddinxveen, The Netherlands).

(c) *Rotating stirrer.*—GFL P24 (GFL, Burgwedel 1, Germany).

(d) *Chemicals.*—Acetonitrile and *n*-hexane were LC reagent grade (Carlo Erba, Milano, Italy). Anhydrous sodium sulfate, sodium chloride, sodium hydroxide, and hydrochloric acid were analytical reagent grade (Carlo Erba). Double-distilled water was filtered through Milli-Q apparatus (Millipore, Molsheim, France) before use.

(e) *Standards.*—Cymiazole analytical standard (99.9%) was donated by Ciba-Geigy (Basel, Switzerland). Stock standard solution (ca 100 ppm) was prepared in acetonitrile. Working standard solutions (0.01–1.00 ppm) were prepared by diluting stock solution with acetonitrile.

Samples

Dissolve 50 g honey in 100 mL water in screw-capped flask. Make solution alkaline with 1M sodium hydroxide to reach pH 9 (ca 400 μL).

Extraction

Add 10 mL *n*-hexane to 10 mL honey aqueous solution in screw-capped test tube. Shake in rotating stirrer for 20 min, and let phases separate (centrifuge, if necessary). Put organic layer into test tube containing 1 g anhydrous sodium sulfate. Transfer 2 mL of the organic layer into 10 mL beaker, and evaporate nearly to dryness under nitrogen stream. Let *n*-hexane evaporate completely in fume hood at ambient temperature. Take up residue in 1 mL acetonitrile, and inject for LC analysis.

Chromatographic Determination

(a) *Procedure.*—Let system equilibrate with mobile phase CH_3CN–0.001N HCl–NaCl (950 mL + 50 mL + 0.3 g/L) at flow rate of 0.8 mL/min until retention times for cymiazole are reproducible (8.43 min). Inject 250 μL analytical sample, and perform analysis at 265 nm (according to UV spectrum of cymiazole).

Received December 9, 1991. Accepted April 28, 1992.

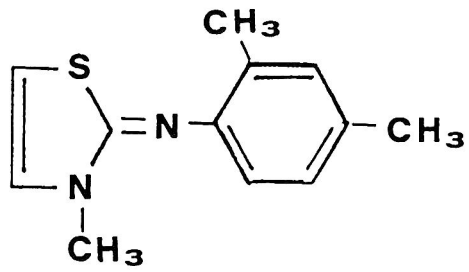


Figure 1. Structure of cymiazole.

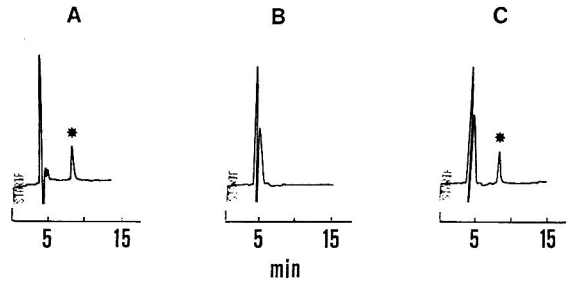


Figure 2. Chromatography of cymiazole (*) on a C_{18} column. Mobile phase, CH_3CN–0.001N HCl–NaCl (950 mL + 50 mL + 0.3 g/L); flow rate, 0.8 mL/min; sample volume, 250 μL; detection, UV at 265 nm; (A) standard solution of cymiazole at 0.01 ppm, (B) extract of untreated honey, and (C) extract of honey fortified with 0.01 ppm cymiazole.

(b) *Preparation of standard curve.*—Prepare standard curve, using external standard method, by plotting concentrations vs peak heights.

Recovery Assays

Recovery assays were performed on 10 mL aliquots of aqueous solutions (pH 9) of untreated honey samples fortified with 0.01, 0.10, and 1.00 ppm cymiazole by adding 100 μL acetonitrile solutions. Samples were extracted as described above. Each recovery assay was replicated 4 times.

Results and Discussion

The separation of cymiazole was initially tried with a normal-phase column (NH_2). However, on this column and with CH_3CN as the mobile phase, the pesticide was poorly retained. Attempts were made, without success, to increase the retention time (R_t) of cymiazole by using other single or mixed solvents of different polarities. Chromatograms obtained at a 0.5 mL/min flow rate from untreated honey samples showed 2 peaks (R_t 6.26 and 8.08, respectively), which were very close to that of cymiazole (R_t 7.06). Under these conditions, if low amounts of cymiazole are measured without a suitable sample cleanup, serious errors could occur. Therefore, a reversed-phase column (C_{18}) was used.

With this column and with CH_2CN–H_2O mixtures (in different ratios) as the mobile phase, cymiazole was not eluted. Water was then replaced in the eluant mixture by 0.001N HCl, but results were unsatisfactory. Cymiazole eluted only when NaCl was added to CH_3CN–0.001N HCl mixtures up to 0.3 g/L. When the percentage of HCl in the mobile phase was increased, retention time and peak sharpness decreased. With a mixture of CH_3CN–0.001N HCl–NaCl (950 mL + 50 mL + 0.3 g/L), chromatograms of untreated honey extract did not show any interfering peaks (Figure 2). Under these operating conditions, the standard curve was built on 6 points by plotting concentration vs peak height (external standard method). Data were processed by a statistical package for least-squares regression. Within the range of 0–1.0 ppm, the standard curve showed good linearity.

Before the extraction with *n*-hexane, the aqueous honey solution was made alkaline at pH 9, because at pH 9 the solubility of cymiazole in water is drastically reduced (by 20% at 150 ppm). This shows a partition coefficient of 700 between *n*-hexane and water. Alkalinization does not cause hydrolysis of cymiazole, because this compound is stable at any pH value. The percent recovery by the extraction described above ranged between 92 and 102%, with a maximum coefficient of variation of 5.6%. Under these conditions, the limit of determination (18) for cymiazole was 0.01 ppm.

The method described in this paper allows the simple and rapid determination of cymiazole residues in honey.

Acknowledgment

This work was supported by grants from Ministero dell'Università e della Ricerca Scientifica, P.R. "Controllo e miglioramento delle produzioni apistiche".

References

(1) Formica, G. (1984) *J. Assoc. Off. Anal. Chem.* **67**, 896–898
(2) Taccheo Barbina, M., De Paoli, M., Spessotto, C., & Marchetti, S. (1985) *Proc. 5 Symp. Chimica degli Antiparassitari*, Piacenza, Italy, June
(3) Klein, E., Weber, W., Hurler, E., & Mayer, L. (1986) *Dtsch. Lebensm. Rundsch.* **82**, 185–188
(4) Stoya, W., Wachendorfer, G., Kary, I., Siebentritt, P., & Kaiser, E. (1986) *Dtsch. Lebensm. Rundsch.* **82**, 217–221
(5) Hemmerling, C. (1987) *Nahrung.* **31**, 835–836
(6) Fabbris, F., Rogledi, R., Cantoni, C., & Palma, R. (1988) *Ind. Aliment.* **27**, 551–553
(7) Taccheo Barbina, M., De Paoli, M., & Spessotto, C. (1988) *Pestic. Sci.* **23**, 59–64
(8) Cozzani, R., & Di Pietrogiacomo, M. (1989) *Ind. Aliment.* **28**, 608–610
(9) Serpe, L., Oliviero, G., & Begliomini, A. (1989) *Ind. Aliment.* **28**, 1173–1174
(10) Stricker, O., Gierschner, K., & Vorwohl, G. (1989) *Dtsch. Lebensm. Rundsch.* **85**, 72–75
(11) Taccheo Barbina, M., De Paoli, M., & Spessotto, C. (1989) *Pestic. Sci.* **25**, 11–15
(12) Taccheo Barbina, M., De Paoli, M., & Valentino, A. (1990) *Pestic. Sci.* **28**, 197–202
(13) Thrasyvoulou, A.T., Infantidis, M.D., Pappas, N.L., & Simmons, K. (1985) *Apidologie* **16**, 89–94

(14) Taccheo Barbina, M., De Paoli, M., Marchetti, S., & D'Agaro, M. (1986) *Proc. of Meeting of EC Experts' Group*, Bad Homburg, Germany, October 15–17, pp. 131–143

(15) Taccheo Barbina, M., De Paoli, M., Barbattini, R., & Chiesa, F. (1988) *Proc. of Meeting of EC Experts' Group*, Udine, Italy, November 28–30, pp. 369–377

(16) Taccheo Barbina, M., De Paoli, M., Chiesa, F., D'Agaro, M., & Pecol, U. (1988) *Proc. of Meeting of EC Experts' Group*, Udine, Italy, November 28–30, pp. 379–386

(17) Thrasyvoulou, A.T., & Pappas, N.L. (1988) *J. Apic. Res.* **27**, 55–61

(18) Thier, H.P., & Zeumer, H. (1987) *Manual of Pesticide Residue Analysis*, Vol. I, VCH, Weinheim, Germany, pp. 37–44

GENERAL REFEREE REPORTS

Committee on Pesticide Formulations and Disinfectants

CIPAC Studies

ALAN R. HANKS
Office of the Indiana State Chemist, Purdue University, 1154 Biochemistry Bldg, West Lafayette, IN 47907-1154

The 36th CIPAC meeting and annual symposium were held in October 1992 at the building of the Swiss Society of Chemical Industries (SGCI) and the Swiss Federal Institute of Technology (ETH), Zurich, Switzerland, respectively. The annual informal Food and Agriculture Organization (FAO) of the United Nations meeting on specifications for pesticides was held in conjunction with the 36th CIPAC meeting and symposium at SGSI. The meetings were sponsored by the Swiss Federal Department of Agriculture (represented by the Swiss Federal Research Station in Wadenswil), the Swiss Society of Chemical Industries (with special support from various member companies), and CIPAC. These meetings were previously scheduled for late May/early June in Bangkok, Thailand; however, in the face of political uncertainties immediately before the original scheduled meeting, it became necessary to cancel the meetings in Bangkok.

Progress was good during the informal FAO meeting on pesticide specifications chaired by F. Sanchez Rösero, Estation Experimental de Zaidin, Granada, Spain. M. Galoux (Station de Phytopharmacie de l'Etat, Gembloux, Belgium) and J. Henriet (Gembloux, Belgium) served as rapporteurs to facilitate the records of the FAO meeting, and the FAO Senior Officer, Pesticides Group, Plant Protection Service, newly responsible for FAO specifications for pesticides, F.-W. Kopisch-Obuch, was able to attend his first informal meeting. Work on the general principles for FAO pesticide specifications, the formal FAO meeting, was scheduled in Rome following the end of the Zurich meeting by F.-W. Kopisch Obuch.

CIPAC Chairman H. Bosshardt (Swiss Federal Research Station, Wadenswil, Switzerland) presided over the CIPAC meeting and served as host for all of the meetings. A. Hourdakis (BENAKI Phytopathological Institute, Athens, Greece) and Alfred Riggenbach (SGCI) shared the duties of symposium chairman, with Alfred Riggenbach providing an outstanding closing summary. The symposium was very good, with many pesticide physical test method presentations, several papers on analytical techniques, and informative talks on a variety of residue monitoring in various countries.

Attendance at these CIPAC and FAO meetings continues to be high, with increasing interest in the quality of pesticide products moving in world trade. The location of the next CIPAC and informal FAO meeting has yet to be finalized, but it is expected to be either in France or Sweden.

During the year, the LC method for methamidophos in formulations was approved first action, CIPAC/AOAC. Also, the CIPAC LC method for phosphamidon cleared review of the AOAC Methods Committee on Pesticides and Disinfectants and was to be reviewed for first action approval by the AOAC Official Methods Board at the time of this writing. The General Referee continues to prepare documents for review and approval containing summaries of CIPAC studies plus the methods studied.

Method actions taken by CIPAC this year are reported in Table 1. Methods reported to be adopted by CIPAC as full methods were previously adopted by CIPAC as provisional, and they were reported by the General Referee last year in some detail if they were found to meet the minimum criteria for review by AOAC. Thus, in the following, only those newly adopted CIPAC provisional methods are reported that are candidates for submission to the Methods Committee for review and approval.

Amitraz.—A collaborative study using GC was conducted on technical material, wettable powders, and emulsion concentrates. Three of 16 laboratories were eliminated for failure to adequately follow the method as provided. A total of 8 results were rejected out of 200 reported across all samples tested following the IUPAC protocol. The report was authored by D. Wooldridge, Schering Agrochemicals Limited, for the Pesticide Analysis Advisory Committee, Great Britain.

Bifenox.—The collaborative study conducted by the Pesticide Analytical Council of France covered the analysis by LC of technical and formulated products and was reported by F. Gomez, Rhône-Poulenc.

Etofenprox.—A collaborative study using GC was conducted on technical materials, emulsifiable concentrates, and wettable powders. Three of 11 laboratories were eliminated from statistical analysis for not following the prescribed analytical program, and a few results from the remaining 8 laboratories were eliminated based on ISO 5725; however, at least 7 laboratories were retained for all samples tested. T. Yanami and R. Mita, Mitsui Toatsu Chemical, Inc., reported the results for the Pesticide Analytical Council of Japan.

Glufosinate-Ammonium.—A collaborative study using LC was conducted on technical grades, soluble concentrates, and an aqueous solution. At least 17 laboratories were retrained for each sample tested after statistical analysis. G. Gorlitz, Hoechst AG, reported the results for the German Pesticide Analytical Council.

Ioxynil Octanoate.—A collaborative study was conducted on emulsifiable concentrates (one also containing Bromoxynil) and technical materials. One Grubbs and one Cochran outlier

Table 1. Summary of the decisions taken at the 36th CIPAC meeting

Code No.	Name	Status of method
73	Dichlobenil	The capillary GC method for dichlobenil technical and formulations, CIPAC/3633, adopted as *full* CIPAC method.
86	Ioxynil octanoate	The packed column GLC method for ioxynil octanoate technical and formulations, CIPAC/3698, adopted as *provisional* CIPAC method.
103	Fentin	The LC method for fentinacetate/hydroxide technical and formulations (including mixtures with maneb), CIPAC/3639, adopted as *full* CIPAC method/referee method for fentin.
171	Oxydemeton-methyl	The LC method for oxydemeton-methyl technical and formulations, *J. Assoc. Off. Anal. Chem.* (1990) **73**, 431, adopted as *full* AOAC/CIPAC method.
201	Methabenzthiazuron	The normal-phase LC method for methabenzthiazuron technical and formulations, CIPAC/3702, approved as *provisional* CIPAC method.
202	Omethoate	The LC method for omethoate technical and formulations, CIPAC/3641, approved as *full* CIPAC method.
278	Iprodione	The LC method for iprodione technical and formulations, CIPAC/3700, approved as *provisional* CIPAC method.
323	Thiabendazole	The LC method for thiabendazole technical and formulations, CIPAC/3645, approved as *full* CIPAC method.
335	Imazalil	The *provisional* GLC method for imazalil technical, CIPAC/3643, adopted as a *full* CIPAC method.
362	Amitraz	The GLC method for amitraz technical and formulations, CIPAC/3686, adopted as *provisional* CIPAC method.
363	Ethiofencarb	The LC method for ethiofencarb technical and formulations, CIPAC/3635, adopted as *full* CIPAC method.
365	Metalaxyl	The GLC method for metalaxyl technical and formulations, CIPAC/3649, adopted as *full* CIPAC method.
371	Bromadiolone	The LC method for bromadiolone technical and formulations, CIPAC/3629, adopted as *full* CIPAC method.
408	Propiconazole	The GLC method for propiconazole technical and formulations, CIPAC/3704, adopted as *provisional* CIPAC method.
413	Bifenox	The LC method for bifenox technical and formulations, CIPAC/3688, adopted as *provisional* CIPAC method.
417	Carbosulfan	The LC method for carbosulfan technical and formulations, CIPAC/3631, adopted as *full* CIPAC method.
437	Glufosinate	The basic ion exchange LC method for glufosinate technical and formulations, CIPAC/3696, adopted as *provisional* CIPAC method.
471	Etofenprox	The GLC method for etofenprox technical and formulations, CIPAC/3691, adopted as *provisional* CIPAC method.

were found in the results of 14 laboratories by ISO 5725. G.C. Buddle and M.J. Fitzmaurice of Rhône-Poulenc Agriculture, Ltd, reported the study for the Pesticide Analytical Advisory Committee, Great Britain.

Iprodione.—The LC method collaborative study was conducted with analysis of technical grade materials, wettable powder, and a suspension concentrate. Five countries were represented among the reports of 13 voluntary participants reporting data from 11 laboratories. One laboratory was eliminated as a straggler on 3 samples and an outlier on 1. Only 2 other data points were eliminated in the report prepared by F. Gomez, Rhône-Poulenc.

Methabenzthiazuron.—Fifteen collaborating laboratories representing 8 countries took part in the study on 5 technical and formulated products. A total of 5 outliers were eliminated, all because of too high a variance. H. Tengler and K.W. Krüger of Bayer AG, Germany, prepared the report for the German Pesticide Analytical Council.

Propiconazole.—Twelve countries were represented among the 22 participating collaborating laboratories that analyzed a technical concentrate and 4 samples of 3 types of formulated products. Fifteen to 16 laboratories provided results for all samples for this study, which was reported by K. Fenkart and J. Stulz from Ciba-Geigy, Switzerland.

Several collaborative studies are planned for next year or are underway and should be reported at the CIPAC meeting next year: α-cypermethrin, β-cyfluthrin, coumatetralyl, dichloroprop, dithianon, fenoxaprop-ethyl, fenpropimorph, fenvalerate, forsethyl-al, pyridate, and teflubenzuron. Thus, CIPAC should have another busy and productive year in 1993. The number of collaborating laboratories from North America and South America involved in CIPAC studies increased in recent years, and others are encouraged to take part.

Recommendations

(1) Adopt as first action the CIPAC method for phosphamidon by LC.
(2) Continue study on all other topics.

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Pesticide Formulations and Disinfectants. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Disinfectants

Submitted by T. J. CZERKOWICZ for ARAM BELOIAN
U.S. Environmental Protection Agency, Biological and Economic Analysis Division, Washington, DC 20460

Hard Surface Carrier Test.—The Associate Referee submitted for publication the results of a collaborative test, and this was published in the *J. AOAC Int.* (1992) **75**, 635. The Hard Surface Carrier Test (HSCT), which replaces the AOAC Use Dilution Test, was adopted as official first action by the AOAC as noted in "Changes in Official Method of Analysis" (1992) *J. AOAC Int.* **75**, 223. The Associate Referee is initiating studies to determine how the HSCT can be used to measure the efficacy of disinfectants in the presence of hard water and organic loads. Continued study is recommended.

Tuberculocidal Test.—Difficulties were encountered in attempting to standardize the cell count and in growing the test *Mycobacterium.* Improvised alternative approaches are being studied to overcome these problems. If these problems can be mitigated or surmounted, the 2 most critical sources of variation in this efficacy test can be resolved. Continued study is recommended.

Virucidal Test.—Laboratory studies were performed to standardize viral concentrations and to determine at what concentrations various disinfectants demonstrate toxicity in recovery media. Inactivation curves were demonstrated to be achievable by using varying concentrations of different disinfectant active ingredients. Work has begun to study the effects of varying test conditions. Continued study is recommended.

Sporicidal Test.—A Cooperative Agreement was led by the Office of Pesticide Programs, EPA, May 1992, to begin studies on updating the AOAC Sporicidal Test. Early efforts are focusing on standardizing the growth media used to propagate bacterial spores, with particular emphasis on replacing the soil extract used to grow *Cl. sporogenes.*

Amendments and Deletions to Methods

The AOAC Use Dilution Test was replaced by the AOAC Hard Surface Carrier Test. In removing the Use Dilution Test, several amendments and deletions are required to the chapter on disinfectants. Included also in this listing are a number of amendments that update the sources of apparatus or media in several methods.

955.11A(b)—Under *Solution B*, second paragraph, add "Bacto Synthetic Broth AOAC may be substituted" to end of paragraph.

955.11A(c)—Delete "(blue-green with bromothymol blue)."

955.11A(d)(3)—Amend the parenthetical address to read "(...Danbury, CT 06810, avail. in 25–50 kg quantities only; or Advanced Lecithin Products, P.O. Box 677, Brookfield, CT 06804)." At end of paragraph, add "Can also be obtained from Difco or BBL."

955.11B(b)—After "...or less;" delete the semicolon and add "(plastic pipettes can be substituted if sterility controls are included for pipettes in the test procedure);". Delete the sentence beginning "Plug medication tubes..." and substitute "Cap tubes with Morton enclosures."

955.11B(c)—Delete the sentence beginning "Blocks of wood...." Delete the word "medication," second sentence.

955.11B(e)—Delete "(formerly called *Bac. typhosus* and...)." Delete "With Ps. aeruginosa PRD 10, proceed...."

955.13–955.15—Delete.

964.02—Delete.

955.16C—After first sentence, add "For *S. aureus*, also follow procedures under **991.48A**(b)."

960.09A(c)—After "(...Danbury, CT 06810..." add "[25–50 kg containers only] or Advanced Lecithin Products, P.O. Box 677, Danbury, CT 06804)."

960.09B—After "...955.12." delete period, substitute semicolon and add "also use procedures under **991.48A**(b) for *Staph. aureus*."

960.09C(c)—Delete narrative after "175 mL" and add ", flint glass."

961.02A—Delete the narrative after "...specified in" and add "**991.47A**(a) and (f); **991.48A**(a); and **991.49A**(a) and (b)." In the second paragraph change "**955.14A**(b)" to "maintained as in **991.48A**(b)," "**955.14A**(b)" to "maintained as in **991.47A**(b)," "**964.02F**" to "maintained as in **991.49A**(c)."

961.02B—Revise sentence beginning "Use apparatus specified..." by deleting the section numbers and substituting "**991.47B**(a), (b), (e), (n), and (o), and in addition:".

961.02B(c)—Add after "...32 × 200 mm" "(Bellco Glass, Inc., P.O. Box B, 340 Edrudo Rd., Vineland, NJ 08360)."

961.02C—After first sentence, first paragraph, add a new sentence: "For *Ps. aeruginosa* follow preparation of culture under **991.49A**(c)."

961.02C—After first sentence, second paragraph, add: "If no time or distance specified use 10 seconds at 1 foot." Delete fourth paragraph. Substitute "For procedures to be followed in assuring standard cultures, for *S. choleraesuis* see **991.47A**(b), for *Staph. aureus* see **991.48A**(b), and for *Ps. aeruginosa* see **991.49A**(c)."

966.04B(f)—Revise text within parentheses to read: "(3, 6.0 metric, silk black braided SA-9G, USP, Ethicon,...)."

966.04C—After the first sentence, first paragraph, add: "Make monthly transfer of *B. subtilis* stock culture on Nutrient Aga. *Clostridia* do not require periodic transfer."

972.04A(b)—Delete "aberrant." Delete "(formerly *Escherichia coli*)."

965.13E(g)—Change "*Streptococcus faecalis*" to "*Enterococcus faecium*." Wherever "*S. faecalis*" appears in the test narrative (8-times), change to "*E. faecium*." This change was made by the American Type Culture Collection.

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Pesticide Formulations and Disinfectants. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Pesticide Formulations: Herbicides

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

Alachlor, Butachlor, Propachlor, Atrazine/Alachlor Mixtures.—Work on the development of new capillary methods for the determination of actives in new formulations is in progress. Minicollaborative studies are set for early 1993.

Bromoxynil.—Continue study.

Chlorophenoxy Herbicides.—Method published in *Journal of the AOAC* in 1991. Collaborative study expected late 1992 or early 1993.

Dicamba.—Continue study on LC methods.

Methazole.—Method approved official first action.

Fluometuron.—Continue study.

Glyphosate.—Methods for determination of active in new formulations is complete. Minicollaborative studies expected early 1993.

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Pesticide Formulations and Disinfectants. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

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

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

Organohalogen Insecticides

Cyfluthrin.—An LC procedure was developed for determination of cyfluthrin in formulated products. The method will be submitted for collaborative study in late 1992.

Cyromazine.—A new method was developed and is ready for collaborative study. The study is expected to be initiated in late 1992.

Carbosulfan.—An LC method (CIPAC/3631) was adopted as a provisional CIPAC method. No AOAC collaborative study is expected.

Carbaryl.—No activity in this area and none expected.

Other Insecticides, Synergists, and Repellents

Allethrins.—No activity. Product no longer being sold. Method **953.05** Titrimetric Method no longer required.

Dipropyl Isocinchomeronate (MGK-Repellant 326).—Continue study.

DDT.—No activity and none expected.

Methomyl.—No activity and none expected. New Associate Referee needed.

Rotenone and Other Rotenoids.—No activity and none expected.

Piperonyl Butoxide.—Work being done by CIPAC.

Alachlor, Butachlor, Propachlor, Atrazine/Alachlor Mixtures.—Work on the development of new capillary methods for the determination of actives in new formulations is in progress. Minicollaborative studies are set for early 1993.

Bromoxynil.—Continue study.

Chlorophenoxy Herbicides.—Method published in *Journal of the AOAC* in 1991. Collaborative study expected late 1992 or early 1993.

Dicamba.—Continue study on LC methods.

Methazole.—Method approved official first action.

Fluometuron.—Continue study.

Glyphosate.—Methods for determination of active in new formulations is complete. Minicollaborative studies expected early 1993.

Recommendations

(1) Drop *DDT, Rotenone and Other Rotenoids, Allethrins, Piperonyl Butoxide, Carbosulfan*, and *Carbaryl* from active AOAC study.
(2) Method **953.05** *Allethrin* (Technical) and *Pesticide Formulations Titrametric Method* to surplus.
(3) New Associate Referees for *Methomyl* and *Oxamyl.*
(4) New General Referee because present General Referee was appointed General Referee for *Pesticide Formulations: Herbicides.*

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Pesticide Formulations and Disinfectants. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Pesticide Formulations: Organophosphorus Insecticides

WILLIAM R. BETKER
Miles, Inc., Agriculture Division, PO Box 4913, Kansas City, MO 64120-0013

The only activity during the past year was the start of one collaborative study, which is now nearing completion. Three methods are being recommended for final action. One method that is final action was superseded by a superior method and is being recommended for surplus action. Two official first action methods that are no longer in general use are being recommended for surplus action. Two official first action methods that are obsolete are being recommended for repeal. Five topics that were completed or are inactive are being discontinued. Two topics are being continued. The following is a status report of the topics assigned to this General Refereeship:

Azinphos-Methyl (Guthion).—Associate Referee Stephen C. Slahck intends to start a minicollaborative study on new formulations of this product. Continue study.

Dichlorvos (DDVP).—Two IR methods (**964.04, 966.07**) were official first action for 28 years. The methods are limited to the formulations used in the original collaborative study that are no longer commercial products. The only U.S. manufacturer of dichlorvos uses a GC method that they believe to be a superior method. The intent to repeal the 2 IR methods was announced in *The Referee*, and no comments have been received. The General Referee recommends that the 2 IR methods be repealed and this topic be discontinued.

Ethoprop.—A collaborative study was started and is nearing completion. Continue study.

Fenitrothion.—The official final action GC method (**985.07**) was superseded by a superior GC method (**989.02**). Announcement to surplus the original method was made in *The Referee*, and we have not received any comments about the recommendation. The General Referee recommends that the old official final action GC method (**985.07**) be surplused and this topic be discontinued.

Fenamiphos (NEMACUR).—Associate Referee Carl Gregg resigned and has not been replaced. Discontinue topic.

Oxydemeton-methyl (Metasystox-R).—The official first action method (**991.05**) was in use for 2 years. Associate Referee Stephen C. Slahck has not received any negative comments. Official final action is recommended. Discontinue study.

Parathion and Methyl Parathion.—The official first action GC methods for parathion (**978.06**) and for methyl parathion (**977.04**) were announced in *The Referee* as being recommended for final action. No comments were received. The LC methods for methyl parathion (**977.05**) and for ethyl parathion (**978.07**) were official first action methods for 22 years. They use a silica column and water-saturated chloroform for the mobile phase, a combination rarely used today. Since GC is the method general used for these compounds, the LC methods are being recommended for surplus action. The above actions were announced in *The Referee*, and no comments have been received. The General Referee recommends official final action for the 2 GC methods and surplus action for the 2 LC methods. Discontinue study.

Recommendations

(1) Adopt as official action the following official first action methods:
    991.05 Oxydemeton-methyl in Formulated Products, Liquid Chromatographic Method.
    977.04 Methyl Parathion in Pesticide Formulations, Gas Chromatographic Method.
    978.06 Parathion in Pesticide Formulations, Gas Chromatographic Method.
(2) Surplus the following official final action method:

985.07 Fenitrothion Technical and Pesticide Formulations, Gas Chromatographic Method.

(3) Surplus the following official first action methods:

977.05 Methyl Parathion in Pesticide Formulations, Liquid Chromatographic Method.

978.07 Parathion in Pesticide Formulations, Liquid Chromatographic Method.

(4) Repeal the following official first action methods:

964.04 DDVP in Pesticide Formulations, Infrared Spectrographic Method. Method I

966.07 DDVP in Pesticide Formulations, Infrared Spectrophotometric Method. Method II

(5) Discontinue the following topics: dichlorvos, fenamiphos, oxydemeton-methyl, parathion, and methyl parathion.

(6) Continue the studies on azinphos-methyl and ethoprop.

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Pesticide Formulations and Disinfectants. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

GENERAL REFEREE REPORTS

Committee on Drugs and Related Topics

Cosmetics

RICHARD M. MONTGOMERY (In lieu of a General Referee)
Unilever Research U.S., Inc., Edgewater, NJ 07020

A General Referee was identified for the topic of Cosmetics, and she began her term on August 1, 1992. She is Ms. Rhonda A. Bayoud of Mary Kay Cosmetics in Dallas, Texas. Her first task will be to revitalize the area and develop the interest and energy that seems to be present in the field, especially from Europe.

There are currently no active projects in this area.

There are 2 methods in Chapter 15, *Official Methods of Analysis*, 15th Ed., that were reviewed and recommended to the Membership for adoption to final action status this year. This leaves only 1 active method in Chapter 15 in first action status. These recommendations are indicated below.

Recommendations

(1) *Water and Ethyl Alcohol in Cosmetics* (***966.22***).—Adopt as final action.
(2) *Zirconium (Soluble) in Antiperspirant Aerosols* (***976.24***).—Adopt as final action.
(3) Discontinue topics in Recommendations 1 and 2.
(4) *Eye Irritants in Cosmetics Constituents* (***973.59***).—Continue first action status.

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Drugs and Related Topics. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Diagnostics and Test Kits

RICHARD M. MONTGOMERY (In lieu of a General Referee)
Unilever Research U.S., Inc., Edgewater, NJ 07020

The status of this topic is under review. There is currently no General Referee and no active projects in this topic.

The most recent report on this topic (*J. Assoc. Off. Anal. Chem.* [1990] **73**, 91) listed 6 methods that were under review. The status of these proposals were reviewed, and it is recommended that these studies be officially discontinued. This recommendation is indicated.

Recommendations

Discontinue the following 6 topics:
(1) *Analytical Release Rate of Drugs from Transdermal Patches* (N. Tymes).
(2) *Automated Microbial Identification System-Vitek* (J. Tardio).
(3) *Automated Microbial Identification System-HP5898A* (L. English).
(4) *Immunological and Diagnostic Assay of Peptides* (U.J. Mehta).
(5) *Hormones and Enzymes* (J.W. Dyminski).
(6) *Tuberculosis and Enteric Infections by Gene Probe* (H. George).

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Drugs and Related Topics. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Drug Residues in Animal Tissues

CHARLIE J. BARNES
U.S. Food and Drug Administration, Division of Veterinary Medical Research, Beltsville, MD 20705

Fenbendazole in Cattle Liver.—No report. A method for benzimidazoles in cattle tissue was collaboratively studied. The method was approved first action for the determination of fenbendazole in cattle liver.

β-*Lactam Residues in Milk by Devotest.—No report.*

β-*Lactams in Milk by LC.—No report. The General Referee recommends this subject be discontinued.*

β-*Lactams in Milk by Competitive Microbial Receptor Assay.—No report. The Associate Referee submitted a collaborative study protocol. The protocol was reviewed and returned to the Associate Referee for additional clarification.*

Enzyme Immunoassay for Antimicrobial Compounds.—No report.

Ethoxyquin by Fluorimetry, ***968.48***.—No report. This method for ethoxyquin in eggs was granted first action status in 1968. I am not aware of any recent reports on its use, and I recommend surplus status for this method.

Sulfamethazine in Milk, Chromatographic Methods.—The Drug Committee approved the submission of the determinative procedure to the Official Methods Board for their vote on adoption first action during the AOAC International 1992 annual meeting.

Sulfamethazine in Plasma.—No report. I recommend that this subject be discontinued.

Sulfonamides in Milk, Chromatographic Methods.—Michael Smedley conducted a collaborative study of a procedure for determining sulfadiazine, sulfathiazole sulfapyridine, sulfamerazine, sulfamethazine, sulfachloropyridazine, sulfadimethoxine, and sulfaquinoxaline at or above the tolerance or concern level in raw cow's milk. Known controls, known fortified controls, blind incurred samples, and blind control samples were used in the study. Eight laboratories, including state and federal laboratories of the United States and a Canadian government laboratory, participated in the study. The results of the study are surprisingly good. I believe the recoveries and interlaboratory and intralaboratory variabilities are satisfactory. The levels of interferences do not preclude satisfactory quantitation at the 10 ppb level and above. I have recommended first action status for this determinative procedure.

Tetracyclines in Tissues, Chromatographic Methods.—The collaborative study is underway.

Recommendations

(1) Discontinue β-*Lactams in Milk by LC and Sulfamethazine in Plasma.*
(2) Surplus *Ethoxyquin by Fluorimetry* (**968.48**).
(3) Continue study on all other subjects.

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Drugs and Related Topics. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Drugs I

RICHARD M. MONTGOMERY (In lieu of a General Referee)
Unilever Research U.S., Inc., Edgewater, NJ 07020

The status of this topic is under review. There is currently no General Referee and no active projects in this topic.

There are 2 methods in Chapter 19, *Official Methods of Analysis*, 15th Ed., that were reviewed and recommended to the Membership for adoption to final action status this year. Acceptance of these recommendations means that all the active methods in Chapter 19 will have final action status. These recommendations are indicated below.

Recommendations

(1) *Acetaminophen in Drug Tablets, LC Method* (***987.12***).—Adopt as final action.
(2) *Diethylpropion Hydrochloride in Drug Substances and Tablets, LC Method* (***988.23***).—Adopt as final action.
(3) Discontinue all topics.

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Drugs and Related Topics. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Drugs II

EDWARD SMITH[1]
U.S. Food and Drug Administration, Center for Drug Evaluation and Research, Washington, DC 20204

Aminacrine.—Associate Referee Elaine A. Bunch reported that she is continuing to investigate LC methods for the determination of aminacrine HCl in drug preparations in the presence of degradation products.

Antihistamines in Combination with Decongestants by LC.—Associate Referee Raja Achari reported the results of the collaborative study of the proposed LC method for the determination of chlorpheniramine maleate, pseudoephedrine HCl and dextromethorphan HBr in liquids and tablets. Excellent re-

coveries and a low standard deviation were obtained for all 3 analytes in liquid cough–cold products. On the basis of the collaborative study results, he is recommending that the proposed method be adopted first action. The Associate Referee is revising the manuscript in concurrence with the committee's recommendations.

Belladonna Alkaloids.—A replacement for this vacant Associate Refereeship is being sought. The scope of this topic would also include the determination of the individual alkaloids and ensure the suitability of the procedure to detect any decomposition product present. The General Referee reported an LC procedure that enables one to directly determine both scopolamine and atropine and the individual enantiomers of each alkaloid.

This was presented at the American Association of Pharmaceutical Scientists meeting in November 1991 (1). The LC methodology for the direct determination of the *d*- and *l*-hyoscyamine that was initially presented (2) was expanded to include other chiral stationary phases and scopolamine. Luis W. Levy of Quito, Ecuador, reported the determination of the enantiomeric purity of scopolamine isolated from plant extract by using achiral/chiral coupled column chromatography (3). He has volunteered to be the Associate Referee for this topic.

Colchicine in Tablets.—Associate Referee Richard D. Thompson reported that he is actively pursuing methodology for the determination of the decomposition product colchicine. No further laboratory work was accomplished this year. He is still planning a collaborative study of an LC method for the detemination of colchicine in tablets containing colchicine in combination with probenecid.

Dicyclomine Capsules.—A new Associate Referee is needed to follow up on the proposed capillary GLC method for dicyclomine in capsules (4) for injections and tablets. The proposed GLC method resolves any decomposition products from the dicyclomine and the internal standard.

Ergot Alkaloids.—A new Associate Referee is being sought for this topic.

Physostigmine and Its Salts.—Associate Referee Norlin W. Tymes reported that he evaluated the official LC method, **982.37** (5, 6), to determine its suitablity for the analysis of decomposed samples of physostigmine containing eseroline and rubreserine prepared according to the method by Yang and Wilken (7). He found no interferences from those compounds, using the official method. He recommended that the topic be discontinued.

Rauwolfia Alkaloids (Reserpine and Rescinnamine).—A new Associate Referee is needed for this topic.

Rauwolfia Serpentina.—A new Associate Referee is needed for this topic.

Recommendations

(1) Adopt as first action the proposed LC procedure for the simultaneous determination of chlorpheniramine maleate, pseudoephedrine HCl, and dextromethorphan HBr in liquid cough–cold products.

(2) Appoint a new Associate Referee, Louis W. Levy, for the topic *Belladonna Alkaloids*.

(3) Declare open any topic that was inactive for an extended period (more than 2 years). New Associate Referees are needed for the following topics: *Dicyclomine*, *Ergot Alkaloids*, *Rauwolfia Alkaloids*, and *Rauwolfia Serpentina*. If no new Associate Referees are identified and appointed, these topics will have to be dropped.

(4) Discontinue the topic *Physostigmine and Its Salts*.

(5) Continue study on all other topics.

References

(1) Smith, E. (1991) Fifth Annual Meeting American Association of Pharmaceutical Scientists, November 17–21, Abstract No. APQ1101; *Pharmaceutical Research* **8**, Supplement (Oct. 1991)

(2) Smith, E. (1988) Third Annual Meeting American Association of Pharmaceutical Scientists, Oct. 31–Nov. 3, Abstract No. AP336; *Pharmaceutical Research* **5**, S-14 (Oct. 1988)

(3) Stalcup, A.M., Faulkner, J.R., Tang, Y., Armstrong, D.W., Levy, L.W., & Regalado, E. (1990) *Biomed. Chromatogr.* **5**, 3–7

(4) Tan, H., Yan, S.I., Ya-Ping, & Thio, A.P. (1989) *J. Chromatogr.* **475**, 381–389; Fourth Annual Meeting American Association of Pharmaceutical Scientists, Oct. 22–26, Abstract No. AP149 *Pharmaceutical Research* **6**, S-13 (Oct. 1989)

(5) *Official Methods of Analysis* (1990) 15th Ed., AOAC, Arlington, VA, pp. 590–591

(6) Tymes, N.W. (1982) *J. Assoc. Off. Anal. Chem.* **65**, 132; (1983) *J. Assoc. Off. Anal. Chem.* **66**, 339

(7) Yang, S.T, & Wilken, L.O. (1988) *Drug Devel. Ind. Pharm.* **14**, 1061–1078

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Drugs and Related Topics. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

† Present address: E. Smith and Associates, 14203 Castaway Drive, Rockville, MD 20853.

Drugs III

RICHARD M. MONTGOMERY (In lieu of a General Referee)
Unilever Research U.S., Inc., Edgewater, NJ 07020

The status of this topic is under review. There is currently no General Referee or active projects under this topic.

There are 2 methods in Chapter 18, *Official Methods of Analysis*, 15th Ed., that were reviewed and recommended to the Membership for adoption to final action status this year. There are 2 more methods in the *First Supplement* to the 15th Edition that were also reviewed and recommended to the

Membership for adoption to final action status. Acceptance of these recommendations means that all of the active methods in Chapter 18 will have final action status. These recommendations are indicated below.

Recommendations

(1) *Xanthine Group Alkaloid Drugs, Microchemical Tests (**960.56**).*—Adopt as final action.
(2) *Hydralazine Hydrochloride in Drug Tablets, Spectrophotometric Method (**989.07**).*—Adopt as final action.
(3) *Clioquinol in Creams and Ointments, LC Method (**990.14**).*—Adopt as final action.
(4) *Penicillin V Potassium in Tablets, LC Method (**990.15**).*—Adopt as final action.
(5) Discontinue all topics.

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Drugs and Related Topics. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Drugs IV

LINDA L. NG
U.S. Food and Drug Administration, Center for Drug Evaluation and Research, Rockville, MD 20857

D- and L-Amphetamines.—The chiral LC method, **988.28**, was adopted final action. Discontinue topic.

Benzodiazepines.—Continue study on flurazepam in bulk drug and capsules currently on first action status. Associate Referee Eileen Bargo is exploring other topics for future collaborative studies.

Dicloxacillin.—Associate Referee Mei-Chick Hsu is working on the protocol for a collaborative study. Continue study.

Heroin.—Associate Referee Charles Clark completed the protocol and initiated the collaborative study on a packed-column GC method for heroin hydrochloride. However, because many laboratories are upgrading to capillary GC equipment, he is in the process of modifying the method to use capillary GC column. A collaborative study will be planned once the method is finalized. Continue study.

Miconazole Nitrate.—Associate Referee Judith Genzale is working on the protocol and will be looking for collaborators for an LC method for miconazole nitrate in creams and suppositories. Continue study.

Piroxicam.—Associate Referee Marie Ines Rocha Miritello Santoro is evaluating piroxicam in various dosage forms available in Brazil. The United States has only the capsule formulation. Methods were developed, and she is planning a collaborative study. Continue study.

Additional Information

An advertisement was placed in *The Referee* for volunteers as Associate Referees. To date, we have received 4 inquiries, 2 from overseas and 2 from the United States. The Associate Referee package and an information letter were mailed to respondents.

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Drugs and Related Topics. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Drugs V

THOMAS G. ALEXANDER
U.S. Food and Drug Administration, Center for Drug Evaluation and Research, HFD–473, Washington, DC 20204

Aminobenzoic Acid and Salicylic Acid Salts in Pharmaceuticals.—The collaborative study was completed, and the Associate Referee, Richard Thompson, is now preparing the data for the report. Continued study is recommended.

Anabolic Steroids Bulk Materials by LC.—The developmental work was published (*J. Assoc. Off. Anal. Chem.* [1990] **73**, 904–926); however, the Associate Referee, Milda Walters, is unable to continue with the assignment. It was envisioned that only a present-absent decision is to be involved. Hopefully, a limited collaborative study involving a few typical steroids will serve the purpose of making this method suitable for official status. I recommend that the topic be continued and an Associate Referee be appointed.

Conjugated Estrogens by LC.—The Associate Referee, Robert Roos, was unable to continue the work. I recommend that the topic be discontinued.

Cromolyn Sodium in Bulk and Dosage Forms.—The Associate Referee, Linda Ng, has completed the preliminary investigation, which indicates that her method is much better suited to regulatory analysis than that which is now in the *United States Pharmacopeia*. She is now preparing the protocol for collaborative study. Continued studied is recommended.

Pentaerythritol Tetranitrate.—Continued first action status of **991.16** is recommended, as is continued study. The Associate Referee is Marvin Carlson.

Progestational Steroids.—Section **971.43** of the *Official Methods of Analysis*, 15th Ed., contains simple dissolve, react, extract, and read colorimetric methods for dimethisterone, medroxyprogesterone acetate, norethindrone, norethindrone acetate, and norethynodrel. There is very limited selectivity to these methods. The development of LC methods for these should be straightforward. I recommend that an Associate Refereeship be established to develop accurate and selective

methods, with the ultimate result that these colorimetric methods be surplused.

Steroids in Tablets.—The Associate Referee, Elaine Bunch, hopes to be able to work on this in the near future. Continued study is recommended.

Recommendations

(1) *Aminobenzoic Acid and Salicyclic Acid Salts in Pharmaceuticals.*—Continue study.
(2) *Anabolic Steroids bulk materials by LC.*—Appoint Associate Referee. Continue study.
(3) *Conjugated Estrogens by LC.*—Discontinue topic.
(4) *Cromolyn Sodium in Bulk and Dosage Forms.*—Continue study.
(5) *Pentaerythritol Tetranitrate.*—Continue first action status of **991.16**, and continue study.
(6) *Progestational Steroids.*—Establish Associate Refereeship(s) for these steroids.
(7) *Steroids in Tablets.*—Continue study.

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Drugs and Related Topics. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Forensic Sciences

STANLEY M. CICHOWICZ
U.S. Food and Drug Administration,
200 C St, SW, Washington, DC 20204

The best way to objectively evaluate the analytical results from a multiple laboratory system is to have a comprehensive quality assurance program that uses validated methods in the analysis of carefully prepared check samples. The solution to the legal nightmare of having to justify results after a failed check sample analysis is a training program built into the QA protocol that immediately corrects deficiencies.

The problem that now exists is not legal or political but one of resources and funding. Everyone knows that better scientific results will come out of laboratories that have strong quality assurance programs and that use validated methods.

Recommendation

(1) I recommend that this refereeship be maintained for 1 year and that it be studied until more resources become available, at which time it can be reinstated.

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Drugs and Related Topics. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Committee on Foods I

Dairy Chemistry

ROBERT L. BRADLEY, JR
University of Wisconsin, Department of Food Science, Madison, WI 53706

Fat in Raw Milk, Babcock Method.—D.M. Barbano reports that this method is still in use by a large number of laboratories across the country. Continued research into the reason for the discrepancy in values between Babcock and Mojonnier, which is approximately 1% of the fat, is ongoing. Tempering bath temperature adjustment may solve the problem. A collaborative study is planned over a wide range of fat concentrations to test corrective procedures.

Nitrogen (Total) in Milk, Kjeldahl Method.—D.M. Barbano reports that this method is being successfully used in many laboratories. No comments were received concerning variance from given performance parameters, particularly those resulting from samples submitted bimonthly to Federal Milk Market Labs.

Nonprotein Nitrogen in Whole Milk, Kjeldahl Method; Protein Nitrogen Content of Milk, Kjeldahl Method and Indirect Method.—D.M. Barbano reports that these methods are being used by a few laboratories with no reports of problems with the method or variances outside performance parameters.

Solids (Total) in Milk by Direct Forced-Air Oven Drying.—D.M. Barbano checked performance of this procedure by use of bimonthly samples submitted to many laboratories. No comments were received that suggest the need for modification.

Solids (Total) in Milk by Forced-Air Oven Drying after Steam Table Predry.—D.M. Barbano knows of some use of this procedure. Again, no comments from users suggest the need for modification.

Solids-not-Fat in Milk by Difference between Total Solids and Fat Contents.—D. M. Barbano received no comments to suggest that alteration of the procedure is needed. This method is used by many laboratories, as well as Federal Milk Market Labs. Within- and between-lab performance considerations are within specifications.

Infrared Spectroscopic Methods for Milk.—D.M. Barbano is proceeding with effort to control variables in this analysis technique before undertaking further study.

Microwave Oven Method for Cheese Moisture.—T. Kierstead reports that an oven remodeling is underway to control analytical repeatability problems in existing equipment. Sampling requirements will be remodeled next, before a collaborative study is undertaken.

Absolute Moisture Content of Dairy Foods.—D.B. Emmons is preparing a collaborative study.

Gerber Method for Fat in Milk.—D.H. Kleyn is in the final stages of organizing a collaborative study to test this European method against the ether extraction method.

Combustion Method for Protein in Dairy Products.—J.G. Sebranek had a collaborative study approved so that the accuracy of the Dumas procedure for milk protein can be ascertained. Results should be forthcoming within a few months.

Iodine in Milk, LC Method.—D.C. Sertl finalized this collaborative study, and it was submitted to the Official Methods Board for consideration.

Fat in Foods, Robotic Mojonnier.—R.L. Bradley is in process of assimilating data from users of this equipment. After statistical review, a determination will be made to ascertain the need for a collaborative study.

Moisture in Cheese.—R.L. Bradley reported that a collaborative study is in the initial planning stage.

Raw Milk Sampling at the Farm.—D.T. Metzger reported that the new sampling technique was successful, with no variations reported outside of given parameters.

No other reports were received from Associate Referees.

Recommendations

(1) Recommend as official final action *Sampling Technique for Barrels (Sampling of Cheese)*, **970.30C**. This procedure was given first action status in 1986. Since that time, it has been used by all U.S. Department of Agriculture and many industry graders and analysts. USDA provided each grader with a stainless device so that penetration into barrels is at proper angle.

(2) Recommend as official final action *Solids (Total) in Milk by Direct Forced-Air Oven Drying*, **990.20**.

(3) Recommend as official final action *Solids (Total) in Milk by Forced-Air Oven Drying after Steam Table Predry*, **990.19**.

(4) Recommend as official final action *Solids-not-Fat in Milk by Difference between Total Solids and Fat Contents* **990.21**.

(5) Recommend as official first action *Iodine in Milk.*

(6) Recommend alteration of **989.04B**(G), *Fat in Raw Milk, Babcock Method.* Some Babcock pipets are not long enough to insert below the junction of the neck and bulb. Alter "Length of delivery tube...100–120 mm" to read "Length of delivery tube (sl longer than bottle neck)...105–125 mm" must not touch milk sample when delivered.

(7) Recommend alteration of methods in Chapter 33 of *Official Methods of Analysis*, where use of an air oven is

indicated to substitute a forced-draft oven. Temperature variance across air ovens can exceed 10°C; therefore, results and variance can be unacceptable.

(8) Recommend alteration in *Water Added in Milk*, **961.07**, from – 0.406°C and –0.598°C to –0.408 and –0.600°C. Use of conversion formula from °H to °C proves this point.

(9) Recommend alteration of *Water Added in Milk*, **961.07B** to reflect corrected weight of NaCl to attain freezing point standards. This should read 0.6889 and 1.0207 g reagent grade NaCl to prepare molal solutions freezing at –0.408 and –0.600°C, respectively.

(10) Recommend that wherever in Chapter 33 reference is given to the use of *Fat in Milk, Roese-Gottlieb Method*, **905.02** that *Fat in Milk, Modified Mojonnier* also be given, because both methods now have AOAC, IDF, and ISO approved status.

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Foods I. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Flavors

DANA A. KRUEGER
Krueger Food Laboratories, Inc., 24 Blackstone St, Cambridge, MA 02139

Additives in Vanilla Flavoring.—Active work in this topic was completed with the acceptance of the LC method for vanilla and related flavor compounds in vanilla as official first action. The General Referee thinks that additional work in this topic would be useful, especially in evaluating the suitability of this methodology for the determination of coumarin, piperonal and other synthetic flavor compounds in vanilla extract and artificial vanilla flavors.

Carbon-14 in Flavoring Materials.—No progress is reported in this topic area.

Vanillin and Ethyl Vanillin.—Associate Referee Sydney Kahan reports that a collaborative study was conducted of an LC method for the determination of vanillin and ethyl vanillin in artificial vanilla flavors and for the determination of ethyl vanillin in vanilla extract.

Licorice Products.—No progress reported in this topic area.

Deuterium NMR Analysis of Vanillin.—No progress reported in this topic area.

Moisture in Vanilla Beans.—No Associate Referee has as yet been appointed to this topic.

A petition was received from the FEMA Technical Committee for the repeal of AOAC method **968.15**, the so-called lead number method for vanilla extract. It is argued that the lead number method is obsolete, imprecise, and requires the use of soluble lead salts, an industrial hygiene problem.

The General Referee agrees that the lead number method is a rather nonspecific method for evaluating the presence or absence of vanilla bean extractables in vanilla extract. The simple imitation vanilla extracts that the method is designed to detect are less frequently seen than in the past, but they are still quite common, particularly in international markets. Whereas most companies in the flavor trade are well-equipped with modern and sophisticated instrumentation and could do without this method, many smaller flavor users are not so equipped and may still find this method beneficial.

I believe that the assertion that the method is imprecise is simply mistaken. Several collaborative studies, some sponsored by FEMA, yielded precise results when compared with many modern instrumental methods.

I agree that the use and disposal of lead reagents has become a larger issue in recent years and that methods requiring the use of lead salts are best avoided where practical. FEMA should strongly urge the flavor industry and its clients to phase out use of this method as a routine quality assurance or specification procedure.

The method is one of well-established reliability and reproducibility from the standpoint of data generation. Further, irrespective of whether or not it should be phased out as a routine procedure, it currently is a routine procedure in widespread use throughout the flavor trade. For this reason, I do not believe that it should be declared surplus at this time. For these reasons, I recommend that no changes be made to the official status of method **968.15** at this time.

Recommendations

(1) In method **920.146A**, the reference to method **955.18D** be replaced by reference to method **942.06B**. This is an editorial correction of an error introduced during the 15th Edition method renumbering.

(2) That methods **925.33C**, **932.11**, **932.10**, **920.147E**, **920.148B**, **920.148C**, and **942.08** be adopted official final action. These methods are all volumetric methods for estimating essential oil content of various extracts and toiletry preparations. The methods have all had official first action status for many years.

(3) That methods **950.44A**, **950.44B**, **920.146A**, **920.147A**, and **920.147B** be adopted official final action. These methods are all pycnometer methods for determining alcohol content of various flavor extracts. The methods have all had official first action status for many years.

(4) That method **920.132** be adopted official final action. This method for determination of methanol in vanilla extract is simply a reference to the official final action method for methanol in distilled spirits. The method has had official first action status for many years.

(5) That method **926.11** be adopted official final action. This is a polarization method for the estimation of citrus oil content of oil-based flavors. The method has had official first action status for many years.

(6) That method **936.10** be adopted official final action. This is a gravimetric method for determination of benzaldehyde in almond extracts. The method has had official first action status for many years.
(7) That method **909.02** be adopted official final action. This is a gravimetric method for determination of benzoic acid in almond extract. The method has had official first action status for many years.
(8) That method **920.146B** be adopted official final action. This is a method for determination of total solids in ginger extract by oven drying. The method has had official first action status for many years.
(9) That method **920.146C** be adopted official final action. This is a qualitative method for determining the presence of ginger in ginger extract. The method has had official first action status for many years.
(10) That method **990.25** be adopted official final action. This is an LC method for determining vanillin and other substances in vanilla extract. The method was adopted official first action in 1990.
(11) That no change of status be made to method **968.15**, the lead number method for vanilla.
(12) That study be continued on all topics.

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Foods I. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Food Additives

THOMAS FAZIO
U.S. Food and Drug Administration, Office of Physical Sciences, Washington, DC 20204

Antioxidants.—Phenolic Antioxidants in Butter Oil, Liquid Chromatographic Method (IDF Collaborative Study), Modification of **983.15**, submitted by Denis Page, was reviewed and accepted by the Committee Statistician and the General Referee. The study is under review for acceptance as official first action status.

Indirect Additives from Packaging.—The following summarizes the activities over the past year in the Associate Referee's laboratory.

Migration Studies

Mixing Effect.—The agitation effect on indirect additive migration of the antioxidants Irganox 1010 and I-1076 from poly(propylene) or from high-density poly(ethylene) appears to be significant for aqueous foods processed below about 135°C. Static migration levels increase 5-fold when mixed end-over-end at 10 rpm. Only a 10% increase occurs in oil simulants at 77°C. The effect is <20% at 135°C for aqueous simulants, and it is not observed for fatty simulants. Food additive petition guidelines may need changing to reflect this effect for low-temperature aqueous food applications.

Irganox 1010 Migration at Elevated Temperatures.—A series of experiments verified that the Irganox 1010 migration rate from poly(propylene) to Miglyol food oil is linearly dependent on the inverse absolute temperature over the range from 50°C to 135°C, and the rate is little affected by partition factors such as mixing. These data will now be used to evaluate barrier properties of this adjuvant polymer system as functions of temperature, polymer crystallinity, and film thickness.

Migration from Crystallizable Polymers.—Experiments were performed to address the question of whether or not migration inversion occurs in crystallizable polymers when they are heated above their glass transition temperature (T_g). The objective of these experiments was to determine if an enhanced migration could be observed at a lower temperature vs migration at a higher temperature in poly(ethylene terephthalate) (PET), which is a crystallizable polymer. The premise behind having a lower migration at a higher temperature is related to the crystallization kinetics in the polymer. If a polymer crystallizes more rapidly at higher temperatures, such an event may actually reduce migration at the higher temperatures below levels experienced at lower temperatures. Migration experiments with PET and CPET were performed at 100°C and 120°C. At these 2 temperatures, the crystallization kinetics are very different. Migration inversion did not occur at these temperatures. At least 3× more migration was observed at 120°C.

The migration increase within this range is significant, because it indicates a major loss of barrier properties. Additional studies are planned on PET barrier properties at elevated temperatures.

Microwave Susceptor Packaging

Non-UV-Absorbing Nonvolatile Migrants From Susceptors.—Supercritical fluid chromatography (SFC) has been demonstrated to be a valuable tool for determining the presence of non-UV-absorbing, nonvolatile migrants from food packaging. Applications to date include determination of aliphatic plasticizers, grease coatings, and adhesive components.

Grease-Proofing Paper Additive.—An analytical procedure was developed that permits the determination of ammonium bis(*N*-ethyl-2-perfluoroalkylsulfonamido ethyl) phosphate salt degradation products produced when paper grease-proofed with this substance is heated (as in microwave susceptor popcorn bags). One of the principal heat degradation products is *N*-ethylperfluorooctanesulfonamido ethanol, which is often referred to as the FOSE alcohol. Early indications are that the salt and the alcohol can now be detected at part-per-million levels in extracts of heated, coated papers.

Paper Decomposition at Elevated Temperatures.—Five paper materials were heated in controlled experiments, and the volatile decomposition products released were analyzed by GC. The paper materials producing the greatest amount of vol-

atile chemicals are dual-ovenable trays and microwave susceptor paperboard.

Further studies on paper decomposition as a function of time and temperature demonstrate that substantially more furfural and total volatiles are formed from heated paper than from equivalent weight of flour or corn meal. Many of these volatiles remain unknown.

Adhesive Decomposition at Elevated Temperatures.—An adhesive component used in dual-ovenable packaging (i.e., diglycidyl ether of bisphenol A [DGEBA]) was found to migrate at levels of 2.3 μg/g or 16 μg/in^2. through its "protective barrier" PET film into a food simulating oil under recommended use conditions. This occurred when the dual-ovenable tray containing the adhesive was heated according to recommended cooking instructions. The dual-ovenable package used in these tests was made with a metallized susceptor film.

Other Studies

Poly(phenylene oxide)/Poly(styrene).—A draft manuscript describing the migration of styrene, benzaldehyde, and 2,6-dimethyl-1,4-benzoquinone (26DMBQ) from poly(phenylene oxide)/poly(styrene) trays to fatty food stimulants is being prepared for publication. Mass spectrometry confirmed the presence of 26DMBQ in migration extracts.

Nylon.—A sensitive method was developed for the determination of residual chemicals in nylon polymers. Initial indications are that, in nylon products such as baking bags, the total residual chemical levels are about 6 mg/g.

Cling Film.—The color component in colored cling film appears attached to the polymer and does not extract in solvent. The film contains about 21% mixed adipate, azelate and aromatic plasticizers, and extractable additives.

Preliminary results of a very limited survey of plasticizers used in cling wraps and container gasket applications reveal the presence of several types of plasticizers, including phthalates, adipates, azelates, epoxidized soy bean oil, and small amounts of unknown aromatic substances.

Benzene in Food.—A number of foods, particularly those thought to contain added or naturally occurring benzoate preservatives, were collected and analyzed for benzene using manual headspace GC and mass-selective detection. This was done to establish a baseline of benzene in foods for comparison with levels that may enter foods by indirect means. Raw mangos, imported from Haiti, and russet potatoes, both raw and microwave baked, were found to be benzene-free. Others included cranberry juice cocktail, 0.5 ppb; BBQ sauce with tomato paste, 5 ppb; taco sauce, 25 ppb; and soft drinks (Coke, Diet Orange Crush, and Diet White Grape), <1 ppb.

Trihalomethanes (THMs) in Soft Drinks.—THMs in soft drinks were analyzed using EPA Method 524.2. This method gives us a 10-fold greater sensitivity for THMs than was previously possible using only GC/FID. Total THMs found ranged from <5 ppb to ca 50 ppb. In order of decreasing concentration in the soft drinks tested, chloroform, bromodichloromethane, and dibromochloromethane were found in all of the soft drinks analyzed.

A number of foods were also analyzed from THMs, as well as benzene and toluene. Of all the foods analyzed, the highest level of THMs was found in soft drinks (97 ppb), and the highest level of toluene residues (75 ppb) was found in canned foods.

Irradiated Foods, Detection Methods.—Associate Referee L.G. Ladomery submitted the following report:

The FAO/IAEA Coordinated Research Program on Analytical Detection Methods for Irradiation Treatment of Foods (ADMIT) will hold its second research coordination meeting in Budapest, June 10–15, 1992. The meeting delegates will review the results of research on a number of methods, qualitative and quantitative, for a variety of food substrates. It should be noted that the methods investigated by the FAO/IAEA Group involve the measurement mostly of physical (e.g., rheological changes, electrical impedance, thermo and chemi-luminescence, electron spin resonance, and NIR), microbiological (e.g., relationship between live-to-dead bacterial count), biological (e.g., ability of a seed or tuber to germinate), or biochemical (e.g., DNA double strand breaks) parameters in irradiated food products. Three methods at an advanced stage studied by the group involve the measurement of chemical analytes (e.g., lipid-derived 2-alkylcyclobutanones and volatiles and *o*-tyrosine from protein). Other chemical methods explored involved the determination of organic peroxides and guanine dimer as indicators of irradiation.

Several methods (ESR, lipid-derived volatiles, and thermoluminescence) are very likely to be prepared as standard methods. These methods would be suitable for further collaborative studies by AOAC and other interested international bodies. An assessment will be made of the state of the art following the meetings, and candidate methods suitable for consideration by AOAC will be identified for collaborative study.

Nitrosamines in Foods.—Associate Referee Sen reports that work continued on the problem of nitrosamine contamination in cured pork products packaged in elastic rubber nettings and on improving the analytical method used for the determination of nonvolatile *N*-nitrosamines in such products. In the total study, 20 such products were analyzed (pork cottage rolls, hams, smoked pork shoulders, etc.) that were procured from local retail outlets from May 1991 to February 1992. Eight of these samples contained fairly high levels (16–48 ppb) of *N*-nitroso-di-*n*-butylamine (NDBA); one contained both NDBA (8 ppb) and *N*-nitrosodiethylamine (10.6 ppb). Fourteen samples contained low to high levels (12–520 ppb; average, 71 ppb) of *N*-nitrosodibenzylamine (NDBZA). All these results are based on the analysis of homogenized meat, not surface samples. Adequate precaution was taken to prevent or minimize artifactual formation during the analysis (e.g., for the determination of volatile *N*-nitrosamines, all the samples were distilled under vacuum at 40–45°C from acidic sulfamic acid solution). The above data suggests that the problem of nitrosamine contamination of such food products still exists, and research continues to try to develop an elastic netting that does not form *N*-nitroso compounds and does not contaminate the food product.

A rapid Celite–basic alumina (deactivated with the addition of 2% water) cleanup technique was developed for the determination of NDBZA in cured pork products. The method gave results comparable with those obtained by a previously published method (*J. Food Sci.* [1988] **53**, 731–738).

Work continues on the development of a rapid LC method for the determination of underivatized, nonvolatile *N*-nitroso compounds in foods. The postcolumn chemical denitrosation technique developed by Havery (*J. Anal. Toxicol.* [1990] **14**, 181–185) is being used for this purpose.

For the ARS laboratory, Associate Referee Pensabene reports that studies were conducted to determine the feasibility of using 3 forms of Alaska pollock (unwashed and washed minced and surimi) as a partial substitute for meat in a nitrite-cured product. The effect of several pre- and postprocessing storage conditions on *N*-nitrosodimethylamine (NDMA) was studied in frankfurters substituted with 15 and 50% unwashed and washed mince and surimi after broiling, a cooking method that favors NDMA formation in this product type. In franks made with unwashed mince that was frozen-stored before processing, NDMA increased during frozen storage and then leveled off after 30 days, whereas little change was observed in franks containing washed mince and surimi. Refrigerated storage of franks for up to 56 days generally showed a decrease in NDMA with all 3 forms of fish. Overall, the use of washed mince and surimi at the 15% substitution level gave mean values less than 2 ppb NDMA, even after boiling. This is considerably lower than the regulatory violative limit of 10 ppb set for nitrosamines in other meat products. The overall mean for NDMA in the uncooked 50% unwashed mince frankfurters was 10.5 ppb and was significantly ($p < 0.05$) higher at 19.7 ppb in the same franks after boiling. This indicates that 50% unwashed mince could not be used as a substitute and that cooking is a major factor in formation of NDMA in this product type.

The addition of cryoprotecting agents, sucrose and sorbitol, to the washed mince to make surimi was the major difference in the 2 forms of fish. Model system experiments, performed first in an aqueous, pH 5.8 buffer and then in a 50% fish-meat simulated frankfurter system, indicated that the combination of sucrose–sorbitol increased NDMA formation, and fructose–sorbitol decreased NDMA formation. Franks prepared with 50% washed mince and containing different carbohydrates showed that sucrose–sorbitol gave higher NDMA values than fructose–sorbitol, even though all the carbohydrates tested gave NDMA results higher than the control.

Polycyclic Aromatic Hydrocarbons.—Work continues in the following 3 areas: (*1*) development of a procedure for the determination of polycyclic aromatic hydrocarbons (PAHs) in smoke flavors, (*2*) investigation of procedures for improving the recently developed procedure entitled "Determination of Polynuclear Aromatic Hydrocarbons in Seafood by Liquid Chromatography with Fluorescence Detection", and (*3*) a study to compare the FDA/Perfetti and NOAA/Krahn method for the GC/MS determination of 18 PAHs.

Our efforts in the coming year will be directed toward selecting a method to be collaboratively studied that uses modern techniques such as LC and GC/MS for the determination of PAHs in foods. Two methods for the determination of PAHs in seafood were validated. One method, "Determination of Polynuclear Aromatic Hydrocarbons in Seafood by Liquid Chromatography with Fluorescence Detection", was accepted by AOAC for publication. "Comparison of Two Methods for the GC/MS Determination of Low Levels (ng/g) of Polynuclear Aromatic Hydrocarbons in Seafood" is currently being prepared for publication. Therefore, it is the Associate Referee's suggestion that one of these improved methods be collaboratively studied in the coming year.

Sulfiting Agents in Foods.—A survey of a wide variety of foods was conducted by the Associate Referee's laboratory by using the optimized Monier-Williams procedure. Various brands of the following foods were analyzed: dried apricots, instant potatoes, shrimp, hominy, grape juice, clam chowder, molasses, sugar, garbanzo beans, fresh potatoes, canned corn, and orange juice. The samples were also analyzed by the formaldehyde capture/LC method that is essentially identical to the Monier-Williams procedure, except that the evolved sulfur dioxide is captured in a formaldehyde solution, and this solution is quantitated by LC. Given the detection limit of 10 ppm established for the optimized Monier-Williams, the results acquired by both methods were in good agreement. This illustrates that the Monier-Williams method is not subject to interference by the foods listed above. Associate Referee Warner hopes to subject the LC method of Warner et al. (*Food Addit. Contam.* [1990] **7**, 575–582) to collaborative study in the next year.

Urethane in Foods.—Associate Referee Canas reports that a collaborative study for the determination of ethyl carbamate (EC), also known as urethane, in alcoholic beverages and foods was successfully completed. The method ("Determination of Ethyl Carbamate in Alcoholic Beverages and Foods by Gas Chromatography with Mass Selective Detection") incorporates many techniques that have now become widely accepted by most EC analyst. These techniques include the use of convenient prepackaged columns for fast extraction of liquids with methylene chloride, capillary GC with mass selective detection for determination and confirmatory identification of EC, and the use of *n*-propyl carbamate as an internal standard. In this study, the collaborators were asked to determine EC in distilled spirits, fortified wine, and soy sauce. The EC levels tested ranged from 40 to 400 ng/g (ppb) in distilled spirits and fortified wines and from 10 to 80 ppb in table wines and soy sauce.

Recommendations

(1) Adopt as official first action the method for determination of polydimethylsiloxane residues in pineapple juice by atomic absorption spectroscopy.

(2) Adopt as official first action the method "Liquid Chromatographic Determination of Nine Phenolic Antioxidants in Butter Oil."
(3) Adopt as official final action the method for NDMA in beer and ale (**982.12**) and the method for NDMA in nonfat dry milk powder (**984.16**).
(4) Continue study on all other topics.

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Foods I. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Meat, Poultry, and Meat and Poultry Products

DAVE SODERBERG
U.S. Department of Agriculture, Food Safety and Inspection Service, Washington, DC 20250

Calcium in Mechanically Separated Poultry and Beef.—Pamela Coleman is newly appointed to this topic. She is trying to develop a method that does not require use of cyanide. The requirement for cyanide in the present method restricts it from being used on-site in meat processing areas.

Comparison of ICP and EDTA methods for Calcium.—Mai Huynh was just appointed Associate Referee for this topic. A study is currently in progress.

Copper Catalyst in Kjeldahl Method for Protein.—Aron Yoffe was appointed to this topic. The data on a copper catalyst was gathered some time ago but was never analyzed in an acceptable manner. A very brief preliminary examination of a copper–titanium dioxide catalyst was also done.

Glutamic Acid and Monosodium Glutamate.—No Associate Referee report received.

Gluten in Meat.—John Skerritt reports that the 1990 AOAC method is becoming widely accepted and has yielded no false-positive or false-negative results to date. A simplified qualitative gluten test was published in *Lancet 2*, 1992. It yielded false-positive results only in vinegar. Apparently, some analysts attempted to increase the sensitivity of the AOAC gluten method by diluting the food extract 1:5 rather than 1:50. This modification may work for analysis of wheat starches but suffers from interferences during the detection of gluten in meat and soy products.

Heme, Nonheme, and Total Iron in Meat, Fish, and Poultry Products.—Arthur Mahoney, Associate Referee for this topic, passed away this year. This topic should be discontinued until another individual can be found to continue it.

Ion Chromatographic Analysis of Meat and Poultry Products.—Mark Paine has been working on a method for several inorganic ions in meat. He reports only slow progress.

LC Methods for Meat and Poultry Products.—Sher Ali reported an extension of the carbamate method to new analytes, a study of the stability of 16 carbamates in liver tissue, and a GC/MS confirmation method for 10 carbamates. He reviewed methods on determination of sulfas by TLC, PAHs by LC, volatiles in chicken by LC, and amines by LC.

LC of Creatinine in Soups and Bouillons.—R. Maeijer reported that method development started during the year and that a preliminary multilaboratory study was done. A collaborative study is anticipated for early 1993.

Near-Infrared Proximate Analysis of Meat Products.—Larry Hand reports no progress this year.

Phosphopyruvic Kinase Assays.—Carl Davis is newly appointed as the Associate Referee for this topic. He developed a field-useable assay. A preliminary multilaboratory validation study is presently underway.

Potentiometric Determination of Sodium and Salt.—R. Maeijer reports that a first trial was completed of a potentiometric method for chloride. Some weaknesses were found and are being further investigated. His working group believes that it is best to do a simple extraction of these elements and to avoid ashing.

Microwave Techniques.—Associate Referee David Fish reports no new progress this year.

Minimum Processing Temperature for Meats.—Work on internal-temperature methods proceeded apace. The Associate Referee for this topic, Grover Pickel, reports that because of a prolonged absence he was unable to keep up with developments. Studies of methods for uncured poultry meat included several enzyme assays, identification of marker proteins by immunoassay, and changes in color and in volatiles produced on cooking. For uncured beef products, lactate dehydrogenase and transaminases were studied. *N*-Acetyl-β-D-glucosaminidase was studied for use in several meats. In addition, refinements to the phosphatase test are being investigated.

Nonmeat Protein in Meat and Poultry Products.—No Associate Referee report received.

Protein Determination in Meat by Combustion Method.—Joseph Sebranek completed a collaborative study. This was accepted by AOAC.

Robotic Methods for Meat and Poultry Products.—Associate Referee Randy Simpson is developing a robotic method with LC for determining sulfa drugs in meat. Development of a robotic method for ivermectin in meat was completed and will shortly be subjected to a 3 analyst validation and peer reviewed in a second laboratory.

Specific Ion Electrode Applications.—No Associate Referee report received.

Supercritical Fluid Methods.—Jerry King was just appointed Associate Referee for this topic. He has been very active in this area and is a well-known pioneer of new devices and techniques.

Total Fat.—A collaborative study of a method for crude fat in meat using the Soxtec analyzer was published by Max Foster, the Associate Referee for fat methods. This procedure was approved as official first action. Foster reports that he is con-

tinuing to take requests for this method and to respond to questions.

Volatiles in Meat and Poultry, and Meat and Poultry Products.—Jeffery Donohue reports continued work on this topic. He reports inconsistent results with static headspace. For dynamic headspace, a cryogenic trap is being installed to facilitate interface between the purge and trap and a capillary gas chromatograph.

Reports from the International Organization for Standardization (ISO) indicate that there are new work item proposals for detection of parasites, acid phosphatase assay, water activity, potentiometric method for chloride at concentrations greater than 0.25% (as NaCl), and performing microbial colony counts in the cold. Revisions were started for pH, Volhard chloride, total fat, free fat, nitrite, and phosphorus. A revised method for moisture content is nearing approval, and a method for L(-)-hydroxyproline is in committee. Microbiological methods for sampling, sample preparation, and for *Salmonella*, coliforms, *Clostridia*, *Enterobacter*, and *Staphylococcus aureus* are in various stages of development.

A completely revised version of the residue chemistry portion of the USDA, FSIS *Analytical Chemistry Laboratory Guidebook* was published this year. The remaining nonresidue methods should be published within a year. The revised methods now include analysis flow charts, chromatograms, hazard information, and quality assurance material.

AOAC Method **990.26**, Hydroxyproline in Meat and Meat Products, published in the First Supplement to the Fifteenth Edition of the *Official Methods of Analysis*, was granted final action status this year.

Recommendations

(1) Discontinue *Heme, Nonheme, and Total Iron in Meat, Fish, and Poultry Products.*
(2) Transfer *Robotic Methods for Meat and Poultry Products* to the Committee on Residues.
(3) Continue all other topics.

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Foods I. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Mycotoxins

PETER M. SCOTT
Health and Welfare Canada, Health Protection Branch, Food Directorate, Bureau of Chemical Safety, Ottawa, ON, K1A 0L2, Canada

Active method development continues, particularly for aflatoxins, fumonisins, ochratoxin A, and immunochemical methods. One AOAC collaborative study was carried out during the past year, and one new Associate Referee was appointed. Reports on the topic areas are as follows.

Aflatoxin M.—The new Associate Referee is Hans P. van Egmond (National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands). He noted studies on performance and application of immunochemically based methodology to determine aflatoxin M_1. A collaborative study was conducted under the auspices the of the International Dairy Federation (IDF), involving 16 participants from 11 countries (1). The method used an immunoaffinity column binding aflatoxin M_1 from the milk passing through the column. Final determination of aflatoxin M_1 was carried out with reversed-phase liquid chromatography (LC) and fluorescence detection. Over the range tested (80–600 ng aflatoxin M_1/kg milk powder), a reproducibility coefficient of variation (CV) of ca 20% was obtained. Recovery experiments were not included in the study; therefore, accuracy data, which are a prerequisite to have the method considered for AOAC approval, could not be derived. Two papers were published on the determination of aflatoxin M_1 using an immunoaffinity column pretreatment system coupled on-line to an LC system (2, 3). A dialysis unit efficiently removed interfering compounds from the system so that the immunoaffinity precolumn could be reused for more than 70 analyses (3). The repeatability of the system was reported to be good (CV = 3%), and the detection limit for aflatoxin M_1 was 10 ng/L, low enough for routine measurements. However, the overall recovery was only 6%.

The 1991 Check Sample Programme for aflatoxin M_1 organized by the International Agency for Research on Cancer (4) involved 125 laboratories that analyzed a lyophilized milk powder sample with a low level of aflatoxin M_1 contamination (overall arithmetic mean found, 0.28 ng/g). The use of AOAC method **974.17**, which has further decreased to ca 8% of participating laboratories, yielded 55% of the results near zero. The limit of detection of this method may not be low enough. Laboratories applying immunoassays (8%) had relatively high results. Laboratories using immunoaffinity columns in combination with LC (6%) obtained results close to the overall mean.

A project to determine the presence of aflatoxins in the U.S. meat supply during the drought year 1988 showed the presence of aflatoxins M_1 and B_1 in pig liver (5). The highest level found for aflatoxin M_1 was 0.44 ng/g.

Hydrated sodium calcium aluminosilicate, which was added to aflatoxin-contaminated diets of dairy cows, was found to reduce the secretion of aflatoxin M_1 in milk by 24–44% (6).

A carry-over experiment was done with Dutch dairy cows, in early and late lactation stages, fed diets naturally contaminated with low levels of aflatoxin B_1 (7). Carry-over percentages up to 6.2% were found, much higher than known thus far. A relation between aflatoxin B_1 intake and aflatoxin M_1 content was found, described by the following formula: aflatoxin M_1 (ng/kg milk) = 1.19 × aflatoxin B_1 intake (μg/cow/day) + 1.9. This means that the production of milk with less then 0.05 ng aflatoxin M_1/g (current European tolerance levels) would require limitation of the average daily intake of aflatoxin B_1 by dairy cows to ca 40 μg.

Rationales for limits on aflatoxin M_1 in milk and milk products are scarce. A recent enquiry (8) showed that only the United States and The Netherlands have declared a risk assessment for aflatoxin M_1. A thorough evaluation of health risk would be highly desirable to provide a scientific basis for regulations on aflatoxin M_1, because the number of countries with (proposed) regulations for aflatoxin M_1 is increasing.

The Codex Committee on Food Additives and Contaminants put forward a maximum level of 0.05 μg/kg (ng/g) for aflatoxin M_1 in liquid milk and agreed to put this value to the 20th session of the Codex Alimentarius Commission in 1993 for adoption (9). Several countries reserved their positions, however.

The Associate Referee recommends the following: (*1*) a new collaborative study to be undertaken in 1993 or 1994 on an immunoaffinity column method for the determination of aflatoxin M_1 in milk, including recovery experiments at very low levels of contamination, in view of the very stringent regulations in some countries for milk used for the production of infant foods; (*2*) further research on the performance and efficiency of solid-phase extraction (SPE) columns in aflatoxin M_1 methods, particularly at very low levels of contamination; (*3*) surplus action status for the official final action method **974.17**; and (*4*) continuation of methods **980.21**, **986.16**, **982.24**, and **982.25** in official final action status.

Aflatoxin Methods.—Associate Referee Mary W. Trucksess (U.S. Food and Drug Administration [FDA], Washington, DC) reported that 2 excellent reviews covering methods of analysis for mycotoxins, including the aflatoxins, were recently published (10, 11). The most significant improvement in the purification step in recent years is the use of SPE, which is quick, solvent-efficient, and economical. A recent example is an SPE/LC method for determination of aflatoxins in peanut meal (12). Many SPE cartridges are available commercially. The analyte may be eluted in a small volume suitable for subsequent injection into a liquid chromatograph, making automation possible.

The number of publications on thin-layer chromatography (TLC) has declined, but this is not necessarily an indication of the extent to which TLC methods are used worldwide. TLC methods are used routinely and extensively, particularly outside the United States, and are only published when applied to new commodities or when improvements in the technique are made. In recent research reports, a microcomputer was interfaced with a fluorodensitometer to simplify data handling (13), and the aflatoxin content of palm kernels was quantified using a bidirectional TLC procedure (14).

LC precolumn derivatization with trifluoroacetic acid was used for the determination of aflatoxins in corn, tree nuts, peanuts, milo, and other commodities after cleanup using multifunctional column filtration (15). A collaborative study of this method was initiated following protocol approval. Letters of invitation were sent to various laboratories that expressed interest in participating. LC postcolumn derivatization with iodine was used for the determination of aflatoxins in 2 peanut butter reference materials (16) and in a collaborative study on a method for aflatoxin B_1 in animal feeding stuffs (17).

A number of immunoassay kits for aflatoxins have been marketed under various trade names (18). Results obtained using these kit methods and those obtained by LC methods for the determination of aflatoxins in corn (19), peanut butter (20), and nuts and nut products (21) are in close agreement. However, the evaluation of these immunoassay methods has been a source of controversy. The various criteria that were considered include sensitivity, specificity, avidity, applicability, stability, procedure, quality control, cost, precision, accuracy, and simplicity (22, 23). A few of the commercially prepared immunochemical testing kits were evaluated by collaborative studies. In some cases, the collaborative study results were no longer valid because the kits were changed. The Agri-Screen kit (**989.06** and **990.32**) was modified to eliminate field problems and to improve its ease of use. The company will conduct an in-house study to evaluate the modified method. Results of this study will be submitted to the Committee on Foods I for approval. Another kit method (the Immuno-Dot Cup, **990.34**) for screening for ≥20 ng/g aflatoxins in cottonseed and peanut butter and for aflatoxins at ≥30 ng/g in corn and raw peanuts was also modified, as reported last year. A follow-up collaborative study was conducted for detecting aflatoxins at ≥20 ng/g in corn, and the results of the study were submitted to the Methods Committee (24). To meet the constant change in technology and manufacturing processes, AOAC International established a new Research Institute to test performance of proprietary test kit methods (25). By means of enzyme-linked immunosorbent assay (ELISA), significant reductions were observed in recovery of aflatoxin B_1 from finely ground peanuts left overnight after spiking (26). This may have important implications for collaborative study samples.

An LC/thermospray mass spectrometric (TSMS) method was developed for the confirmation of aflatoxins in peanuts (27). The detection limits were 60, 40, 100, and 100 pg for aflatoxins B_1, B_2, G_1, and G_2, respectively. A TSMS method was used to characterize the reaction products of aflatoxins B_1 and G_1 with iodine in methanol–water (28); iodine and a methoxy group are added to the double bond of the furan ring.

Sampling and sample preparation are labor-intensive and remain problems that need to be solved to take advantage of the new rapid methods. Immunoaffinity cleanup columns require manual operation (29). However, automated aflatoxin analysis is in its infancy. A partially automated robotic system was coupled with a disposable immunoaffinity column after extraction, filtration, and dilution (30). A method for screening for several mycotoxins (including aflatoxins) in foods and feeds that incorporates an on-line sample cleanup was reported earlier (31).

These methods are very expensive and require LC (4 pumps and 2 switching valves) or robotic systems (>$50,000), in addition to costly SPE and immunoaffinity columns. A recent development is the use of an on-line LC affinity column cleanup, coupled with column switching to an analytical column, and postcolumn derivatization using electrochemically generated bromine (32). The packing in the LC affinity column is a macroporous hydrophilic copolymer of 2-hydroxyethyl methacrylate and ethylene dimethacrylate. The entire automated LC procedure takes about 30 min. The LC affinity column support can be used for more than 100 injections.

Because of new laws, laboratory and environmental safety and their applications have received a lot of attention. A recent paper describes safety training and the use of personal protection equipment; summarizes safety guidelines for handling mold cultures and moldy commodities, analysis of test samples, and preparation and administration of contaminated diets for animal studies; and reviews methods for destruction of toxins in waste products, equipment, work surfaces, and laboratory space (33). The authors also discuss procedures to avoid pollution of the work environment and the use of biological safety cabinets and fume hoods.

The regulatory levels of aflatoxins that were adopted by more than 50 countries are tending toward lower limits, and they differ widely. In some cases, the legal limit and the acceptance criterion are also different. For example, in the United Kingdom the legal limit for aflatoxin B_1 in dried figs is 5.0 ng/g, and the acceptance criterion is 2.5 ng/g, which provides a safety margin allowing for uncertainties in aflatoxin distribution (34).

The Associate Referee recommends that (*1*) the modified "Immuno-Dot Cup" (Signal Accucup) ELISA, which was collaboratively studied for corn, be adopted as official first action for screening aflatoxins B_1 and G_1 at ≥20 ng/g corn; (*2*) work continue on development of automated aflatoxin methods; (*3*) the collaborative study of the multifunctional column LC method for aflatoxins in corn, peanuts, almonds, pistachio nuts, and Brazil nuts be completed; (*4*) the best method for aflatoxin in dried fruits (including figs) be selected and collaboratively studied; and (*5*) aflatoxin methods be selected and collaboratively studied as new regulations may require.

Alternaria Toxins.—Associate Referee Angelo Visconti (National Research Council, Bari, Italy) drew attention to a new multiauthor book, co-edited by the Associate Referee, on the biology, plant diseases, and metabolites of *Alternaria* (35). Chemical and biological data for 71 *Alternaria* metabolites, both mycotoxins and phytotoxins, are collected in a chapter of this book (36). Continuation of work in Poland on natural occurrence of *Alternaria* toxins in cereals led to a report of alternariol and/or alternariol methyl ether in 56 of 114 samples of kernels or chaff from "black heads" of wheat, rye, triticale, barley, and oats (37). Further evidence for the widespread occurrence of toxigenic *Alternaria* is provided by reports on the isolation of *Alternaria* strains producing tenuazonic acid, alternariol, alternariol methyl ether, altenuene, and altertoxin I from cereal grains in some Mediterranean countries (38) and from cereals, potatoes, tomatoes, and rapeseed in Poland (39).

Recent work on analytical methods for *Alternaria* mycotoxins was limited to fungal cultures. A simple procedure was developed for purification of tenuazonic acid from *Alternaria alternata* isolated from *Beta vulgaris* and for quantitation of the toxin based on its chelation of Fe^{3+} (40). A reversed-phase LC system for the detection of tenuazonic and related tetramic acids, including *allo*-tenuazonic acid, in fungal cultures was developed using a column packed with deactivated, end-capped C_{18} silica with a high carbon load (41). Reversed-phase LC of the *Alternaria* pigments alterporriols D and E (42) and the phytotoxin tentoxin (43) was also reported.

Some interest was shown by mycotoxicologists toward AAL toxin, a phytotoxin produced by *Alternaria alternata* f. sp. *lycopersici*, because of its structural similarity to the fumonisins. Although no toxic effects were observed in rats (44), AAL toxin was cytotoxic (44, 45). Mutagenicity of another *Alternaria* toxin, stemphyltoxin III, was reported (46). *Alternaria alternata* and, more specifically, altemariol methyl ether were proposed to cause human esophageal cancer (47). Continued study on methods for altemariol methyl ether and related compounds is recommended.

Citrinin.—Associate Referee David M. Wilson (University of Georgia, Tifton, GA) reported no method development during the last year. Experiments on thermal decomposition and detoxification of citrinin under various moisture conditions were published (48, 49).

Cyclopiazonic Acids.—Associate Referee Joe W. Dorner (U.S. Department of Agriculture (USDA), Dawson, GA) noted continued research on the toxicity, mode of action, and natural occurrence of α-cyclopiazonic acid (50–53). Of particular toxicological interest is a report stating that concentrations as low as 100 ng/g in the diet of channel catfish resulted in a significant decrease in growth of the fish (50). Cyclopiazonic acid was found at a level of 10 μg/g in sunflower seed screenings associated with conception problems and feed refusal in sows (52) and at concentrations of 25–250 ng/g in 4 of 7 truckloads of corn (53). In methodology, an ELISA for cyclopiazonic acid that measured 30 pg to 2 ng of the mycotoxin was applied to the analysis of cultures of various fungi (54); it remains to be seen whether the method can be applied to the analysis of commodities such as corn, peanuts, and feed. The LC method referred to last year was published (55). Recoveries of cyclopiazonic acid from corn and peanuts were 72–84%.

Ergot Alkaloids.—Associate Referee George M. Ware (FDA, New Orleans, LA) reported that one additional laboratory was sent samples to allow completion of the collaborative study on a method for ergot alkaloids in wheat and rye. He noted that a survey was conducted over a period of 6 years to determine the prevalence and concentrations of ergot alkaloids in the Canadian food supply (56). The following pharmacologically active alkaloids were determined by LC with fluorescence detection in over 400 samples of grain foods: ergonovine, ergotamine, ergocornine, α-ergokryptine, and ergocristine. Rye flour was the most contaminated food, with a mean total ergot alkaloid concentration of 220 ng/g. Wheat flour total alkaloid concentrations were much lower (mean = 23 ng/g) than those of rye flour. The

predominant alkaloids found were ergotamine and ergocristine.

Several papers pertaining to methodology for ergot alkaloids were published during the last year. The use of cyclodextrins for separation of ergot alkaloids by capillary zone electrophoresis was reported (57). Good resolution of racemic ergot alkaloids was achieved using a background electrolyte containing either β-cyclodextrin or γ-cyclodextrin. A gas chromatographic (GC)/mass spectrometric (MS) method for the quantitation of ergotamine in human plasma was developed (58). A sensitive ELISA for clavine alkaloids, ergopeptide alkaloids, and simple lysergic acid derivatives was used to identify endophyte-infected tall fescue (59). A spectrophotometric method was reported for determining 6 pharmacologically active ergot alkaloids in pharmaceutical preparations (60). Ergot alkaloids reacted with ninhydrin in sulfuric acid medium containing traces of iron(III) ions to produce a stable red product that had a maximum absorbance at 494 nm.

Fumonisins.—Associate Referee Ronald D. Plattner (USDA, Peoria, IL) reported that, in the past year, research effort devoted to fumonisins increased rapidly. Awareness of the presence of fumonisins, particularly in corn, and of the potential for these mycotoxins to cause severe animal losses increased. A special issue of *Mycopathologia* (**117**[1], 1992) contains collected research papers on fumonisins presented at the 2-day conference in Raleigh, NC (April 24–25, 1991). In methods of analysis and confirmation of fumonisins, much work is being done.

Thermospray, electrospray, fast-atom bombardment (FAB)/MS, and FAB/MS/MS were reported for fumonisins, and FAB/MS/MS was suggested as a useful screening procedure to detect the presence of fumonisins in food and crop extracts (61). The Associate Referee is aware of work in several laboratories using FAB/MS and MS/MS for the confirmation of fumonisins at sub μg/g levels. The production of deuterium-labeled fumonisin B_1 was reported (62). A FAB/MS method for quantitating fumonisins in corn extracts was developed in the Associate Referee's laboratory. The method uses labeled fumonisin B_1 as an internal standard. Full details of this method were reported at the 1992 meeting of AOAC International.

Production of polyclonal (63) and monoclonal (64) antibodies to fumonisin B_1 was reported. These antibodies cross react with fumonisins B_2 and B_3 but not with hydrolyzed fumonisins. One manufacturer is offering an antibody affinity column to test for fumonisins. This affinity column is being evaluated by the Associate Referee to determine its suitability as a cleanup step for analysis of fumonisins in corn and corn products.

It appears that fumonisin levels in suitably cleaned-up extracts of corn can be reliably measured by fluorescence LC with derivatives formed by *o*-phthaldialdehyde, fluorescamine, naphthalene-2,3-dicarboxaldehyde, and 4-fluoro-7-nitrobenzofurazan. Use of the latter reagent was reported at the 1991 Annual Meeting of AOAC International (65). The method using *o*-phthaldialdehyde was recently optimized and includes determination of fumonisin B_3 as well as fumonisins B_1 and B_2 (66); this reagent was also used in analysis of plasma and urine for fumonisin B_1 (67).

The Associate Referee believes that the most critical parts of all reported methods are extraction and cleanup. He recommends continued evaluation of published methods with particular emphasis on these steps before beginning a formal AOAC collaborative study.

Immunochemical Methods.—Associate Referee James J. Pestka (Michigan State University, East Lansing, MI) reported that 2 new approaches for generating deoxynivalenol antibodies and implementing these in ELISA were published (68, 69). These new assays enable detection of deoxynivalenol at the nanogram/milliliter level. Methods for generating polyclonal and monoclonal antibodies to the fumonisins using cholera toxin as a carrier protein were also described (63, 64) (see *Fumonisins*). The limit of detection of fumonisin B_1 in ELISA using these antibodies is 50–100 ng/mL. An ELISA for cyclopiazonic acid was reported (54) (see *Cyclopiazonic Acids*). John et al. (70) described an approach for preparing tenuazonic acid proteins for antibody production. New approaches for ochratoxin A immunoaffinity column cleanup and immunoassay were also reported (71, 72). Details for immunochromatography of fusarochromanone mycotoxins were provided by Yu and Chu (73).

A number of comparative studies were performed on immunochemical detection of aflatoxins, including ELISA (19–21, 26, 74–76) (see *Aflatoxin Methods*) and studies on immunoaffinity column protocols (3, 30). There was extensive investigation on the detection of aflatoxin adducts in clinical samples (77–80).

Schneider et al. (81) described the development of test strip enzyme immunoassays (EIA) for the rapid detection of aflatoxin B_1, aflatoxin M_1, ochratoxin A, T-2 toxin, diacetoxyscirpenol, 3-acetyldeoxynivalenol, roridin A, and zearalenone using an antibody-coated nylon membrane. Using a corresponding toxin–horseradish peroxidase conjugate in a direct competitive assay, dot color development of toxin-positive test strips was visually discernible from that of the negative control. The results of the visual evaluation were compared with that of instrumental reflectance measurements of the test strips. The visual detection limits of the test strip assays for these mycotoxins were 0.6, 0.2, 2, 1, 0.2, 10, 15, and 5 ng/mL in buffer. By using a simple extraction procedure, aflatoxin B_1, ochratoxin A, T-2 toxin, and zearalenone in spiked corn samples were detected by test strip EIA at levels of 15, 100, 20, and 80 ng/g, respectively.

Ochratoxins.—Associate Referee Stanley Nesheim (FDA, Washington, DC) noted that, although the new official first action LC method **991.44** for ochratoxin A (82) was not adopted for analysis of pig kidney, other methods are available. One of these appears to be very simple and affords excellent recovery of 93–106% in the 1–10 ng/g range (83). This method was developed by the authors after they investigated 4 other published LC fluorometric methods and found them to suffer from interferences or poor recoveries. The method also performed well in a second laboratory. The Associate Referee proposes to conduct a collaborative study of this method applied to pig liver and kidney. Several other variations of LC methods have appeared recently. The method of Guerrero et al. (84) was applied

to 100 different grains and processed products. It mainly addresses the application of 2 extraction methods to different materials. Takeda et al. (85) isolated ochratoxin A from pig serum for LC determination using C_{18} Sep-Pak. A fast LC method for determination of ochratoxin A in serum gave better recoveries (87–94%) than the enzymic spectrophotometric method (62–67%) at concentrations of 5–50 ng/mL serum (86). Another method used ion pairing and alkaline pH to achieve enhanced fluorescence response for ochratoxin A in plasma (87). An LC method for analysis of buffered rumen fluid was also published (88).

GC of ochratoxin A after methylation with diazomethane was reported for the first time (89). Identification and quantitation were by GC/negative chemical ionization MS, and the limit of detection was 0.1 ng/g in foods. A radioimmunoassay kit capable of determining ochratoxin A in pig serum (detection limit, 0.1 ng/mL) and pig tissue (detection limit, 1 ng/g) was applied to a survey of pigs in Czechoslovakia (90). Methods for determining ochratoxin A were reviewed by van Egmond (91) in a chapter of a book that contains many papers on ochratoxin A that cover its toxicology, risk assessment, analysis, natural occurrence, and worldwide regulation (92). Natural occurrence of ochratoxin A continues to be reported, particularly in pig products and in human blood. Holmberg et al. (93) correlated the ochratoxin A content of swine blood and moisture of barley at harvest for different years. Holmberg et al. (94) found that *Penicillium verrucosum* was more common in the feed of ochratoxin A positive herds than in that of negative herds and reported that 38% of the 63 *P. verrucosum* isolates produced ochratoxin A. Ochratoxin A was also detected in dried figs (95).

More countries continue to join Denmark in regulating ochratoxin A contamination (96). For example, Czechoslovakia set a tolerance of 5 ng/g for children's food and 20 ng/g for adults (90). The 37th report (1990) of the Joint FAO/WHO Expert Committee on Food Additives proposed a provisional tolerable daily intake (TDI) of 16 ng/kg body weight (97). The Nordic Working Group on Food Toxicology and Risk Evaluation concluded that, based on its carcinogenicity, the highest TDI of ochratoxin A is 5 ng/g body weight (98). These figures compare with the 0.2–4.2 ng/kg body weight TDI calculated from the carcinogenicity data by Kuiper-Goodman and Scott (99). Daily intake of ochratoxin A in Germany, calculated from random analysis of food samples (cereals other than corn, cereal products, and sausages) and consumption data, was estimated to be 1 ng/kg body weight, with a further 2 ng/kg body weight for people who eat corn products (100). The IUPAC Commission V1.1 report "Ochratoxin A: a Review" was also published (101).

The Associate Referee recommends collaborative study of the new LC method for ochratoxin A in pig kidney and liver.

Trichothecenes.—Associate Referee Robert M. Eppley (FDA, Washington, DC) reported that the number of papers relating to the chemistry and analysis of trichothecenes decreased over the last 2 years. There is some interest in immunoassay procedures (see *Immunochemical Methods*). A fluorescent hydrazone derivative for trichothecene detection was investigated (102). The test is based on the rapid formation of a molecular association complex rather than a chemical reaction. Detection limits ranged from 50 ng to 4 μg in spot tests on cellulose. A procedure involving derivatization with coumarin-3-carbonyl chloride for determination of T–2 toxin and its hydrolysis products by LC with fluorescence detection had minimum detectable amounts of 0.8–2 ng (103). In other work on LC of trichothecenes, retention data for deoxynivalenol, nivalenol, 3- and 15-acetyldeoxynivalenol, and 7-deoxydeoxynivalenol were reported (104). Eight fatty acid esters and 2 glucosides of deoxynivalenol were synthesized and analyzed by reversed-phase LC; the 3- and 15-glucosides could not be separated (105). A search for these compounds in wheat was reported to be underway. The advantages of LC/frit-FAB high-resolution MS for identification and monitoring trichothecenes without derivatization and a comparison of plasmaspray, thermospray, and FAB interfacing techniques were presented (106, 107). A GC/ion trap MS method for determination of 6 trichothecenes in cereals was also described (108). There were a few more surveys of natural occurrence of trichothecenes (109–112) and of *Fusarium* species for characterization of types of trichothecene produced (113, 114).

The Associate Referee recommends that (*1*) studies be continued on the development of methods for the determination of trichothecenes in grains and foods and (*2*) two methods be considered for collaborative study, one capable of quantitatively determining 5–6 of the more frequently occurring trichothecenes and the other a quantitative ELISA for deoxynivalenol. An editorial correction needs to be made to **986.18C**: the extraction solvent is $CHCl_3$–EtOH (8 + 2).

Zearalenone.—Associate Referee Glenn A. Bennett (USDA, Peoria, IL) noted several publications during the past year on zearalenone and related metabolites. Stereoselective reduction of zearalenone and zearalanone by baker's yeast to give different ratios of the respective α/β alcohols was ascribed to different conformations of the macrocyclic ring in aqueous solution (115). Conformations of zearalenone, α-zearalenol, and β-zearalenol in acetonitrile solution and of zearalenone and zearalanone in deuterated benzene–acetone solution were determined from circular dichroism and nuclear magnetic resonance spectra, respectively (116, 117).

In an assessment of zearalenone exposure in the Hungarian population based on analysis of wheat and its products, daily intake did not exceed 0.2 μg/kg body weight (118). In 1.7% of the samples, >40 ng/g zearalenone was detected. Zearalenone was also detected in rice and soybeans in Korea (119) and in moldy amaranth grain in Argentina (120). An epidemiological study in Germany on sickness in swine concluded that zearalenone contamination in the feed was considered by the authors to be hazardous at levels of 20–50 ng/g, and the recommended limit in rations should be 10 ng/g (121). Hydrated sodium calcium aluminosilicate reduced some of the toxicity of dietary zearalenone to mink (122). Physical methods (sieving and dehulling) can reduce zearalenone concentrations in grains (123), and it was also shown that zearalenone production in high-moisture corn (27%) is inhibited in 20–60% carbon dioxide atmospheres (124).

A test strip immunoassay developed for several mycotoxins (see *Immunochemical Methods*) was able to detect 80 ng/g of

zearalenone in spiked corn (81). An important development in LC of zearalenone is the use of postcolumn reaction with aluminum chloride to give a 5-fold increase in fluorescence response without significantly affecting the level of background interference from cereal and feed coextractives (125). A GC method for determination of zearalenone, α-zearalenol, and β-zearalenol in cereals using an ion-trap mass-selective detector was also described (126). The limit of detection was about 1 ng/g for all 3 mycotoxins, determined as their trimethylsilyl derivatives. GC of zearalenone as its heptafluorobutyrate derivative was used in a method for grain analysis (127).

The Associate Referee recommends continued study on rapid screening methods for zearalenone and on methods for bound or "unextractable" zearalenone.

Recommendations

(1) Assign surplus status to the official final action method **974.17** for determination of aflatoxin M_1 in dairy products.
(2) Adopt as official first action the modified Immuno-Dot Cup (Signal Accucup) ELISA for screening aflatoxins B_1, B_2, and G_1 at ≥20 ng/g in corn.
(3) Make an editorial change to **986:18**; the extraction solvent in **986.18C** should be $CHCl_3$–EtOH (8 + 2).
(4) Continue study on all topics.

References

(1) Tuinstra, L.G.M.T., Roos, A.H. & van Trijp, J.M.P. (1992) *DLO-State Institute for Quality Control of Agricultural Products Report 92.14*, Wageningen, The Netherlands
(2) Farjam, A., de Vries, R., Lingeman, H., & Brinkman, U.A.T. (1991) *Int. J. Environ. Anal. Chem.* **44**, 175–184
(3) Farjam, A., van de Merbel, N.C., Nieman, A.A., Lingeman, H., & Brinkman, U.A.T. (1992) *J. Chromatogr.* **589**, 141–149
(4) Friesen, M. (1992) *Preliminary Report on the Statistical Analysis of Results Obtained for the Analysis of Aflatoxin M_1 in 91-3 Lyophilized Milk, Carried Out in 1991*, International Agency for Research on Cancer, Lyon, France
(5) Stubblefield, R.D., Honstead, J.P., & Shotwell, O.L. (1991) *J. Assoc. Off. Anal. Chem.* **74**, 897–899
(6) Harvey, R.B., Phillips, T.D., Ellis, J.A., Kubena, L.F., Huff, W.E., & Peterson, H.D. (1991) *Amer. J. Vet. Res.* **52**, 1556–1559
(7) Veldman, A., Meijs, J.A.C., Borggreve, G.J., & Heeres-van der Tol, J.J. (1992) *Anim. Product.* **55**, 163–168
(8) Stoloff, L., van Egmond, H.P., & Park, D.L. (1991) *Food Addit. Contam.* **8**, 213–221
(9) *Draft Report Codex Committee on Food Additives and Contaminants*, The Hague, The Netherlands, 23–28 March, 1992
(10) Scott, P.M. (1991) in *Analysis of Oilseeds, Fats and Fatty Foods*, J.B. Rossell, & J.L.R. Pritchard (Eds), Elsevier Applied Science, London, England, pp. 141–184
(11) Ellis, W.O., Smith, J.P., Simpson, B.K., & Oldham, J.H. (1991) *Crit. Rev. Food Sci. Nutr.* **30**, 403–439
(12) Roch, O.G., Blunden, G., Coker, R.D., & Nawaz, S. (1992) *Chromatographia* **33**, 208–212
(13) Whitaker, T.B., Dickens, J.W., & Slate, A.B. (1990) *Peanut Sci.* **17**, 96–100
(14) Nawaz, S., Coker, R.D., & Haswell, S.J. (1992) *Analyst* **117**, 67–74
(15) Wilson, T.J., & Romer, T.R. (1991) *J. Assoc. Off. Anal. Chem.* **74**, 951–956
(16) Gilbert, J., Sharman, M., Wood, G.M., Boenke, A., & Wagstaffe, P.J. (1991) *Food Addit. Contam.* **8**, 305–320
(17) van Egmond, H.P., Heisterkamp, S.H., & Paulsch, W.E. (1991) *Food Addit. Contam.* **8**, 17–29
(18) Council for Agricultural Science and Technology (CAST) (1989) *Mycotoxins: Economic and Health Risks. Task Force Report No. 116*, p. 61
(19) Beaver, R.W., James, M.A., & Lin, T.Y. (1991) *J. Assoc. Off. Anal. Chem.* **74**, 827–829
(20) Ward, C.M., & Morgan, M.R.A. (1991) *Food Addit. Contam.* **8**, 9–15
(21) Azer, M., & Cooper, C. (1991) *J. Food Prot.* **54**, 291–294
(22) Trucksess, M.W., & Page, S.W. (1990) in *Biodeterioration Research 3*, G.C. Llewellyn & C.E. O'Rear (Eds), Plenum Press, New York, N.Y. pp. 161–173
(23) Koeltzow, D.E., & Tanner, S.N. (1990) *J. Assoc. Off. Anal. Chem.* **73**, 584–589
(24) Trucksess, M.W., & Stack, M.E. (1992) *Program & Abstracts 106th AOAC Int. Ann. Meet.* August 31–September 3, Cincinnati, OH, p. 81
(25) *The Referee* (1991) AOAC International, Arlington, VA, **15**(9), pp. 1,10
(26) Figueira, A.C., Taylor, K.D.A., Barlow, P.J., & Morgan, M R.A. (1991) *Food Agric. Immunol.* **3**, 13–19
(27) Hurst, W.J., Martin, R.A., Jr, & Vestal, C.H. (1991) *J. Liq. Chromatogr.* **14**, 2541–2550
(28) Holcomb, M., Korfmacher, W.A., & Thompson, H.C., Jr (1991) *J. Anal. Toxicol.* **15**, 289–292
(29) Candlish, A.A.G., Haynes, C.A., & Stimson, W.H. (1988) *Int. J. Food Sci. Technol.* **23**, 479–485
(30) Sharman, M., & Gilbert, J. (1991) *J. Chromatogr.* **543**, 220–225
(31) Chamkasem, N., Cobb, W.Y., Latimer, G.W., Salinas, C., & Clement, B.A. (1989) *J. Assoc. Off. Anal. Chem.* **72**, 336–341
(32) Urano, T., Trucksess, M.W., & Page, S.W. (1991) *Program & Abstract 105th AOAC Ann. Int. Meet.* August 12–14, Phoenix, AZ, p. 139
(33) Trucksess, M.W., & Richard, J.L. (1992) in *Natural Toxins: Toxicology, Chemistry and Safety*, R.F. Keeler, N.B. Mandava, & A.T. Tu (Eds), Alaken, Inc., Fort Collins, CO, pp. 337–345
(34) Sharman, M., Patey, A.L., Bloomfield, D.A., & Gilbert, J. (1991) *Food Addit. Contam.* **8**, 299–304
(35) *Alternaria: Biology, Plant Diseases and Metabolites* (1992) J. Chelkowski & A. Visconti (Eds), Elsevier Science Publishers B.V., Amsterdam, The Netherlands
(36) Montemurro, N., & Visconti, A. (1992) in *Alternaria: Biology, Plant Diseases and Metabolites*, J. Chelkowski & A. Visconti (Eds), Elsevier Science Publishers B.V., Amsterdam, The Netherlands, pp. 449–557
(37) Chelkowski, J., & Grabarkiewicz-Szczsna, J. (1992) in *Cereal Grain. Mycotoxins, Fungi and Quality in Drying and*

Storage, J. Chelkowski (Ed.), Elsevier Science Publishers B.V., Amsterdam, The Netherlands, pp. 67–76

(38) Logrieco, A., Bottalico, A., Solfrizzo. M., & Mule, G. (1990) *Mycologia* **82**, 501–505

(39) Zajkowski, P., Grabarkiewicz-Szczsna, J., & Schmidt, R. (1991) *Mycotoxin Res.* **7**, 11–15

(40) Robeson, D.J., & Jalal, M.A.F. (1991) *J. Inorg. Biochem.* **44**, 109–116

(41) Shephard, G.S., Thiel, P.G., Sydenham, E.W., Vleggaar, R., & Marasas, W.F.O. (1991) *J. Chromatogr.* **566**, 195–205

(42) Suemitsu, R., Horiuchi, K., Ohnishi, K., & Kubota, M. (1990) *J. Chromatogr.* **503**, 282–287

(43) Suemitsu, R., Horiuchi, K., Ohnishi, K., Hidaka, T., & Horiuchi, M. (1991) *J. Chromatogr.* **539**, 211–214

(44) Mirocha, C.J., Gilchrist, D.G., Shier, W.T., Abbas, H.K., Wen, Y., & Vesonder, R.F. (1992) *Mycopathologia* **117**, 47–56

(45) Shier, W.T., Abbas, H.K., & Mirocha, C.J. (1991) *Mycopathologia* **116**, 97–104

(46) Davis, V.M., & Stack, M.E. (1991) *Appl. Environ. Microbiol.* **57**, 180–182

(47) Liu, G.T., Qian, Y.Z., Zhang, P., Dong, Z.M., Shi, Z.Y., Zhen, Y.Z., Miao, J., & Xu, Y.M. (1991) in *Relevance to Human Cancer of N-Nitroso Compounds, Tobacco Smoke and Mycotoxins*, I.K. O'Neill, J. Chen, & H. Bartsch (Eds), International Agency for Research on Cancer, Lyon, France, pp. 258–262

(48) Kitabatake, N., Trivedi, A.B., & Doi, E. (1991) *J. Agric. Food Chem.* **39**, 2240–2244

(49) Trivede, A.B., Doi, E., & Kitabatake, N. (1992) *Biosci. Biotechnol. Biochem.* **56**, 423–426

(50) Jantrarotai, W., & Lovel, R.T. (1990) *J. Aquatic Anim. Health* **2**, 255–260

(51) Riley, R.T., & Showker, J.L. (1991) *Toxicol. Appl. Pharmacol.* **109**, 108–126

(52) Ross, P.F., Rice, L.G., Casper, H.H., Crenshaw, J.D., & Richard, J.L. (1991) *Vet. Hum. Toxicol.* **33**, 284–285

(53) Lee, Y.J., & Hagler, W.M., Jr (1991) *J. Food Sci.* **56**, 871–872

(54) Hahnau, S., & Weiler, E.W. (1991) *J. Agric. Food Chem.* **39**, 1887–1891

(55) Urano, T., Trucksess, M.W., Matusik, J., & Dorner, J.W. (1992) *J. AOAC Int.* **75**, 319–322

(56) Scott, P.M., Lombaert, G.A., Pellaers, P., Bacler, S., & Lappi, J. (1992) *J. AOAC Int.* **75**, 773–779

(57) Fanali, S., Flieger, M., Steinerova, N., & Nardi, A. (1992) *Electrophoresis* **13**, 39–43

(58) Feng, N., Minder, E.I., Grampp, T., & Vonderschmitt, D.J. (1992) *J. Chromatogr. Biomed. Appl.* **575**, 289–294

(59) Shelby, R.A., & Kelley, V.C. (1991) *Food Agric. Immunol.* **3**, 169–177

(60) Zakhari, N.A., Hassan, S.M., & El-Shabrawy, Y. (1991) *Acta Pharm. Nord.* **3**, 151–154

(61) Korfmacher, W.A., Chiarelli, M.P., Lay, J.O., Jr, Bloom, J., Holcomb, M., & McManus, K.T. (1991) *Rapid Commun. Mass Spectrom.* **5**, 463–468

(62) Plattner, R.D., & Shackelford, D.D. (1992) *Mycopathologia* **117**, 17–22

(63) Azcona-Olivera, J.I., Abouzied, M.M., Plattner, R.D., Norred, W.P., & Pestka, J.J. (1992) *Appl. Environ. Microbiol.* **58**, 169–173

(64) Azcona-Olivera, J.I., Abouzied, M.M., Plattner, R.D., & Pestka, J.J. (1992) *J. Agric. Food Chem.* **40**, 531–534

(65) Scott, P.M., & Lawrence, G.A. (1992) *J. AOAC Int.*, **75**, 829–934

(66) Sydenham, E.W., Shephard, G.S., & Thiel, P.G. (1992) *J. AOAC Int.* **75**, 313–318

(67) Shephard, G.S., Thiel, P.G., & Sydenham, E.W. (1992) *J. Chromatogr.* **574**, 299–304

(68) Usleber, E., Märtlbauer, E., Dietrich, R., & Terplan, G. (1991) *J. Agric. Food Chem.* **39**, 2091–2095

(69) Mills, E.N.C., Alcock, S.M., Lee, H.A., & Morgan, M.R.A. (1990) *Food Agric. Immunol.* **2**, 109–118

(70) John, A.E., Coker, R.D., Blunden, G., Rogers, D.J., & Crabb, T.A. (1991) *Food Agric. Immunol.* **3**, 179–184

(71) Nakajima, M., Terada, H., Hisada, K., Tsubouchi, H., Yamamoto, K., Uda, T., Itoh, Y., Kawamura, O., & Ueno, Y. (1990) *Food Agric. Immunol.* **2**, 189–195

(72) Kawamura, O., Sato, S., Nagura, M., Kishimoto, S., Ueno, I., Sato, S., Uda, T., Ito, Y., & Ueno, Y. (1990) *Food Agric. Immunol.* **2**, 135–143

(73) Yu, J., & Chu, F.S. (1991) *J. Assoc. Off. Anal. Chem.* **74**, 655–660

(74) Hongyo, K.-I., Itoh, Y., Hifumi, E., Takeyasu, A., & Uda, T. (1992) *J. AOAC Int.* **75**, 307–312

(75) Goto, T. (1990) *Rep. Nat. Food Res. Inst.* No. 54, pp. 35–43

(76) Adachi, Y., Hara, M., Kumazawa, N.H., Hirano, K., Ueno, I., & Egawa, K. (1991) *J. Vet. Med. Sci.* **53**, 49–52

(77) Groopman, J.D., Hasler, J.A., Trudel, L.J., Pikul, A., Donahue, P.R., & Wogan, G.N. (1992) *Cancer Res.* **52**, 267–274

(78) Groopman, J.D., Zhu, J., Donahue, P.R., Pikul, A., Zhang, L., Chen, J.-S., & Wogan, G.N. (1992) *Cancer Res.* **52**, 45–52

(79) Olubuyide, I.O., Makarananda, K., Judah, D.J., & Neal, G.E. (1991) *Int. J. Cancer* **48**, 468–472

(80) Harrison, J.C., & Garner, R.C. (1991) *Carcinogenesis* **12**, 741–744

(81) Schneider, E., Dietrich, R., Märtlbauer, E., Usleber, E., & Terplan, G. (1991) *Food Agric. Immunol.* **3**, 185–193

(82) Nesheim, S., Stack, M.E., Trucksess, M.W., Eppley, R.M., & Krogh, P. (1992) *J. AOAC Int.* **75**, 481–487

(83) Scott, P.M., Kanhere, S.R., Canela, R., Lombaert, G.A., & Bacler, S. (1991) *Prehrambeno-Tehnol. Biotehnol. Rev.* **29**, 61–64

(84) Guerrero, H.G., Bagneris, R.W., Shih, B.Y., Jordan, V.A., & Carter, L., Jr (1991) Personal communication

(85) Takeda, N., Akiyama, Y., & Shibasaki, S. (1991) *Bull. Environ. Contam. Toxicol.* **47**, 198–203

(86) Beker, D. & Radic, B. (1991) *J. Chromatogr.* **570**, 441–445

(87) Breitholtz, A., Olsen, M., Dahlbäck, Å, & Hult, K. (1991) *Food Addit. Contam.* **8**, 183–192

(88) Lerch, C.M., & Müller, H.-M. (1990) *Chromatographia* **30**, 424–427

(89) Jiao, Y., Blaas, W., Rühl, C., & Weber, R. (1992) *J. Chromatogr.* **595**, 364–367

(90) Fukal, L. (1991) *Dtsche. Lebensmittel-Rundsch.* **87**, 316–319

(91) van Egmond, H.P. (1991) in *Mycotoxins, Endemic Nephropathy and Urinary Tract Tumours*, M. Castegnaro, R. Pleština, G. Dirheimer, I.N. Chernozemsky, & H. Bartsch

(Eds), International Agency for Research on Cancer, Lyon, France, pp. 57–70

(92) *Mycotoxins, Endemic Nephropathy and Urinary Tract Tumours* (1991) M. Castegnaro, R. Pleština, G. Dirheimer, I.N. Chernozemsky, & H. Bartsch (Eds), International Agency for Research on Cancer, Lyon, France

(93) Holmberg, T., Breitholtz, A., Bengtsson, A., & Hult, K. (1990) *Acta Agric. Scand.* **40**, 201–204

(94) Holmberg, T., Breitholtz-Emanuelsson, A., Häggblom, P., Schwan, O., & Hult, K. (1991) *Mycopathologia* **116**, 169–176

(95) Özay, G., & Alperden, I. (1991) *Mycotoxin Res.* **7**, 85–91

(96) van Egmond, H.P. (1991) in *Mycotoxins, Endemic Nephropathy and Urinary Tract Tumours*, M. Castegnaro, R. Pleština, G. Dirheimer, I.N. Chernozemsky, & H. Bartsch (Eds), International Agency for Research on Cancer, Lyon, France, pp. 331–336

(97) Herrman, J.L. (1991) in *Mycotoxins, Endemic Nephropathy and Urinary Tract Tumours*, M. Castegnaro, R. Pleština, G. Dirheimer, I.N. Chernozemsky, & H. Bartsch (Eds), International Agency for Research on Cancer, Lyon, France, pp. 327–329

(98) The Nordic Working Group on Food Toxicology and Risk Evaluation (1991) *Health Evaluation of Ochratoxin A in Food Products*, Nordic Council of Ministers, Copenhagen, Denmark

(99) Kuiper-Goodman, T., & Scott, P.M. (1989) *Biomed. Environ. Sci.* **2**, 179–248

(100) Frank, H.K. (1991) in *Mycotoxins, Endemic Nephropathy and Urinary Tract Tumours*, M. Castegnaro, R. Pleština, G. Dirheimer, I.N. Chernozemsky, & H. Bartsch (Eds), International Agency for Research on Cancer, Lyon, France, pp. 77–81

(101) Pohland, A.E., Nesheim, S., & Friedman, L. (1992) *Pure Appl. Chem.* **64**, 1029–1046

(102) Novak, T.J., & Quinn-Doggett, K. (1991) *Anal. Lett.* **24**, 913–924

(103) Cohen, H., & Boutin-Muma, B. (1992) *J. Chromatogr.* **595**, 143–148

(104) Lanin, S.N., & Nikitin, Y.S. (1991) *J. Chromatogr.* **558**, 81–88

(105) Savard, M.E. (1991) *J. Agric. Food Chem.* **39**, 570–574

(106) Kostiainen, R., Matsuura, K., & Nojima, K. (1991) *J. Chromatogr.* **538**, 323–30

(107) Kostiainen, R. (1991) *J. Chromatogr.* **562**, 555–562

(108) Schwadorf, K., & Müller, H.M. (1991) *Chromatographia* **32**, 137–142

(109) Hietaniemi, V., & Kumpulainen, J. (1991) *Food Addit. Contam.* **8**, 171–172

(110) Park, J.-C., Zong, M.S., & Chang, I.-M. (1991) *Food Addit. Contam.* **8**, 447–451

(111) Lauren, D.R., Agnew, M.P., Smith, W.A., & Sayer, S.T. (1991) *Food Addit. Contam.* **8**, 599–605

(112) Russell, L., Cox, D.F., Larsen, G., Bodwell, K., & Nelson, C.E. (1991) *J. Anim. Sci.* **69**, 5–12

(113) Greenhalgh, R., Miller, J.D., & Visconti, A. (1991) *J. Agric. Food Chem.* **39**, 809–812

(114) Miller, J.D., Greenhalgh, R., Wang, Y., & Lu, M. (1991) *Mycologia* **83**, 121–130

(115) Šunjic, V., Gelo, M., Rusman, S., DiGrazia, L., & Clauti, G. (1991) *Enzyme Microbiol. Technol.* **13**, 344–348

(116) Gelo, M., Raza, Z., Šunjic, V., Guo, J., & Snatzke, G. (1991) *Tetrahedron: Asymmetry* **2**, 1005–1010

(117) Cordier, C., Gruselle, M., Jaouen, G., Hughes, D.W., & McGlinchey, M.J. (1990) *Magn. Reson. Chem.* **28**, 835–845

(118) Koudela, S., Soós, K., Sohár, J., & Biró, G. (1990) *Acta Aliment.* **19**, 229–235

(119) Lee, Y.-W., Kim, J.-G., Chung, D.-H., Roh, P.-U., & Pestka, J.J. (1991) *Mycotoxin Res.* **7**, 69–72

(120) Bresler, G., Vaamonde, G., & Brizzio, S., (1991) *Int. J. Food Microbiol.* **13**, 75–80

(121) Ewald, C., Rehm, A., & Haupt, C. (1991) *Berl. Münch. Tierarztl. Wochenschr.* **104**, 161–166

(122) Bursian, S.J., Aulerich, R.J., Cameron, J.K., Ames, N.K., & Steficek, B.A. (1992) *J. Appl. Toxicol.* **12**, 85–90

(123) Trenholm, H.L., Charmley, L.L., Prelusky, D.B., & Warner, R.M. (1991) *J. Agric. Food Chem.* **39**, 356–360

(124) Paster, N., Blumenthal-Yonassi, J., Barkai-Golan, R., & Menasherov., M. (1991) *Int. J. Food Microbiol.* **12**, 157–166

(125) Hetmanski, M.T., & Scudamore, K.A. (1991) *J. Chromatogr.* **588**, 47–52

(126) Schwadorf, K., & Müller, H.-M. (1992) *J. Chromatogr.* **595**, 259–267

(127) Luo, Y., Zheng, J., & Yang, J. (1991) *Environ. Chem.* **10**, 59–63

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Foods I. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Plant Toxins

SAMUEL W. PAGE
U.S. Food and Drug Administration, Natural Products and Instrumentation Branch, Washington, DC 20204

Little progress in the collaborative study of analytical methods for plant toxins was made during the past year. The major limiting factor continues to be the lack of sufficient reference standards to carry out collaborative studies. Attempts by the General Referee's laboratory to obtain some of these materials under contract were not successful. Other approaches to obtain these reference materials are being explored.

Glucosinolates.—(D. Ian McGregor, Agriculture Canada Research Station, Saskatoon, SK). Several available methods for glucosinolates are under consideration for collaborative study.

Hydrazines.—(Joseph M. Betz, Natural Products and Instrumentation Branch, FDA, Washington, DC). The initiation of the collaborative study of an LC method for agaritine in *Agaricus bisporus* (1) awaits the completion of the synthesis of sufficient agaritine for use as reference standards.

Hypoglycin in Ackee Fruit.—(G. William Chase, Center for Nutrient Analysis, FDA, Atlanta, GA). The final interlaboratory studies and the collaborative study of a method (2) for hypoglycin A in canned ackee fruit cannot be completed until additional quantities of the hypoglycins are obtained.

Phytoestrogens.—(Shia S. Kuan, Natural Toxins Research Center, FDA, New Orleans, LA). An improved method for phytoestrogens in soy products is undergoing interlaboratory evaluation. A collaborative study of this method will be initiated following successful completion of these studies.

Pyrrolizidine Alkaloids.—(Robert M. Eppley, Natural Products and Instrumentation Branch, FDA, Washington, DC). A revised procedure for the determination of pyrrolizidine alkaloids in comfrey is undergoing ruggedness testing. This procedure is being used in a survey of commercial comfrey products. A collaborative study of this method is being planned.

Steroidal Alkaloids.—(Allen S. Carman, Natural Toxins Research Center, FDA, New Orleans, LA). The revised protocol for the collaborative study of a method (3) for α-solanine and α-chaconine in *Solanum tuberosum* was approved. As soon as additional quantities of potatoes with the required levels of these alkaloids are prepared, the collaborative study will be completed.

Recommendations

(1) Initiate collaborative study of LC method for phytoestrogens in soy-based infant formulas. Continue study in other areas.
(2) Initiate collaborative study of GC method for pyrrolizidine alkaloids in comfrey. Continue study in other areas.
(3) Complete collaborative study of LC method for solanine and chaconine in potatoes. Continue study in other areas.
(4) Continue study in all other Associate Refereeship areas.

References

(1) Stijve, T., Fumeaux, R., Philippossian, G. (1986) *Dtsch. Lebensm. Rundsch.* **82**, 243–248
(2) Chase, G.W., Jr, Landon, W.O., Jr, Gelbaum, L.T., & Soliman, A.-G.M. (1989) *J. Assoc. Off. Anal. Chem.* **72**, 374–377
(3) Carman, A.S., Jr, Kuan, S.S., Ware, G.M., Francis, O.J., Jr, & Kirschenheuter, G.P. (1986) *J. Agric. Food Chem.* **34**, 279–282

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Foods I. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Seafood Toxins and Seafood Products

JAMES M. HUNGERFORD
U.S. Food and Drug Administration, 22201 23rd Dr. SE, PO Box 3012, Bothell, WA 98041-3012

Cell Bioassays for Seafood Toxins.—Associate Referee, Ronald Manger, of the Seafood Products Research Center, U.S. Food and Drug Administration (FDA), Bothell, WA, submitted the following report:

Monitoring programs for marine toxins in commercially important seafood were limited in large part to mouse bioassays (1). Increasingly, these methods were criticized by advocates of animal welfare and because of the cost, sensitivity, and effort required for the assays. In light of these concerns, there is an impetus for development of alternative biological assays. These alternative bioassays are not intended to replace the standard mouse assay but to provide a complementary method that will reduce the use of animals and provide additional data that may have been limited by total reliance on the animal test method.

Alternative bioassays can be based on in vitro methods using animal cells in culture or bacteria. It is essential that these cell-based assays satisfy the following basic criteria: In vitro assays should respond within the dose range of the suspected or known in vivo effect; the assay should exhibit low inherent variability and, ideally, should give a linear dose response curve; and the assay should be rapid and relatively simple.

For purposes of clarity, existent cell-based assays are reviewed here. Special attention is focused on the following marine toxicity categories: paralytic shellfish poisoning, neurotoxic shellfish poisoning, diarrhetic shellfish poisoning, and ciguatera fish poisoning.

A number of cell-based assays were recently introduced for the detection of toxins associated with paralytic shellfish poisoning (PSP). One of the most promising of these methods, developed by Kogure et al. (2), is a tissue culture assay for tetrodotoxin, saxitoxin, and related toxins. The mouse neuroblastoma cell line Neuro-2A can be readily grown and maintained in tissue culture. Cells treated with veratridine in the presence of ouabain rapidly swell, and cell death occurs from an uncontrolled influx of sodium. The assay is based on the ability of tetrodotoxin, saxitoxin, or gonyautoxin to antagonize this effect by blocking the sodium channel. The reported sensitivity of this assay is 3nM, or approximately 1×10^{-3} mouse units. The neuroblastoma assay was recently modified by Jellett et al. (3) for semiautomated determinations using a microplate reader. This modified assay has a reported detection limit that is 100-fold more sensitive than the standard mouse bioassay for saxitoxin. The cell-based neuroblastoma assay is sensitive, simple, and economical. Furthermore, this method allows for rapid screening of a large number of samples.

A promising test method is the Microtox assay (Microbics Corp., Carlsbad, CA). This method is based on a short-duration metabolic inhibition test using a standardized suspension of lu-

minescent bacteria (4). Bruno et al. (5) reported the detection of saxitoxin with the Microtox assay from a toxic algal bloom. Their reported sensitivity for pure saxitoxin was 1.28 μg/mL (EC_{50}), which compares favorably with the mouse bioassay; however, the Microtox assay is almost 1000-fold less sensitive than the neuroblastoma assay discussed above. Advantages of this method include its rapidity, which is typically less than 30 min, and the availability of the test organisms as a freeze-dried reagent that is immediately usable upon rehydration.

A competitive displacement assay for the detection of saxitoxin and tetrodotoxin was developed by Davio and Fontelo (6). These toxins are detected on the basis of binding to the sodium channel in isolated rat brain membranes. Although animals are required for isolation of target membranes, there is the potential that a suitable cell line could be identified or developed for use instead. The present displacement assay has a sensitivity in line with that of the neuroblastoma bioassay, it is simple and rapid, and the only major equipment requirement is a liquid scintillation counter.

Diarrhetic shellfish poisoning (DSP) is attributed to a number of dinoflagellate toxins. Of these DSP toxins, okadaic acid has become the focus of intense study because of its unique inhibition of cellular protein phosphatases PP1 and PP2 (7). Fortunately, the effect upon phosphorylated, controlled cellular mechanisms can be assessed by a number of in vitro methods. The reported cellular effects are numerous and include the following: okadaic acid-induced cytotoxicity in cultured cerebellar neurons at concentrations as low as 0.5nM (8), rapid increase of phosphorylated proteins in treated hepatocytes (9), reversion of phenotype in cells transformed by oncogenes (10), stimulation of IL-1 synthesis in isolated peripheral blood mononuclear cells (11), and induction of prolonged backward swimming in *Paramecium* (12). From this wide repertoire of in vitro effects, a DSP screening method for shellfish appears to be feasible.

Neurotoxic shellfish poisoning (NSP) is caused by a group of toxins, brevetoxins, produced by the dinoflagellate associated with Florida's red tide. Isolated rat brain synaptosomes were used by Baden et al. (13) to examine in vitro the potency of brevetoxins. In this competition assay, 50% displacement values (ED_{50}) ranged from ca 3 to 16 nm for various brevetoxins. These investigators mention the potential of developing a similar assay based on using neuroblastoma tissue culture. There is reason to believe this is a feasible approach based on earlier studies of PbTx-1 induced influx of ^{22}Na in veratridine treated neuroblastoma cells (14).

Ciguatoxin and maitotoxin are principal components associated with ciguatera. Ciguatoxin affects the sodium channel (15) and forms the basis for recent studies of sodium-dependent effects on calcium mobilization in cultured neuroblastoma/glioma (NG108-15) cells (16). Ciguatoxin induced a tetrodotoxin-sensitive increase in cytosolic calcium levels, which suggests a possible cell-based detection method.

Maitotoxin was shown to induce a profound Ca^{2+} influx in cultured rat pheochromocytoma cells (PC12) (17) and to cause significant release of norepinephrine and dopamine from cells at concentrations as low as 0.5 ng/mL (18). The potential for a practical cell-based assay is suggested by the recent results of Durand-Clement et al. (19), in which 3T3 fibroblasts, N18 neuroblastoma cells, and rabbit chondrocytes showed stimulation of calcium uptake in the presence of maitotoxin as assessed with the fluorescent probe Fura-2.

Many of the assays discussed above show potential as routine bioactivity screening tests and offer the basis for development of enhanced methods. However, improvements in sensitivity, selectivity, and rapicity will be required before they are accepted alongside the standard mouse bioassay. A number of potential developments could bring about the general use of cell-based methods. It is apparent that the current status and potential development of in vitro bioassays for marine toxins is intimately tied with the selection of appropriate target cells. It is also possible that the sensitivity and selectivity of these bioassays may improve with either the screening of alternative cell lines or with selected subclones generated from the original target cells. In addition, it may be possible to measure alternative cellular parameters in appropriate target cells that are indirectly affected by marine toxins but that afford a rapid and easily measured dose-dependent change. One possible area of examination would be the adaptation of standard cytotoxicity assays, such as colorimetric assays of cellular dehydrogenase activity or respiration (20, 21), perturbations of ATP levels from baseline (22), or any of the many labeled precursor assays for protein, DNA, or RNA synthesis. Most likely, these secondary effect assays will not provide the rapidity of the mouse bioassay, but they do offer the potential of providing an economical and simple method of obtaining complementary data from a large number of samples. Although the development of routine cell-based bioassays for use in regulatory screening of consumable seafood is in the early stages of development, the potential of a complementary method to the mouse bioassay is enormous.

Chemometric Methods.—Associate Referee Bradley J. Tenge of the Seafood Products Research Center, FDA, Bothell, WA, submitted the following report:

Pattern recognition (PR), a topic in the field of chemometrics, is investigated for it usefulness for species identification and decomposition identification of seafood species. This past year species identification was the focus of this referee project. The research team (23) working on the project focused first on the species identification of rockfish (genus *Sebastes*) and red snapper (*Lutjanus campechanus*), because rockfish is fraudulently substituted for red snapper in certain markets. This practice is prohibited by the Food, Drug, and Cosmetic Act, As Amended, Section 403(b).

Several other pairs of species, where one species is fraudulently substituted for another, include the following (using the common names): alaskan pollock for true cod, yellowtail for mahi mahi, and oreo dory or john dory for orange roughy (24).

Development of a PR method requires the sampling of biochemical profiles from fish samples that can serve as typical patterns for a given species or a given group of species. If a pattern is unique to a species, it can be considered a fingerprint. Biochemical patterns can include, for example, protein profiles (25–28), fatty acid profiles from neutral lipids (this study), cellular fatty acid profiles (29), and DNA restriction fragment lengths (30) or sequences (31, 32).

Generic chemometric software, equipped with PR modules, is used to mathematically classify certain types of patterns, such as chromatograms or densitograms. This classification discerns if the various species in question can be discriminated from each other (i.e., form separate classes along species lines). The PR software is then used to predict the classification of unknown samples. For example, PR can be used to determine whether or not rockfish species can be discriminated from red snapper on the basis of fatty acid methyl esters (FAME) profiles from neutral lipids sampled from the flesh or protein profiles sampled from the flesh. Computer-based PR for plant or animal species identification was successfully used elsewhere and serves as a precedent for these PR studies (29, 33, 34).

Once a data base is generated and validated, unknown samples can have their species classified rapidly. For a FAME analysis, the species can be determined in as little as 3 h after receipt of the fish sample. This includes homogenization, extraction, derivatization, chromatography, peak report generation, and computer-based PR prediction of the species using the data base.

Several official AOAC methods are available for fish species identification using electrophoretic methodology (35); however, the official methods do not yet include methodology that uses species confirmation via computer data base methodology applied to densitometric profiles or images of the gel output. For example, confirmation of species could be done using PR through peak matching for output generated using the method **980.16**. These existing AOAC official methods are qualitative in nature and recommend only visual inspection of the developed gel.

In this current study, PR is applied to data bases of known rockfish samples and known red snapper samples. One data base (also known as a training set) contains the FAME profiles from gas chromatography (GC) for this set of rockfish and red snapper. The FAME profiles are generated using a BF_3–MeOH transesterification method (36) after a methylene chloride extraction for neutral lipids from homogenized fish flesh of a section of each sample. The peak area report for each GC/FAME sample lists the same 19 FAME peak areas for each GC sample. The peak reports are entered into the data base.

Another data base that is concurrently being investigated in conjunction with Beckman, Inc., contains the capillary electrophoresis (CE) molecular weight (MW) output for rockfish and red snapper samples. CE using a capillary gel for sizing was reported previously (37), and this particular set of data uses a newly developed capillary gel for sizing (Beckman, Inc.) (38). For the capillary electrophoresis that produces a MW profile, these regions are integrated for each sample. The set of peak areas reported for the same MW regions for each sample is the set of variables entered in the spreadsheet of the data base.

Regardless of the data base, each row (or "record") contains a known sample's profile (for instance, one can think of the 19 FAME peak areas as a "digitized chromatogram"). Each column (or "field") corresponds to a specific variable, a certain FAME at a given normalized retention time for the GC/FAME, or a certain MW range for the protein capillary electrophoresis output. The same set of variables (retention times, MW ranges) are collected for each sample of the respective data base.

Current development on this project has progressed through the data base ("training set") formation for a set or rockfish and red snapper. The first level of PR to be applied to the data sets involves the determination of whether or not a pattern exists; if a pattern exists, the generation of a mathematical model allows the estimation of species identity of the training set samples using this same model. Only the GC/FAME data were tested in this manner: With the same 7 FAMEs (variables) selected from the profiles, all of 17 red snapper were correctly identified; all of 79 rockfish were correctly identified using the Soft Independent Modeling of Class Analogy algorithm (39, 40), as embodied in the interactive chemometric software package, Pirouette 1.1 (Infometrix, Inc). One outlier was deleted from the data base before this estimation of species identity was made; no false positives or false negatives were observed. Many more samples' chromatograms and peak area reports were collected and will be added to this FAME rockfish/red snapper data base.

The second level of PR to be applied is a performance study, in which prediction of species identity of fish meat is performed using the mathematical model generated for the data base. The unknowns are fish samples that are not used to build the data base. With FAME analyses, this step is particularly important to discern the data base's applicability for correct prediction of an unknown's true class (species) when caught in waters or seasons different from the data base samples. The positive hits for species identity, false positives, and false negatives are recorded in the performance study to evaluate the applicability of the data base. Diet and/or geography will be a factor in the practicality of the data base, because fatty acid profiles are affected by diet. To investigate this issue, samples caught in different waters will be included among the unknowns included in the performance study.

The envisioned collaborative study for this rockfish/red snapper data base would test the ability of the PR mathematical model from the data base to predict the identity of species provided to the collaborating laboratory. The laboratory would extract the neutral lipids from the fillet flesh, derivatize to the methyl ester, and collect the gas chromatogram. Software would be provided by the Associate Referee with oversight by the committee statistician and General Referee. The validity of the method would be described by the statistics for positive hits, false positives, false negatives, and "unable to classify" for each unknown relative to its true identity. A probability could then be estimated for the occurrence of positives hits, false positives, false negatives, and "unable to classify" in routine use of the method. These statistics/probability considerations are similar to pass/fail statistics for microbiological tests and other pass/fail tests (41).

In actual use of such a data base, one envisions a family of PR data bases, each of which discriminates between 2 species or a set of species. A preclassification step can be taken by the experienced analyst concerning which initial data base should be used. On the basis of the appearance of the flesh, regulatory sample history will indicate if one should focus on a rockfish/red snapper discrimination or discrimination between a

different set of species. A decision tree can then be applied to guide the analyst through which data base to use based on the response of "unable to classify" until a positive hit is achieved.

One of the benefits of this project is to provide the capability of multiple confirmation of species identity for species that are problems for species substitution. Isoelectric focusing (IEF) protein data are also being collected for rockfish and red snapper, with the goal of providing peak-matching computer data bases for IEF speciation. A library of DNA sequence information of polymerase chain reaction amplified sequences for the set of rockfish and red snapper is being developed by W. Hill of FDA's Seafood Products Research Center (42). The advantage of FAME data bases or CE/MW data bases is that sampling is fast. For cases that carry the greatest promise of legal action, multiple confirmation could be carried out with IEF-peak matching and/or DNA sequencing.

Ciguatoxins, Biochemical Method.—Associate Referee Doug Park of the University of Arizona, Tucson, AZ, reported that a minicollaborative study of the solid-phase immunobead assay (Ciguatect™) was conducted to provide a preliminary assessment of interlaboratory precision. The kit, which detects polyether toxins associated with ciguatera poisoning, was used in the study to screen toxic and nontoxic fish obtained from fishing areas around the Hawaiian Islands. The Ciguatect test kit was optimized for application to field/marketplace screening of ciguatoxic fish. The presence or absence of ciguatera-related toxins is determined by binding the toxins to a membrane attached to a plastic strip and exposing the toxin-laden membrane to a monoclonal antibody-colored latex bead complex that has a high specificity for ciguatera-related toxins. The intensity of the color on the membrane denotes the presence of the toxins in the fish or fish extract.

Twelve parrot, surgeon, and amberjack fish fillets and fish extract test portions containing various concentrations of toxins were used in the minicollaborative study. Before distribution of the test samples, toxic components in the fish were confirmed by extraction, column purification, and toxicity testing using the brine shrimp (*Artemia* sp.) assay. Okadaic acid was used to standardize both the S-PIA and brine shrimp assays. Four laboratories completed the study. In the determination of ciguatoxin and related polyether toxins in the parrot, surgeon, and amberjack fish fillets, the relative standard deviations for repeatability (RSD_r) were 13.5, 9.0, and 4.3%, respectively, and the relative standard deviations for reproducibility (RSD_R) were 44.4, 29.7, and 14.3%, respectively, for concentrations ranging from 1–4 ng/test strip. For determination of ciguatoxin and related polyether toxins in parrot, surgeon, and amberjack fish extracts, the RSD_r values were 5.8, 4.8, and 3.7%, respectively, and the RSD_R values were 11.9, 9.9, and 7.6%, respectively, for concentrations ranging from 3–5 ng/test strip. These statistical results are comparable with those obtained in interlaboratory studies evaluating thin-layer chromatography (TLC), enzyme-linked immunosorbant assay (ELISA), and affinity column methods for aflatoxins in various commodities (43–48).

Although prevalent in tropical waters (49–54), ciguatera is a world health problem. Unfortunately, all assays developed for the ciguatoxins, including alternative immunoassays (55), are compromised because none of the ciguatoxins are available in sufficient quantity to allow their use in collaborative studies. Okadaic acid, which is available commercially and was associated with some outbreaks of ciguatera, cross-reacts with the antibody used in the test kit. In the Ciguatect kit, okadaic acid is used for standardization, and the toxin levels referred to above are expressed in nanograms of okadaic acid.

Ciguatera by Liquid Chromatography (LC).—Associate Referee Robert W. Dickey (Fishery Research Facility, Division of Seafood Research, Office of Seafood, Dauphin Island, AL) reported that 2 LC/fluorometric methods for the ciguatoxins were evaluated (56, 57). The methods are based on the derivatization of ciguatoxins possessing C-1 primary hydroxyls (e.g. ciguatoxin-1, the principal toxin identified from moray eel in the South Pacific). The fluorescent reagents used were 1-anthroylnitrile and 7-diethylaminocoumarin-3-carbonyl azide. Whereas derivatization of ciguatoxin-1 using either reagent appears to proceed successfully, the quantum yield of the respective derivatives do not appear to provide parts per billion levels of sensitivity, which will be required for regulatory analyses from seafood matrixes. Fluorometric methods were not reported for the detection of ciguatoxin-1 precursors (reportedly produced by *Gambierdiscus toxicus*) and several congeners of ciguatoxin-1 that lack primary hydroxyls. Further exploratory efforts are needed in the development of highly sensitive LC methods for the determination of ciguatoxins.

Conjugated Dienes as Indicators of Decomposition.—Associate Referee Judith Kryznowek of the Northwest Fisheries Science Center, NMFS, Gloucester, MA, submitted the following report:

For fatty fish species, the rate and extent of lipid oxidation often determine the acceptability of the product. The presence of polyenoic fatty acids in fish offers many sites for rapid oxidation. In anticipation of increasing seafood inspection, a rapid (and ideally, automated) test for oxidative decomposition is needed.

Ultraviolet (UV) spectroscopy at a wavelength of 230 nm has traditionally been used to investigate the formation of conjugated dienes, but background absorbance at this wavelength makes measurements somewhat unreliable. Parr and Swoboda (58) describe a method assaying for "conjugable oxidation products" that allows for a distinction between dienoic or polyunsaturated precursors. The method is labor-intensive. Brown and Snyder (59) investigated an LC method using UV detection for conjugated dienes in soy oils. Takagi et al. (60) wrote an intriguing paper on enzymatic oxidation of various fatty acids with the formation of chromophores at longer wavelengths for fish oils.

An LC instrument equipped with a diode array detector (DAD) is being used in our laboratory to extend these previous methods. Advantages of this LC/DAD approach include the information-rich retention and spectral patterns and accurate subtraction of background signals.

Diarrhetic Shellfish Toxins.—Associate Referee Takeshi Yasumoto of Tohoku University, Japan, submitted the following report on the toxins responsible for diarrhetic shellfish poisoning (DSP):

A research group in Canada isolated a new toxin from mussels collected in Ireland and determined the structure as 31-methyl-dinophysistoxin-1 (61). Accordingly, the toxin was named dinophysistoxin-2 (DTX-2). DTX-2 can be called a hybrid of okadaic acid and dinophysistoxin-1 (61). Another new member to be added to the DSP family is 7-*O*-palmitoyl-okadaic acid, confirmed to be present in Irish mussels (62). Occurrence of new toxins of yet unknown structures was also confirmed in mussels from the Adriatic Sea (62). Likewise, disagreements were observed between the LC results and mouse assay results on Chilean mussels. The toxin profiles in these mussels could be much more complex in some specimens than suggested in previous work. Evidently, further effort is needed to elucidate toxin profiles in shellfish from various waters.

A new LC/fluorometric method for the determination of okadaic acid in shellfish and phytoplankton was described (63). Following aqueous-methanolic extraction and partitioning into chloroform from water, sample extracts are taken up in acetonitrile and derivatized with 1-bromoacetylpyrene. The resulting pyrenacyl okadate derivative (fluorescence-tagged okadaic acid) is enriched by solid-phase extraction, following the method of Lee et al. (64). Deoxycholic acid is used as internal standard, and separation is carried out in the reversed-phase mode on a C_{18} column (alternative internal standards are being explored to address probable coelution of DTX-1 and deoxycholic acid). An on-column detection limit of 0.1 ng was obtained, and okadaic acid levels as low as 62 ng/g were easily detected in oysters (*C. virginica*).

Determination of diarrhetic toxins in mussel tissues by ion-spray LC/MS was reported by Pleasance and Quilliam (65). The method is advantageous over existing fluorometric methods by virtue of its simplicity in sample preparation and reliability of identification. A method that combines the fluorometric LC method with MS analysis was also investigated. The method seems promising. Regrettably, the usefulness of the method is limited to those labs that have access to the expensive ion-spray interface.

Immunoassay is regarded as a desirable testing method for monitoring shellfish toxicity because of its simplicity and rapidity. The immunoassay kit developed for DSP testing by the UBE Company of Japan has the drawback of no cross-reactivity between the antibody and dinophysistoxin-3 (DTX-3). Hamano recently reported a new monoclonal antibody that cross-reacts with DTX-3 (66). Details of the cross-reactivity were not given in the report. Nevertheless, a test kit using the new antibody is expected to become commercially available.

Domoic Acid.—Associate Referee Michael A. Quilliam of the Marine Biosciences Institute, NRC Canada, submitted the following report on domoic acid:

Domoic acid (67), the toxin responsible for the 1987 incident of amnesic shellfish poisoning (ASP) in eastern Canada, once again made the headlines with 2 further incidents, this time on the West Coast of the United States. In September 1991, hundreds of pelicans and cormorants in Monterey Bay, CA, died after eating anchovies contaminated with domoic acid. The anchovies became laden with domoic acid after grazing on the pennate diatom *Pseudonitzschia australis*, a newly identified producer of domoic acid (68). The possibility of herbivorous finfish acting as vector for marine toxins is obviously now of considerable concern. Two months later in an apparent outbreak of ASP, several people became ill after consuming razor clams contaminated with domoic acid. Razor clams sampled in the immediate area had levels of domoic acid of over 100 μg/g. The crab fishery was closed briefly after domoic acid was discovered in Dungeness crab viscera (69). The source of domoic acid in Washington has not yet been established.

Production of domoic acid by the diatom *Nitzschia pungens f. multiseries* (the organism responsible for the 1987 Canadian incident) was studied in detail (70), and axenic cultures of the organism were shown to produce the toxin (71). Other known producers of domoic acid now include the species *Nitzschia pseudodelicatissima* (72) and *Pseudonitzschia australis* (67). The uptake, disposition, and depuration of domoic acid by blue mussels was investigated (73, 74).

The toxicology and pharmacology of domoic acid were studied extensively (75–90). The initial regulatory level for the toxin in Canada was set at 20 μg/g edible tissue, pending the outcome of animal studies. Any lowering of the regulatory level will obviously have serious implications for analytical methodology. The solubility of domoic acid in water and non-aqueous solvents was studied (91), and further information on the NMR of the toxin was published (92).

The AOAC first action procedure for domoic acid of Lawrence et al. (93) was taken through a collaborative study (94). This method uses the AOAC acid extraction procedure for PSP toxins, followed by LC separation with UV detection at 242 nm. This method has the advantage of providing an extract for both PSP and ASP analysis. However, extractions based on either boiling water or dispersion into aqueous methanol were previously found to give superior extraction efficiencies (95) and were used in some laboratories performing only domoic acid monitoring (96). A recently developed method that found wide acceptance is based on aqueous methanol extraction followed by cleanup on a (derivatized silica) strong-anion exchange cartridge (97). The high chemical selectivity of the cleanup and the potential for preconcentration facilitate a detection limit of 30 ng/g with LC/UV detection. The method was shown to be suitable for the determination of domoic acid in mussels, clams, crabs, and unsalted anchovies. A full AOAC collaborative study is now planned to evaluate the interlaboratory precision of the method.

Confirmatory procedures based on GC/MS of *N*-trifluoroacetyl-*o*-silyl derivatives (98), ion-spray LC/MS (99, 100), and LC/UV of the butyl isothiocyanate derivative (101, 102) have appeared in the literature. Capillary electrophoresis with either UV or MS detection was shown to be a useful analytical method (100, 103). A specific antibody to domoic acid was developed and used for both ELISA and radioimmunoassay (RIA) determinations of domoic acid in serum and urine (104). A domoic acid calibration solution (DACS-1) and a mussel tissue reference material (MUS-1) were made available through the Marine Analytical Chemistry Standards Program of the National Research Council of Canada (105).

Fish Flesh in Frozen Coated Fishery Products by End-scrape Method.—Associate Referee Jane Fox-Dobson of the National Marine Fisheries Service, Gloucester, MA, submitted a preliminary protocol for a collaborative study. The study will evaluate the method of Warren and Allhands (106) for determining the fish flesh content of frozen, coated, fishery products. Data collected will be compared with the known fish flesh content before storage at 0°F. The study is designed to determine what effect direct handling of the product has on the determination of fish flesh content for the purpose of modifying the method and to reevaluate the 2% variability factor presently allowed.

Flow Injection Analysis.—Associate Referee James M. Hungerford of the Seafood Products Research Center, FDA, Bothell, WA, reported that flow injection analysis (FIA) is enjoying increasing use in seafood research and in rapid screening. Seafood samples, because of their complexity and variability, have in the past required extensive sample cleanup prior to batch or chromatographic analysis. In contrast, modern FIA methods for seafood analysis require only minimal sample handling. These methods owe their success to 2 fundamentally different approaches to the challenging sample matrix found in seafoods and other biological materials. These powerful techniques are gas-diffusion FIA and the manipulation of chemical kinetics.

Gas-diffusion FIA uses a macroporous and hydrophobic membrane of poly(tetrafluoroethylene) or polyproylene as a gas-selective barrier between the sample-transporting carrier stream and an acceptor stream. In the analysis of seafoods, this method was applied to sulfites as volatile sulfur dioxide (107) and gaseous decomposition products, such as total volatile acids and total volatile bases (108), which are easily detected by their acidic or basic properties. These methods have the same advantages of all gas-diffusion FIA procedures in their ability to automatically move analyte vapors or volatile analyte-conversion products away from interfering biological materials in crude unpurified solutions and into clean detection streams. This approach, especially well-suited to the analysis of crude extracts, allows impressive reductions in sample handling. Precise control of timing allows reproducible detection of the transferred analyte, even though the diffusion process is not allowed to reach completion.

Analysis of nonvolatile analytes is more challenging, because the gas-diffusion approach is ruled out. In this case, the precise timing of FIA was used to manipulate chemical kinetics. In the flow-injection determination of histamine, high levels of naturally occurring histidine, which must be removed from finfish extracts in other procedures, are effectively ignored by fine-tuning, kinetics-derived selectivity (109). Continuing episodes of scombroid poisonings have only increased the demand for rapid histamine-screening methods, and the FIA method for histamine was incorporated into a national FDA training course, "Analytical Techniques for Seafood." The highly selective chemistry used in this method was coupled to a cartridge containing Sepharose-bound diamine oxidase (DAO). This approach allowed simultaneous detection of histamine and histaminase inhibitors in scombrotoxic fish (110). Given the generality of the FIA approach (111) and a demonstrated ability to reduce sample handling, FIA will continue to find new applications in seafood analysis. Rapid FIA methods in food microbiology were recently reviewed (112).

Neurotoxic Shellfish Poisons.—Associate Referee Daniel G. Baden of the Rosenthal School of Marine & Atmospheric Sciences, Miami, FL, reported that, during the past year, several aspects of neurotoxic shellfish poisoning resulting from brevetoxins were advanced. These deal with isolation and characterization of the specific receptor for the brevetoxins in nerve tissues (113), publication of a specific ELISA for the detection of brevetoxin in a variety of matrixes (114), commencement of a collaborative test for the ELISA, and undertaking of an interlaboratory calibration of all available assays for the detection and quantitation of brevetoxins.

A collaboration between the University of Miami and the University of Washington Department of Pharmacology resulted in the photoaffinity labeling and localization of the brevetoxin receptor in excitable membranes. This work, conducted as part of the research of V. Trainer at the University of Miami, indicated that the brevetoxin receptor is associated with the alpha-subunit of voltage-sensitive sodium channels. More specifically, Trainer's work illustrated that brevetoxins bind specifically to domain IV of the subunit. Using the photoaffinity-brevetoxin covalent conjugate constructed in Miami in Baden's laboratory and the unique anti-sodium channel peptide antibodies produced in W. Catterall's laboratory in Seattle, the localization work was accomplished. Further work, not yet published but outlined at the Fourth International Conference on Ciguatera, indicates that further progress is being made on brevetoxin binding in reconstituted sodium channel in artificial vesicles. Identification of the specific amino acid sequence that binds brevetoxin should provide the basic information necessary to develop a binding assay that reflects toxicity.

Brevetoxin ELISA was developed and reported in *Toxicon* (114). The publication indicates that sensitivity of the assay is approximately 40 pg, and linearity is exhibited between 40 pg and 40 ng per microtiter plate well. The assay was applied to the detection of brevetoxin in dinoflagellate cells and seawater, and progress is being made with respect to detection in biological matrixes. The assay, which uses brevetoxin affinity column matrixes to purify the antibody, does not cross-react with okadaic acid, unlike other tests that detect polyether toxins. A recent, unpublished facet to this research is the finding that clones of the ciguatoxin-producing dinoflagellate *Gambiardiscus toxicus* also cross-react in the assay. This finding, when coupled with the knowledge that the antibodies cross-react with ciguatoxic fish, lend credibility that the assay will be of use in the detection of ciguatoxin in fish flesh.

With funding from the Saltonstall-Kennedy Program from the Department of Commerce, the Chiral Corporation of Miami, Florida, is undertaking collaborative testing of the brevetoxin ELISA for AOAC. Eight laboratories were selected across the United States and include several FDA and National Marine Fisheries Service laboratories, the U.S. Army in Fort Detrick, private industry, and universities. The testing, presently underway, is still seeking another 3 or 4 laboratories to act as participants. Interested parties may contact Chiral Corpora-

tion at 1110 Brickell Ave, Suite 407, Miami, FL 33131 (Fax 305-539-1010). Collaborative testing should be complete by the end of 1992.

In another collaborative effort, all available assays for the detection of brevetoxins in seawater and seafood will be calibrated and compared. Furthermore, the potential for toxin accumulation under both induced and natural conditions will be evaluated. This work is also funded by the Saltonstall-Kennedy Program of the Department of Commerce. The following laboratories are participating: Florida Department of Natural Resources, Marine Research Institute, St. Petersburg, FL; Mote Marine Laboratory, Sarasota, FL; and, acting as lead agency, Chiral Corporation, Miami, FL. Using a variety of techniques ranging from LC, TLC, radioimmunoassay, ELISA, and affinity chromatography, the participants are seeking to gain insight into the optimal assays for toxin detection and quantitation. The study, in its second month, will be gathering data and evaluating techniques throughout 1992, with final results to be tallied in the first quarter of 1993. The study, which boasts to be the first major collaboration of its type to include state, private foundation, and corporate entities, is the culmination of continuing collaboration between C. Tomas (Florida Department of Natural Resources, Marine Research Institute), R. Pierce (Mote Marine Laboratory), and D. Baden (Chiral Corporation). A descriptive and instructional video program is being produced as part of the program.

In summary, brevetoxins and neurotoxic shellfish poisoning continue to be examined in detail. Results of the various facets of the work should be reported in the months to come in a variety of peer-reviewed journals.

Oxytetracycline in Farm-Raised Fish.—Associate Referee Stephen W. Hadley of the Seafood Products Research Center, FDA, Bothell, WA, submitted the following report:

Antibiotics are currently used in the aquaculture industry to cure and prevent the spread of diseases. A worldwide growth in fish farming operations coupled with an increase in seafood consumption within the United States have heightened concerns about the potential for antibiotic residue contamination in seafood products derived from these fish farming operations. The use of antibiotics in the aquaculture industry is regulated within the United States. For example, regulation established a tolerance level of 0.1 ppm for oxytetracycline (OTC) residues in the raw edible tissues of salmonids and catfish (CFR 21:556.500). OTC is the most widely used antibiotic in the aquaculture industry. To begin a more aggressive monitoring program that evaluates OTC residues in aquaculture-derived seafood products, reliable and rugged analytical methods specific to OTC must be available for regulatory use.

A variety of LC methods using UV detection were developed for the determination of OTC residues in fish tissue samples (115–121), including salmonids (122, 123). The instrumental detection limit for OTC using conventional UV detection is 2 ng injected (instrumental detection limit is defined here as the amount of OTC standard injected that gives a signal-to-noise ratio of 3:1). Instrumental detection limits for OTC reported in the literature range from 5 to 40 ng injected. To quantitate OTC by using UV detection at the tolerance level of 0.1 ppm in muscle tissue, OTC must be concentrated and purified from crude muscle extracts before LC evaluation. Several methods in the literature use C_{18} solid-phase extraction (SPE) techniques to concentrate and purify OTC from muscle extracts and to lower overall detection limits. Recoveries of OTC from C_{18} SPE cartridges may vary considerably. Therefore, several of the reported methods use an internal standard such as tetracycline or 4-epitetracycline to ensure that recovery of OTC from the column is acceptable. The problem with the C_{18} SPE columns appears to be related to differences (between lots) in the manufacture of the columns. The reason for this lot-to-lot variability is unclear but may be related to variations in the level of silanol end-capping of the C_{18} column matrix.

Variable recoveries of tetracyclines from C_{18} SPE cartridges indicate that a simplified sample cleanup should be pursued. Our approach has been to increase the sensitivity of detection by a factor of 10–20 over conventional UV detection, so that on-cartridge preconcentration is unnecessary. To this end, a high-sensitivity LC method using postcolumn derivatization and fluorescence detection is under development.

Although a postcolumn fluorescence method using an OTC–Eu(III)–EDTA complex was published (124) and modified (125), the instrumental detection limits for these methods were roughly equivalent to conventional UV detection. Fluorescence quenching of lanthanide metal ion complexes in aqueous solution can lead to a substantial decrease in the intensity of the fluorescence signal. However, fluorescence quenching can be controlled by manipulation of the chemical environment surrounding the metal ion complex in solution. Particularly important is the chemical nature of the coordinating ligands directly bonded to the metal ion. Because lanthanide ions are quite large, they have multiple sites available for coordination. Coordination of a single OTC molecule to a Eu(III) ion leaves several additional sites remaining on the ion available for coordination by other ligands. Thus, by changing the ligands coordinated to the OTC–Eu(III) complex, the fluorescence signal can be enhanced.

We have been evaluating the fluorescence properties of several new OTC–Eu(III)–ligand complexes where the polyaminocarboxylate ligand EDTA was replaced with beta-diketone ligands. Several beta-diketone ligand complexes have been studied, including OTC–Eu(III)–ACAC (ACAC, acetylacetone), OTC–Eu(III)–HFACAC (HFACAC, hexafluoroacetylacetone) and OTC–Eu(III)–THD (THD, tetramethylheptanedione). Fluorescence properties of the OTC–Eu(III)–ACAC complex (as measured in a conventional scanning spectrofluorimeter) suggested that a 25-fold improvement in the detection of this complex was achievable when compared with the OTC–Eu(III)–EDTA complex. The fluorescence properties of the ACAC complex were also superior to those of the HFACAC complex. The THD complex did not form a fluorescent complex under the experimental conditions.

Several problems were encountered when the Eu(III)–ACAC reagent system was applied as an LC postcolumn reagent. The combination of Eu(III) and ACAC in an aqueous buffer at basic pH proved to be unstable, apparently because of alkaline hydrolysis of the Eu(III) ion to insoluble Eu(III) ox-

ides. Unlike EDTA, ACAC does not complex to Eu(III) strongly enough to prevent metal ion hydrolysis.

Currently, we are evaluating several polycarboxylate ligands, including citrate, tartrate, and polyacrylate, in addition to several simple carboxylate ligands, such as acetate and trifluoroacetate. The utility of these complexes as postcolumn reagents is being studied.

Paralytic Shellfish Poisons, LC Methods.—Associate Referee Sherwood Hall of the Office of Seafoods, FDA, Washington, DC, submitted the following report:

The naturally occurring saxitoxins fall into 3 categories with differing net charges; the range in acidic solution is from +2 to zero. This, coupled with the polar nature of these substances and their multiplicity, has made the saxitoxins a formidable separation challenge. LC methods for them employ several different separation strategies, all based on ion pairing of the highly charged analytes. One extreme is that of Oshima et al. (126), which is based on 3 separate sets of isocratic LC conditions tailored to the 3 distinct charge categories. The other extreme is that of Sullivan and Wekell (127), in which a single gradient run is used to separate the group A and group B toxins, although a separate run is still required for the group C toxins. A hybrid approach, being developed by Thielert (personal communication), employs a stepped transition between different isocratic conditions for separation of groups A and B in a single run.

All these methods employ the postcolumn oxidation of the saxitoxins to yield fluorescent degradation products, which then can be detected at useful levels. Stability of the output is limited by variation in the yield of fluorescent products; yield is quite sensitive to conditions. The choice of oxidant is critical and merits further development. Periodate solutions have proven useful but can develop troublesome precipitates if systems are not cleaned frequently.

Oxidation of the saxitoxins to give fluorescent products prior to LC is an alternative with significant possible advantages, as well as problems. With no postcolumn reaction system, the plumbing is much simpler, and the purinoid degradation products are, in principle, more amenable to high-resolution separation than the highly charged parent compounds. However, there is not a 1:1 mapping of toxin to oxidation product: some substituents are lost, thus losing distinctions among toxins, and multiple derivatives can be produced from single toxins. Nevertheless, the possibilities remain attractive. Such an approach was recently studied by Lawrence and Menard (102).

Efforts to produce reliable reference standards of the saxitoxins for LC were continued. The production of the pure toxins is now fairly routine; however, concentrations found in a standard solution after a practical storage period remain difficult to define due to epimerization. A calibration technique was developed for standardizing mixtures of the 11-hydroxysulfate toxins using primary standards of known net concentration but indeterminate epimeric purity. This will make it more practical to produce and maintain such standards.

Volatile Amines (TMA, DMA) by Gas Chromatography.—Associate Referee Ronald C. Lundstrom of National Marine Fisheries Service, Gloucester, MA, reports that no additional progress was made in this area, and additional participants are needed before a collaborative study can be initiated.

Collaborative Studies

Drained Weight of Block-Frozen Peeled or Deveined Shrimp.—Associate Referee William D. Chauvin of the American Shrimp Processors Association, New Orleans, LA, submitted results of a collaborative study of a modified method for determination of drained weight of block-frozen peeled or deveined shrimp. The study was supported by SK Award NA86NC-H-06177.

Species of shrimp used in the study included *Penaeus setiferus* (Gulf White), *Peneaus duorarum* (Gulf Pink), and *Penaeus aztecus* (Gulf Brown). Six laboratories returned complete results from the 4-round study. Each of the participants was sent 4 sets of samples consisting of 6 samples each. The 6 samples were actually 3 pairs of blind duplicates. Statistical analysis revealed excellent results, with repeatability coefficients of variation (CVs) ranging from 0.12 to 2.81% and reproducibility CVs ranging from 1.01 to 4.65%. official first action is recommended. The General Referee concurs.

Recommendations

(1) Initiate full collaborative study of the solid-phase immunobead assay (Ciguatect kit) for polyethers associated with ciguatera.
(2) Appoint new Associate Referee for the subject Paralytic Shellfish Poisons—Immunoassay Methods.
(3) Adopt as official first action the modified method for determination of drained weight of block-frozen peeled or deveined shrimp.
(4) In the next edition of *Official Methods of Analysis*, in the first action LC method for domoic acid in mussels using the AOAC PSP-extraction procedure (93), add the following statements at the end of the procedure: "Ensure baseline resolution of L-tryptophan from domoic acid; adjust mobile phase composition accordingly. Determine recoveries of domoic acid at the 20 ppm level."
(5) Submit the LC-SAX method for domoic acid (ref. 97, plus current modifications) to collaborative study.

References

(1) Evans, M.H. (1972) *Int. Rev. Neurobiol.* **15**, 83
(2) Kogure, K., Tamplin, M.L., Simidu, U., & Colwell, R.R. (1988) *Toxicon* **26**, 191–197
(3) Jellett, J.F., Marks, L.J., Stewart, J.E., Dorey, M.L., Watson-Wright, W., & Lawrence, J.F. (1992) *Toxicon* **30**, 1143–1156
(4) Bulich, A.A. (1979) *Aquat. Tox.*, L.L. Markings and R.A. Kimerle (Eds), American Society for Testing and Materials, pp. 98–106
(5) Bruno. M., Gucci, P.M.B., Pierdominici, E., Ioppolo, A., & Volterra, L. (1990) *Toxicon* **28**, 1113–1116
(6) Davio, S.R., & Fontelo, P.A. (1984) *Anal. Biochem.* **141**, 199–204

(7) Cohen, P., Holmes, C.F.B., & Tsukitani, Y. (1990) *Trends. Biochem. Sci.* **15**, 98–102

(8) Fernandez, M.T., Zitko, V., Gascon, S., & Novelli, A. (1991) *Life Sci.* **49**, 157–162

(9) Haystead, T.A.J., Sim, A.T.R., Carling, D., Honnor, R.C., Tsukitani, Y., Cohen, P., & Hardie, D.G. (1989) *Nature* **337**, 78–81

(10) Sakai, R., Ikeda, I., Kitani, H., Fujiki, H., Takaku, F., Rapp, U., Sugimura, T., & Nagao, M. (1989) *Proc. Natl. Acad. Sci. USA.* **86**, 9946–9950

(11) Hokama, Y., Scheuer, P.J., & Yasumoto, T. (1989) *J. Clin. Lab. Anal.* **3**, 215–221

(12) Proud, C.G. (1986) *Trends Biochem Sci.* **11**, 73–77

(13) Baden, D.G., Mende, T.J., Szmant, A.M., Trainer, V.L., Edwards, R.A., & Roszell, L.E. (1988) *Toxicon* **26**, 97–103

(14) Catterall, W.A., & Risk, M.A. (1981) *Mol. Pharmacol.* **19**, 345–348

(15) Bidard, J.N., Vijverberg, H.P., Frelin, C., Chungue, E., Legrand, A.M., Bagnis, R., & Lazdunski, M. (1984) *J. Biol. Chem.* **259**, 8353–8357

(16) Molgo, J., Comella J.X., Shimahara, T., Morot Gaudry-Talamarmain, Y., & Legrand, A.M. (1992) Fourth international conference on ciguatera fish poisoning, Institut Territorial de Recherches Medicales Louis Malarde, Tahiti

(17) Takahashi, M., Ohizumi, Y., & Yasumoto, T. (1982) *J. Biol. Chem.* **257**, 7287–7289

(18) Takahashi, M., Masahiro, T., & Ohizumi, Y. (1983) *J. Biol. Chem.* **258**, 10944–10949

(19) Durand-Clement, M., Legay, C., Ramdi, H., & Lievremont, M. (1992) Fourth international conference on ciguatera fish poisoning, Institut Territorial de Recherches Medicales Louis Malarde, Tahiti

(20) Mosman, T. (1983) *J. Immun. Meth.* **65**, 55–63

(21) Buskirk, H.H., Crim, J.A., Van Giessen, G.J., & Petering, H.G. (1973) *J. Natl. Cancer. Inst.* **51**, 135–138

(22) Garewal, H.S., Ahmann, F.R., Schifman, R.B., & Celniker, A. (1986) *J. Natl. Cancer Inst.* **77**, 1039–1045

(23) Species identification project team: B. Tenge, S. Kwolek, N-L. Dang, J. Barnett. (Seafood Products Research Center and Science Branch, Seattle District, FDA); F. Fry, (Center for Food Safety and Applied Nutrition, FDA); J. Nolan (Beckman Instruments, Inc., Fullerton, CA); S. Ramos (Infometrix, Inc., Seattle, WA)

(24) Personal communication with Jim Barnett, Organoleptic Specialist, Science Branch, Seattle District, FDA

(25) Lundstrom, R. (1980) *J. Assoc. Off. Anal. Chem.* **63**, 69–73

(26) Lundstrom, R. (1983) *J. Assoc. Off. Anal. Chem.* **66**, 974–980

(27) Hamilton, W. (1982) *J. Assoc. Off. Anal. Chem.* **65**, 119–122

(28) An, H. (1989) *J. Food Sci.* **54**, 253–257

(29) Sasser, M. (1990) Technical Note #101, MIDI, Inc., Newark, DE

(30) Bermingham, E., Forbes, S., Friedland, K., & Pla, K. (1991) *Can. J. Fish. Aquat. Sci.* **48**, 884–893

(31) Bartlett, S., & Davidson, W. (1991) *Can. J. Fish. Aquat. Sci.* **48**, 309–317

(32) Bartlett, S., & Davidson, W. (1992) *BioTechniques* **12**, 408–411

(33) Lavine, B., & Carlson, D. (1987) *Anal. Chem.* **59**, 468A–470A

(34) Valcarce, R., Smith, G., Stevenson, D., & Asay, K., (1990) *Chemomet. Intell. Lab. Sys.* **9**, 95–105

(35) *Official Methods of Analysis* (1990) 15th Ed., AOAC, Arlington, VA, pp. 883–886

(36) Morrison, W., & Smith, L. (1964) *J. Lipid Res.* **5**, 600; also personal communication with J. Hudson, NMFS, Seattle, WA

(37) Cohen, A., & Karger, B. (1987) *J. Chromatog.* **397**, 409–417

(38) Personal communication with Judith Nolan, Bioanalytical Applications, Beckman, Inc.

(39) Wold, S. (1976) *Patt. Recog.* **8**, 125–139

(40) Wold, S., & Sjostrom, M. (1977) in *Chemometrics, Theory and Application*, B.R. Kowlaski (Ed.), American Chemical Society, Washington, DC, pp. 243–282

(41) Wehr, H.M. (1987) *J. Assoc. Off. Anal. Chem.* **70**, 348–349

(42) Personal communication with W. Hill, Research Microbiologist, Seafood Products Research Center

(43) Park, D.L., Miller, B.M., Nesheim, S., Trucksess, M.W., Vekich, A., Bidigare, B., McVey, J.L., & Brown, L.H. (1989) *J. Assoc. Off. Anal. Chem.* **72**, 638–643

(44) Royal, S.C. (1982) *Morbidity and Mortality Weekly Reports.* **31**, 391

(45) Stack, M.E. (1974) *J. Assoc. Off. Anal. Chem.* **57**, 871–874

(46) Shotwell, O.L., & Stubblefield, R.D. (1972) *J. Assoc. Off. Anal. Chem.* **55**, 781–788

(47) Trucksess, M.W., Stack, M.E., Nesheim, S., Page, S.W., Albert, R.H., Hansen, T.J., & Donahue, K.F. (1991) *J. Assoc. Off. Anal. Chem.* **74**, 81–88

(48) Waltking, A.E. (1970) *J. Assoc. Off. Anal. Chem.* **53**, 104–113

(49) Bagnis, R. (1970) *Clin Toxicol.* **3**, 579–583

(50) Bagnis, R.A., Kuberski, T., & Langier, S. (1979) *Am. J. Trop. Med. Hyg.* **28**, 1067–1073

(51) Banner, A.H. (1965) *Hav. Med. J.* **24**, 353–361

(52) Lewis, N.D. (1986) *Soc. Sci. Med.* **23**, 983–993

(53) Maharaj, S.R., Desai, P., Kulkarni, P., & Pinnock, M.A. (1986) *W. I. Med. J.* **35**, 321–323

(54) Park, D.L., Miller, B.M., Hart, L.P., Yang, G., McVey, J., Page, S.W., & Brown, L.H. (1989) *J. Assoc. Off. Anal. Chem.* **72**, 329–335

(55) Park, D.L. (1992) "Evolution of methods for assessing aquatic product safety: Ciguatera," Presented at the Institute of Food Technologists Annual Meeting, June 20–24, New Orleans, LA

(56) Legrand, A.M., Fukui, M., Cruchet, P., Ishibashi, Y., & Yasumoto, T. (1992) *Proc. 3rd Int. Conf. on Ciguatera Fish Poisoning*, T. Tosteson (Ed.), Polyscience Publications, Quebec, Canada, pp. 25–32

(57) Dickey, R.W., Bencsath, F.A., Granade, H.R., & Lewis R.J. (1992) in *Abstract of the 4th Int. Conf. on Ciguatera Fish Poisoning*, May 4–7, Tahiti (Manuscript submitted for proceedings)

(58) Parr, L.C., & Swoboda, P.A.T. (1976) *J. Food Technol.* **11**, 1

(59) Brown, H.B., & Snyder, H.E. (1982) *J. Am. Oil Chem. Soc.* **59**, 280

(60) Takagi, T., Wakasa, N., & Miyashita, K. (1987) *J. Am. Oil Chem. Soc.* **64**, 1320

(61) Hu, T., Doyle, J., Jackson, D., Marr, J., Nixon, E., Pleasance, S., Quilliam, M.A., Water, J.A., & Wright, J.L.C. (1992) *J. Chem. Soc. Commun.* **30**, 41

(62) Zhao, J., Lembeye, G., Cenci, G., Wall, B., & Yasumoto, T. *Proc. 5th Int. Conf. on Toxic Phytoplankton*, in press

(63) Dickey, R.W., Granade, H.R., & Bencsath, F.A. *Proc. 5th Int. Conf. on Toxic Phytoplankton*, in press

(64) Lee, J.S., Yanagi, T., Kenma, R., & Yasumoto, T. (1987) *Agric. Biol. Chem.* **51**, 887–891

(65) Pleasance, S., & Quilliam, M.A. (1991) *Mass Spectr.* **6**, 121–127

(66) Hamano, Y. (1991) *Osaka Daigaku Igaku Zasshi (Bull. Med. School Osaka Univ.)* **43**, 67–81

(67) Wekell, M.M. (1991) *J. Assoc. Off. Anal. Chem.* **74**, 137–141

(68) Fritz, L., Quilliam, M.A., Wright, J.L.C., Beale, A.M., & Work, T.M. (1992) *J. Phycology* in press

(69) Luscotoff, S. (1993) *J. Assoc. Food Drug Off.*, submitted

(70) Bates, S.S., DeFreitas, A.S.W., Milley, J.E., Pocklington, R., Quilliam, M.A., Smith, J.C., & Worms, J. (1991) *Can. J. Fish. Aquat. Sci.* **48**, 1136–44

(71) Douglas, D.J., & Bates, S.S. (1992) *Can. J. Fish. Aquat. Sci.* **49**, 85–90

(72) Martin, J.L., Haya, K., Burridge, L.E., & Wildish, D.J. (1990) *Mar. Ecol.: Prog. Ser.* **67**, 177–182

(73) Madhyastha, M.S., Novaczek, I., Ablett, R.F., Johnson, G., Nijjar, M.S., & Sims, D.E. (1991) *Aquat. Toxicol.* **20**, 73–81

(74) Novaczek, I., Madhyastha, M.S., Ablett, R.F., Johnson, G., Nijjar, M.S., & Sims, D.E. (1991) *Aquat. Toxicol.* **21**, 103–118

(75) Glavin, G.B., Pinsky, C., & Bose, R. (1990) *Brain Res. Bull.* **24**, 701–703

(76) Grimmelt, B., Nijjar, M.S., Brown, J., Macnair, N., Wagner, S., Johnson, G.R., & Amend, J.F. (1990) *Toxicon* **28**, 501–508

(77) Perl., T.M., Bedard, L., Kotsatsky, T., Hockin, J.C., Todd, E.C.D., & Remis, M.D. (1990) *N. Engl. J. Med.* **322**, 1775–1780

(78) Stewart, G.R., Zorumski, C.F., Price, M.T., & Olney, J.W. (1990) *Exp. Neurol.* **110**, 127–138

(79) Sutherland, R.J., Hoesing, J.M., & Whishaw, I.Q. (1990) *Neurosci. Lett.* **120**, 221–223

(80) Teitelbaum, J.S., Zatorra, R.J., Carpenter, S., Gendron, D., Evans, A.C., Gjedde, A., & Cashman, N.R. (1990) *N. Engl. J. Med.* **322**, 1781–1787

(81) Tryphonas, L., Truelove, J., Todd, E., Nera, E., & Iverson, F. (1990) *Food Chem. Toxicol.* **28**, 707–715

(82) Tryphonas, L., & Iverson, F. (1990) *Toxicol. Pathol.* **18**, 165–169

(83) Tryphonas, L., Truelove, J., Nera, E., & Iverson, F. (1990) *Toxicol. Pathol.* **18**, 1–9

(84) Tryphonas, L., Truelove, J., & Iverson, F. (1990) *Toxicol. Pathol.* **18**, 297–303

(85) Bruni, J.E., Bose, R., Pinsky, C., & Glavin, G. (1991) *Brain Res. Bull.* **26**, 419–424

(86) Dakshinamurti, K., Sharma, S.K., & Sundaram, M. (1991) *Neurosci. Lett.* **127**, 193–197

(87) Strain, S.M., & Tasker, R.A.R. (1991) *Neuroscience* (Oxford) **44**, 343–352

(88) Tasker, R.A.R., Connell, B.J., & Strain, S.M. (1991) *Can. J. Physiol. Pharmacol.* **69**, 378–382

(89) Chiamulera, C., Costa, S., Valero, E., & Reggiani, A. (1992) *Pharmacol. Toxicol.* (Copenhagen) **70**, 115–120

(90) Petrie, B.B., Pinsky, C., Standish, N.M., Bose, R., & Glavin, G.B. (1992) *Pharmacol. Biochem. Behav.* **41**, 211–214

(91) Falk, M., Seto, P., & Walter, J.A. (1991) *Can. J. Chem.* **69**, 1740–1744

(92) Falk, M., Walter, J.A., Seto, P.F., & Leek, D.M. (1991) *Spec. Publ., R. Soc. Chem.* **94**, 323–324

(93) Lawrence, J.F., Charbonneau, C.F., & Menard, C., Quilliam, M.A., & Sim, P.G. (1989) *J. Chromatogr.* **462**, 349–356

(94) Lawrence, J.F., Charbonneau, C.F., & Menard, C. (1991) *J. Assoc. Off. Anal. Chem.* **74**, 68–72

(95) Quilliam, M.A., Sim, P.G., McCulloch, A.W., & MacInnes, A.G. (1989) *J. Environ. Anal. Chem.* **36**, 139–54

(96) Gilgan, M.J. (1992) Fisheries & Oceans Canada, Halifax, NS, personal communication

(97) Quilliam, M.A., Xie, M., & Hardstaff, W.R. (1991) (Technical Report No. 64) Halifax, NS: Institute for Marine Biosciences, National Research Council Canada

(98) Pleasance, S., Xie, M., LeBlanc, Y., & Quilliam, M.A. (1990) *Biomed. Environ. Mass Spectrom.* **19**, 420–427

(99) Quilliam, M.A., & Pleasance, S. (1991) in *Actas du Colloque sur les Biotoxines Marines,* J.M. Fremy (Ed.), Centre National d'Etudes Veterinaires et Alimentaires, Paris, France, pp. 131–135

(100) Quilliam, M.A., Ayer, S.W., Pleasance, S., Sim, P.G., Thibault, P., & Marr, J.C. (1992) in *Seafood Science and Technology,* E.G. Bligh (Ed.), Fishing Book News, Oxford, United Kingdom, pp. 376–386

(101) Lawrence, J.F., & Menard, C. (1991) *J. Chromatogr.* **550**, 595–601

(102) Lawrence, J.F., & Menard, C. (1991) *Fresenius J. Anal. Chem.* **339**, 494–498

(103) Pleasance, S., Thibault, P., & Kelly, J. (1992) *J. Chromatogr.* **591**, 325–339

(104) Newsome, H., Truelove, J., Hierlihy, L., & Collins, P. (1991) *Bull. Environ. Contam. Toxicol.* **47**, 329–334

(105) Hardstaff, W.R., Jamieson, W.D., Milley, J.E., Quilliam, M.A., & Sim, P.G. (1990) *Fresenius J. Anal. Chem.* **338**, 520–525

(106) Warren, J., & Allhands, T. (1971) *J. Assoc. Off. Anal. Chem.* **54**, 640–642

(107) Sullivan, J.J., Hollingworth, T.A., Jr, Wekell, M.M, Newton, R.T., & LaRose, J.E. (1986) *J. Assoc. Off. Anal. Chem.* **69**, 542–546

(108) Wekell, M.M., Hollingworth, T.A., & Sullivan, J.J. (1987) *Proc. Int. Symp. on Seafood Quality Determination*, D.E. Kramer & J. Liston (Eds), Elsevier, New York, NY, pp. 17-25

(109) Hungerford, J.M., Walker, K.D., Wekell, M.M., LaRose, J.E., & Throm, H.R. (1991) *Anal. Chem.* **62**, 1971–1981

(110) Hungerford, J.M., & Arefyev, A.A. (1992) *Anal. Chim. Acta* **261**, 351–359

(111) Ruzicka, J., & Hansen, E.H. (1989) *Flow Injection Analysis*, Elsevier Science Publishing Co., Inc., New York, NY

(112) Lemieux, L., Puchades, R., & Simard, R.E. (1991) *Lebensm. Wiss. Technol.* **24**, 189–197

(113) Trainer, V.L., Thomsen, W.J., Catterall, W.A., & Baden, D.G. (1991) *Mol. Pharmacol.* **40**, 988–994

(114) Trainer, V.L., & Baden, D.G. (1991) *Toxicon* **29**, 1387–1394

(115) Long, A.R., Hsieh, L.C., Malbrough, M.S., Short, C.R., & Barker, S.A. (1990) *J. Assoc. Off. Anal. Chem.* **73**, 864–867

(116) Rogstad, A., Hormazabal, V., & Yndestad, M. (1988) *J. Liq. Chromatogr.* **11**, 2337–2347

(117) Iversen, B., Aanesrud, A., Kolstad, A.K., & Rasmussen, K.E. (1989) *J. Chromatogr.* **493**, 217–221

(118) Eivindvik, K., & Rasmussen, K.E. (1989) *J. Liq. Chromatogr.* **12**, 3061–3071

(119) Moats, W.A. (1990) *J. Assoc. Off. Anal. Chem.* **73**, 343–346

(120) Ueno, R., Uno, K., Kubota, S.S., & Horiguchi, Y. (1989) *Nippon Suisan Gakkaishi* **55**, 1273–1276

(121) Bjorklund, H. (1988) *J. Chromatogr.* **432**, 381–387

(122) Reimer, G.J., & Young, L.M. (1990) *J. Assoc. Off. Anal. Chem.* **73**, 813–817

(123) Aoyama, R.G., McErlane, K.M., Erber, H., Kitts, D.D., & Burt, H.M. (1991) *J. Chromatogr.* **588**, 181–186

(124) Wenzel, T.J., Collette, L., Dahlen, D., Hendrikson, S., & Yarmaloff, L, (1988) *J. Chromatogr.* **433**, 149–158

(125) Duggan, J.X. (1991) *J. Liq. Chromatogr.* **14**, 2499–2525

(126) Oshima, Y., Sugino, K., & Yasumoto, T. (1989) in *Mycotoxins and Phycotoxins 88*, S. Natori, K. Hashimoto, & Y. Ueno (Eds), Elsevier, Amsterdam, The Netherlands, pp. 319–326

(127) Sullivan, J.J., & Wekell, M.M. (1987) in *Seafood Quality Determination*, D.E. Kramer & J. Liston (Eds), Elsevier Science Publishing Co., Inc., New York, NY, pp. 357–371

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Foods I. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

GENERAL REFEREE REPORTS

Committee on Foods II

Alcoholic Beverages

RANDOLPH H. DYER
Bureau of Alcohol, Tobacco, and Firearms Laboratory, 1401 Research Blvd, Rockville, MD 20850

The AOAC-ASE (American Society of Enologists) Liaison Officer, Art Caputi, is initiating an effort to arrange the adoption by AOAC of OIV (Office International de la Vigne et due Vin) methods. An arrangement similar to the cooperative agreement of AOAC and AAOC (American Association of Cereal Chemists) seems entirely appropriate.

Art Caputi's method "Glycerol in Wine and Grape Juice, Liquid Chromatographic Method" was adopted first action by the Official Methods Board at their meeting on August 15, 1991.

Mark Schwiesow is the new AOAC-ASBC (American Society of Brewing Chemists) Liaison Officer. The ASBC method "Alcohol and Original Gravity Content in Beer, SCABA Method" submitted for AOAC adoption first action was delayed for additional clarification of performance specifications and system suitability, and it will be resubmitted at a later date.

ASBC contacted AOAC with regard to fill methods for malt beverages. At the request of ASBC, AOAC was asked to review updated submissions of "Total Contents of Bottles and Cans by Calculation from Measured Net Weight" and "Total Contents of Cans of Known Tare Weight." ASBC also asked AOAC to delete the method "Deliverable Contents of Bottles and Cans by Calculation From Measured Net Weight." All 3 methods received interim first action in December 1988 and official first action in the Spring of 1989. The 2 former methods were updated to reflect the results from recent studies that showed a more accurate and theoretically sound calculation on the effect of carbon dioxide on beer density. Deletion of the latter method was requested, because ASBC showed no interest and would not include the method into ASBC *Methods of Analysis*. Currently, these requests are under consideration and evaluation.

Ben Canas was reappointed Associate Referee for "Ethyl Carbamate in Alcoholic Beverages and Foods, Gas Chromatographic-Mass Selective Detection Method." The protocol for a collaborative study was approved.

Alan Reisig was appointed Associate Referee to test and study analytical methods for "Lead in Wines."

Recommendation

(1) Continue study on all topics.

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Foods II. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Cereals and Cereal Products

RALPH H. LANE
University of Alabama, Department of Nutrition and Hospitality Management, Tuscaloosa, AL 35487-0158

β-D-Glucan in Cereal Products.—As requested by the Committee on Foods II, Associate Referee Lou Zygmunt completed the revisions on his collaborative study concerning the enzymatic analysis of (1 → 3)(1 → 4)-β-D-glucan in cereal products. Revisions include the following: adding enzyme activity assessment methods that immediately follow the β-D-glucan method, stating the sieve material specification, and deleting trade names of ready-to-eat cereals used as test materials in the investigation. The method, approved and recommended earlier in the year by the General Referee and Foods II Statistician, is now ready for submission by the Foods II Committee to the Official Methods Board for consideration at the 106th Annual meeting in Cincinnati for adoption first action.

Crude Protein in Cereals and Oilseeds by Combustion Analysis.—Associate Referee Ron Bicsak completed the revisions requested by the General Referee, Foods II Statistician, and Committee on Foods II concerning his collaborative study on crude protein in cereal grains and oilseeds by the combustion method. The method will be recommended by the Foods II Committee to the Official Methods Board at the 106th Annual Meeting in Cincinnati for adoption first action.

Fat Acidity in Flour.—A study was continued in the General Referee's laboratory to determine if fat-soluble pigments (carotenes) interfere with perception of the visual endpoint of phenolphthalein during fat acidity analysis by the official method (**939.05**). An earlier work (*J. Assoc. Off. Anal. Chem.* [1990] **73**, 626–627) indicated that higher acidity values may result in a titration medium of alcohol alone, as compared with the toluene–alcohol medium prescribed in **939.05**. Duplicate cereal samples (whole wheat flour, rye flour, brown rice flour, and corn meal) were refluxed 6 h in petroleum ether and evaporated to dryness. One duplicate was dissolved in toluene–alcohol–phenolphthalein (TAP) (official method) and the other in alcohol–phenolphthalein (AP). Titration with standard

0.0178M KOH to the endpoint of phenolphthalein was followed potentiometrically, noting the pH at the visual endpoint (faint pink). Although the difference in pH between TAP and AP at the visual endpoint of the whole wheat flour extracts approached significance ($P = 0.09$), only in the case of corn meal did the pH at the visual endpoint actually differ ($P < 0.01$). No differences ($P > 0.05$) were noted in flour acidity values between the 2 titration media (TAP and AP) except for brown rice flour, which produced a higher value ($P < 0.001$) in AP. Additional data are needed to determine if the removal of toluene from the titration medium affects fat acidity values.

Iron in Flour.—Associate Referee Jim Martin successfully completed the investigation concerning the reduction of points for the concentration curve from 11 to 4 for the colorimetric analysis of iron in flour (**944.02B**). The General Referee and Foods II Committee Statistician approved the study and recommended it to the Foods II Committee for adoption first action.

Gliadin in Gluten-Free Products.—The review of the gliadin in gluten-free products collaborative study by Associate Referee W. Hekkens is near completion. Pending revision, approval was received from the General Referee and the Foods II Committee Statistician. The Associate Referee is currently finishing final manuscript revisions for subsequent submission to the Committee on Foods II.

Gluten in Foods.—John H. Skerritt, Associate Referee, reports that his first action method for the determination of gliadin/gluten in gluten-free products is gaining acceptance among analysts. He continues to work with this research topic, having published related articles on a simplified qualitative test for gluten in foods and comparisons between Kjeldahl protein (prescribed as the official test for gluten in gluten-free products by Codex Alimentarius) and gluten protein by his ELISA technique. Because the Kjeldahl method determines reduced nitrogen expressed as protein, whereas the ELISA method measures gliadin/gluten specifically, the Associate Referee believes that the ELISA technique should be adopted as the defining method for gluten in gluten-free foods by Codex.

Minerals in Cereal Products.—Associate Referee Yasmin Neggers performed a preliminary study in which a general, straightforward ashing method was compared with official ashing methods (**922.03** and **936.07**) for cereal products. The method differs from the official methods in that no ashing aids other than demineralized water are used, and ashing temperatures above 450°C are avoided. Preliminary results, which were inconclusive, were presented in Seattle last fall to the AACC Mineral Technical Committee. The committee indicated that ashing procedures are matrix-dependent and that one method alone may not be appropriate for all cereal matrixes. The Associate Referee is continuing the work, with input from members of the AACC Minerals Technical Committee.

Near-Infrared Reflectance Analysis for Protein in Wheat Flour.—Donald E. Koeltzow, Associate Referee, in support of adoption of AACC Methods 39-10 and 39-11, and NIR methods for protein in wheat and wheat flour that did not meet AOAC protocol with respect to method validation, furnished the General Referee an existing data set from the USDA-FGIS NIR "blind sample" program in lieu of initiating a collaborative study. The data set included triplicate protein analyses from 12 to 14 laboratories of 5 wheat samples. Statistical analysis by the Foods II Committee Statistician indicated that the data set performed well with respect to repeatability and reproducibility, even though the samples were not sent out simultaneously and the same operator may not have performed all analyses. Moreover, means from NIR protein analysis compared quite well with means by the official Kjeldahl method. The General Referee and the Foods II Committee Statistician recommend approval of this data set as adequate method validation in support of the adoption of AACC Method 39-10, Near-Infrared Reflectance Method for Protein Determination of Wheat and AACC Method 39-11, Near-Infrared Reflectance Method for Protein-Wheat Flour, as first action AOAC methods.

Phytate in Foods.—No activity.

Recommendations

(1) Adopt as first action the β-D-glucan method.
(2) Adopt as first action the crude protein combustion method.
(3) Continue study on the removal of toluene from the fat acidity method (**939.05**).
(4) Approve adoption of reducing the number of points on the standard concentration curve from 11 to 4 for the iron in flour method (**944.02B**).
(5) Approve adoption of the gliadin in gluten-free products method as first action pending revisions suggested by the GR.
(6) Continue work on general ashing method for cereal products.
(7) Approve recommendation to accept USDA-FGIS data set as method validation for AACC Methods 39-10 and 39-11.

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Foods II. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Dietary Fiber

LEON PROSKY
U.S. Food and Drug Administration, Division of Nutrition, Laurel, MD 20708

Recommendations

(1) Accept as official first action the direct method for soluble dietary fiber (SDF). This will complete the methodology for the determination of total dietary fiber (TDF),

insoluble dietary fiber (IDF), and SDF by independent phosphate buffer methods.

(2) Accept as official first action the nonenzymatic method for TDF, which may be used in the analysis of foods that contain little or no starch (B. Li).

(3) Accept as official first action the rapid gravimetric method for TDF by Mongeau and Brassard.

(4) Continue the collection and evaluation of data for a chemical method for the determination of dietary fiber (O. Theander).

(5) Terminate the Associate Refereeship for the urea–enzyme method for dietary fiber due to inactivity during the last 3 meetings of AOAC.

(6) Official first action method **991.43** is being used in many places in North America and Mexico for food analysis. The method is currently being evaluated for fiber labeling purposes in Europe and Australia.

(7) A physiological enzyme method is currently being evaluated for use in the determination of dietary fiber components such as resistant starch, nonstarch polysaccharides (cellulose, hemicellulose, and others) and lignin.

(8) A dietary fiber definition and analysis survey was organized. The purpose of this survey is to provide general guidelines for various committees throughout the world to delineate dietary fiber definitions and methodology issues. The survey results will be ready in 1993 and published in *J. AOAC Int.*

(9) Because of environmental concerns and the health risks associated with mercury in the protein method, it is suggested that laboratories switch to copper sulfate or copper sulfate–titanium mixes for total Kjeldahl nitrogen determination. Many laboratories already have successfully switched to these catalysts, and this should be noted in the official method for TDF.

(10) An Associate Referee is being sought for the investigation of a number of matrixes where defatting is necessary before determining TDF and where petroleum ether is not sufficient to remove all the fat. The matrixes include mayonnaise, caramel, marinades, frosted desserts, TV dinners, etc.

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Foods II. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Fats and Oils

DAVID FIRESTONE
U.S. Food and Drug Administration,
Division of Contaminants Chemistry, Washington, DC 20204

Emulsifiers.—Associate Referee Theresa Lee reported that James Liu of Ross Laboratories developed a method using normal-phase LC with evaporative, light-scattering detection (ELSD) for separation of major neutral lipid classes including free fatty acids, sterols, monoglycerides, diglycerides, triglycerides, and steryl esters. The method can be used to quantitate monoglyceride content in commercial emulsifiers. Results obtained with the LC/ELSD method agreed well with those obtained by GC and supercritical fluid chromatography (SFC) methods.

The Associate Referee reviewed a method for mono- and diglycerides and other lipid components (1) that involves determination of the silyl ether derivatives by capillary column GC, as does the IUPAC Method for mono- and diglycerides (2). Resolution of analytes (fatty acid methyl esters [FAME], monoglycerides, diglycerides, triglycerides, free sterols, and sterol esters) and the precision of the method were very good, which was indicated by standard deviations reported by the authors. However, accuracy and calibration were not discussed. Although baseline shift and column stability at 350°C (maximum temperature, programmed temperature operation) is a concern, the chromatogram in the report did not show any baseline shift.

Van Oosten et al. (3) reported good separations of a mixture of mono-, di-, and triglycerides by density-programmed capillary SFC on a nonpolar stationary phase (DB-5; temperature, 110°C).

Hydrogenated Fats.—Associate Referee W.M.N. Ratnayake, Food Directorate, Sir Frederick G. Banting Research Centre, Health and Welfare Canada, will initiate a collaborative study of a combined GC/IR method (4–7). Total trans unsaturation in partially hydrogenated fats is determined by an IR procedure similar to that described by Madison et al. (8). Capillary GC provides the proportions of trans 18:2; trans, trans 18:2; and trans 18.3. The trans 18.1 content is calculated from a mathematical formula that allows calculation of trans 18:1 content. The cis 18:1 content is obtained as the difference between total 18:1 fatty acids and trans 18:1. GC is performed on a 100 m × 0.25 mm fused silica column coated with SP-2560 or another suitable cyanoalkylsiloxane, polar, stationary phase.

The Italian Technical Commission of the Oil and Fat Industry, Subcommission on Animal and Vegetable Fats, recently published a capillary column method for determining trans unsaturated fatty acids in fats and oils that specifies use of a 50 m column coated with cyanopropylsilicone (9). I prepared a book chapter reviewing methodology for determination of trans fatty acids (10).

Lower Fatty Acids.—The topic has no Associate Referee. Precht (11) reported detection of as low as 2–5% foreign fats

(vegetable oils and fats) in milk fat by GC analysis of triglycerides and observation of specific triglyceride ratios.

Marine Oils.—Associate Referee, R.G. Ackman, completed work on validation of the capillary column GC method for determination of fatty acid composition of marine oils (12), and the method was adopted first action. The Associate Referee is continuing investigations of fish oils and fish lipids. Preliminary investigation of oxidized lipids in fish meal, in cooperation with the International Association of Fish Meal Manufacturers, suggested that the recovered lipid (gravimetric determination) did not equate well with metabolizable energy.

Newton et al. (13) reported a method for determination of petroleum contaminants in fish products by capillary GC after separation of the contaminants by steam distillation. Not cited by Newton was the first application of steam distillation for separation of petroleum contaminants in fish (14).

The Associate Referee will re-examine the Bligh and Dyer lipid extraction method for marine matrixes following the report of Cabrini et al. (15), which showed that 2 other procedures extracted considerably more total lipids from fish tissues than the Bligh and Dyer method (none of the 3 methods tested recovered neutral lipids, phospholipids, and lipophilic antioxidants with the same efficiency).

According to the supplier, Omegawax 320 capillary columns available from Supelco are optimized to ensure reproducibility of column polarity for analysis of fish oil fatty acid methyl esters. Oxygen-resistant Carbowax columns, such as the Quadrex CW capillary columns, are now available for analysis of marine oil fatty acids.

Olive Oil Adulteration.—Associate Referee E. Fedeli and colleagues (16) performed an LC study of olive oil triglycerides as a means of determining oil quality and observing changes produced by enzymatic activity and industrial treatment (e.g., refining). UV detection at 215, 230, and 268 nm (observation of conjugated isomers in the triglycerides) was used to determine changes caused by autoxidation of an oil or produced by refining.

Cozzoli et al. (17) performed a collaborative study of an LC method (RP-18 column, acetone–acetonitrile [1 + 1] mobile phase) for analysis of olive oil triglycerides and mixtures of olive oil–vegetable oil triglycerides. LC analysis of triglycerides is especially useful for detecting small quantities of linoleic-rich vegetable oils (e.g., sunflower oil) in olive oil, because olive oils contain 0.5% or less of trilinolein compared with substantially higher levels in the linoleic-rich oils. For 9 laboratories that performed the collaborative study, the between-labs coefficients of variation (%) were 51 (0.2% TG [triglyceride]), 30 (0.5% TG), 20 (1% TG), 10 (3% TG), 5 (10% TG), 3 (20% TG), and 2 (50% TG).

Grob et al. (18) reported a coupled LC/GC method for determination of free erythrodiol in olive oil that resembles an LC/GC method developed by Grob et al. (19) for determination of free and esterified sterols and wax esters in olive oil and other vegetable oils. Erythrodiol is present in solvent-extracted, refined olive oils at levels several times as high as levels found in virgin olive oils. Lanzon et al. (19) presented a GC method to the International Olive Oil Council for detection of refined olive oil in virgin olive oil based on determination of stigmasta-3,5-diene, which is present at 3–100 ppm in refined olive oil but is not present in significant quantities in virgin olive oil. Stigmasta-3,5-diene is produced by dehydration of β-sitosterol during refining due to heat treatment and use of bleaching clays in the same way that cholesta-3,5-diene is formed from cholesterol in refined butterfat (20).

Oxidized Fats.—Associate Referee M.M. Blumenthal is continuing investigation of methods of analysis of frying fats that include quick tests for on-site determination of frying fat quality. The Associate Referee is planning collaborative studies of several quick test kits developed in his laboratory. In addition, he has carried out a survey to determine which analytical methods were used by or were of interest to industrial frying operations. For inspection of fresh oils, the following characteristics were of interest: % FFA; % trans acids; iodine value; ppm phosphorous; totox value (anisidine value + 2 [peroxide value]); % mono-, di-, and triglycerides; odor and taste; FAME profile; and Lovibond color (red, yellow, and blue). For recycled frying oils, the following should be determined: ppm soaps (alkaline materials); oxidation products and polymers by LC; sensory evaluation; and ppm trace metals (iron, copper, sodium, potassium, magnesium, and calcium). In-use inspection should include determination of total polar materials. Also, parts per million of water, food leachates (sugars, protein residues, and electrolytes such as citric acid), and filter aid leachates, such as phenolics from paper binders, may be of interest. Safety tests include smoke point, flash point, ignition point, and soluble acrolein and acrolein concentration in workplace air. Stability tests include determination of antioxidants in packaging materials, sensory evaluation of the fried foods, and off-odor development in reheating and microwave treatment.

Nakamura and Maeda (21) described a simple assay for lipid hydroperoxides based on triphenylphosphine oxidation and LC. Total hydroperoxides in lipid samples are determined by direct measurement of triphenylphosphine oxide, which is produced in the reduction of hydroperoxides to hydroxy compounds, by using LC with UV detection. Oishi et al. (22) described a rapid coulometric method for measurement of peroxide value in edible oils and fats in which iodine, which is produced by the reaction of the iodide ion and peroxide in the oil, is electrochemically reduced at a carbon-felt electrode. The authors observed that light affected the peroxide–potassium iodide reaction, increasing the liberation of iodine and indicating that the test should be performed in diffuse daylight or in artificial light shielded from a direct light source. I recommend that **965.33** (Peroxide Value of Oils and Fats) be revised to specify that the test be performed in diffuse daylight or shielded light.

Miyashita et al. (23) described a simple, rapid method for quantitative determination of aldehydes in autoxidized vegetable and fish oils. The analysis is based on the reaction of *N,N*-dimethyl-*p*-phenylenediamine with aldehydes in the presence of acetic acid. The reaction products are determined by visible absorption at 400, 460, and 500 nm, which are the approximate absorbance maxima for alkanals, alkenals, and alkadienals, re-

spectively. Each aldehyde class is estimated in mixtures via equations developed by a 3 × 3 matrix inversion of the relevant molar absorptivities.

Nourooz-Zadeh and Appelqvist (24) described methodology for isolation and quantitation of sterol oxides in vegetable oil and other plant-based foods. Vegetable oil or extracted lipids are chromatographed on a Lipidex 5000 column, and the sterol oxide fraction is enriched by passage through an NH_2 cartridge. The sterol oxide fraction is then derivatized to TMS ethers and analyzed by capillary column GC on a chemically bonded methyl silicone column. Schwartz and Rady (25) reported a relatively simple method for quantitation of hydroxy fatty acids in lipids. A test portion is derivatized with pyruvic acid chloride 2,6-dinitrophenylhydrazone in the presence of triethylenediamine, the derivatives are fractionated on alumina, and the hydroxy fatty acid fraction is evaluated spectrophotometrically at 404 nm.

Pork Fats in Other Fats.—The topic has no Associate Referee.

Sterols and Tocopherols.—Associate Referee R.J. Reina is validating a new method for capillary GC determination of sterol and triterpene diol content of vegetable oils. β-Cholestanol is used as an internal standard.

Pioch et al. (26) reported a rapid method for quantitative determination of sterols in margarine foods by dissolving or suspending a test portion in DMF without prior fat extraction, isolating (solvent extraction) the unsaponifiable fraction, using a micro method for silylation of the sterols, and then analyzing by capillary column GC.

Other Topics.—The Commission on Oils, Fats and Derivatives, Applied Chemistry Division, IUPAC, performed a collaborative study of an LC method for determination of benzo(*a*)pyrene in oils and fats (27). The Commission adopted the method as Method 2.608. I recommend adoption as first action of IUPAC Method 2.608.

Commission on Oils, Fats, and Derivatives, Applied Chemistry Division, IUPAC.—The 45th meeting of the Commission was held in Budapest, Hungary, September 8–11, 1992. The meeting was chaired by Commission Chairman, Albrecht Dieffenbacher. Nine Working Groups (WG) reported on their respective activities: WG 2/87, Trans Fatty Acids by Capillary GC; WG 2/88, Cross Contamination in Bulk Shipments; WG 3/88, Residual Chlorinated Hydrocarbons in Fats and Oils; WG 5/88, Detection of Thermally Treated Oils; WG 7/88, Oxidation of Oils and Fats; WG 1/89, Determination of Cadmium by AAS; WG 1/91, Determination of Chlorophyll (Spectrophotometric Method); WG 2/91, Rapid Determination of Unsaponifiable Matter; WG 3/91, Environmental Impact of Standard Methods; WG 7/88 will publish a final report, and a new Working Group (WG 4/92) will investigate automated, conductivity-based methods for determining oil stability. Adoption was recommended of the spectrophotometric method for determining chlorophyll at 670 nm. Low recoveries of phytosterols were obtained with the rapid method for determination of unsaponifiable matter. The method requires additional work to improve recoveries.

New Working Groups include WG 1/92, which will study determination of fatty acid composition including determination of iodine value by calculation from GC data; WG 2/92, which will recommend replacements for toxic solvents in standard methods; WG 3/92, which will seek to minimize the reagents in the method for acid value; and WG 4/92, which will study methods for determining stability of oils and fats.

The next meeting of the Commission will be held in Lisbon, Portugal, August 6–8, 1993, during the 37th General Assembly of the International Union of Pure and Applied Chemistry.

Recommendations

(1) Revise **965.33** (Peroxide Value of Oils and Fats) by adding a caution that the test be carried out in diffuse daylight or in artificial light shielded from a direct light source.
(2) Adopt as first action the IUPAC method for determination of benzo(*a*)pyrene in oils and fats by LC.
(3) Continue study on all other topics.

References

(1) Mariani, C., Bondioli, P., Venturini, S., & Fedeli, E. (1991) *Riv. Ital. Sostanze Grasse* **68**, 549–551
(2) Bruschweiler, H., & Dieffenbacher, A. (1991) *Pure Appl. Chem.* **63**, 1153–1162
(3) Van Oosten, H.J., Klooster, J.R., Vandeginste, B.G.M., & de Galan, L (1991) *Fat Sci. Technol.* **93**, 481–487
(4) Ratnayake, W.M.N., & Beare-Rogers, J.L. (1990) *J. Chromatogr. Sci.* **28**, 633–639
(5) Ratnayake, W.M.N., Hollywood, R., O'Grady, E., & Beare-Rogers, J.L. (1990) *J. Am. Oil Chem. Soc.* **67**, 804–810
(6) Ratnayake, W.M.N., Hollywood, R., & O'Grady, E. (1991) *Can. Inst. Food Sci. Technol. J.* **24**, 81–86
(7) Ratnayake, W.M.N., & Pelletier, G. (1992) *J. Am. Oil Chem. Soc.* **69**, 95–105
(8) Madison, B.L., DePalma, R.A., & D'Alonzo, R.P. (1982) *J. Am. Oil Chem. Soc.* **59**, 178–181
(9) Technical Commission of the Oil and Fat Industry, Subcommission on Animal and Vegetable Fats, Method UNI 22032 (1991) *Riv. Ital. Sostanze Grasse* **68**, 646–650
(10) Firestone, D., & Sheppard, A. (1992) *Advances in Lipid Methodology-One*, W.W. Christie (Ed.), The Oily Press, Ayr, Scotland, Chapter 8
(11) Precht, D. (1991) *Fat Sci. Technol.* **93**, 538–544
(12) Joseph, J.D., & Ackman, R.G. (1992) *J. AOAC Int.* **75**, 488–506
(13) Newton, J.M., Rothman, B.S., & Walker, F.A. (1991) *J. Assoc. Off. Anal. Chem.* **74**, 986–990
(14) Ackman, R.G., & Noble, D. (1973) *J. Fish. Res. Board Can.* **30**, 711–714
(15) Cabrini, L., Landi, L., Stefanelli, C., Barzanti, V., & Sechi, A M. (1992) *Comp. Biochem. Physiol.* **101B**, 383–386
(16) Cortesi, N., Rovellini, P.A., & Fedeli, E. (1992) *Riv. Ital. Sostanze Grasse* **69**, 1–6
(17) Cozzoli, O., Cortesi, N., Gigliotti, C., Bocca, A., Mattei, A., Berti, G., Pierattini, G., Amelio, M., Sarti, E., & Tiscornia, E. (1991) *Riv. Ital. Sostanze Grasse* **68**, 543–548
(18) Grob, K., Biedermann, M., & Laubli, T. (1989) *J. High Resolut. Chromatogr.* **12**, 49–50

(19) Lanzon, A., Cert, A., & Albi, T. (1991) Instituto de la Grasa y sus Derivados, Seville, Spain, personal communication
(20) Roderbourg, H., & Kuzdzal-Savoie, S. (1979) *J. Am. Oil Chem. Soc.* **56**, 485–493
(21) Nakamura, T., & Maeda, H. (1991) *Lipids* **26**, 765–768
(22) Oishi, M., Onishi, K., Nishijima, M., Nakagomi, K., Nakazawa, H., Uchiyama, S., & Suzuki, S. (1992) *J. AOAC Int.* **75**, 507–510
(23) Miyashita, K., Kanda, K., & Takagi, T. (1991) *J. Am. Oil Chem. Soc.* **68**, 748–751
(24) Nourooz-Zadeh, J., & Appelqvist, L.-A. (1992) *J. Am. Oil Chem. Soc.* **69**, 288–293
(25) Schwartz, D.P., & Rady, A.H. (1992) *J. Am. Oil Chem. Soc.* **69**, 170–173
(26) Pioch, D., Lozano, P., Frater, C., & Graille, J. (1991) *Rev. Fr. Corps Gras* **38**, 381–386
(27) Hendrikse, P.W., & Dieffenbacher, A. (1991) *Pure Appl. Chem.* **63**, 1659–1666

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Foods II. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Fruits and Fruit Products

FREDERICK E. BOLAND
U.S. Food and Drug Administration, Division of Food Chemistry and Technology, Washington, DC 20204

Adulteration of Apple Juice.—Thomas A. Eisele was recently appointed Associate Referee to take the place of John Heuser, who resigned. Both Eisele and Heuser recently published a method entitled "A Rapid Method for Separation and Quantitation of D-Malic Acid in Fruit Juice," (*J. Food Sci.* [1990] **55**, 1614–1616). Eisele plans to collaboratively study this method, which is summarized as follows: An LC method using an aqueous mobile phase containing the chiral ligand-exchanger CU II-L-valine complex at pH 5.5 with a polystyrene divinylbenzene copolymer column was used to resolve D-malic acid in apple, pear, and concord grape juices. D-Malic acid was detected and quantitated at 330 nm in less than 15 min per sample. The detection limit appeared to be 2 mg/100 mL D-malic acid in 12° brix juice, or 0.33% total malic acid in a typical apple juice containing 0.6 g/100 mL. I recommend continued study.

Fruit Acids.—Associate Referee E.D. Coppola developed an LC method to determine major organic acids in apple juice and cranberry juice cocktail that is now official final action (**986.13**). The method continues to be used by his firm to enlarge their data base on cranberry juice and to determine major organic acids in other juices (raspberry, grape, and grapefruit).

Coppola's group experienced resolution problems in using the 2-column system referenced in "Liquid Chromatographic Determination of Major Organic Acids in Apple Juice and Cranberry Juice Cocktail: Collaborative Study" (Coppola, E.D., & Starr, M.S. [1986] *J. Assoc. Off. Anal. Chem.* **69**, 13). Specifically, they were still able to obtain acceptable separation of quinic acid from malic acid and malic acid from citric acid, but separation of citric acid from fumaric acid was not as satisfactory as they had originally observed. They believe that, over the years, column manufacturing has changed slightly and resulted in this resolution loss.

Several months of experimental trials resulted in using KGCQ-324, 5 μm, 23 × 4.0 mm id guard column followed by 2 ymc aq 303-5 HPLC columns, 25 cm × 4.6 mm i.d., 5 μm, flow 0.6 mL/min, and 0.2M potassium dihydrogen phosphate buffer adjusted to pH 2.4. Very satisfactory separation was obtained between citric acid and fumaric acid and among all major acids. Total analysis time was 50 min. A 1-column system, using 0.8 mL/min flow and 0.5M phosphate buffer, can also be used for routine, fast separations. The analysis time is 20 min, and resolution between citric and fumaric acid is acceptable.

The method used by Coppola's firm and their data base on organic acids (quinic mainly) for calculating percent cranberry juice continue to be used by state and private laboratories to assess cranberry juice content and authenticity of cranberry products. The firm will continue to keep current on better and simpler ways to assay major organic acids in fruit juice and to validate any promising new methods. Internally, his organization periodically assesses the precision of their method and the reproducibility of determining percent cranberry juice in blind samples of specific composition. The precision is good.

They are preparing a manuscript for submission to *J. AOAC Int.* entitled "Cranberry Composition Including Nondomestic Varieties. Data Base-Authenticity Parameters." This will update the quinic acid values and show differences in composition varieties.

The Associate Referee recommends continued study on this topic, and I concur with his recommendation.

Adulteration of Orange Juice by Pulpwash and Dilution.—Betsy Woodward, Florida Department of Agriculture, was recently appointed Associate Referee. Continued study is recommended.

Fruit Juices, Identification and Characterization.—Associate Referee Ronald E. Wrolstad, Oregon State University, reports that the interest and need for improved methodology in detecting adulteration of fruit juices is substantiated by the activities of the Technical Committee for Juice and Juice Products (TCJJP). A board meeting was held March 20th, 1992, in Corvallis, OR, and an open meeting of the board is scheduled for August 1, 1992, in Cincinnati, OH, in conjunction with the AOAC annual meeting.

Wrolstad served as chairman in 1991–1992, and Carla Barry, Agriculture Canada, is chairwoman for 1992–1993. Inquiries for membership on the TCJJP should be directed to secretary-treasurer Betsy Woodward, Florida Department of Agriculture.

National Food Processors Association (NFPA), in cooperation with the U.S. Food and Drug Administration (FDA), is in the second year of a 3-year investigation on the composition of

authentic apple juice. The study incorporates collection of authentic samples from the major apple juice processing regions in the world and collaborative analysis of several constituents, such as sugars, nonvolatile acids, metals, and phenolics.

The Associate Referee recommends continued study, and I concur with his recommendation.

Determination of Naringin and Neohesperidin in Orange Juice.—Associate Referee Widmer reports the following: Occasionally, the price of grapefruit juice falls significantly below that of orange juice, to the point where a processor may add grapefruit juice to orange juice to increase profits. Juice blends of sweet orange (*Citrus sinensis*) and grapefruit (*Citrus paradisi*) may be distinguished from blends of sweet orange and other cultivars containing naringin. The FDA standard of identity for frozen concentrated orange juice allows juices from *Citrus sinensis* to contain 10% juice from *Citrus reticulata* or *Citrus reticulata* hybrids and 5% juice from *Citrus aurantium*. Legal blends may be distinguished from adulterated blends by measuring the naringin/neohesperidin ratio in the juice. Whereas juices from cultivars of *C. sinensis* do not contain detectable levels of naringin or neohesperidin, naringin/neohesperidin ratios range from 14 to 83 in grapefruit juice, 1.3 to 2.5 in juices from *C. aurantium*, and are always <1 in juices of *C. reticulata* hybrids containing naringin.

The collaborative study showed the method to be sensitive for naringin and neohesperidin detection down to 5 ppm. Provided nonabsorbing filters are used for sample preparation, the method is adequate to detect the presence of grapefruit juice in orange juice using lower limits of 10 ppm naringin and <3 ppm neohesperidin detected for confirmation. This would represent grapefruit juice addition of 1–3%. However, should interferences occur in the elution area of neohesperidin, grapefruit juice addition could falsely be accepted as addition of juice from sour orange. The method is acceptable provided a small number of false negatives can be tolerated.

In the past year, different mobile phase compositions were evaluated in a somewhat successful attempt to minimize peak tailing on problem analytical columns and to eliminate the small interfering peaks. Once the conditions have been established, another collaborative study will be conducted using the new parameters.

The Associate Referee recommends continued study on this topic, and I concur with his recommendation.

Sodium Benzoate in Orange Juice.—In the national survey of commercial single-strength and reconstituted frozen concentrated orange juice, 0.6.–226.2 ppm benzoic acid was found in 13 samples, and 0.9–6.9 ppm sorbic acid was found in 2 samples. Four samples showed both benzoate and sorbate. A total of 1120 samples were tested from July 1, 1991, through June 30, 1992.

For the collaborative study of benzoic acid in orange juice, the initial draft of the AOAC collaborative study was prepared and submitted to AOAC methods committee, the General Referee, and the statistician. Also, a copy of the report was sent to the 10 participating laboratories. The first revised draft was submitted in response to the suggestions issued by the AOAC statistician. The General Referee, committee statistician, and safety advisor accepted the draft. A final draft was forwarded to the methods committee on Foods II for final approval. Comments from 6 reviewers from the committee were received. Changes were made to address the issues raised by committee members, and valid suggestions were incorporated in a revised final draft. This revision was submitted to the methods committee on Food II.

The Associate Referee recommends continued study, and I concur with his recommendation.

Stable Carbon Isotope Ratio Analysis of Fruit Products.—No activity was reported. Continued study is recommended.

Application of Computer Pattern Recognition Analysis of Trace Elements for Determination of Geographical Origin of Orange Juice.—Associate Referee Seif Nikdel reports that he developed 2 neural network applications that determine whether orange juice complies with the U.S. government labeling regulations and the Florida State quality regulations.

The reason for the project was that the Florida Department of Citrus has financial incentive programs for resellers outside of the state to sell "pure Florida" orange juice. The state checks batches of orange juice to make sure the reseller is telling the truth.

The network trains on a data base that took 2 years to develop. A contractor collected samples of oranges grown in Florida, California, Arizona, Brazil, Mexico, and Belize. Samples from Costa Rica were obtained through U.S customs in Miami. The Associate Referee analyzed the samples for the presence of 15 minerals, including sodium, calcium, and potassium, using inductively coupled plasma atomic emission spectrometry to measure the amounts of minerals in each sample.

The data base was first used with a statistical program called Arthur. This took a year to develop. Arthur accurately characterized the country of origin but could only be operated on a Vax minicomputer, because it required the entire training data base to be available in computer memory for test identification. Unlike a neural network, Arthur had no learning ability.

After developing Arthur, the Associate Referee learned about neural networks and decided the problem could better be handled by neural network. He used NeuroShell along with a NeuroBoard to train on the data base. The neural network's success rate was greater than Arthur's and made classifications faster. The neural network system can be run on a personal computer, because it does not require the data base to be held in memory.

The first neural network determines the state or country where the oranges were raised, thereby providing information for the processor to comply with the U.S. customs requirement to list on the container the country of origin for each juice lot.

The second neural network determines whether the juice was fresh-squeezed from the orange without anything added ("single-strength orange juice" in the technical jargon) or whether it contains pulpwash, a by-product that is left when the juice is squeezed from the orange.

Florida State law prohibits inclusion of pulpwash during the preparation of frozen concentrated orange juice processed within Florida. However, Florida processors are allowed to make and sell pulpwash to be added to beverages. Each year, Florida processors produce an average of 30 million pounds of pulpwash, which sells for $0.90/lb and yields $27 million in

revenue. Sodium benzoate must be added to pulpwash in Florida as a tracer for monitoring orange juice.

Presently, UV-vis absorption and room temperature fluorescence excitation and emission spectra are used to detect adulteration and approximate the pulpwash content when it is >10%.

A neural network was built to distinguish between juice lots that were pure orange juice and those that contained 1, 5, 10, and 100% pulpwash. Inductively coupled plasma atomic emission spectrometry was used to analyze samples for mineral concentrations. The measurements were then fed to the neural network, which determined whether the samples contained pulpwash. Neural network was able to separate a class of orange juice with 1% pulpwash content from another class with 100% pure orange juice.

The Associate Referee recommends continued study, and I concur with his recommendation.

Recommendation

Continue study on all topics.

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Foods II. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Nonalcoholic Beverages

JOHN M. NEWTON
U.S. Food and Drug Administration, 50 United Nations Plaza, San Francisco, CA 94102

Richard Thomson, Associate Referee for coumarin in vanilla-flavored beverages, will be presenting a paper at the 106th AOAC international meeting. The paper describes an LC procedure that measures coumarin, vanillin, and ethyl vanillin in 40 products representing 14 brands of vanilla extract.

The Associate Referee for safrole in sassafras root, Marvin Carlson, is in the process of obtaining various sassafras products for his LC method. He has already obtained 6 samples.

Francis J. Farrell recently collaborated his method through the ISO for the determination of ash content of instant tea. The method was collaborated using 5 tea powder samples using 19 laboratories worldwide and was adopted as an official method through ISO. Farrell would like to submit the method and data from the ISO study for review and possible adoption by AOAC.

The Associate Referee for caffeine and methylxanthine in nonalcoholic beverages, M. Newton, recommends that work continue on this subject.

Recommendations

(1) Appoint an Associate Referee on the analysis of quinine.
(2) Study the collaborative results from Francis J. Farrell's ISO method for determining the crude fiber in instant tea as a possible AOAC method.
(3) Continue study on all topics.

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Foods II. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Processed Vegetable Products

THOMAS R. MULVANEY
U.S. Food and Drug Administration, Division of Food Chemistry and Technology, Washington, DC 20204

LC Determination of Sugars in Processed Vegetables.—Associate Referee Peter H. Yu submitted the following report: Experiments conducted in the past year focused on eliminating interference in the region of the sugar peaks. The first experiments used the silver nitrate precipitation technique to remove salt, a calcium carbonate precipitation technique coupled with a low-level sugar extraction method (using 80% ethanol and evaporation of the alcohol by rotary evaporation), and an ion-exchange method (minicolumn and cartridge of AG 50 W × 8 cation exchanger). Best results were found with the ion-exchange method. The second experiments used Maxi-Clean IC-Ag cartridges to remove salts from the samples. Promising results were also found. The next phase will involve a detailed study of the ion-exchange methods (both the AG 50 W × 8 and the Maxi-Clean IC-Ag) using spikes at several levels to determine percent recoveries of sugar samples. If the results are satisfactory, a final report together with the LC protocol will be written and submitted to the AOAC methods committee for approval for a collaborative study.

pH Determination.—No favorable response was received to efforts to obtain an Associate Referee for this topic. Study of new instrument developments is needed (e.g., alternative reference electrodes and cleanable junction systems).

Sodium Chloride.—Associate Referee J. Anderson Williams completed a review of methodology and a determination of current needs. He reported that the widely used, currently available methodology reflects a level of technical sophistication appropriate to this application and meets current and anticipated needs. Therefore, he recommended no further study at this time.

Total Solids by Microwave Moisture Methods.—Associate Referee Henry B. Chin amended this official first action status method by adding the instrument warmup and calibration pro-

cedures that were included in the collaborative study on the determination of total solids in processed tomato products by microwave drying oven (*Official Methods of Analysis* [1992] 15th Ed., Supplement 3, **985.26**). Results of a related study were published by Wang (*J. Assoc. Off. Anal. Chem.* [1987] **70**, 758–759). Review and comments are requested.

Water Activity in Foods.—The Associate Referee reported no progress, and he will no longer be available for this topic after September 1992. Study of new instrument developments is needed.

Other

Intrinsic Chemical Marker Measurement of Heat Applied in Aseptic Processing of Particulates.—Results of research from the leading laboratory scientists in this field were recently summarized. The results are encouraging. Validation of the important relationship between the formation of the markers and the destruction of the microorganisms remains to be completed (Kim, H., Taub, I.A., Richardson, M., Kustin, K., & Ross, E. [1992] "Intrinsic Chemical Markers for Validating the Sterility of Aseptically Processed Particulates, "*Proceedings of R & D Associates Meeting, Activities Report*, **441**, 120–129; Research and Development Associates for Military Food and Packaging Systems, Inc., San Antonio, TX).

Recommendations

(1) *LC Determination of Sugars.*—Continue study.
(2) *pH Determination.*—Appoint new Associate Referee. Continue study.
(3) *Sodium Chloride.*—Discontinue topic.
(4) *Total Solids by Microwave Moisture Methods.*—Continue study.
(5) *Water Activity in Foods.*—Appoint new Associate Referee. Continue study.

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Foods II. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Spices and Other Condiments

JAMES E. WOODBURY
14382 Holt Ave, Santa Ana, CA 92703

Capsicum Spices and Oleoresins: Pungency.—Associate Referee Mark Parrish, McCormick & Co., reports that a preliminary study was completed. The protocol for the collaborative study comparing methods is being prepared.

Curcumin in Turmeric.—Associate Referee Ted Lupina, Kalsec, Inc., reports that more labs are sought for the collaborative study.

Moisture in Dried Spices and Vegetables.—Associate Referee Lou Sanna, California Chili and Spice, reports that a collaborative study is in progress.

Volatile Oil in Cassia: Sample Preparation.—Associate Referee Phil Guarino, McCormick & Co., reports that a collaborative study was completed and a final report is being prepared.

Sumac in Oregano.—Associate Referee Jonathon Yentis, Tone Brothers, Inc., reports a method was completed, and study is continuing.

Water Activity of Spices.—Associate Referee Susan Schena, Cal-Compack Foods, reports a collaborative study is in progress.

Recommendations

(1) Recommend continuation of study of *Capsicum Spices and Oleoresins: Pungency.*
(2) Recommend continuation of study of *Curcumin in Turmeric.*
(3) Recommend continuation of study of *Moisture in Dried Spices and Vegetables.*
(4) Recommend continuation of study of *Volatile Oil in Cassia: Sample Preparation.*
(5) Recommend continuation of study of *Sumac in Oregano.*
(6) Recommend continuation of study of *Water Activity of Spices.*
(7) Recommend AOAC method **987.07** (Piperine in Pepper Preparations) be revised to indicate "60 mesh" instead of "30 mesh" as a minor change based on in-house data.

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Foods II. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Sugars and Sugar Products

MARGARET A. CLARKE
Sugar Processing Research Institute, Inc.,
1100 Robert E. Lee Blvd, New Orleans, LA 70124

Corn Syrup and Sugar Products.—Report from Associate Referee Raffaele R. Bernetti will follow.

Enzymatic Methods in Sugar Analysis.—Associate Referee Guenther Henniger recommends continued studies.

Glucoamylase (Amyloglucosidase) Enzyme Activity.—Associate Referee Michael T. Elder completed his collaborative study and is preparing the report for publication.

Honey.—Associate Referee Jonathan W. White reports that, in the past year, a modified protocol was developed for the isolation and purification of the honey protein required for the internal standard isotope ratio method for honey analysis. It was tested as required by the AOAC and added to the collaborative study manuscript.

Method **978.17** for SCIR analysis of honey specifies use of batchwise instrumentation for the analysis: combustion and analysis are carried out in separate equipment. A recent advance in isotope ratio instrumentation is the continuous-flow automated ^{15}N ^{13}C analyzer. Sample preparation and analysis are completely automated and performed in ca 6 min with no attention by the operator. This greatly reduces costs and possible errors in sample combustion and transfer of gases. There are at least 22 installations of this equipment in the United States and Canada, as well as at least 61 elsewhere.

The British company (Europa Scientific Ltd.) that manufactures the equipment analyzed hundreds of honey samples, many by the new internal standard procedure that the Associate Referee provided to them. The desirability of qualifying the instrumentation for this use by AOAC was mentioned in a recent paper in *J. AOAC Int.* from Europa. A set of samples used in the collaborative test of the ISIRA method for C-4 sugars in honey was analyzed by Europa, without any indication of the previous results.

The Associate Referee recommends that, because this study meets the requirements of a "method modification" by showing equivalent performance for the 2 procedures, the use of ANCA-MS as an alternative procedure be approved. I concur with this recommendation.

Lactose Purity Testing.—Associate Referee Janice R. Saucerman reports no new progress at this time.

Liquid Chromatographic Methods.—A new Associate Referee is needed.

Maple Sap, Maple Syrup, and Maple Products.—Lynn Whalen, SMI Consulting, Inc., former Associate Referee for this topic until 1989, agreed to resume duties as Associate Referee.

Methods Standardization.—Associate Referee Mary A. Godshall reports no new progress at this time.

NIR Analysis of Sugars.—Associate Referee Cynthia McDonald-Lewis is preparing a method for collaborative study. The preliminary method (Application of NIR to Sugars Analysis—Comparison with Traditional Methods) will be presented at the 106th AOAC International Annual Meeting.

Oligosaccharides.—Associate Referee George Steinle reports that a study on analysis of raffinose in molasses by high-performance anion chromatography is in progress.

Polarimetric Methods.—Associate Referee Ronald Plews reports no new activities.

Stable Isotope Ratio Analysis.—Associate Referee Landis W. Doner reports that his study, approved official first action in 1991, on "Detecting Adulteration of Frozen Concentrated Orange Juice with Sugar-Beet Derived Syrups on the Basis of $^{18}O/^{16}O$ Measurements in Water" is awaiting publication in *J. AOAC Int.*

Sugars in Cereals.—Associate Referee Lucien Zygmunt reports trials with high-performance anion chromatography on sugars in cereals and experiments on separations of mono-, di-, tri-, and tetrasaccharides to meet labeling requirements.

Sulfites-Screening Methods.—Associate Referee Richard Riffer reports the following 2 new approaches to sulfite analysis in foods that may be applicable to sugar products: a sulfite selective enzyme electrode [*J. Biotechnol.* (1991) **18**(1–2), 93–102] and a pulsed-amperometric system for sulfite in beer [*J. Am. Soc. Brew. Chem.* (1992) **50**, 1–3].

Visual Appearance of Sugar by Color, Turbidity, and Reflectance.—Associate Referee Margaret A. Clarke reports a planned collaborative study on combined color and turbidity determination in raw sugar samples.

Weighing, Taring, and Sampling.—Associate Referee Michael Steele reports no new activity at this time.

Recommendations

(1) The addition of the procedure of ANCA-MS as a method modification, an automated instrumental procedure for analysis **978.17**, C-4 plant sugars in honey, is recommended. Results comparing analyses by the current and proposed additional methods were reported.
(2) Continue study on all other topics.

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Foods II. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Vitamins and Other Nutrients

MIKE J. DEUTSCH
U.S. Food and Drug Administration, Division of Nutrition, Washington, DC 20204

Thirteen papers in this topic area were presented as a poster session at the 106th annual meeting of the Association. Four additional nutrient papers were published in the Journal during the last year.

Analysis of Milk-Based Infant Formula.—Phase V (Folic Acid, Pantothenic Acid, Vitamin E, and Vitamin A) was made official first action.

The Official Methods Board is reorganizing the Food Committees. Foods Committee II is evaluating methodology for nutrient labeling purposes. When this is finished, many Associate Referee assignments will be made and collaborative studies will be initiated.

Recommendation

Continued study on all topics.

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Foods II. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Committee on Residues

Metals and Other Elements

STEPHEN G. CAPAR
U.S. Food and Drug Administration,
Division of Contaminants Chemistry, 200 C St, SW,
Washington, DC 20204

As Associate Chapter Editor, I reviewed Chapter 9, "Metals and Other Elements at Trace Levels in Foods," *Official Methods of Analysis* (1990) 15th Ed., for the appropriateness of the methods with respect to currently used analytical techniques. The chapter is in need of a major update to analytical methods using modern analytical equipment and to reduce chemical wastes. To begin the update, a number of the methods are recommended for surplus action at the end of this report. Most of these methods use colorimetric detection and do not detect the analyte at current levels of interest.

New analytical methods for the chapter should be sought that use modern analytical equipment. The analytical techniques commonly used for trace elements in foods are flame atomic absorption spectrometry (AAS), electrothermal atomization AAS (also referred to as graphite furnace AAS), inductively coupled plasma atomic emission spectrometry (ICP AES), and electroanalytical techniques. For example, the following additions should be made to the chapter: An electrothermal atomization AAS method is especially needed for analysis of foods for Pb and other elements such as Cd, Cr, and Ni; a method using the multielement capabilities of ICP AES should be included in the chapter for analysis of foods for trace elements, particularly elements of nutritional interest; methods using commercial vapor generation equipment for determination of As, Se, and Hg with either AAS or ICP AES would be welcome additions, especially for analysis of seafood; modern food mineralization procedures, such as microwave digestion, should also be pursued.

Volunteers to assist on updating the methods outlined above will be appreciated.

The analytical method for leachable cadmium and lead from earthenware is in Chapter 9 (**973.32**). However, the subject index to *Official Methods of Analysis* directs the reader to page 232, method **975.05** in Chapter 8, which in turn directs the reader to method **973.32** in Chapter 9. Two different designations for the same method leads to confusion when referencing the analytical method. In addition, method **975.05**, *Cadmium and Lead in Earthenware*, erroneously directs the reader to method **973.33**, *Arsenic in Meat and Poultry*.

Changes in the concentration of Pb standard solutions in method **973.32**, *Cadmium and Lead in Earthenware*, were approved in 1987. The change was replacement of a 20 μg/mL Pb standard solution with a 3 μg/mL Pb solution. The affect of this change to the Determination section [**973.32D**(a)] was not addressed at that time. The instructions in this section to dilute samples containing >20 μg Pb/mL should be changed to >15 μg Pb/mL, because this is now the highest standard level. The instructions to prepare a 3 μg Pb/mL solution instead of a 20 μg Pb/mL solution should be deleted, because the change to the method accommodates this instruction.

Two new Associate Referees were appointed during the past year, and one topic was transferred to the General Referee topic Metals and Other Elements. All Associate Referees were sent letters requesting their annual reports. Their responses follow.

Atomic Absorption Spectrophotometry (AAS).—Associate Referee Milan Ihnat reports that 10 new natural matrix Agricultural/Food Reference Materials were characterized with respect to their elemental compositions via an extensive interlaboratory characterization (certification) campaign. The inherent philosophy applied to the arrival at best-estimate and informational concentration values was the expert analyst-independent method approach. Consequently, chemical analyses were conducted involving the initiating laboratory and analysts in 71 cooperating laboratories. Thirteen major classes of independently different analytical methods were applied, which led to 13 000 results for a large number of nutritionally, toxicologically, and environmentally pertinent elements. In-depth assessment of results using technical and statistical criteria, as well as judgment, led to 278 best-estimate and informational concentration values for 34 elements, with each material having typically 21 best estimates and 7 informational values. The majority (213) of these are best-estimate values with associated uncertainties representing a 95% confidence interval for a single future observation; 85 are informational values. Concentrations are available for Al, As, B, Ba, Br, Ca, Cd, Cl, Co, Cr, Cs, Cu, F, Fe, Hg, I, K, Mg, Mn, Mo, N, Na, Ni, P, Pb, Rb, S, Sb, Se, Sr, Ti, V, W, and Zn in bovine muscle powder, whole egg powder, microcrystalline cellulose, wheat gluten, corn starch, corn bran, whole milk powder, durum wheat flour, hard red spring wheat flour, and soft winter wheat flour. A wide range of matrixes and elemental contents are represented, including nutritionally and agriculturally important B, I, N, P, and S and 2 products with low elemental (blank) levels. These materials are available from the Standard Reference Materials Program, National Institute of Standards and Technology, Gaithersburg, MD 20899. Certificates of Analysis contain complete information regarding material preparation, composition, certification, and concentration values with associated uncertainties.

Fluorine.—Associate Referee Robert Dabeka did not report on this topic.

Graphite Furnace-Atomic Absorption Spectrophotometry (GF-AAS).—Associate Referee Robert Dabeka did not report on this topic.

Lead in Calcium Supplements.—Paul H. Siitonen was appointed Associate Referee on October 31, 1991. Methodology was developed to analyze a wide variety of Ca health food supplements for Pb. The analysis involves a combination dry-ash/wet digestion of the supplements, followed by quantitation of Pb by GFAAS. Supplements are dry-ashed at 450°C, mineralized with a mixture of HNO_3 and H_2O_2, and transferred to volumetric flasks with 1N HNO_3; $(NH_4)_2HPO_4$ is added as a matrix modifier to a final concentration of 0.5%. GFAAS analysis, performed at 283.3 nm, employs Smith-Hieftje background correction. Analysis of a variety of Ca supplement matrixes fortified with 5.00 μg Pb yielded recoveries averaging 103 ± 11% (n = 20). Results from analyses of National Institute of Standards and Technology's Standard Reference Material 1572 Citrus Leaves averaged 13.2 ± 0.6 μg Pb/g (certified value, 13.3 ± 2.3 μg Pb/g). The method's limit of quantitation ranged from 0.05 to 0.20 μg Pb/g on the basis of test portions of 2.0 to 0.5 g, respectively.

Neutron Activation Analysis (NAA).—Associate Referee William C. Cunningham reported continued development of a method for the determination of Na in biological materials. The method was rewritten to exclude less common NAA laboratory procedures. An interlaboratory method trial is expected to be completed by next year. Cunningham is the AOAC Liaison Officer to the American Society for Testing and Materials' Task Group on Nuclear Methods of Chemical Analysis. He continues to actively participate with this Group on issues related to the development of standard NAA methods. Cunningham also reports that the infant formula reference material discussed last year continues to be characterized for inorganic and organic nutrients and was designated by the National Institute of Standards and Technology as Standard Reference Material 1846.

Organotin in Foods.—Associate Referee Allen Uhler did not report on this topic.

Organometallics in Fish.—Associate Referee Walter Holak did not report on this topic. Two laboratories submitted comments on the official first action method **990.04**, Mercury (Methyl) in Seafood Liquid Chromatographic-Atomic Absorption Spectrophotometric Method. The comments were forwarded to Holak for his review and consideration for changes to the method.

Lead in Wines.—Associate Referee Alan Reisig did not report on this topic. This topic was transferred from the Committee on Foods II.

Graphite Furnace Atomic Absorption Spectrophotometric Determination of Lead and Cadmium Released from Ceramicware.—Susan C. Hight was appointed Associate Referee on May 4, 1992. An interlaboratory trial is underway to evaluate a GFAAS method for determination of Pb released from ceramicware into 4% acetic acid. The purpose of the trial is to assess the clarity of the method's written instructions, the precision and accuracy of the method when used by 3–4 different laboratories, and Pb levels found in controls (method blanks) prepared by participating laboratories. Participating laboratories are tentatively scheduled to begin analyses in Summer 1992. An AOAC collaborative study for Pb and Cd in leach solutions will be conducted upon successful completion of the interlaboratory trial.

Recommendations

(1) Designate spectrophotometric method **964.16**, *Antimony in Food*, as surplus.
(2) Designate Kjeldahl flask digestion method **963.21**, *Arsenic in Food*, as surplus.
(3) Designate molybdenum blue method **942.17**, *Arsenic in Food*, as surplus.
(4) Designate silver diethyldithiocarbamate method **952.13**, *Arsenic in Food*, as surplus.
(5) Designate molybdenum blue method **973.33**, *Arsenic in Meat and Poultry*, as surplus.
(6) Designate dithizone method **945.58**, *Cadmium in Food*, as surplus.
(7) Designate colorimetric method **960.40**, *Copper in Food*, as surplus.
(8) Designate colorimetric method **944.07**, *Fluorine on Apples and Pears*, as surplus.
(9) Designate colorimetric method **935.51**, *Lead on Apples and Pears*, as surplus.
(10) Designate general dithizone method **934.07**, *Lead in Food*, as surplus.
(11) Designate colorimetric dithizone method **952.14**, *Mercury in Food*, as surplus.
(12) Designate colorimetric method **944.09**, *Zinc in Food*, as surplus.
(13) Continue official first action status of the liquid chromatographic-atomic absorption spectrophotometric method for methyl mercury in seafood (**990.04**).
(14) Adopt as official final action the graphite furnace atomic absorption spectrometry method for copper, iron, and nickel in edible oils and copper and iron in edible fats (**990.05**).
(15) Make editorial change to method **975.05**, *Cadmium and Lead in Earthenware*, by eliminating the reference to see method **973.33**.
(16) Remove method **975.05**, *Cadmium and Lead in Earthenware*, from Chapter 8 of the *Official Methods of Analysis* (1990) 15th Ed.
(17) Modify method **973.32**, *Cadmium and Lead in Earthenware*, by changing **973.32D**(a), second paragraph, first sentence from "Dil. samples contg >20 μg Pb/mL with 4% HOAc." to "Dil. sample contg >15 μg Pb/mL with 4% HOAc."
(18) Modify method **973.32**, *Cadmium and Lead in Earthenware*, by changing **973.32D**(a), second paragraph, last sentence from "Det. Pb as above, except substitute 3.0 mL and 3 μg/mL for 20.0 mL and 20 μg/mL, resp., in **973.32B**(c)(*2*)." to "Det. Pb as above."
(19) Continue study on all other topics.

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Residues. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Multiresidue Methods

LEON D. SAWYER
U.S. Food and Drug Administration,
Division of Contaminants Chemistry, 200 C St SW,
Washington, DC 20204

Comprehensive Multiresidue Methodology.—(Associate Referee S. Mark Lee, California Department of Food and Agriculture, Sacramento, CA.) No report from the Associate Referee was received.

Fumigants.—(Associate Referee James L. Daft, U.S. Food and Drug Administration [FDA], Kansas City, MO.) During the past year, the Associate Referee successfully adapted a manual headspace analytical procedure to detect methyl bromide residues in selected food items. The procedure is based on the automated closed system described by the Associate Referee on Methyl Bromide [*J. AOAC Int.* (1992) **75**, 328–334]. The manual headspace procedure will also be published in *J. AOAC Int.* in the upcoming year. The Associate Referee will continue with plans to collaboratively study the multifumigant method that was submitted for protocol approval 3 years ago. The protocol was approved at that time, but the study was not completed because of the priority placed on the methyl bromide investigations.

Low Moisture–High Fat Samples.—(Associate Referee Gregory Beard, Hershey Chocolate USA Laboratory Services, Hershey, PA.) The Associate Referee reported no work on this topic was accomplished this year.

Miniaturized Multiresidue Methods for Fat Containing Foods.—(Associate Referee D. Ronald Erney, FDA, Pesticides and Industrial Chemicals Research Center, Detroit, MI.) By using an adaptation of the miniaturized milk method described in last year's report, the Associate Referee was able to recover tetrahydrophthalimide (THPI—metabolite of captan) at ca 80% from fortifications of 0.05, 0.10, and 0.20 ppm. Minor changes in the partitioning step and an additional cleanup by elution through a diol cartridge were necessary. Quantitation was performed with an N/P detector after chromatographing THPI on a 20 m DB-17 wide-bore column at 160°C.

Gas chromatographic "enhancement" effects were also reported on last year and were investigated further. The Associate Referee reported that responses of certain polar chemicals that are chromatographed in the presence of sample matrix are routinely greater than the response of the same chemical amount chromatographed in "pure" solvent. His investigations concluded that the enhancement was conclusively the result of a chromatographic adsorptive effect on the "pure" chemical versus the other postulated cause, detector response enhancement from matrix materials. He plans to attempt eliminating the adsorption by adding chemical modifiers (not blank matrix extracts) to standard solutions.

Miniaturized Multiresidue Methods for Nonfatty Foods.—(Associate Referee Charles H. Parfitt, Jr, FDA, Washington, DC.) The miniaturized method reported on and presented at last year's AOAC meeting demonstrated comparable recoveries of 7 pesticides from 6 crops, and 6 incurred pesticides in 3 crops were obtained using both **985.22** and the proposed method. This information was published in an FDA Laboratory Information Bulletin. The comparison study used the same extraction step described in **985.22**; 100 g is extracted with 200 mL acetone. Investigations this past year focused on miniaturizing the extraction step.

Extraction efficiency comparisons among **985.22** and 25 g/50 mL miniaturized approaches were conducted using crops with incurred residues. The miniaturized extraction approaches were as follows: Waring blender with a 1 pint cup followed by filtering, Polytron homogenization in a 125 mL Erlenmeyer flask followed by filtration, and Polytron homogenization followed by centrifuging. Both filtration procedures gave comparable results, and the centrifuge procedure was found to be impractical and was abandoned. Comparisons of extraction efficiencies among **985.22** and the 2 filtration procedures with incurred parathion in lemons are given in Table 1.

Blending and homogenization results were comparable and indicated either approach could be used, but blending was judged as more convenient because of cleaning considerations. All additional comparison extraction studies used the blending approach.

Further comparisons of extraction efficiencies between **985.22** and blending 25 g in 1 pint cups were carried out with pears containing methamidophos and kiwi fruit containing pirimiphos methyl and diazinon. In addition to extraction comparisons, 20 mL aliquots of **985.22** extractant were analyzed by the miniaturized cyrogenic solvent exchange (CSE), and the results (average of duplicates) are given in Table 2.

The Associate Referee noted that results from 3 of the 4 residues extracted by the 25 g/50 mL approach were ca 10% higher than those obtained with **985.22**. Methamidiphos in pears was the exception to the trend, and this result was ca 20% lower. The lower result, in this instance, appears to be related to a poorer partitioning ratio in the CSE approach, because portions of the same **985.22** extractant were analyzed both ways and the CSE results are in close agreement. Plans are to continue the method comparisons with additional crops that contain incurred residues to see if the apparent trend continues. A current inventory of 12 different crops and 14 different incurred organohalogenated and organophosphorus pesticides are available for the study comparisons.

Investigation of a sweep codistillation (Unitrex™) cleanup of extracts prepared by the miniaturized CSE procedure was initiated. It was shown that with special treatment of the distillation tubes, employment of a specialized sample introduction

Table 1. Comparative parathion extraction: 985.22 and miniaturized

Method	Weight, g	Acetone, mL	Mean, ppm (n = 4)	RSD, %
985.22	100.0	200.0	1.08	3.5
Blender	25.0	50.0	1.18	2.2
Polytron	25.0	50.0	1.17	3.6

technique, and use of silica gel G (10% H_2O deactivated) as the trapping medium, quantitative recoveries of methamidophos, acephate, omethoate monocrotophos, dimethoate, parathion, methyl chlorpyriphos mercabam, and ethion were obtainable. Four commodities were included in this recovery/cleanup investigation; lemons, lettuce, cabbage, and onions. Significant visual and chromatograhical cleanup improvements were reported with the application of this step.

Plans to initiate method adaptation and recovery information with *N*-methylcarbamates were delayed because of problems obtaining a functional LC system. A system is currently being rebuilt and should be available for these investigations in the near future.

In an activity not directly related to miniaturization, last year the Associate Referee gave a poster presentation at the AOAC meeting on an interlaboratory study of wide-bore capillary GC quantitation using phosphorus-selective flame photometric detection of 23 organophosphorus pesticides added to 5 different crop extracts prepared by **985.22** methodology. Both methyl silicone (DB-1) and phenyl methyl silicone (DB-17) columns were evaluated. Also, different and dissimiliar injection volumes/concentrations were included in the study. The results of this study were drafted into manuscript format for submission to *J. AOAC Int.* Assuming favorable peer review and publication, the DB-17 column will be recommended as an addition to **985.22** as an alternative GC column to DEGS for this class of pesticides.

Supercritical Fluid Extraction of Pesticide Residues in Food.—(Associate Referee, Marvin L. Hopper, FDA, Total Diet Research Center, Lenexa, KS.) Research efforts of the Associate Referee were targeted toward the development of a multivessel, large-volume supercritical fluid extractor using CO_2 as the extractant. His efforts resulted in a working prototype that can extract 6 samples (volumes to 100 mL) simultaneously. Problems with pump failure and contamination from valves and commercial-grade CO_2 were encountered. Contamination problems were eliminated by changing the valves and adding a filter system to the CO_2 inlet. Pump failure is still a problem, and the Associate Referee is working closely with the manufacturer in an effort to extend the pump durability. The pump must cycle every 3 s and deliver 30 L/min gaseous CO_2 to the 6 extraction vessels.

Validation of the extraction efficiency was performed by using comparative fat amounts obtained from pork sausage, peanut butter, cheddar cheese, and corn chips that were extracted by Soxhlet (**960.39**), FDA Pesticide Analytical Manual Vol. I [PAM I/§211.13 (f), (k), and (c)], and SFE. Mean percent fat values and %RSDs obtained by the 3 approaches are given in Table 3.

Another validation comparative study was performed with previously analyzed samples (cheddar cheese, saltine crackers, sandwich cookies, and cooked ground beef) that were solvent-extracted and known to contain pesticide residues. These samples were extracted by SFE, and the resultant fat was cleaned up by the same gel permeation/Florisil chromatographic procedures originally used. Residue amounts were determined by the same GC procedures. Comparative findings are given in Table 4.

The potential for cross-contamination between vessels during extraction and cross-contamination between a following series of extractions was investigated. Butter, fortified at 100 ppm *cis*-chlordane and malathion, was extracted in 3 vessels simultaneously with 3 vessels containing unfortified butter. After a 15 mL solvent flush of the plumbing followed by N_2 drying, unfortified butter was extracted in the 3 vessels that contained 100 ppm fortifications. Complete recoveries of *cis*-chlordane and malathion (range, 93–100%) were obtained, and no carryover to any of the unfortified butter was detected. A series of 6 butter samples fortified at 0.1 ppm *cis*-chlordane and malathion were also extracted, and all recoveries were within a range of 95–107%.

Sweep Codistillation.—(Associate Referee Barrie Magor, Australian Government Analytical Laboratorites, Melbourne, Australia.) The Associate Referee is preparing letters of invitation to laboratories that may be interested in collaboratively studying the sweep codistillation (SCD) cleanup procedure. He anticipates between 10–15 laboratories will be eager to participate. Protocols for the planned study should to be forwarded for review in the near future. The planned study is to be separate from the Australian National Pesticide Residue Proficiency Testing Program (NPRPTP), as originally recommended.

The results of behavior studies with linuron, nonachlor, chlorfenvinphos, captan, mirex, coumaphos, and ronnel during routine SCD cleanup operations were reported. Nonachlor, mirex, and ronnel were routinely recovered at ≥80%. An increase in distillation temperature and changes in trap elution solvent were necessary to obtain good coumaphos recoveries.

Table 2. Comparative extraction and phase separation: 985.22 and miniature

Method	Extract/solvent exchange	Pears, methamidophos, ppm	Kiwi, diazinon, ppm	Kiwi, pirimphos methyl, ppm
985.22	100 g/200 mL, 80 mL/shake out	0.464	0.018	0.241
Combined	100 g/200 mL, 20 mL/CSE	0.388	0.018	0.224
Miniature	25 g/50 mL, 20 mL/CSE	0.374	0.020	0.264

Table 3. Percent fat extracted (%RSD, n = 6)

Sample	960.39	PAM I	SFE
Pork sausage	30.55 (4.75)	29.83 (1.55)	29.83 (1.32)
Peanut butter	50.32 (0.39)	49.29 (0.60)	49.51 (0.44)
Cheddar cheese	33.85 (3.13)	33.94 (3.46)	33.26 (1.14)
Corn chips	31.32 (0.62)	31.80 (0.76)	31.51 (0.46)

Linuron, chlorfenvinphos, and captan produced erratic recoveries under conditions favorable to most organochlorine (OC) pesticides, and investigative work is continuing to achieve optimum conditions for these additional pesticides.

A comparative study of the performance of SCD, GPC, and Florisil column cleanup of animal fats fortified at 0.1 mg/kg with 17 OC pesticides was conducted at the Associate Referee's laboratory during the past year. It was concluded that equivalent results were obtained with all 3 procedures.

Synthetic Pyrethroids.—(Associate Referee—Vacant.)

Recommendations

(1) *Comprehensive Multiresidue Methodology.*—Continue investigation/validation of reported sample weight concentration relationship with "salting out" procedures and publish findings reported on last year. Continue with and finalize investigations to improve *N*-methylcarbamate recoveries and proceed with an interlaboratory study of a "complete" CDFA method. If successful, prepare a report of the results along with a proposed plan for collaborative study for review.

(2) *Fumigants.*—Publish methyl bromide manual headspace method and proceed with previous (1990) plans to conduct both an interlaboratory trial and a collaborative study following the approved protocol.

(3) *Low Moisture–High Fat Samples.*—Draft in AOAC official method format the GPC method currently being studied by the Associate Referee, along with recovery data for all pesticides and commodities that were evaluated, for review and comment by the General Referee.

(4) *Miniaturized Multiresidue Methods for Fat Containing Foods.*—Finish THPI investigations and publish results. Investigate chemical modifiers that may be added to standard solutions to minimize chromatographic adsorption effects. If a successful modifier can be identified, continue development of the miniaturized milk method with emphasis on its application to polar pesticides and metabolites. If recoveries can be demonstrated to be consistently achievable at ≥80% at levels approximating 0.0005 ppm, proceed with plans to initiate the collaborative study process.

(5) *Miniaturized Multiresidue Methods for Nonfatty Foods.*—Complete small-scale extraction study and continue developing recovery information with selective *N*-methylcarbamates, pyrethroids, and triazoles as planned. Proceed with plans to conduct an interlaboratory trial that will include representative chemicals from all classes of pesticides that can be successfully recovered.

(6) *Supercritical Fluid Extraction of Pesticide Residue in Food.*—The Associate Referee has clearly shown that multivessel, large-volume SFE is both possible and practical. His research is currently directly toward a specific goal of achieving a solventless fat extraction procedure for a variety of food types; a goal that appears achievable in the near future. Because the extractor being used is a prototype, it is recommended that the Associate Referee pursue commercialization of the unit to advance the availability of this promising technique. It is further recommended the Associate Referee continue with work reported on prior to this year with SFE extraction studies of pesticide residues from nonfat-containing foods.

(7) *Sweep Codistillation.*—Submit a protocol and a method formatted in AOAC style for the planned collaborative study to the General Referee for comment and statistical

Table 4. Comparative residue findings: solvent and SFE extractions

Sample	Pesticide	Original, ppm	SFE mean (n = 6), ppm	SFE RSD, %
Cheddar cheese	Heptachlor epoxide	0.001	0.0012	4.3
	Dieldrin	0.001	0.0025	10.2
Saltine crackers	Methyl chlorpyrifos	0.023	0.0291	5.7
	Malathion	0.019	0.0316	4.5
Sandwich cookies	Malathion	0.031	0.0372	3.6
Cooked ground beef	*p,p'*-DDE	0.007	0.0090	2.8

review. Incorporate any changes that may be necessary, and proceed with plans to collaboratively study the procedure. Submit for publication the results of the comparative cleanup study with animal fats/OC pesticides using SCD, GPC, and Florisil procedures.

(8) *Synthetic Pyrethroids.*—Appoint a new Associate Referee to this topic area. After appointment, the new Associate Referee should prepare plans for an interlaboratory trial of the method that was developed.

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Residues. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Organohalogen Pesticides

BERNADETTE M. MCMAHON
U.S. Food and Drug Administration, Division of Pesticides and Industrial Chemicals, Washington, DC 20204

There are currently 5 topic areas under the General Refereeship on organohalogen pesticides. The current status of each topic area follows:

Chlorinated Dioxins.—(David Firestone, U.S. Food and Drug Administration [FDA], Washington, DC.) The Associate Referee prepared his annual review of presentations and publications related to analysis of polychlorinated dibenzo-*p*-dioxins and furans. Many of the references in this year's review were presented at the DIOXIN '91 Conference held in Research Triangle Park, NC, September 1991.

Because very low levels (parts per trillion and lower) of these residues are of interest, most of the reviewed methods use sophisticated instrumentation, such as high-resolution GC coupled with high-resolution MS, quantitation by isotope dilution techniques, and pattern recognition, to distinguish among residues from different environmental sources. Concerns about analytical quality and procedures for assuring quality are increasingly noticeable in publications related to these analyses.

Included among the publications reviewed are several that report residues in samples of fish, crustaceans, cow's milk, food composites, and human milk and blood. These papers, describing work done in Sweden, Canada, U.S. Great Lakes, Germany, and The Netherlands, represent international concern over residues in the environment. In addition, several studies of residues in paper, pulp, wastewater effluents, and sludge are included.

Chlorophenoxy Alkyl Acids and Pentachlorophenol.—(Vacant.) The former Associate Referee, Marvin Hopper, published a description of the method for acids and phenols (1). No indication exists, however, that the topic is likely to lead to a collaborative study in the near future.

Methyl Bromide.—(Joseph Ford, U.S. Department of Agriculture, Gulfport, MS.) The Associate Referee and his colleagues published a paper describing a method for the analysis for methyl bromide in nuts (2). The method is a headspace technique that uses partitioning coefficients (amount of methyl bromide in headspace/amount of methyl bromide in sample) calculated in advance for each of the 11 types of nuts involved in the study. The totally closed system combines sealed blender jars with GLC determination by means of a series of valves. Headspace sampling is computer-actuated for automation. Up to 12 blenders can be included in the system simultaneously. Routine limit of quantitation is 0.02 ppm, with 0.2 ppb possible.

Using this system, the Associate Referee's laboratory analyzed 1132 samples of nuts; only 3 residues were found, the highest being 0.03 ppm. The lack of residues verified expectations, because the nuts were heat processed.

The Associate Referee previously considered organizing a symposium on methyl bromide for an AOAC International meeting, but he was unable to devote the time required for a symposium on all fumigants; the latter topic was preferred by the Association. He continues to believe that a symposium devoted to methyl bromide alone is appropriate. Current interest in this chemical stems not only from the possibility of its appearing as a residue in foods but also from the concern over its alleged ozone-depleting characteristics. The Associate Referee proposed that his agency investigate the possibility that exposure to excited oxygen could degrade methyl bromide as it is released from fumigation chambers, a measure designed to minimize atmospheric pollution by methyl bromide.

The Associate Referee still plans to organize a collaborative study of a method for methyl bromide; he continues to work with James Daft, FDA, Kansas City District, in this endeavor. No protocol has yet been submitted for such a study.

PCB Determination by Measurement of Specific Congeners.—(Kimmo Himberg, Crime Laboratory, National Bureau of Investigation, Helsinki, Finland.) No report was received from this Associate Referee, and no correspondence was received during the past year.

PCBs in Blood.—(Virlyn Burse, Centers for Disease Control, Atlanta, GA.) The Associate Referee's long-standing intent to perform an additional collaborative study on the official final action method for PCB in blood, **990.07**, has not yet been funded by his agency. The study would extend the method to include analysis for chlorinated pesticides using ^{13}C-labeled standards. The Associate Referee will present a poster at the International Meeting in Cincinnati on congener-specific PCB quantitation using the **990.07** method.

Recommendations

(1) Continue to monitor progress on the development of methods for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and for hexachloro-, heptachloro-, and octachloro-*p*-dioxins and dibenzofurans in foods.

(2) Discontinue the topic on chlorophenoxy alkyl acids and pentachlorophenol; the topic can be reestablished in the

future if an Associate Referee can be found to perform a collaborative study for these residues.

(3) Develop a protocol for collaborative study of a method for methyl bromide in nuts and spices; submit the protocol for approval through the established process.

(4) Describe a complete method for PCB determination by measurement of specific congeners, including extraction and cleanup steps. Perform experiments to show the compatibility of the extraction, cleanup, and determinative techniques. Prepare a protocol for an interlaboratory study of this method. Perform the interlaboratory study, and if successful, prepare a collaborative study protocol.

(5) Proceed with study of the official final action method for PCBs in blood serum, **990.07**, as it is applied to the analysis for pesticides in blood serum. Develop a protocol for collaborative study of this application of the method.

(6) Continue the official first action status of the method for pentachlorophenol in gelatin, **985.24**.

References

(1) Hopper, M.L., McMahon, B., Griffitt, K.R., Cline, K., Fleming-Jones, M.E., & Kendall, D.C. (1992) *J. AOAC Int.* **75**, 707–713

(2) Ford, J.H., Legendre, M.G., Ladner, D.L., Dawson, J.A., & Raymond, C. (1992) *J. AOAC Int.* **75**, 328–333

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Residues. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Organonitrogen Pesticides

W. HARVEY NEWSOME
Health & Welfare Canada, Health Protection Branch, Food Research Division, Ottawa, ON, K1A 0L2, Canada

Five Associate Referee reports were received. David Northrup was appointed Associate Referee for the topic of *Organonitro Pesticides*. Associate Referees are required for an additional 11 topics.

Anilazine.—An Associate Referee is required to select a method for anilazine residues in fruit and vegetables and subject it to an interlaboratory trial.

Benzimidazole–Type Fungicides.—An Associate Referee is required to study a method for benomyl, thiophanate methyl, and carbendazim that converts all compounds to carbendazim and subsequently determines it by LC or GLC.

Captan and Related Fungicides.—Associate Referee Dalia Gilvydis reports that the interlaboratory study of her method for captan, captafol, and folpet in tomatoes, cucumbers, and apples was published (*J. Assoc. Off. Anal. Chem.* [1991] **74**, 830–835). Additional collaborators are required for a full collaborative study to proceed.

Carbamate Herbicides.—An Associate Referee is required to select and test a method for the carbamate herbicides asulam, desmedipham, and phenmedipham.

Carbamate Insecticides.—Associate Referee M. Sher Ali did not submit a report.

Carbofuran.—An Associate Referee is required to conduct a collaborative study of a method for carbofuran phenolic metabolites and 3-hydroxycarbofuran glucoside residues in crops and for carbofuran and its carbamate and phenolic metabolites in milk and meat.

Chlorothalonil.—An Associate Referee is required to study and test collaboratively a method for chlorothalonil and its 4-hydroxy metabolite in food crops.

Daminozide and 1,1-Dimethylhydrazine (UDMH).—No data was received on the interlaboratory study conducted by the National Food Processors Association using the method of Conditt et al. (*J. Assoc. Off. Anal. Chem.* [1988] **71**, 735–739).

Diquat and Paraquat.—Associate Referee Brian Worobey reports that his manuscript describing a collaborative study of an LC method for diquat and paraquat in potatoes was revised and the study was accepted by the Committee and Official Methods Board as interim official first action.

Dithiocarbamate Fungicides.—An Associate Referee is required to select and test a method for the differentiation and quantitative determination of dimethyl and ethylenebisdithiocarbamates in foods.

Glyphosate.—An Associate Referee is required to conduct a collaborative study of a method such as that described by Cowell (J. Agric. Food Chem. [1986] **34**, 955–960) for glyphosate and its metabolite, (aminomethyl)phosphonic acid.

Maleic Hydrazide.—An Associate Referee is required to study a GLC or LC method for maleic hydrazide residues.

Organonitro Pesticides.—Associate Referee David Northrup reports that conditions were established for the separation of the organonitro compounds 4-nitrophenol, 2,4-dinitrophenol, DNOC, and dinoseb by LC. An acetonitrile–phosphate buffer (pH 2.7) gradient and electrochemical detection were used. Recovery studies of the analytes using C_{18} solid-phase extraction (SPE) cartridges for cleanup showed good yields of DNOC and dinoseb but only a 50% yield for 2,4-dinitrophenol. 4-Nitrophenol was not recovered. Application of the acetone extraction and methylene chloride partitioning procedure (PAM I, sec. 232.4), followed by the SPE cleanup, to samples of oranges, apples, or corn indicated insufficient cleanup to remove interferences. Future work will involve the use of additional cleanup techniques.

Sodium o-Phenylphenate.—An Associate Referee is required to select a method for *o*-phenylphenol residues and conduct a collaborative study of it.

Substituted Ureas.—Associate Referee Ronald Luchtefeld reports that additional collaborators are required to initiate a collaborative study of his postcolumn photolytic LC method for urea herbicides.

Thiolcarbamate Herbicides.—An Associate Referee is required to conduct a collaborative study of a residue method for thiolcarbamates, such as the steam distillation method described in *Analytical Methods for Pesticides and Plant Growth Regulators*, vol. XIII (1984) for EPTC and butylate.

s-Triazines.—Associate Referee Stephen Clegg reports that his method for *s*-triazines in corn, which involves extraction into acetonitrile–water followed by partitioning into chloroform and determination by GC with a nitrogen-specific detector, requires additional cleanup. The procedures being examined include SPE cartridges composed of alumina, florisil, and C_{18} reversed-phase.

Recommendations

(1) Obtain a sufficient number of laboratories to conduct a collaborative study of the Associate Referee's method for captan, captafol, and folpet.
(2) Prepare a set of instructions to collaborators prior to initiation of a collaborative study of the LC method for carbamate insecticides in liver. Conduct an interlaboratory trial of the method.
(3) Discontinue the topic of *Daminozide and 1,1-Dimethylhydrazine*, because the registration of daminozide for use on North American food crops was withdrawn by the manufacturer.
(4) Adopt the method developed and tested by the Associate Referee for diquat and paraquat in potatoes as official first action. Continue study to broaden the scope of the method.
(5) Continue development of suitable cleanup methods for selected organonitro pesticides in food crops. Attempt to expand the number of compounds that are included in the method.
(6) Continue the investigation of more efficient cleanup procedures for the determination of triazines in food crops. Conduct recovery studies and ruggedness testing of the resulting method.
(7) Obtain additional collaborators and initiate study of the Associate Referee's multiresidue LC method (*J. Assoc. Off. Anal. Chem.* [1987] **70**, 740) for urea herbicides in onions and potatoes.
(8) Appoint Associate Referees for anilazine, benzimidazole-type fungicides, carbamate herbicides, carbofuran, chlorothalonil, dithiocarbamate fungicides, glyphosate, maleic hydrazide, sodium *o*-phenyl phenate, and thiolcarbamate herbicides.

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Residues. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Radioactivity

EDMOND J. BARATTA
U.S. Food and Drug Administration, Winchester Engineering and Analytical Center, Winchester, MA 01890

Cesium-137.—Methods for determining cesium-137 in milk, foods, and biological materials at lower levels are being considered. (Also, search for an Associate Referee is continuing.)

Iodine-131.—A collaborative study for the determination of iodine-131 in milk at very low levels was completed. The report "Collaborative Study for the Determination of Iodine-131 at Low Levels in Milk" is being presented at the 106th AOAC International Meeting and Exposition in Cincinnati, OH, on September 1, 1992. The study was submitted to the Committee on Residues.

Plutonium-239.—The nominee for Associate Referee was approved by her supervisor. Her appointment by AOAC International is expected soon. A method was selected for collaborative study. It will be tested before submission for the study.

Radium-228.—The report for the collaborative study was expected in December, 1991. I will try again to make contact. If this fails, I will write up and submit the results of the collaborative study to the Committee on Residues for possible interim first action status.

Strontium-89 and -90.—Search for and appoint a new Associate Referee for this topic. The new referee will prepare a protocol for the collaborative study of the method of Baratta and Reavey (*J. Agric. Food Chem.* **17**, 1337–1339) for determining strontium-89 and -90 in foods. This will be done with the approval of the General Referee and Statistician.

Tritium.—The selection made for an Associate Referee for this topic had to decline because of pressure related to his present assignments. Search was renewed for a new Associate Referee for this topic.

Recommendations

(1) Recommend the collaboratively tested "Method for Iodine-131 at Low-Levels in Milk" for interim first action.
(2) Submit the study for radium-228 when the report is completed.
(3) Search for new Associate Referees for *Cesium-137*, *Strontium-89 and -90*, and *Tritium*.

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Residues. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Committee on Microbiology and Extraneous Materials

Cosmetic Microbiology

ANTHONY D. HITCHINS
U.S. Food and Drug Administration, Division of Microbiology, HFF-234, Washington, DC 20204

This report presents the status of 3 topic areas of current interest: collaborative measurement of preservative efficacy in nonsolid cosmetics, recovery of microbes from cosmetics, and research on methods for measuring preservative efficacy in solid cosmetics.

Collaborative Evaluation of the Cosmetic Toiletry and Fragrance Association's (CTFA) Guideline Method for Determination of Preservative Efficacy in Nonsolid Cosmetics and Toiletries.—Neil Machtiger (Vicks Research Center) assumed the Associate Refereeship of this collaborative study from R.J. Spielmaker (Amway Corp.), who oversaw much of the necessary planning and preliminary research. The final study was temporarily delayed by unexpected difficulty in formulating an unpreserved control for one of the products, but this has been achieved. A preliminary, 2-laboratory test of all the formulations to be used in the final study is planned shortly by using an independent method, the standard *U.S. Pharmacopeia* method, so as not to compromise the final study. The collaborative study itself will evaluate the CTFA method for testing the efficacy of preservation of non-eye area, water-miscible cosmetic and toiletary formulations. Five different products (shampoo, hair conditioner, oil–water emulsion type moisturizer, water–oil emulsion type moisturizer, and high sun protection factor suncream) with and without preservatives will be tested in duplicate by about 15 collaborators against 4 pools of challenge microorganisms (American Type Culture Collection strains). Challenged products will be sampled at 7, 14, and 28 days after inoculation with $1–9 \times 10^6$ colony-forming-units per gram. The data will be analyzed according to the statistical method for qualitative studies described by F.D. McClure (*J. Assoc. Off. Anal. Chem.* [1990] **73**, 953–960).

Isolation of Microbes from Cosmetics.—No Associate Refereeships were established in this topic area, although there is a need for rapid quantitative methods for recovering stressed and unstressed bacteria from preserved cosmetics. Investigators interested in collaborative studies of the isolation of microbes from cosmetics should contact the General Referee. There is a crucial need to evaluate the performance of neutralizers in the microbiological analysis of cosmetics. In the related area of disinfectant neutralizers, S.R. Rachui, S.V.W. Sutton, and D.K. Brannan (*Abstr. Genl. Mtg. Amer. Soc. Microbiol.* [1992] Q-351 p. 394) found that no single neutralizing medium was effective for all disinfectants for all microbes. It is likely that a similar situation exist, at least to some extent, with cosmetic preservative neutralizers.

M.L. Vance, M.C. Roach, and D.K. Brannan (*Abstr. Gen. Mtg. Amer. Soc. Microbiol.* [1992] Q-348 p. 393) attempted to validate the semiquantitative swab technique for quantifying microbes in consumer products in order to make it more reliable. They found that "more robust" neutralizing media and product dilution for consistent sampling were the most desirable improvements.

A.D. Hitchins, J. McCarron, and T.T. Tran revised the cosmetic microbiology methodology chapter for the FDA's *Bacteriological Analytical Manual*, and it was published in 1992 by AOAC International. The major changes involve an emphasis on the quantitation by direct plating (or by MPN analysis) of diluted samples and on the identification of isolates by commercially available identification kits.

Preservative Efficacy in Solid Cosmetics.—Investigators interested in a collaborative study of the direct-contact bacterial challenge method for evaluating solid cosmetic preservative efficacy (T.T. Tran et al. *Intl. J. Cosmetic Science* [1990] **12**, 175–183) should contact the General Referee. (**Note**: After this report was submitted, AOAC approved the appointment of T.T. Tran as Associate Referee to test and study analytical methods for "Preservatives Efficacy in Solid Cosmetics.")

The direct contact method uses the survival D-value method of D.S. Orth. Interestingly, he recently reported (*Cosmetics & Toiletries* [1991] **106**, 45–51) that this analytical method can support standards criteria more stringent than the standards criteria used in the conventional cosmetic preservative efficacy tests, and that it even allows for the pseudomonad "rebound" effect. The research used inadequately, marginally, and satisfactorally preserved emulsions as test cosmetics.

The following useful review articles covering cosmetic microbiology and preservation were recently published:

(1) Cosmetic preservation (Sharpell, F. & Manowitz, M. [1991] *Disinfection, Sterilization, and Preservation*, S.S. Block [Ed.], Lea & Febiger, Malvern, PA).

(2) Microbial degradation of preservatives (Hugo, W.B. [1991] *Int. Biodeterior.* **27**, 185–194).

(3) Identification and determination of antimicrobial agents (DeKruijf, N., & Schouten, A. [1991] *Parfum. Kosmet.* **72**, 386–398).

(4) Cosmetic preservative reference materials (Weyland, J.W., & Wagstaffe, P.J. [1991] *Parfum. Kosmet.* **72**, 750–765).

Recommendation

Continue study of all topic areas.

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Microbiology and Extraneous Materials. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Drug- and Device-Related Microbiology

ANA M. PLACENCIA
U.S. Food and Drug Administration, Minneapolis, MN

Steam Biological Indicators.—(Robert R. Berube, Associate Referee.) The single, greatest limitation in performance testing of biological indicators is the sensitivity of the growth promotion of the nonstressed spore population and the stressed spore population. The emphasis has been on media and medium supplements. What is needed is a "benchmark" medium that will give reproducible results; a chemically-defined medium may be a possibility. Also, the idiosyncracies of the test technique may need to be spelled out.

Sporicidal Testing of Disinfectants/Sterilants.—(James W. Danielson, Associate Referee.) To improve the AOAC sporicidal method for testing disinfectants, work was done to replace culture filtrates of soil extract nutrient broth and soil extract egg meat medium using water suspensions of *Bacillus subtilis* spores and *Clostridium sporogenes* spores. A collaborative study will be conducted using *B. subtilis* var. *niger* spores in a water suspension for inoculating carriers. A collaborative study using a clean spore suspension of *Cl. sporogenes* will also be done.

Ethylene Oxide Biological Indicators (BIs).—(Christopher Demitrius, Associate Referee.) A study was conducted to determine the effect of temperature upon decimal reduction value (D-value). The manuscript is in draft form for future publication. The performances of European (United Kingdom and Denmark) and American BIs were studied and correlated. Data were presented in an ASM poster session in 1991, "Comparison of European and U.S. Biological Indicators for Ethylene Oxide Sterilization." A manuscript is being prepared for a future publication. A study is being planned using American and Denmark BIs, which are known for their protection from ethylene oxide lethality with salt.

Chemical Indicators.—(Marvin L. Hart, Associate Referee.) Little time was spent in AOAC activities, but considerable involvement, which can be shared with AOAC members, was devoted to writing standards on chemical indicators as a co-chair of a sub-TAG with the Association for the Advancement of Medical Instrumentation and as a U.S. delegate on a chemical indicator working group with the International Organization for Standardization. These standards include chemical indicators for steam, ethylene oxide, dry heat, steam/formaldehyde, and irradiation sterilization processes. Draft documents will be circulated in 1992, and adoption of these standards in 1993 is anticipated.

Packaging Integrity for Medical Devices.—(Ana M. Placencia, Associate Referee.) The following 2 manuscripts were submitted for publication and/or presentation: Placencia, A.M., & Peeler, J.T. (1992) "Proposed Standards for Evaluating Medical Device Packaging Materials Using Microbial Methods—An Update", TAPPI Polymers, Laminations and Coatings Conference, September 8–11, 1992, Orlando, FL; and Placencia, A.M., & Peeler, J.T. (1992) "Dry Test Method for Rating the Penetration of Viruses through Porous Materials Used in Protective Clothing by Health Care Workers" in *Performance of Protective Clothing*, J.P. McBriarty & N.W. Henry (Eds), Vol. 4, ASTM STP 1133, American Society for Testing and Materials, Philadelphia, PA.

A laboratory information bulletin entitled "Methods for Testing Medical Device Packages for Micro-leaks" was submitted for publication. An AOAC collaborative study is being planned for the near future on this subject.

An AOAC/ASTM collaborative study on the Exposure Chamber Method (microbial method for package integrity) is planned for August 1992. The method was modified to incorporate one flow meter per port and to increase the bacterial challenge.

The Associate Referee participated in an ASTM collaborative study entitled "Standard Test Method for Resistance of Protective Clothing Materials to Penetration by Biological Fluids."

Endotoxins by Limulus Amebocyte Lysate.—(Christine W. Twohy, Associate Referee.) No work was accomplished on this project this year but methods development will resume next year.

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Microbiology and Extraneous Materials. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Filth and Extraneous Materials in Foods and Drugs

JACK L. BOESE
U.S. Food and Drug Administration,
Division of Microbiology, Washington, DC 20204

(1) Aerator, water, **945.75B(a)**: Replace first 2 sentences with "Modify to produce fine, even spray of H_2O by removing screen(s). If aerator has more than 1 disk, use only the 1 with small (ca 1 mm) holes." The next sentence "(Available ... 00200.)" is unchanged. After it, add "Unit may be securely attached to faucet with heavy rubber tubing (wall thickness ca 3.2 mm) to provide suitable working distance from sieve and allow some movement of sprayer head."
(2) Alkaline Phosphatase in Foods, Electrophoresis Detection Method: Continue study.
(3) Baked Goods with Fruit and Nut Tissues, Light Filth by Flotation Method: Continue study.
(4) Basil (Unground), Light Filth by Flotation Method: Continue study.
(5) Bean Paste, Light Filth by Flotation Method: Continue study.
(6) Cheese, Filth by Sieving Method: New topic.
(7) Chocolate and Chocolate Products, Light Filth by Flotation Method: Continue study.
(8) Coffee (Ground), Light Filth by Flotation Method: Continue study.
(9) Condimental Hot Sauces, Light Filth by Flotation Method: Continue study.
(10) Condimental Sauces Containing Soy Sauce, Thickeners, and Spices, Light Filth by Flotation Method: Continue study.
(11) Crabmeat, Shrimp, and Tuna (Canned), Light Filth by Brine Flotation Method: Discontinue topic.
(12) Fish Paste and Sauces, Light Filth by Flotation Method: Continue study.
(13) Grain Products, Mammalian Feces by Chemical Detection Method: Continue study.
(14) Grain Products, Light Filth by Flotation Method: Continue study.
(15) Grain Products, Mammalian Feces Detection by Alkaline Phosphatase Method: Discontinue topic.
(16) Grains (Whole), Internal Infestation by Cracking Flotation Method: Continue study.
(17) Grains (Whole), Internal Infestation by ELISA Method: Continue study.
(18) Grains and Seeds (Whole), External Light Filth by Flotation Method: Continue study.
(19) Rodent Gnawing of Packaging Materials and Foods, Salivary Amylase Test: Continue study.
(20) Soybean Curd, Light Filth by Flotation Method: Continue study.
(21) Spices, Mammalian Feces by Chemical Detection Method: Continue study.
(22) Urine on Grains, Magnesium Uranyl Acetate Test (first action), **962.28**; *J. Assoc. Off. Anal. Chem.* (1963) **46**, 685: Recommended for surplus status.
(23) Urine Stains on Foods and Containers, Chemical Detection Methods: Continue study.
(24) Vegetable Products (Dehydrated), Light Filth by Flotation Method: Continue study.
(25) Other topics: Add the following publications to the list of General References in Chapter 16, Extraneous Materials: "Key for Identification of Mandibles of Stored-Food Insects" (1985) AOAC, 2200 Wilson Blvd, Arlington, VA 22201-3301; "Ecology and Management of Food Industry Pests" (1991) AOAC, 2200 Wilson Blvd, Arlington, VA 22201-3301; and "Insect and Mite Pests in Food: An Illustrated Key" (1991) USDA, Agricultural Handbook No. 655.

Flotation methods for the extraction of light filth from fish products containing spice developed by Larry Glaze and light filth from sauces containing soy sauce, thickeners, and spices by Marvin Nakashima were adopted first action by the Official Methods Board. A sieving method for filth in tofu by Marvin Nakashima was also adopted first action. The methods will be published in the fourth supplement to *Official Methods of Analysis*, 15th Ed.

The collaborative study of a flotation method for light filth in whole wheat flour by Larry Glaze and John Bryce was completed, and the method is being recommended for adoption as first action. Collaborative study of a flotation method for light filth in bean paste is being conducted by John Bryce.

Marvin Nakashima was appointed Associate Referee for the new topic Filth in Cheese by Sieving Method. Patricia A. Valdes was appointed Associate Referee for the existing topic Urine Stains on Foods and Containers by Chemical Detection Methods. On the basis of the recommendation of Associate Referee George Ziobro, the topic Grain Products, Fecal Contamination by Gas Chromatography Detection Method was changed to Grain Products, Mammalian Feces by Chemical Detection Method.

The following topics are being discontinued: Grain Products, Mammalian Feces Detection by Alkaline Phosphatase Method—Harriet Gerber; and Crabmeat, Shrimp, and Tuna (Canned), Light Filth by Brine Flotation Method—James Barnett. Study will continue on all other topics.

The principal method for analyzing large (ca 50 g) laboratory samples of grains for urine is currently the magnesium uranyl acetate test, **963.28**, which has the important disadvantage of using a highly toxic reagent in spray form. The urease–bromothymol blue–agar test, **972.41**, may be considered as an alternative method for large samples (Figure **945.88**). However, even though it has greater safety, specificity, and reliability than the magnesium uranyl acetate test, it is designed primarily for the analysis of small numbers of grains. A study by Patricia Valdes to modify the method for use with larger sample sizes is nearing completion. The magnesium uranyl acetate test will be recommended for surplus sta-

tus based on its limited current use and the availability of an improved method in the near future.

An editorial change is needed to help clarify the description of the *Aerator, water,* **945.75B(a)**. As written, the aerator itself is described but not the modified apparatus, which is intended to provide a spray of water rather than an aerated stream in the wet-sieving step of filth methods. The number of screens and disks used in different models of aerators varies, and directing the analyst to simply remove the "lower screen" is no longer adequate. In addition, no information is given about how to attach the aerator, or sprayer, unit to the faucet to provide a suitable working distance from the sieve. Sinks, faucets, and sprayers vary too much to provide specific instructions, but mention of a simple system in common use may be helpful. With these points in mind, an editorial change to *Aerator, water,* **945.75B(a)** is recommended as follows. Replace first 2 sentences with "Modify to produce fine, even spray of H_2O by removing screen(s). If aerator has more than 1 disk, use only the 1 with small (ca 1 mm) holes." The next sentence "(Available ... 00200.)" is unchanged. After it, add "Unit may be securely attached to faucet with heavy rubber tubing (wall thickness ca 3.2 mm) to provide suitable working distance from sieve and allow some movement of sprayer head."

Three recent and very useful publications will be recommended for addition to the list of General References in the Extraneous Materials chapter of the *Official Methods of Analysis*: "Key for Identification of Mandibles of Stored-Food Insects" by Diane McClymont Peace, and "Insect and Mite Pests in Food: An Illustrated Key" and "Ecology and Management of Food Industry Pests" by J. Richard Gorham, Editor.

Recommendations

(1) Discontinue study on the following topics: Grain Products, Mammalian Feces Detection by Alkaline Phosphatase Method and Crabmeat, Shrimp, and Tuna (Canned), Light Filth by Brine Flotation Method.
(2) Continue study on all other topics.
(3) Surplus the first action method Urine on Grain, Magnesium Uranyl Acetate Test, **963.28**.
(4) For the apparatus *Aerator, water,* **945.75B(a)**, make editorial changes as described.
(5) Add the following publications to the list of General References in Chapter 16: Extraneous Materials: "Key for Identification of Mandibles of Stored-Food Insects" (1985) AOAC, 2200 Wilson Blvd, Arlington, VA 22201-3301; "Ecology and Management of Food Industry Pests" (1991) AOAC, 2200 Wilson Blvd, Arlington, VA 22201-3301; and "Insect and Mite Pests in Food: An Illustrated Key" (1991) J. Richard Gorham, Editor; USDA, Agricultural Handbook No. 655.

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Microbiology and Extraneous Materials. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Food Microbiology—Dairy

J. RUSSELL BISHOP
Virginia Polytechnic Institute and State University, Food Science and Technology, Blacksburg, VA 24061

The Dairy Microbiology section of AOAC is on the verge of initiating 3 collaborative studies.

There are presently 4 collaborative studies underway or at various steps of completion, with another 3 collaborative or precollaborative studies to be initiated in the near future. Those collaborative studies presently "in the system" are as follows:

(*1*) *Listeria monocytogenes* in Milk and Dairy Products: IDF Method, Robert M. Twedt and Anthony D. Hitchins, FDA, Washington, DC. A collaborative study is underway concerning the recovery of viable *Listeria monocytogenes* from milk and dairy products (Camembert, Limburger, skim milk powder, and ice cream). The test portion was homogenized with *Listeria*-selective liquid enrichment medium and cultured at 30°C for 48 h. The enrichment culture was then subcultured onto a solid isolation medium at 37°C for 48 h. Suspected *Listeria* colonies were identified by appropriate conventional morphological, physiological, and biochemical tests. The 5 dairy matrixes were spiked with *L. monocytogenes* at 2 levels: 12 and 120 colony-forming units (CFU)/25 g. Each of the 18 collaborating laboratories analyzed 15 blind test portions from each matrix, comprising 5 replicates at each spiking level and 5 uninoculated controls, for a total of 1350 analyses. The specificity of the method was 100%; its sensitivity was 90–100% at the high-spiking level and 89–98% at the low-spiking level, except for Limburger, for which it was only 68%. No specificity or sensitivity differences were observed between laboratories for all matrixes at the high-spiking level and for all except Limburger at the low-spiking level. The calculated 50% detection limits for all products except Limburger and for Limburger itself were 1.6 and 4.1 CFU/25 g, respectively. The method is proposed for adoption as interim first action.

(*2*) Enumeration of Total Bacteria in Raw and Pasteurized Milk by Reflectance Colorimetry, Gary H. Richardson, Utah State University/Wescor, Logan, UT. Seven laboratories participated in a collaborative study to compare a reflectance colorimetric (RC) bioactivity monitor (Omnispec™4000) method to the standard plate counter (SPC) method for estimation of total bacteria in raw and homogenized pasteurized milk. Each collaborator analyzed 12 samples by the SPC method and 24 samples (the same 12 samples in blind duplicate) by the RC method. For the 6 primary collaborators, the RC $RSDR_r$ was 1.7% and the RC RSD_R was 4.5%. The SPC RSD_R was 20.8%. For 17 collaborators, the RSD_r was 3.9% and the RSD_R was 5.9%. The RC method is proposed for official first action.

(*3*) *Salmonella* Detection from Milk Products by Motility Enrichment on Modified Semisolid Rappaport-Vassiliadis Medium (MSRV), Robert Bolderdijk, Jacobs Suchard Corporate Microbiological Services, Montezumalaan, Belgium. The use of motility enrichment on MSRV is being compared to the cul-

tural procedure of the *Salmonella* method for dried milk products in FDA's *Bacteriological Analytical Manual.*

(*4*) Comparison of the Fossomatic 360 to the Fossomatic 215, David McKenna, Foss Food Technology, Eden Prairie, MN. The accuracy and precision of the Fossomatic 250/300/360 series of somatic cell counters were compared to those of the Fossomatic 215. The Fossomatic 215 was granted first action approval by AOAC in 1978 and Final Approval in 1984. A second-generation cell counter, the Fossomatic 90, was granted Final Approval in 1989. This demonstrates that the method has been well tested and has satisfied all the requirements of the Association. Since then the manufacturer, Foss Electric, has introduced a third generation of equipment that is being distributed as 3 different models: Fossomatic 250, Fossomatic 300, and Fossomatic 360. The introduction of these instruments as different models was purely a marketing decision, because in effect, they are different versions of the same instrument. The only differences are that they are packaged differently and perform at different speeds to satisfy different customer requirements. This new generation of equipment uses exactly the same methodology that has twice been granted final approval, but to take advantage of advances in instrumentation technology the following modifications were introduced in the new designs: more efficient autosampling, digital circuitry instead of analogue circuitry used in the Fossomatic 215, slope and bias adjustments added to the circuitry with automatic discriminator control, and reagent recipe adjustment.

The first objective is to demonstrate that these changes have in no way affected the accuracy and precision of the method. The second objective is to demonstrate, using in-house data, that the preservative Bronopol can be used in place of potassium dichromate without affecting the accuracy of precision of the method.

To prove these points, researchers will make a statistical comparison between the Fossomatic 215s in the approval study, 2 Fossomatic 215s still being used on a daily basis, and 6 instruments of the Fossomatic 250/300/360 series. They will also examine how all these instruments perform against the manufacturer's operating specification.

The collaborative studies include:

(*1*) Standard Plate Count and Coliform Count in Milk and Dairy Products by Impedance Microbiology (Malthus), Philip Coombs, Radiometer America, Westlake, OH.

(*2*) Comparison of the Somacount 300 to the Fossomatic 215 and 90, Bent Lyder, Bentley Instruments, Chaska, MN.

(*3*) Coliform Detection and Enumeration in Milk by Impedance Microbiology (Bactometer).

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Microbiology and Extraneous Materials. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Food Microbiology—Nondairy

WALLACE H. ANDREWS
U.S. Food and Drug Administration,
Division of Microbiology, Washington, DC 20204

Collaborative Studies

Listeria, Comparison of Culture Methods.—Because the U.S. Food and Drug Administration (FDA) has regulatory jurisdiction for seafoods and for dairy foods (that cross state lines) and the U.S. Department of Agriculture (USDA) is responsible for red meats and poultry, the choice of using either the FDA or USDA procedure as the reference culture method in prior and current AOAC *Listeria* collaborative studies was determined by the foods used. Thus, the FDA method (1) was used as the reference culture method in determining the effectiveness of a rapid test kit for detecting *Listeria* in cheese, whereas the USDA method (2) would be used as the standard if ground beef were the food being analyzed.

Eleven Nordic laboratories participated in a collaborative study, conducted under the auspices of the Biology Division, National Food Administration, Uppsala, Sweden, of methods for the detection of *L. monocytogenes* in blue-and-white mold cheese and in canned corned beef. A comparison was made of the following 3 factors: enrichment by FDA procedure vs enrichment by USDA procedure, presence or absence of KOH treatment on enriched culture prior to plating, and plating on lithium chloride–phenylethanol–moxalactam (LPM) agar vs Oxford agar.

There was no statistically significant difference in the effectiveness between the FDA and USDA enrichment procedures for the detection of *L. monocytogenes*. Moreover, KOH treatment of the enriched culture did not significantly increase the detection rate of the organism, and Oxford agar was superior to the LPM agar. For cheese, the detection level of the methods was below 0.2 *L. monocytogenes* organisms per g, whereas for corned beef, the detection level was below 5 *L. monocytogenes* organisms per g.

On the basis of the results from this collaborative study, a method was recommended for adoption as official first action that consisted of enrichment by either the FDA or USDA procedure, absence of KOH treatment of the enriched culture, and subculturing on Oxford agar. I do not concur because of numerous discrepancies with AOAC-recommended guidelines for conducting collaborative studies.

Listeria, GENE-TRAK Systems Colorimetric DNA Hybridization Method.—GENE-TRAK Systems (Framingham, MA) developed a colorimetric DNA hybridization (DNAH) assay for the rapid detection of *Listeria* species in dairy products, meats, seafoods, and environmental samples. The mechanism of action involves solution hybridization between target ribosomal RNA (rRNA) molecules and synthetic deoxyribonucleotide probes directed against *Listeria*-specific rRNA se-

quences. Following hybridization, probe:target hybrids are captured onto a solid support (plastic dipstick) by means of hybridization between a polydeoxyadenylic acid tail present on one of the probes (capture probe) and a polydeoxythymidilic acid molecule bound to the solid support. Detection of hybridization is achieved by using a colorimetric detection system composed of an antibody–enzyme conjugate that recognizes and binds to a hapten attached to one of the probes (detector probe). Addition of a substrate–chromogen combination results in the formation of a colored end product that is measured photometrically.

In a precollaborative study, 1300 samples that represented 15 food types were analyzed in parallel by both the DNAH procedure and the conventional culture method (FDA procedure for dairy products and seafoods and the USDA procedure for meats). Samples included foods artificially contaminated with one of 15 *Listeria* strains (representing 5 species), naturally contaminated products, and negative controls.

Of 660 inoculated and negative control dairy and seafood samples, the FDA culture method detected 354 positives, and the DNAH procedure detected 393 positives, 391 of which were confirmed. The DNAH method was statistically equivalent to the FDA method for 8 of the 9 food types tested. In some trials with cheddar cheese, a statistically higher number of positive samples was obtained with the DNAH method than with the FDA method.

Of 540 inoculated and control meat samples, the USDA culture method detected 261 positives and the DNAH procedure detected 378 positives, all of which were confirmed. The DNAH procedure was statistically equivalent to the culture method for 3 of the 6 products tested. In some trials with roast beef, frankfurters, and fermented sausage, a statistically higher number of positive samples was obtained with the DNAH procedure than with the USDA method.

Of 100 samples that were supposed to be naturally contaminated with *Listeria*, the culture method detected 84 positives, and the DNAH procedure detected 86 positives. The 2 methods were reported to be statistically equivalent.

In a subsequent collaborative study conducted by Associate Referee Michael Curiale, 19 laboratories compared the colorimetric DNAH procedure with the FDA and USDA culture methods for the detection of *Listeria* in 2% fluid milk, Brie cheese, crabmeat, frankfurters, roast beef, and raw ground pork. Replicate samples containing various concentrations of *Listeria* were examined for each of these 6 food types.

Listeria was detected in 774 samples with the DNAH method and in 772 samples with the culture methods recommended by FDA and USDA. The DNAH and culture methods were in agreement for 668 samples containing *Listeria* and for 306 samples not containing *Listeria*. The overall rate of agreement between the DNAH and culture methods was 82.3%.

On the basis of these results, the Associate Referee recommends that the colorimetric DNAH method for the detection of *Listeria* in all foods be adopted official first action, and I concur.

Listeria, Listeria-Tek Enzyme Immunoassay.—The Listeria-Tek system (Organon Teknika Corporation, Durham, NC) is a colorimetric, monoclonal enzyme immunoassay (EIA) for *Listeria*. With this assay, the final result is read spectrophotometrically.

In a precollaborative study conducted by Associate Referee Michael Curiale, a total of 1135 naturally contaminated, artificially inoculated, and uninoculated samples was comparatively analyzed by both the EIA procedure and the conventional culture methods recommended by FDA and USDA. The conventional culture methods detected 708 (62.4%) confirmed-positive samples, whereas the EIA procedure detected 781 (68.8%) positive samples, of which 762 (67.1%) were confirmed.

In a subsequent collaborative study involving 25 collaborators, the EIA and the conventional FDA/USDA culture procedures were compared for the detection of *L. monocytogenes* in 2% fluid milk, Brie cheese, crabmeat, frankfurters, roast beef, and frozen shelled shrimp. Replicate samples containing various concentrations of *Listeria*, as well as uninoculated control samples, were examined for each of these 6 food types.

L. monocytogenes was detected in 484 samples with the EIA procedure and in 465 samples with the culture procedures. Identical results with the EIA and culture procedures were obtained for 386 positive samples and for 457 negative samples. The overall agreement between the EIA and culture procedures was 82.6%.

On the basis of the results from the precollaborative and collaborative studies, the Associate Referee recommends that this colorimetric, monoclonal EIA for the detection of *L. monocytogenes* in dairy foods, seafoods, and meat products be adopted official first action. I do not concur, and I have recommended that the segment of the collaborative study concerned with the analysis of shrimp be repeated because of the relatively large number of laboratories yielding statistically outlying results and because of the relatively high numbers of false-positive results.

Salmonella, Alternative Two-Step Immunodiffusion Method.—The immunodiffusion method, **989.13**, was adopted as an official first action method in 1988. This AOAC approved immunodiffusion method, hereafter referred to as the "1-step" immunodiffusion method, requires a 24 h preenrichment of the food sample before inoculation and incubation of the 1-2 TEST unit (BioControl Systems, Inc., Bothell, WA).

The effectiveness of the unit for detecting *Salmonella* in raw flesh foods and other highly contaminated products was reported to be increased by inclusion of a selective enrichment step (separate from and in addition to the selective enrichment that occurs in the unit) before inoculation of the unit and extension of the minimum incubation time of the unit from 8 h to 14–30 h. Tetrathionate broth is the sole selective enrichment used and is incubated for 24 h at 42°C in a water bath. This modified immunodiffusion method is hereafter known as the "2-step" immunodiffusion method.

Associate Referee Russell Flowers recently conducted a series of studies to validate the 2-step immunodiffusion method. First, a precollaborative study was conducted in which 1075 samples, representing 20 food types, were comparatively analyzed by the reference culture method, **967.25–967.28**, and the 2-step immunodiffusion method. False-negative rates for the reference culture method and the immunodiffusion procedure

were 3.4 and 2.2%, respectively. In addition, naturally contaminated and artificially contaminated samples, representing 10 food types, were analyzed by the 1-step immunodiffusion method, the 2-step immunodiffusion method, and the reference culture method. In both phases of this precollaborative study, all methods were reported to be statistically equivalent.

In a subsequent collaborative study involving 35 laboratories, a comparison was made of the immunodiffusion methods and the reference culture method for the detection of *Salmonella* in selected foods. When the culture method, **967.25–967.28**, and the 1-step immunodiffusion method, **989.13**, were compared, there were false-negative rates of 0.3 and 0.9%, respectively. The Associate Referee reported a 99.3% agreement between the 2 methods. When the culture method, **967.25–967.28**, and the 2-step immunodiffusion method were compared, there were false-negative rates of 0.6 and 0.0%, respectively. The Associate Referee reports a 99.6% agreement between the 2 methods.

Another study performed by the Associate Referee to validate the immunodiffusion methods included an in-house comparison of the 3 methods for the analysis of 10 food types thought to pose the greatest challenge or to be the most problematical for the immunodiffusion methods. In a final validation study, involving several laboratories, a sequential series of dilutions of *Salmonella* was analyzed by the 3 methods so as to reach an endpoint of detection. For these latter 2 studies, the Associate Referee reported equivalency between the reference culture and immunodiffusion methods.

On the basis of all of the above studies, the Associate Referee recommends that the 1-step immunodiffusion method, **989.13**, be revised to increase the incubation time of the unit to 14–30 h. It is further recommended that the enrichment protocol for raw flesh foods and highly contaminated products be modified so as to include a selective enrichment step in tetrathionate broth incubated at 42°C. In essence, the 1-step immunodiffusion method is being recommended for processed foods and the 2-step procedure for raw flesh foods and highly contaminated products. I abstain.

Salmonella, Modified Semisolid Rappaport-Vassiliadis Method for Cocoa and Chocolate.—A procedure for the rapid detection of *Salmonella* was developed that is based on the immobilization of motile cultures in modified semisolid Rappaport-Vassiliadis (MSRV) medium.

There are 2 variations of the procedure. With the first variation (direct motility enrichment), 3 drops of an 18–20 h sample preenrichment are inoculated onto a MSRV plate. With the second variation (indirect motility enrichment), the sample is preenriched for 24 h and selectively enriched for 8 h in selenite cystine broth and in tetrathionate broth with brilliant green dye. Three drops from each selective enrichment are inoculated onto a MSRV plate. Inoculated plates are incubated at 42 ± 0.5°C for 24 h (direct motility enrichment) or 16 h (indirect motility enrichment). Motile *Salmonella* organisms, if present, migrate to form a gray-white, turbid zone extending from the inoculation site on the agar surface. A bacterial suspension from the edge of the migration is used for culture confirmation by the reference method, **967.26–967.28**.

Associate Referee John Milas conducted a collaborative study in which 13 laboratories compared the 2 MSRV procedures and the reference culture method, **967.25–967.28**, for the recovery of *Salmonella* from samples of cocoa powder and 3 types of chocolate (milk, sweet, and dark). For each of these food types, 5 samples contained a high inoculum level (0.18–0.96 *Salmonella* organisms per gram on the day of initiation of analyses), 5 samples contained a low inoculum level (0.02–0.23 *Salmonella* organisms per g), and 5 samples contained uninoculated negative control samples.

There was no significant difference in the proportion of samples positive by the MSRV procedures and the reference culture method. The sensitivity rate (percent of positive samples correctly classified) was 98.1% for the MSRV procedure and 94.9% for the reference culture method. The specificity rate (percent of negative samples correctly classified) was 100.0% for both methods.

On the basis of the results from this collaborative study, the Associate Referee recommends that the MSRV procedures (direct motility enrichment and indirect motility enrichment) for the detection of *Salmonella* in chocolate and cocoa be adopted official first action, and I concur.

Salmonella, Salmonella-Tek Enzyme Immunoassay.—Several modifications of this colorimetric monoclonal enzyme immunoassay (EIA) (Organon Teknika Corporation, Durham, NC), **986.35** and **987.11**, were proposed. These modifications are as follows: incubation of tetrathionate broth and M broth at 42 ± 0.5°C, addition of 10 μg/mL novobiocin to the M broth, elimination of the need to agitate the microtiter plates during antibody capture, and elimination of the requirement for centrifugal concentration of the M broth culture for all food enrichments. The purpose of the first 2 objectives is to reduce the number of false-positive reactions by minimizing the growth of competitor organisms, whereas the second 2 objectives are intended to make the assay a more practical one.

To verify that the elevated temperature and the use of novobiocin were not inhibitory to *Salmonella*, a preliminary study was conducted comparing the growth of 226 *Salmonella* strains, representing 158 serotypes, in M broth without novobiocin at 35°C with that in M broth with novobiocin at 42°C. Only one strain did not grow in M broth with novobiocin at 42°C. Moreover, analysis of 1001 random food samples, sent to the Associate Referee's laboratory for analysis, demonstrated a significantly higher false-negative rate with the reference culture method, **967.25–967.28**, than with the modified EIA procedure. In addition to these samples, 10 replicates of each of 10 naturally contaminated samples were analyzed by the culture method and the modified EIA method. No significant differences were observed between the 2 methods.

Subsequent to this preliminary study, Associate Referee Karl Eckner conducted a precollaborative study comparing the modified EIA procedure with the reference culture method for the detection of *Salmonella* in 1200 food samples. The incidence of agreement between the 2 methods was 96.7%. The false-negative incidence was 1.5% for the modified EIA and 5.3% for the reference culture method.

In addition to this precollaborative study, the Associate Referee conducted a collaborative study. Seventeen laboratories participated in a study comparing the modified EIA method and the reference culture method for the detection of *Salmonella* in 5 artificially contaminated foods (nonfat dry milk, dried egg, soy flour, ground black pepper, and milk chocolate) and in naturally contaminated raw ground turkey. For each of the artificially contaminated food types, 5 samples contained a high inoculum level (0.023–>1.1 *Salmonella* organisms per g on the day of initiation of analyses), 5 samples contained a low inoculum level (0.003–0.023 *Salmonella* organisms per g), and 5 were uninoculated negative control samples. Naturally contaminated samples of turkey contained either a high (0.460) or low (0.093) level of *Salmonella* organisms per g.

The Associate Referee reported perfect agreement between the 2 methods for the analysis of nonfat dry milk, dried egg, ground black pepper, and milk chocolate. Ninety-eight percent agreement between the 2 methods was observed with samples of soy flour and 76–85% agreement, depending on the level of contamination, with samples of turkey. There were 7 false-negative results with the modified EIA method and 27 false-negative reactions with the reference culture method.

On the basis of these results, the Associate Referee recommends that the modified EIA for the detection of *Salmonella* in all foods be adopted official first action. Because an inadequate number of samples of black pepper and milk chocolate were analyzed, I recommended that these segments of the collaborative study be repeated.

Staphylococcal Enterotoxin, Tecra Enzyme Immunoassay.—The current AOAC method, the microslide gel double diffusion test, **976.31**, is a chromatographic toxin selection procedure requiring about 7 days for completion. This assay can detect staphylococcal enterotoxin at a concentration as low as 30–60 ng toxin per g food.

The Tecra staphylococcal enterotoxin visual immunoassay (Biotech Australia, Roseville, N.S.W., Australia) was designed to provide a rapid, sensitive, and specific alternative to the current official final action method. The EIA is performed in a "sandwich" configuration. High-affinity "capture" antibodies to the toxins are adsorbed onto the surfaces of wells of plastic microtiter trays. If staphylococcal enterotoxins are present in the sample, they will be captured by the adsorbed antibodies. All other material in the sample is washed away. The "sandwich" is completed by the addition of enzyme-labeled antibodies, i.e., conjugate, specific for staphylococcal enterotoxins. The enzyme's presence, in turn, is detected when it converts the colorless substrate to a green color.

The test can be performed manually and the results determined visually within 4 h. The use of an automated plate reader is optional.

Associate Referee Reginald Bennett conducted a collaborative study in which 10 food samples were sent to each of 15 collaborators. Five samples contained staphylococcal enterotoxin at concentrations ranging from 4 to 10 ng per g food; the other 5 foods (of the same food type as those inoculated with toxin) contained no enterotoxin (controls). All collaborators analyzed 5 replicates of each of the 10 food samples.

Foods containing toxin were positive, with absorbance (A = 414 ± 10) readings of >0.200; toxin-free foods were negative (A ≤ 0.200). Overall, 15 of 15 collaborators correctly performed 780 visual determinations and 780 absorbance readings. For the toxin concentrations used in this study, the sensitivity rate (the probability that the method will classify a test solution as having toxin, given that the test sample is known to contain toxin) was 1.00 (100%). The specificity rate (the probability that the method will classify a test solution as being free of toxin, given that the test sample is free of toxin) was also 1.00 (100%). False-positive and false-negative rates for the method were zero.

On the basis of the results from this collaborative study, the Associate Referee recommends that the colorimetric polyclonal EIA for the detection of staphyloccal enterotoxin in foods be adopted official first action, as an alternative to the microslide gel double diffusion test, **976.31**, and I concur.

Total Coliforms and Escherichia coli, ColiComplete Discs.—The ColiComplete Substrate Supporting Disc (SSD) system (BioControl Systems, Inc., Bothell, WA) is reported to simultaneously determine the total coliform and *Escherichia coli* counts within 24–48 h. This system uses a small disc impregnated with substrates that can be used with lauryl tryptose broth. The disc can be used either in an MPN or a presence/absence format.

In the MPN fermentation method, lactose is metabolized by the enzyme β-galactosidase to produce carbon dioxide gas. This gas is trapped in the inverted fermentation vial and indicates the potential presence of coliforms.

In the SSD procedure, 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside is the indicator nutrient. A water-soluble indigo blue derivative is formed by cleavage of 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside into the 5-bromo-4-chloroindoxyl intermediate, which, in turn, undergoes oxidation to yield an indigo blue dimer. Any blue color on the disc or in the surrounding medium is considered a positive reaction for total coliforms.

The ColiComplete disc also contains the substrate 4-methyl umbelliferyl β-D-glucuronide (MUG). If *E. coli* organisms are present, then its enzyme, glucuronidase, will cleave the MUG reagent into a glucuronide nutrient portion and methylumbelliferone, which fluoresces under long-wave ultraviolet light.

In a precollaborative study conducted earlier, Associate Referee Philip Feldsine demonstrated comparability between the SSD procedure and the reference MPN method, **966.23–966.24**, for the enumeration of total coliforms and *E. coli* in a wide variety of foods. Subsequently, a collaborative study was performed in which the 2 methods were compared for the analysis of 6 food types. Twenty-nine laboratories participated in the study.

Data were analyzed separately for total coliform bacteria and for *E. coli*. Mean log MPN counts were determined by the SSD procedure and the reference MPN method. Results were then analyzed for repeatability, reproducibility, and mean log MPN statistical equivalence. In general, the 2 methods were statistically equivalent.

On the basis of the results from the precollaborative and collaborative studies, the Associate Referee recommends that the substrate supporting disc procedure for the enumeration of total coliforms and *E. coli* in all foods be adopted official first action, and I concur. At the time of preparation of this report, the Methods Committee has also concurred, and the procedure will be considered for adoption at the August 1992 meeting of the Official Methods Board.

Associate Referee Reports

Automated Conductance Methods.—The Malthus Microbiological Analyzer (Malthus Instruments Ltd., West Sussex, UK) detects changes in the electrical conductance of media caused by the growth and metabolism of microorganisms. These changes in electrical conductance were shown to be directly proportional to changes in the number of microorganisms in the broth medium. The time needed to observe a detectable change in conductance is referred to as the detection time and is related to the number of organisms initially present in the sample. The greater the number of organisms initially present, the shorter the detection time. In addition to being able to provide an estimate of microbial numbers as a measurement of bacteriological quality, the conductance method can be used to perform presence/absence tests for pathogens.

An automated conductance method for the detection of *Salmonella* in foods was approved as an official first action method, **991.38**. Associate Referee Donald Gibson reported that automated conductance methods for the detection of *Listeria monocytogenes* and *Campylobacter* spp. in foods were developed, and a precollaborative evaluation of the *L. monocytogenes* procedure will be initiated in the near future. Moreover, the Associate Referee reports that the conductance instruments are in use in research programs or in predictive microbiological modelling for food safety.

Hydrophobic Grid Membrane Filter Methods.—Associate Referee Phyllis Entis was in contact with several laboratories using the hydrophobic grid membrane filter/MUG (ISO-GRID) method (QA Life Sciences, Inc., San Diego, CA) for the enumeration of total coliforms and *E. coli* in foods, **990.11**, and she received no adverse comments about the performance of the method. Therefore, the Associate Referee recommends that this official first action method be adopted official final action, and I concur.

Listeria, Assurance Enzyme Immunoassay.—The Assurance *Listeria* System (BioControl Systems, Inc., Bothell, WA) is a colorimetric EIA configured in a microwell format. Associate Referee Philip Feldsine conducted a precollaborative study and a specificity study comparing the method to the FDA method or to the USDA method, depending upon the food type being analyzed. A manuscript reporting these results is being prepared. Additionally, a collaborative study protocol was approved by the Methods Committee.

Listeria, Identification by Gas Chromatography of Cellular Fatty Acids.—The MIDI Microbial Identification System (Hewlett Packard Company, Avondale, PA) is a computer-assisted microbial identification system that identifies microorganisms based on cellular fatty acids. Microorganisms are grown under standardized conditions, their cellular fatty acids are extracted, and these extracts are chromatographed using capillary GC. The resulting fatty acid patterns are then compared to a data base prepared using known strains. This comparison is made by a computer, and the 3 most likely species are provided to the analyst.

Associate Referee Linda English completed a precollaborative study of the MIDI Microbial Identification System for identifying *Listeria* species. The original broth used in developing the library was modified to improve the identification of *L. monocytogenes* and *L. innocua*. The Associate Referee is currently preparing a report of the results from the precollaborative study.

Listeria, MICRO-ID System.—The procedure using this system (Organon Teknika Corporation, Durham, NC) was adopted official first action by the Official Methods Board at its May 1992 meeting.

Listeria, TECRA Visual Immunoassay.—A protocol for a precollaborative study of this assay (Biotech Australia, Roseville, N.S.W., Australia) was approved by the General Referee.

Listeria, Vitek AutoMicrobic GPI and GNI System.—The procedure using this system (McDonnell Douglas, Hazelwood, MO) was adopted official first action by the Official Methods Board at its May 1992 meeting.

Pectin Gel Methods.—With methods in place for determining the aerobic plate counts in foods, **988.18**, and the total coliform counts in dairy products, **989.11**, Associate Referee Jonathan Roth reported the development of a new pectin gel (Colichrome 2) medium (RCR Scientific, Inc., Goshen, IN) for differentiating total coliforms from fecal coliforms. A quantitative confirmed test result is available within 24 h after initiation of analysis. A precollaborative study of this medium is currently in progress.

Salmonella, Assurance Enzyme Immunoassay.—The procedure using this assay (BioControl Systems, Inc., Bothell, WA) was adopted official first action by the Official Methods Board at its January 1992 meeting.

Salmonella, GENE-TRAK Systems Colorimetric DNA Hybridization Assay.—The procedure using this assay (GENE-TRAK Systems, Framingham, MA) was adopted official first action by the Official Methods Board at its August 1991 meeting.

Salmonella, Oxoid Method.—This test uses a 24 h preenrichment and a 24 h selective enrichment, the latter occurring in the Oxoid culture vessel (Oxoid Limited, Hampshire, UK) with 5 compartmentalized media. Test reactions indicative of *Salmonella* are screened by the Oxoid *Salmonella* Latex Test, which uses *Salmonella* somatic and flagellar antibodies. Positive results are confirmed by the reference culture method, **967.25–967.28**.

In a precollaborative study, equivalency was reported between the Oxoid culture procedure and the reference culture method for the detection of *Salmonella* in 20 types of artificially contaminated foods and in 3 types of naturally contaminated foods. Subsequently, a collaborative study was performed, and Associate Referee Karl Eckner reported that the results are being collated and a manuscript is being prepared.

Vibrio vulnificus, Identification by Gas Chromatography of Cellular Fatty Acids.—Bacteria grown under controlled laboratory conditions exhibit constant, but unique, fatty acid profiles that can be used to differentiate genera, species, and even subspecies. GC can be used to determine qualitative differences (the presence or absence of particular fatty acids) or quantitative differences in fatty acid amounts. These fatty acids profiles are, in turn, used to distinguish various organisms.

To identify bacteria by GC, several steps are required. Cultures are grown under carefully controlled conditions. Cells are harvested and then saponified to release the fatty acids. These fatty acids are methylated to increase their volatility. Next, the methyl esters of the fatty acids are extracted from the acidified aqueous solution. Before injection into the gas chromatograph, the organic extract is washed with a dilute base. The washed extract can be analyzed by GC with a nonpolar capillary column and a flame ionization detector. Cellular fatty acid profiles of unknown cultures are automatically searched against reference profiles stored in a computer-generated library to identify the unknown culture.

Associate Referee Warren Landry conducted a collaborative study during the past year on the identification of *Vibrio vulnificus* by cellular fatty acid composition using the Hewlett Packard 5898A Microbial Identification System (Hewlett Packard Company, Avondale, PA). The results from the collaborators were received and are being evaluated by the Associate Referee.

In related activities, the Associate Referee made presentations on the identification of *Alteromonas* species, *Edwardsiella tarda*, and *Bacillus thuringiensis* subspecies by fatty acid composition at the Aquaculture 92, American Society for Microbiology, and Institute of Food Technology meetings, respectively.

Recommendations

(1) Adopt as official final action the official first action method Total Coliform and *Escherichia coli* Counts in Foods, Hydrophobic Grid Membrane Filter/MUG (ISO-GRID) Method, **990.11**.

(2) Continue study on the following topics:
   a. *Aerobic Plate Counts of Foods, Perfilm Methods*
   b. *Aerobic Plate Counts, Determination Using Redigel Media*
   c. *Bacillus cereus, Enzyme Immunoassay for Enterotoxins*
   d. *Bacillus cereus Enterotoxin, Microslide Gel Double Diffusion Test*
   e. *Clostridium perfringens, Recovery from Marine Environment by Iron Milk Test*
   f. *Listeria, Assurance Enzyme Immunoassay*
   g. *Listeria, GENE-TRAK Colorimetric DNA Hybridization Method*
   h. *Listeria, Identification by Gas Chromatography of Cellular Fatty Acids*
   i. *Listeria, Listeria-Tek Assay*
   j. *Listeria, MICRO-ID System*
   k. *Listeria, Tecra Enzyme Immunoassay*
   l. *Listeria, Vitek AutoMicrobic System*
   m. *Salmonella, Assurance Enzyme Immunoassay*
   n. *Salmonella, GENE-TRAK DNA Hybridization Method*
   o. *Salmonella, Hydrophobic Grid Membrane Filter (ISO-GRID) Method*
   p. *Salmonella, Immunodiffusion Method*
   q. *Salmonella, Malthus Automated Conductance Method*
   r. *Salmonella, Modified Semisolid Rappaport-Vassiliadis Method for Cocoa and Chocolate*
   s. *Salmonella, Oxoid Method*
   t. *Salmonella, Q-TROL Enzyme Immunoassay Method*
   u. *Salmonella, Salmonella-Tek Enzyme Immunoassay*
   v. *Salmonella, Tecra Immunoassay*
   w. *Salmonella, Escherichia coli, and Other Enterobacteriaceae, MICRO-ID System*
   x. *Salmonella, Escherichia coli, and Other Enterobacteriaceae, Vitek AutoMicrobic GNI System*
   y. *Staphylococcal Enterotoxin, Tecra Enzyme Immunoassay*
   z. *Staphylococcus aureus, GENE-TRAK Colorimetric DNA Hybridization Method*
   aa. *Total Coliforms and Escherichia coli, ColiComplete Discs*
   bb. *Total Coliforms and Escherichia coli, Petrifilm Methods*
   cc. *Vibrio vulnificus, Identification by Gas Chromatography of Cellular Fatty Acids*

(3) Transfer the Associate Referee topic "Aerobic Plate Counts, Reflective Colorimetry Method for Automated Microbiology" to the General Refereeship for Dairy Microbiology.

(4) Discontinue the following topics:
   a. *Bactoscan Methods*
   b. *Escherichia coli in Chilled and Frozen Foods, MUG Test*
   c. *Escherichia coli in Shellfish, MUG Test*
   d. *Listeria, DNA Probe Method*
   e. *Salmonella in Chocolate*
   f. *Vibrio cholerae, Elevated Temperature Enrichment Method*

References

(1) U.S. Food and Drug Administration (1984) *Bacteriological Analytical Manual*, 6th Ed., AOAC, Arlington, VA

(2) U.S. Department of Agriculture (1989) *FSIS Method for the Isolation and Identification of Listeria monocytogenes from Processed Meat and Poultry Products, Laboratory Communication No. 57*, Revised May 24, 1989, USDA, Beltsville, MD

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Microbiology and Extraneous Materials. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992

Water Microbiology

ALFRED P. DUFOUR
U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Cincinnati, OH 45268

I received two proposals for collaborative study of methods for water microbiology. One plan submitted by Kristen Brenner proposed the evaluation of a membrane filter method for simultaneous enumeration of coliforms and *Escherichia coli* in drinking water on surface water samples.

The second plan submitted by James Messer is a protocol for testing the efficiency of microbiological water purifiers for removing viruses, bacteria, and protozoa from contaminated water. Both plans will be submitted for review by the Committee on Microbiology and Extraneous Materials.

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Microbiology and Extraneous Materials. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Committee on Feeds, Fertilizers, and Related Materials

Antibiotics in Feeds

HUSSEIN S. RAGHEB
Purdue University, Dept of Biochemistry,
West Lafayette, IN 47907

Bacitracin in Feeds.—Associate Referee Anil Desai, A.L. Laboratories, Inc., conducted a collaborative study to evaluate the double extraction method for analysis of bacitracin MD in feeds by the microbiological plate assay. Four swine and 4 poultry feeds, each containing 22, 44, and 88 g/ton, were assayed in duplicate by 11 laboratories. The Grubbs test showed no outliers. For each laboratory and feed concentration combination, there were no day-to-day differences. The Student's *t*-test showed no significant differences between feeds for the same level of fortification. The overall coefficients of variation (CVs) between collaborators was 15–20%.

The within-laboratory precision (s_r) variances were calculated for each feed and each level of fortification. The approximate standard deviations were 2 for both feeds containing 22 g/ton, 5 for 44 g/ton, and 8 for 88 g/ton. Comparable values for standard deviations for among-laboratory (s_L) precision were ca 3.5, 6, and 12 g/ton for the 3 feed concentrations and the 2 feed types. In many cases, the magnitude of within-laboratory variance equaled that of among-laboratory precision.

Neomycin in Feeds.—Associate Referee G.L. Stahl, Upjohn Company, carried out a validation study for the analysis of neomycin base in 2 swine and 2 cattle feeds (swine and cattle feeds at 140 g/ton and 280 g/ton each) and 2 premixes at concentrations of 1.4 g/lb and 7.0 g/lb. Three laboratories participated in the analyses of the 6 feeds in duplicate, on 6 different days, by the NaCl–Tris method described before (Stahl, G.L., & Kratzer, D.D. [1984] *J. Assoc. Off. Anal. Chem.* **67**, 863). The range of recovery was 82–109% for laboratory 1, 85–114% for laboratory 2, and 78–134% for laboratory 3. The average CVs for all feeds were 7.5, 10.5, and 12.8% for laboratories 1, 2, and 3 respectively. The plate-to-plate variation (within sample) was the largest source of variance for all collaborators. As percentage of the total variance, plate variation accounted for 78.6% for laboratory 1, 62% for laboratory 2, and 43.5% for laboratory 3. This supports the statement that perhaps diffusion assays are not suitable for this antibiotic (*J. AOAC Int.* [1992] **75**, 163).

Chlortetracycline (CTC) and Oxytetracycline (OTC) in Feeds.—Associate Referee Mary Lee Hasselberger, Nebraska Department of Agriculture (Associate Referee on CTC and OTC), conducted an interlaboratory study of a concentrate and a finished feed containing CTC–HCl. The purpose was to evaluate precision of the microbiological plate assays using plate-to-plate variation and deviation of individual standard points from the calculated values. Results showed plate-to-plate variation to be within 7% and deviations of standard concentrations to be within 3%. Results from laboratories showed good precision: 7 of 11 observed CVs of 6% for feeds labeled 100 g CTC–HCl/ton and 3% for feeds labeled 2 g/lb concentrate. This is in agreement with recent results (CV, 8%) on CTC–HCl analysis by 24 laboratories on AAFCO Sample No. 9223. The Associate Referee will repeat the collaborative study in the near future and urge the collaborators to evaluate their performance based on the previous guides recommended.

A similar study on OTC analysis in feeds showed CV of 5% for complete feed at 100 g/ton (7 of 10 laboratories) and CV of 10% for the 4 g/lb concentrate. The OTC samples were analyzed by both microbiological and LC methods. For the complete feed, results were 71 g/ton by the plate method and 69.5 g/ton by LC. Comparable results for the concentrate were 3.6 g/lb by the microbiological method and 3.4 g/lb by LC. The Associate Referee will continue the studies.

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Feeds, Fertilizers, and Related Materials. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Drugs in Feeds

ROBERT L. SMALLIDGE
Office of Indiana State Chemist, Purdue University,
1154 Biochemistry Bldg, West Lafayette, IN 47907-1154

A list of topics for which there is an assigned Associate Referee follows. Included is a discussion of the status of each subject.

Amprolium.—R.L. Smallidge, Associate Referee. A collaborative study based on the reversed-phase ion-pair method (*J. Assoc. Off. Anal. Chem.* [1988] **71**, 251–255) is planned for 1993.

Carbadox.—Alicia Henk, Associate Referee. No plans were made for a collaborative study. Most laboratories are satisfactorily using variations of the Thorpe method (*J. Chromatogr. Sci.* [1988] **26**, 545–550) or the Lowie et al. method (*J. Assoc. Off. Anal. Chem.* [1983] **66**, 602–605). Aerts et al. car-

ried out an interlaboratory study for carbadox by an adaptation of the Lowie method (*J. Assoc. Off. Anal. Chem.* [1988] **71**, 484–490); this study is being reevaluated to determine if it satisfies AOAC collaborative study guidelines.

Chlortetracycline (LC Method).—Mary Lee Hasselberger, Associate Referee. Studies in the Associate Referee's laboratory have shown good correlation between LC and the official microbiological assay for high-level feeds and premixes. It has not been possible to satisfactorily resolve CTC and epi-CTC peaks from matrix peaks in low-level feeds. Work published by Jeffrey Duggan (*J. Liq. Chromatogr.* [1991] **14**, 2499–2525) and presented at the Midwest Regional AOAC meeting may help to improve the selectivity of the CTC-LC assay for low-level feeds. The study showed that postcolumn addition of alkaline europium resulted in a selective CTC–europium complex that could be measured in either a fluorometric or luminescence detector. The Associate Referee plans to share samples with Duggan to determine if this technique could be adapted to the analysis of low medication levels of tetracyclines in feeds.

Ethopabate.—Joseph Hillebrandt, Associate Referee. With a return to the feed industry in 1992, the Associate Referee hopes to have more time to pursue planning and conducting a collaborative study.

Furazolidone and Nitrofurazone.—Lori L. Rhodig, Associate Referee. These drugs were removed from the marketplace in many countries (Canada still markets them). The Associate Referee feels that this topic should become inactive. I agree and recommend that this topic be discontinued. We thank Rhodig for her contributions as a resource person in this area.

Lasalocid (LC Method).—Alexander MacDonald, Associate Referee. The Associate Referee discussed different approaches to the analysis of lasalocid, with particular emphasis on problems of extraction from mineral matrixes, at the *Antibiotics and Drugs Workshop* that preceded the 105th International AOAC Meeting in Phoenix, AZ, August 11, 1991. Work to determine the optimum extractant/extraction procedure continues. Work also continues to find an optimum reversed-phase chromatographic system for the analysis. The Associate Referee reported that lasalocid can be extracted from many sample matrixes with methanol, but for mineral matrixes, acidified methanol (0.1% HCl in methanol) is the preferred extractant.

Melengestrol Acetate.—Raymond Davis, Associate Referee. There is no current method development for MGA in feeds in his laboratory. Dennis McCurdy (FDA) reported at the Association of American Feed Control Officials meeting, Indianapolis, IN, August 1992, that the U.S. Food and Drug Administration (FDA) was going to pursue development of an LC method for MGA in feeds.

Monensin and Narasin (LC Method).—Mark R. Coleman, Associate Referee. A paper co-authored by the Associate Referee, "Determination of Monensin in Raw Material, Premix, and Animal Feeds by Liquid Chromatography with Correlation to Microbiological Assay," was published in 1992 (*J. AOAC Int.* **75**, 272–279). The Associate Referee submitted a monensin collaborative study protocol for AOAC approval. The collaborative study will be conducted in the fall of 1992. The method to be studied is a reversed-phase, postcolumn derivatization LC method with vanillin as the postcolumn reagent. A manuscript reporting the in-house studies to validate an LC method for narasin will be submitted for publication.

Morantel Tartrate.—Linda Werner, Associate Referee. An LC method developed for the analysis of this drug was submitted to FDA for approval. No progress is reported in evaluating questions raised by the FDA review.

Nifursol.—Ellen Jan De Vries, Associate Referee. During this past year, this topic was added to the *Drugs in Feeds* topic area, and Ellen De Vries was appointed Associate Referee. He reports that an LC method was used successfully in his laboratory for more than 3 years, and a manuscript detailing the in-house validation of the method will soon be submitted to *J. AOAC Int.* The Associate Referee reports that he has a preliminary commitment from several potential collaborators in Europe and North America and plans a collaborative study in 1993.

Oxytetracycline (LC Method).—Mary Lee Hasselberger, Associate Referee. The Associate Referee has a paper, "Assay of Oxytetracycline in Animal Feed by Liquid Chromatography and Microbiological Plate Assay," accepted for publication. Studies involving a few other laboratories indicated that many reversed-phase LC columns do not satisfactorily resolve the analyte from furazolidone, riboflavin, and small sample matrix peaks. This problem will be further studied in the fall, with plans for a full collaborative study in 1993.

Pyrantel Tartrate.—Joyce Konrardy, Associate Referee. Progress was made on a new method for pyrantel tartrate. If fine-tuning, ruggedness testing, and in-house validation progress as expected, a collaborative study should be possible in 1993.

Roxarsone.—Inactive topic. The official spectrophotometric method (**971.47**) for roxarsone analysis requires the recovery of 30.0 mL extract for further processing after extraction. With some matrixes, this is not possible; however, if both the sample weight and the volume of extractant are doubled, 30.0 mL extract can easily be recovered. I recommend that the following parenthetical note be added after the second sentence in the section, *Preparation of Sample* (**971.47 D**): (Doubling of the sample weight, the phosphate extractant volume, and the extraction vessel volume is necessary to recover 30.0 mL extract from some matrixes).

Sampling.—John H. Gallagher, Associate Referee. The Associate Referee resigned because of a change of job responsibilities. An attempt will be made to find a new referee who has the background and time to study sampling of bulk and bag materials and preparing a representative laboratory sample. Most current AOAC sampling procedures were based on proximate analyses and are not necessarily suited for the nature and variety of low-level medicaments. When the current sampling procedures (**965.16, 950.02**) were made official, the variety of feed pellets found in the marketplace today did not exist. Another problem that has evolved in recent years is the difficulty of obtaining a representative sample from feeds to which medicaments are added in concentrated, protected particles rather than as powders. A study of mills in use for particle size reduc-

tion and mixing procedures in subsample preparation would be beneficial to those working with feed medicaments.

Sulfadimethoxine and Ormetoprim.—Alex MacDonald, Associate Referee. A manuscript reporting the in-house validation of an LC method for the simultaneous determination of these 2 drugs in poultry and fish feeds, with the Associate Referee as co-author, was submitted to FDA for approval and to AOAC for review and publication. Plans for an AOAC collaborative study depend upon FDA action. FDA's *Laboratory Information Bulletin* No. 3593 contains preliminary validation work on the "Determination of Sulfadimethoxine and Ormetoprim in Catfish Feed using HPLC" by K. Paul and J. Meyer of the FDA Denver District Laboratory. This is a simple extract, clarify, and inject, reversed-phase method that should be given consideration for more rigorous validation and ruggedness testing in different sample matrixes.

Sulfa Drug Residues in Feeds.—Valerie Reeves, Associate Referee. Continue study.

Sulfamethazine and Sulfathiazole in Premixes & Finished Feeds .—Dwight M. Lowie, Associate Referee. Lowie reports that efforts to develop a single LC/UV method for the analysis of both medicated-feed-level sulfamethazine and sulfathiazole continues to be unproductive. A reversed-phase LC/UV method involving extraction with aqueous acetonitrile followed by cleanup on a cation exchange cartridge was published: "Determination of Sulfadimidine [Sulfamethazine] in Medicated Animal Feeds" (1992) *Analyst* **117**, 817–822.

A collaborative study of the LC postcolumn derivatization method (*J. Assoc. Off. Anal. Chem.* [1988] **71**, 710–717) for sulfamethazine in medicated complete feeds was initiated by myself and should be completed by the end of the year.

Addresses for the Associate Referees are listed under the *Committee on Feeds, Fertilizers, and Related Topics* elsewhere in this issue or in the *AOAC Membership Directory*. Contact the specific Associate Referee, the General Referee, or AOAC with questions about, or comments on, drugs in feeds methods, especially Official Methods with limitations or errors. Anyone with an improved method for the analysis of drugs in feeds is encouraged to submit a manuscript of the in-house validation of that method to *J. AOAC Int.*

Recommendations

(1) *Amprolium in Feeds.*—Change current topic name to *Amprolium* for list uniformity. Continue study.
(2) *Carbadox.*—Continue study.
(3) *Chlortetracycline (LC Method).*—Continue study.
(4) *Ethopabate.*—Continue study.
(5) *Furazolidone and Nitrofurazone.*—Discontinue topic.
(6) *Lasalocid by HPLC* .—Change current topic name to *Lasalocid (LC Method)* for list uniformity. Continue Study.
(7) *Melengestrol Acetate.*—Continue study.
(8) *Monensin and Narasin Chromatographic Method.*—Change current topic name to *Monensin and Narasin (LC Method)* for list uniformity. Continue study.
(9) *Morantel Tartrate.*—Continue study.
(10) *Nifursol.*—Continue Study.
(11) *Oxytetracycline in Feeds by Liquid Chromatographic Methods.*—Change current topic name to *Oxytetracycline (LC Method)* for list uniformity. Continue study.
(12) *Pyrantel Tartrate.*—Continue study.
(13) *Roxarsone.*—Add parenthetical note to **971.47D** as recommended in text.
(14) *Sampling.*—Appoint new Associate Referee. Continue study.
(15) *Sulfadimethoxine and Ormetoprim.*—Continue study.
(16) *Sulfa Drug Residues in Feeds.*—Continue study.
(17) *Sulfamethazine and Sulfathiazole in Premixes and Finished Feeds.*—Continue study.

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Feeds, Fertilizers, and Related Materials. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Fertilizers and Agricultural Liming Materials

PETER F. KANE
Office of the Indiana State Chemist, Purdue University, West Lafayette, IN 47907

Trace fertilizer analysis by ICP is an area that needs method development. David Averitt of IMC in Mulberry, FL, expressed an interest in being appointed the Associate Referee in this area. I will recommend his appointment pending indication of company support of his effort.

Sulfur in fertilizers, **980.02**, has some method shortcomings. The elemental S portion of the method is imprecise, especially as the level of elemental S increases. Samples with very high-level elemental S are biased low. A relatively new sulfur product that contains 40 or 41% sulfur and trace elements in fritted glass, so as to be slow release, gave erratic and low results. I wrote an outline of the problem in *The Referee*. It is hoped an Associate Referee will be found to work on possible solutions.

Sampling.—George Latimer completed a collaborative study on a fluid sampling tube for fertilizers. The method successfully navigated the approval process and is now before the Official Methods Board for final review for first action. I asked Associate Referee Doug Caine to consider incorporating new International Organization for Standardization procedures for fluid fertilizer sampling into the existing AOAC fluid sampling procedures.

Sample Preparation.—Associate Referee Robert Beine is in the process of preparing a paper describing various procedures that could be used to measure the performance of the

splitting, grinding, and mixing operations involved in sample preparation. Hopefully, discussion of these procedures will lead to the collaboration of performance criteria, which will be incorporated into official sample preparation procedures.

Nitrogen by Combustion.—Associate Referee Don Tate submitted a collaborative study for review, which is currently before the Official Methods Board and scheduled for first action consideration at its next meeting. Tate is now doing method development with a liquid sampler for the combustion instrument and attempting to devise a procedure using volumetric measurement of liquids for direct injection into the analyzer.

Phosphorus.—Associate Referee Joe Gliksman conducted a collaborative study on an new extraction for the available phosphoric acid method, and he is recommending the method for first action approval. I concur. Gliksman is currently investigating additional questions raised during the collaborative study. He also anticipates a new investigation upgrading the automated colorimetric detection of both available and total phosphoric acid extracted solutions.

Potassium.—Natalie Newlon was appointed Associate Referee for K_2O by flame photometry. She evaluated new instrumentation for the automated flame photometric method, and she is working on a paper describing how well the new instrumentation met the performance specifications in the official method.

The area of K_2O by ICP needs to be addressed.

Free and Total Water.—No report.

Slow-Release Mixed Fertilizers.—The Associate Referee position is vacant. I had hoped to appoint Joe Trimm as Associate Referee to replace Stan Katz, but Joe was not able to arrange acceptance of the position. However, Joe is active in the area and intends to do much of the work of an Associate Referee, because this is closely related to his area of expertise.

Manganese in Feed and Fertilizer Ingredients.—Associate Referee Ronald Baker is in process of revising into AOAC style a wet chemistry method for manganese, which is more precise than atomic absorption for very high-level materials. He intends to pursue a collaborative study comparing the method to atomic absorption.

Urea and Methylene Ureas.—Associate Referee Thomas Parham reports no activity in his area this year.

Iron.—Associate Referee James Silkey reports no progress on the method for chelated iron in mixed fertilizer. He hopes to schedule work on the project this fall.

Recommendations

(1) In January, I recommended that the following methods be changed from first action to final action status: **974.01**, Sodium in Fertilizers, Flame Photometric Method; **983.04**, Sodium in Fertilizers, Atomic Absorption Spectrophotometric Method; **983.05**, Aluminum in Aluminum Sulfate-Type Soil Acidifiers, Atomic Absorption Spectrophotometric Method; **973.03**, Particle Size Range of Peat, Mechanical Analysis; **973.05**, Sand in Peat; **973.06**, Nitrogen (Total) in Peat; **973.07**, Volume, Peat; and **973.08**, Volume Weight, Water-Holding Capacity, and Air Capacity of Water-Saturated Peat Materials.

(2) Continue study on all other topics.

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Feeds, Fertilizers, and Related Materials. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Nutrients in Soils

THOMAS L. JENSEN
Nebraska Dept of Agriculture, 3703 S. 14th St,
Lincoln, NE 68502

The major accomplishment in this area has been the preparation for a collaborative study on soil pH. Several months ago, a committee was appointed by the Soil Science Society of America (SSSA) to develop 4 methods to be used in the study. The Associate Referee has taken the methods recommended by the committee and developed a protocol for conducting the collaborative study. The protocol was submitted to the methods committee. Collaborators have been enlisted.

In conjunction with the pH study, I have been in contact with the U.S. Department of Agriculture, National Soil Survey Laboratory, which will be supplying samples for this study. The Soil Survey Laboratory has access to a wide variety of samples that will be of great use in this and future collaborative studies.

In other areas, preliminary plans are being made to validate methods to determine cation exchange capacity in soils. The SSSA committee is also prioritizing future nutrient areas for validation. Also, interest in additional areas was shown by outside sources.

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Feeds, Fertilizers, and Related Materials. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Tobacco

WILLARD W. WEEKS
North Carolina State University, Crop Science Dept, PO Box 7620, Raleigh, NC 27695

I contacted each of the Associate Referees with the tobacco division, and no one has anything to report at this time. Maurice Snook, PhytoChemical Research Center, USDA, ARS, SAA, reports that the polyphenol collaborative study he is conducting is still in progress, although he has only received data from 2 of his collaborators at this time.

Michael Ogden of Reynolds Tobacco Company reports that at this time there is no collaborative work being conducted on environmental tobacco smoke. There is, however, an article that should appear in the next issue of *J. AOAC Int.* concerning environmental tobacco smoke.

The alkaloid study using the autoanalyzer to determine total alkaloids from tobacco is still underway. At this time, the collaborators are trying to analyze 10 tobacco samples that are representative of all different tobacco types in the tobacco industry.

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Feeds, Fertilizers, and Related Materials. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Veterinary Analytical Toxicology

P. FRANK ROSS
U.S. Department of Agriculture, Ames, IA

The method for cholinesterase by the pH method (1) was approved first action. Results of the collaborative study will be reported in the *Journal of AOAC International*. A collaborative study of zinc in liver by atomic absorption (AA) was completed, and the report was submitted for approval.

The Association of Veterinary Laboratory Diagnosticians (AAVLD)/American College of Veterinary and Comparative Toxicologists (AAVCT)/AOAC Advisory Committee on Veterinary Analytical Toxicology met at the annual meeting of the AAVLD, October 26, 1991, in San Diego, CA. Its annual report was published (2).

The Ninth Annual Workshop on Veterinary Analytical Toxicology was held at the Midwest Regional AOAC meeting, June 9–10, 1992, Champaign, IL. Oral presentations and posters were given on the following topics: clenbuterol, alkyl mercury by afterglow detection, cholinesterase activity in blood and brain, multiresidue insecticide screening, nitrate/nitrite methods, xenobiotic metabolites in animals, ICP analysis of tissues, TLC screening for ionophores, diagnostic toxicology data bases, and several fumonisin topics. The Tenth Annual Workshop is planned for the Midwest Regional meeting, June 1993, in Des Moines, IA. Scientists interested in participation should contact me.

The fumonisin mycotoxins continue to be of interest to diagnosticians. Reports on chemical, toxicological, and mycological studies (3–22) have added significantly to the knowledge base for the ubiquitous mycotoxins. Readers are encouraged to review the General Referee Report on Mycotoxins published in this volume for further information on fumonisins.

Historically, committee and workshop activities have been a significant portion of this report. This year is no exception. Activities remained strong, as witnessed by the wide range of topics at the above-mentioned meetings. A major effort to improve communications through the use of electronic mail is currently underway. Using Internet and/or Bitnet networks, several diagnostic toxicology sections are routinely exchanging information. Support for continued improvement in methods is still strong, as witnessed by the Toxicology Cooperative Check Sample Program sponsored by the AAVLD/AAVCT/AOAC committee. During 1991, a series of check samples for mycotoxins, ionophores, and nitrate were supplied to interested laboratories. Laboratories were asked to use their method of choice and return results, which were discussed at the annual committee meeting. Similar activity for 1992 is currently underway, with the addition of vitamins A and E to the program.

Activities in topic areas varied during the past year. Budget, time, and other factors significantly influenced the work. I wish to thank the Associate Referees for a job well done. Summaries of some topics follow.

Antibiotic Screening Methods.—Associate Referee Wynne Landgraf (National Veterinary Services Laboratories, Ames, IA) proposed a study on the TLC method for screening feeds for monensin. A previous interlaboratory study with 3 samples (2 containing monensin and 1 blank) was generally encouraging. Recovery problems were resolved. The protocol is pending approval by the appropriate methods committee.

The Associate Referee recommends continued study.

Cholinesterase, Colorimetric Method.—Associate Referee Karen Harlin (University of Illinois, Urbana, IL) reports that the colorimetric method for whole blood cholinesterase (**991.10**) received no adverse feedback.

The Associate Referee recommends continued first action status.

Cholinesterase, pH Method.—Associate Referee Paula Martin Imerman (Iowa State University, Ames, IA) reports that the report on the collaborative study of the pH method on whole blood cholinesterase (1) was approved first action.

The Associate Referee recommends continued first action status.

Nitrates and Nitrites.—Associate Referee Mike Carlson (University of Nebraska, Lincoln, NE) reported on various studies of nitrate and nitrite methodologies. The following is a

summary abstracted from his presentation at the 1992 Midwest AOAC Meeting, June 8, 1992, Champaign, IL. One phase involved a limited study (1) to determine if forage nitrate results were affected by 2 methods of extraction: boiling or shaking the specimen in extracting solution. Three forage nitrate specimen pools were made by thoroughly mixing forage specimens with similar nitrate contents that were submitted to and analyzed at the University of Nebraska-Lincoln, Veterinary Diagnostic College. The pooled specimens were analyzed by an AOAC-collaborated potentiometric method to verify nitrate content; the specimens were designated as low (<2000 ppm nitrate), medium (5000–10 000 ppm nitrate), and high (>10 000 ppm nitrate). Portions of each forage pool were submitted to the collaborating laboratories, and each specimen was analyzed after extraction by boiling or shaking in extraction solution in 3 replicates. A statistically significant difference was detected for method of extraction; the nitrate content found after extraction by boiling was consistently greater than that found after extraction by shaking. The magnitude of the difference depended upon the laboratory and the method used for quantitation.

The Associate Referee recommends further study.

A second phase involved a study to determine the following: if significant differences occurred in nitrate and nitrite results for biological fluids obtained by 2 laboratories, if differences depended upon the matrix being analyzed, and if differences depended upon nitrate and nitrite contents. Treatment structure for the experiment was a $2 \times 4 \times 4 \times 4$ factorial with treatment factors being lab (NP, University of Nebraska-Lincoln), matrix (serum, heparinized plasma, fetal ocular fluid, water), nitrate spike (0, 10, 20, 50 μg nitrate/mL), and nitrite spike (0, 0.20, 0.50, 1.00 μg nitrite/mL). Treatment allocation was completely random and according to a balanced incomplete block design using 8 blocks each consisting of 16 experimental units. Defining contrasts were selected so as not to confound any of the main or 2-way interaction effects. One lab performed the analyses using a high-performance ion chromatographic (HPIC) system with UV detection of nitrate and visible spectrophotometric detection of nitrite after postcolumn derivitization. The other laboratory used an automated cadmium reduction, diazo-coupling spectrophotometric system. Spike recovery was calculated for each specimen by using the mean nitrate and nitrite concentrations found in unspiked specimens. Nitrate and nitrite concentrations and spike recoveries were compared using analysis of variance. Statistically, significant treatment effects found for nitrate detected were matrix × nitrate spike, lab, matrix, nitrate spike, and nitrite spike ($P < 0.05$). The only significant treatment effect found for nitrite detected was nitrite spike ($P = 0.0001$). The magnitudes of the differences detected, other than for effects containing nitrate spike, were about 3 μg nitrate/mL.

Significant effects found for nitrate recovery were matrix × nitrate spike, lab × matrix, matrix, and nitrite spike. The only significant effect found for nitrite recovery was for lab nitrate recoveries; nitrite recoveries for serum, plasma, and ocular fluid were all between 90 and 102%; recoveries for water were at least 85%. Matrix × nitrate effect for nitrate recovery indicated that serum and plasma behaved comparably, as did ocular fluid and water, but serum and plasma behaved differently from ocular fluid and water, which suggests that quality control specimens should be matched to the matrix being analyzed. The automated Cd-reduction system, as used, was not as sensitive for nitrite as was the HPIC system. The data indicate that differences of up to 5 μg nitrate/mL may occur at nitrate concentrations less than 10 μg nitrate/mL.

The Associate Referee Recommends continued study.

Pesticides in Toxicological Samples.—Associate Referee H.M. Stahr (Iowa State University, Ames, IA) reports successful application of the method for chlorinated hydrocarbons in blood to the analysis of blood for pyrethroid insecticides. Deltamethrin was recovered from blood of animals given relatively low doses. Further study is planned. As previously reported, the Associate Referee continues to evaluate the highly sensitive and selective elemental plasma detector and its application to tissues from toxicology cases.

The Associate Referee recommends continued study.

Selenium in Animal Tissue.—Associate Referee Karen Harlin resigned because of a job change. She recommends that study continue.

Sodium Monofluoroacetate.—Associate Referee H.M. Stahr reports on a limited response from potential collaborators for a study using the fluoride electrode for the indirect determination of sodium monofluoroacetate in tissues. Several laboratories are currently using the method with excellent results. The Associate Referee renewed his call for collaborators.

The Associate Referee recommends continued study.

Zinc in Animal Tissues.—Associate Referee Dana Perry (University of Arizona, Tucson, AZ) reports that he received no negative feedback concerning the serum zinc method (**991.11**). He completed a collaborative study of the AA method for zinc in liver, and the report was submitted for approval. Results of the collaborative study were presented at the 1991 annual meeting in Phoenix, AZ.

The Associate Referee recommends continued first action status for the serum zinc method and adoption of first action status of the AA method for zinc in liver.

Recommendations

(1) *Antibiotic Screening Methods.*—Continue study.
(2) *Cholinesterase Colorimetric Method.*—Continue first action status of the colorimetric method for whole blood cholinesterase (**991.10**).
(3) *Cholinesterase pH Method.*—Continue first action status of the pH cholinesterase method.
(4) *GC/MS Methods.*—Continue study.
(5) *Fluoride in Animal Tissue.*—Continue study.
(6) *Lead in Animal Tissue.*—Continue study.
(7) *Multielement Analysis by ICP.*—Continue study.
(8) *Multiple Anticoagulant Screening.*—Continue study.
(9) *Natural Products.*—Continue study.
(10) *Nitrates and Nitrites.*—Submit protocol for a collaborative study on dip-stick method for forage nitrate.

(11) *Pesticides in Toxicological Samples.*—Continue study.
(12) *Selenium in Animal Tissue.*—Assign new Associate Referee.
(13) *Sodium Monofluoroacetate.*—Continue study.
(14) *Vitamins A and E.*—Continue study.
(15) *Vitamins D and K.*—Retire topic.
(16) *Zinc in Animal Serum.*—Continue first action status of the method serum zinc (**991.11**).

References

(1) Michel, H.D. (1967) *J. Lab. Clin. Med.* **34**, 222–225
(2) *Veterinary Analytical Toxicology Advisory Committee Report* (January 1992) American Association of Veterinary Laboratory Diagnosticians Newsletter, PO Box 6023, Columbia, MO, pp. 17–18
(3) Colvin, B.M., & Harrison, L.R. (1992) *Mycopathologia* **117**, 79–82
(4) Kellerman, T.S., Marasas, W.F.O., & Thiel, P.G. (1990) *Onderstepoort J. Vet. Res.* **57**, 269–275
(5) Leslie, J.F., Pearson, C.A.S., Nelson, P.E., & Toussoun, T.A. (1990) *Phytopathology* **80**, 343–350
(6) Leslie, J.F., Plattner, R.D., Desjardins, A.E., & Klittich, C.J.R. (1992) *Phytopathology* **82**, 341–345
(7) Leslie, J.F. (1991) *Phytopathology* **81**, 1058–1060
(8) Nelson, P.E., Plattner, R.D., Shackelford, D.D., & Desjardins, A.E. (1991) *Appl. Environ. Microbiol.* **57**, 2410–2412
(9) Norred, W.P., Plattner, R.D., Vesonder, R.F., Hayes, P.M., Bacon, C.W., & Voss, K.A. (1990) *Toxicologist* **10**, 165
(10) Osweiler, G.D., Ross, P.F., Wilson, T.M., Nelson, P.A., Witte, S.T., Carson, T.L., & Nelson, H.A. (1992) *J. Vet. Diagn. Invest.* **4**, 53–59
(11) Plattner, R.D., Weisleder, D., Shackelford, D.D., Peterson, R.E., & Powell, R.G. (1992) *Mycopathologia* **117**, 23–28
(12) Plattner, R.D., Ross, P.F., Reagor, R., Stedelin, J., & Rice, L.G. (1991) *J. Vet. Diagn. Invest.* **3**, 357–358
(13) Plattner, R.D., Norred, W.P., Bacon, C.W., Voss, K.A., Peterson, R., Shackelford, D.D., & Weislder, D. (1990) *Mycologia* **82**, 698–702
(14) Rottinghaus, G.E., Coatney, C.E., & Minor, H.C. (1992) *J. Vet. Diagn. Invest.* in press
(15) Ross, P.F., Rice, L.G., Plattner, R.D., Osweiler, G.D., Wilson, T.M., Owens, D.L., Nelson, H.A., & Richard, J.L. (1991) *Mycopathologia* **114**, 129–132
(16) Ross, P.F., Rice, L.G., Reagor, J.C., Osweiler, G.D., Wilson, T.M., Nelson, H.A., Owens, D.L., Plattner, R.D., Harlin, K.A., Richard, J.L., Colvin, B.M., & Banton, M.I. (1991) *J. Vet. Diagn. Invest.* **3**, 238–241
(17) Ross, P.F., Rice, L.G., Osweiler, G.D., Nelson, P.E., Richard, J.L., & Wilson, T.M. (1992) *Mycopathologia* **117**, 109–114
(18) Sydenham, E.W., Shephard, G.S., Thiel, P.G., Marasas, W.F.O, & Stockenstrom, W. (1991) *J. Agric. Food Chem.* **39**, 2014–2018
(19) Thiel, P.G., Shephard, G.S., Sydenham, E.W., Marasas, W.F.O, Nelson, P.E., & Wilson, T.M. (1991) *J. Agric. Food Chem.* **39**, 109–111
(20) Thiel, P.G., Marasas, W.F.O., Sydenham, E.W., Shephard, G.S., Gelderblom, W.C.A, & Nieuwenhuis, J.J. (1991) *Appl. Environ. Microbiol.* **57**, 1089–1093
(21) Wilson, T.M., Ross, P.F., Owens, D.L., Rice, L.G., Green, S.A., Jenkins, S.J., & Nelson, H.A. (1992) *Mycopathologia* **117**, 115–120
(22) Yoo, H., Norred, W.P., Wang, E., Merrill, J., Riley, R.T. (1992) *Toxicol. Appl. Pharmacol.* **114**, 9–15

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Feeds, Fertilizers, and Related Materials. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Committee on Environmental Quality

Cooperative Studies

JOSEPH R. DONNELLY
Lockheed Engineering & Sciences Co., 980 Kelly Johnson Dr., Las Vegas, NV 89119

Cooperative studies over the past year involved joint U.S. Environmental Protection Agency (EPA) and AOAC interlaboratory studies of EPA methods. These interlaboratory studies were conducted following AOAC guidelines for collaborative method validations. Studies 1–6 (below) were conducted through efforts by AOAC Associate Referees Kenneth W. Edgell (Bionetics Corporation, Cincinnati, OH), James E. Longbottom (EPA, Environmental Monitoring and Support Laboratory, Cincinnati, OH), and Viorica Lopez-Avila (Mid-Pacific Environmental Laboratory, Mountain View, CA). Robert Miller (University of California, Davis) was appointed Associate Referee for inorganic elements in plant tissues and nitrates in soils.

Progress on individual cooperative studies is summarized below:

(1) *Determination of Chlorinated Acids in Groundwater by Gas Chromatography/Electron Capture Detection.*—Kenneth W. Edgell, Elizabeth J. Erb, and James E. Longbottom. The results of the collaborative study were presented at the AOAC 105th International Annual Meeting in Phoenix, AZ, August 1991. The method was recommended for official first action at the AOAC 106th International Annual Meeting in Cincinnati, OH, August 1992.

(2) *Determination of Ethylene Thiourea (ETU) in Water by Gas Chromatography with Nitrogen/Phosphorus Detection.*—Kenneth W. Edgell, Elizabeth J. Erb, Viorica Lopez-Avila, and James E. Longbottom. The results of the collaborative study were presented at the AOAC 105th International Annual Meeting in Phoenix, AZ, August 1991. The method was recommended for official first action at the AOAC 106th International Annual Meeting in Cincinnati, OH, August 1992.

(3) *Determination of Pesticides in Groundwater by Liquid Chromatography with Ultraviolet Detection.*—Kenneth W. Edgell, Elizabeth J. Erb, Viorica Lopez-Avila, and James E. Longbottom. This method was approved as official first action in the January 1992 meeting of the Official Methods Board.

(4) *Determination of 1,2-Dibromoethane (EDB) and 1,2-Dibromo-3-chloropropane (DBCP) in Water by Gas Chromatography.*—Kenneth W. Edgell and James E. Longbottom. This method is based on EPA Method 504. The method was recommended for official first action at the AOAC 106th International Annual Meeting in Cincinnati, OH, August 1992.

(5) *Determination of Carbonyl Compounds by High-Performance Liquid Chromatography (HPLC).*—Kenneth W. Edgell and James E. Longbottom. The collaborative study was completed in October 1991, and the report will be presented at the AOAC 107th International Annual Meeting.

(6) *Dissolved Hexavalent Chromium in Drinking Water, Groundwater, and Industrial Wastewater Effluents, Ion Chromatographic Method.*—This method was submitted by K.W. Edgell for AOAC review.

(7) *Metals in Water, Soil, Sludge, and Fly Ash by Inductively Coupled Plasma/Mass Spectrometry (ICP/MS).*—The collaborative study was completed, and results will be presented by J.R. Donnelly at the AOAC 107th International Annual Meeting.

(8) *Microwave Digestion of Plant Samples for Inorganic Elemental Analysis.*—Robert O. Miller. Preliminary testing of the method was completed. The selection process was initiated for collaborative study laboratories.

Recommendations

Adopt as official first action the following methods:

(1) *Determination of Chlorinated Acids in Groundwater by Gas Chromatography/Electron Capture Detection.*

(2) *Determination of Ethylene Thiourea (ETU) in Water by Gas Chromatography with Nitrogen/Phosphorus Detection.*

(3) *Determination of 1,2-Dibromoethane (EDB) and 1,2-Dibromo-3-chloropropane (DBCP) in Water by Gas Chromatography.*

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Environmental Quality. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Organics in Water

VIORICA LOPEZ-AVILA
Midwest Research Institute, California Operations,
625-B Clyde Ave, Mountain View, CA 94043

I served as Co-Associate Referee on the collaborative studies described under the General Refereeship on Cooperative Studies.

Associate Referee Topics

Two Associate Referees were appointed: Shirley Gee, University of California, Davis, CA, will serve as Co-Associate Referee with Robert Harrison, Immunosystems Inc., Scarborough, ME, for study on "Determination of Atrazine in Water by Enzyme Immunoassay," and David Herzog, Ohmicron Corp., Newtown, PA, will serve as Associate Referee for study on "Measurement of Atrazine in Water by Magnetic-Particle-Based Enzyme Immunoassay."

Diquat and Paraquat.—An Associate Referee needs to be appointed.

Determination of Carbonyl Compounds in Waters by High-Performance Liquid Chromatography.—Collaborative study was completed (see Cooperative Studies).

Thermospray LC/MS Analysis of Carbamates.—Tammy Jones completed a minicollaborative study on the calibration of the thermospray LC/MS system for the determination of 3 *N*-methylcarbamates, 3 *N*-methylcarbamoyl oximes, 2 substituted urea pesticides, and 1 ester of a substituted carbamic acid.

Future Studies.—I am pursuing EPA-Cincinnati funding for a study on an alternative solid-phase extraction method using the Empore membrane disks. The procedure is less labor-intensive and uses substantially less glassware and organic solvent than the conventional procedures. The list of analytes has not been defined yet. Suggestions to pursue the organochlorine pesticides were received. Craig Markell of 3M expressed interest in serving as Co-Associate Referee.

This report of the General Referee was presented at the 106th AOAC International Annual Meeting, August 31–September 2, 1992, at Cincinnati, OH. The recommendations were reviewed by the Committee on Environmental Quality. See the report of the committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and its supplement, *Changes in Official Methods of Analysis*, 3rd supplement, 1992.

Journal Information

The Association

The primary objective of AOAC International is to obtain, improve, develop, test, and adopt precise, accurate, and sensitive methods for analysis of foods, vitamins, food additives, pesticides, drugs, cosmetics, plants, feeds, fertilizers, hazardous substances, air, water, and any other products, substances, or phenomena affecting the public health and safety, the economic protection of the consumer, or the protection of the quality of the environment; to promote uniformity and reliability in the statement of analytical results; to promote, conduct, and encourage research in the analytical sciences related to foods, drugs, agriculture, the environment, and regulatory control of commodities in these fields; and to afford opportunity for the discussion of matters of interest to scientists engaged in relevant pursuits.

AOAC *Official Methods* are methods that have been validated by an AOAC-approved collaborative study, recommended by the appropriate AOAC General Referee, Methods Committee, and the Official Methods Board, and adopted and published according to the Bylaws of the Association. Published papers that include such methods are distinguished by the words Collaborative Study in the title and by footnotes that indicate Association actions.

Membership in AOAC is open to all interested persons worldwide. Sustaining memberships are available to any government agency, private company, or association interested in supporting an independent methods validation program.

European Representatives. For information about AOAC and its publication, persons outside the U.S. may also contact the following: Margreet Lauwaars, PO Box 153, 6720 AD Bennekom, The Netherlands, telephone 31-8389-18725; Derek C. Abbott, 33 Agates Lane, Ashtead, Surrey, KT21 2ND, UK, telephone 44-3722-74856.

The Journal

The *Journal of AOAC International* (ISSN 1060-3271) is published bimonthly by AOAC International, Suite 400, 2200 Wilson Blvd, Arlington, VA 22201-3301 USA. Each volume (one calendar year) will contain about 1200 pages. The scope of the *Journal* encompasses the development and validation of analytical procedures pertaining to the physical and biological sciences related to foods, drugs, agriculture, and the environment. Emphasis is on research and development of precise, accurate, and sensitive methods for the analysis of foods, food additives and supplements, contaminants, cosmetics, drugs, toxins, hazardous substances, pesticides, feeds, fertilizers, and the environment.

Methods. The scientific validity of published methods is, of course, evaluated as part of the peer-review process. However, unless otherwise stated, methods published in contributed papers in the *Journal* have not been adopted by AOAC and are not AOAC Official Methods.

Manuscripts. Prepare manuscripts double spaced throughout and carefully revise before submission; 4 copies should be submitted to Managing Editor, AOAC International, Suite 400, 2200 Wilson Blvd, Arlington, VA 22201-3301 USA. AOAC reserves the right to refuse manuscripts produced on dot matrix printers of less than letter quality.

Reprints. Authors may order reprints of articles when they return desktop published proofs. An order form and schedule of rates is included with each author proof. Readers who wish to obtain individual reprints should contact authors directly.

Responsibility. AOAC and the Editors assume no responsibility for statements and opinions expressed by authors of manuscripts published in the *Journal*.

Subscriptions

Journal subscriptions are sold by the volume (one calendar year). Rates for 1992 are as follows: Members: $120.00 in U.S.; $158.00 outside U.S. Nonmembers: $175.00 in U.S.; $213.00 outside U.S. Two year subscriptions are also available. Airmail $98.00 additional per year. Claim for copies lost in the mail will not be allowed unless received within 30 days of the date of issue for U.S. subscribers or 90 days for all others. Claimants must state that the publication was not received at their recorded address. Address requests for replacement copies to AOAC International, Suite 400, 2200 Wilson Blvd, Arlington, VA 22201-3301 USA. For subscribers outside the U.S., copies of the *Journal* lost in transit cannot be replaced without charge because of uncertain mailing conditions.

Change of Address. Notification should include both old and new addresses, with mail code, and be accompanied by a mailing label from a recent issue. Allow 4 weeks for change to become effective. Subscribers outside the U.S. should use airmail for notification.

Microfilm. Volumes on microfilm are available from Princeton Microfilm Corp., PO Box 2073, Princeton, NJ 08540.

Single Issues. Information on single issues is available from AOAC.

Copying

Copyright. All articles published in the *Journal* are protected by copyright. Persons requiring copies of *Journal* articles beyond the number allowed by the fair use provisions of the 1978 U.S. copyright law may request permission to copy directly from AOAC or may make the required copies and pay $1.00 per copy through the Copyright Clearance Center, Inc., 21 Congress St, Salem, MA 01970. Articles which are copied and royalties paid through the Copyright Clearance Center must be identified by the following code: 0004-5756/87$1.00, indicating the International Standard Serial Number assigned to *J. AOAC Int.*, the year, and the copying fee per copy. Information on the use of the Copyright Clearance Center is available from the Center.

Please Note. Except for photocopies prepared as described above, no part of this *Journal* may be reproduced in any form or stored in any microform or electronic form without the written permission of the Association.

Advertising

Placement. All space reservations and all advertising copy are due 1½ months in advance of publication at AOAC International, Suite 400, 2200 Wilson Blvd, Arlington, VA 22201-3301 USA. Advertising inserted 3, 6, or 12 times within 1 year of first insertion earns frequency discount rates. Contact Marilyn Blakely, AOAC, for size requirements and schedule of rates.

Policy. Advertising is prepared by the advertisers and has been reviewed for compliance with the AOAC Policy on Advertising in Association Publications. AOAC publication of an advertisement is not an endorsement or approval, expressed or implied, by the Association or the Editors of any service, product, or claim made by the manufacturer.

New Products. Notices of new products are prepared from literature received from manufacturers and other companies and are published only as a service to readers. Publication of notices of new products is not intended as an AOAC endorsement or approval of any service, product, or claim made by the manufacturers.

11/92

The 106th AOAC Annual International Meeting & Exposition

Cincinnati Convention Center
Cincinnati, Ohio

AUGUST 30 - SEPTEMBER 2, 1992

Highlighting
Chemical and Biological Analysis

REPORTS AND TRANSACTIONS

RECOMMENDATIONS FOR OFFICIAL METHODS

Committee on Pesticide Formulations and Disinfectants

RICHARD H. COLLIER, CHAIRMAN
Purdue University, Center for Environmental and Regulatory Information, 1231 Cumberland Ave, Suite A, West Lafayette, IN 47907-1317

PETER BLAND
ICI Americas, Inc., Western Research Center, 1200 S. 47th St, PO Box 4023, Richmond, CA 94804-0023

WARREN BONTOYAN
Maryland Dept. of Agriculture State Chemist, 50 Harry S. Truman Pkwy, Annapolis, MD 21401

THADDEUS J. CZERKOWICZ
U.S. Environmental Protection Agency, Bldg 306, BARC, Beltsville, MD 20705

ARTHUR H. HOFBERG
Ciba-Geigy Corp., Analytical Section, PO Box 18300, Greensboro, NC 27419

MARGARET A. NEMETH, COMMITTEE STATISTICIAN
Monsanto Agricultural Co., 800 N. Lindbergh Blvd, St. Louis, MO 63167

HARRY OSTAPENKO, SAFETY ADVISER
Heinz USA, 1062 Progress St, Pittsburgh, PA 15212

FRAN PORTER
Florida Department of Agriculture and Consumer Services, Division of Chemistry, Tallahassee, FL 32301

STEPHEN C. SLAHCK, SECRETARY
Mobay Corporation, Miles Inc., Agriculture Division, PO Box 4913, Kansas City, MO 64120-0013

The Committee on Pesticide Formulations and Disinfectants met on August 30, 1992, during the Annual AOAC International meeting held in Cincinnati, OH. Portions of the meeting were attended by several General Referees whose topics are assigned to the Committee.

The committee discussed the needs for increasing public relations with major industrial representatives. This should include a personal visit from the staff of AOAC International with the Directors of Research to encourage their support both as volunteers and as sustaining members. These firms are primary sources of expertise for referees. Additionally, it is recommended that institutions that have been sustaining members for an extended period (e.g., 10 years) should be provided an attractive plaque recognizing their commitment of time and resources.

The Committee decided to contact personnel of major industrial producers of pesticides to encourage participation as Associate Referees for compounds they produce. Currently, some major companies are inactive in this Committee's area.

The Committee recommends that the AOAC International make presentations at national meetings of other scientific associations (e.g., American Chemical Society) to acquaint their membership with the collaborative study process. Special attention should be given to making a presentation at the IUPAC International Meeting on Pesticides to be held in Washington, DC, July 1994.

The Committee again discussed the issues of equivalency in methodology. The Committee voted to endorse listing specific suppliers of analytical reference standards and identifying all stationary phases that were used to obtain acceptable results in GC or LC collaborative studies. The fundamental source of analytical reference standards is the company that manufactures the active ingredient. These standards are typically available at no charge from these sources. Standards that are sold by third parties are usually not "certified" by the manufacturers. These third sources are generally not acquainted with typical impurities or problems associated with their evaluation.

In the case of GC and LC phases, again the Associate Referee is usually a representative from the manufacturer, who is familiar with all impurities and the manufacturing process for the material. A particular chromatographic phase is selected to provide the ultimate specificity for the active ingredient, resolving it from all manufacturing and potential degradation impurities. Although slightly different phases may be used by a collaborator for a study, these results are evaluated statistically to determine if the phase used was acceptable.

Use of a generic label to identify GC or LC phases is unreasonable, because other phases, although advertised to be similar, have not been evaluated by the Associate Referee or collaborators. Providing a system performance test, or criteria under which additional phases would be acceptable, would involve documenting the impurities in a manufactured active ingredient. This information is always considered proprietary and would not be released.

The Committee recommends that Protocol and Collaborative Study review packets contain a top sheet that is designed to be signed and telefaxed back to the AOAC office. Its purpose is to inform the office that the packet was actually received and that it is actively being addressed. Both of these issues have been problems in the past.

The Committee realigned the following topics: Herbicides I and II were combined to become simply **Herbicides**; Carba-

*An asterisk before a recommendation indicates that official action on that recommendation resulted in its appearance in *Changes in Official Methods.*

The recommendations submitted by the Committee on Pesticide Formulations and Disinfectants were adopted by the Association.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and *Changes in Official Methods of Analysis,* 3rd supplement (1992).

mate Insecticides and Substituted Urea Insecticides, Organothiophosphorous Insecticides, Other Organophosphorus Insecticides, Organohalogen Insecticides, and Other Insecticides, Synergists and Insect Repellents were combined to become **Insecticides, Synergists and Repellents**; and Fungicides with Rodenticides and Miscellaneous Pesticides were combined to become **Fungicides and Rodenticides**. The Committee recommends these alignments for the Pesticide Formulation Chapter of the 16th Edition of the *Official Methods of Analysis*.

The Committee is currently seeking a General Referee to fill the vacancy for Fungicides and Rodenticides. Additional committee members are being sought to fill vacancies, particularly people with experience in the disinfectant area.

The Committee decided to consider providing new volunteers with an educational package specifically oriented toward developing pesticide formulations methodology. A previously available package will be reviewed by the next meeting.

Pesticide Formulations: Carbamate Insecticides and Substituted Urea Insecticides

*(1) *Allethrin*: (a) Surplus titrimetric method (**953.05**). (b) Discontinue study.
(2) *Azinphos-methyl* (*Guthion*): Continue study.
(3) *Benzene Hexachloride and Lindane*: Discontinue topic.
(4) *Carbaryl*: Discontinue study.
(5) *Carbosulfan*: Discontinue study.
(6) *Cyfluthrin*: Continue study.
(7) *Cyromazine*: Continue study.
*(8) *Dichlorvos* (*DDVP*): (a) Repeal the infrared methods (**964.04** and **966.07**). (b) Discontinue topic.
(9) *Dipropyl Isocinchomeronate* (*MGK Repellant 326*): Continue study.
(10) *Ethoprop*: Continue study.
(11) *Fenamiphos* (*Nemacur*): Discontinue study.
*(12) *Fenitrothion*: (a) Surplus GC method (**985.07**). (b) Discontinue study.
(13) *Methomyl*: Continue study.
*(14) *Methyl Parathion and Parathion*: (a) Surplus LC methods **977.05** and **978.07**. (b) Recommend adoption of the GC methods **977.04** and **978.06** as official final action. (c) Discontinue study on both topics.
(15) *Oxamyl*: Continue study.
*(16) *Oxydemeton-methyl* (*Metasystox-R*): (a) Recommend adoption of the LC method as official final action (**991.05**). (b) Discontinue study.
(17) *Rotenone and Other Rotenoids*: Discontinue study.

Pesticide Formulations: CIPAC Studies

(1) *Bensultap*: Discontinue topic.
(2) *Bentazon*: Continue topic.
(3) *Carbetamide*: Discontinue topic.
(4) *Cyhexatin*: (a) Continue official first action status of the CIPAC-AOAC LC method (**988.02**). (b) Continue study.
(5) *Cyanazine*: (a) Continue official first action status of the LC method for maneb with fentin (**991.32**). (b) Continue study.
(6) *DDT*: (a) Continue the official first action status of the LC method (**991.04**). (b) Continue study.
(7) *Deltamethrin*: (a) Continue official first action status of the CIPAC-AOAC LC method (**991.03**). (b) Continue study.
(8) *Dichlobenil*: Discontinue topic.
(9) *Dimethoate*: Discontinue topic.
(10) *Edifenphos*: Discontinue topic.
(11) *λ-Cyhalothrin*: Discontinue topic.
(12) *Malathion*: Discontinue topic.
(13) *Maneb in Fentin Formulations*: Continue official first action status of the CIPAC-AOAC method (**991.33**). (b) Continue study.
(14) *Methamidophos* (*Tamaron, Monitor*): (a) Continue official first action status of the CIPAC-AOAC LC method. (b) Continue study.
(15) *Parathion and Methyl Parathion*: Discontinue topic.
(16) *Pencycuron* (*Monceren*): Discontinue topic.
(17) *Phosphamidon*: Continue study.
(18) *Pirimiphos-Methyl*: (a) Continue the official first action status of the GC method (**991.34**). (b) Continue study.
(19) *Temephos*: Discontinue topic.
(20) *Triadimenol* (*Baytan*): Discontinue topic.

Pesticide Formulations: Fungicides

(1) *Benomyl*: (a) Continue official first action status of the LC method (**984.09**). (b) Continue study.
(2) *Brodifacoum*: Continue study.
(3) *Carboxin and Oxycarboxin*: Continue study
(4) *Chlorothalonil*: Continue study
(5) *Dithiocarbamate Fungicides*: Continue study.
(6) *Tebuconazole* (*Folicur*): Continue study.
(7) *Triphenyltin* (*Fentin*): (a) Continue official first action status of the GC method (**984.04**). (b) Continue study.
(8) *Sampling*: (a) Continue official first action status of the sampling procedures for fertilizers (**984.03**) as applied to pesticide–fertilizer mixtures. (b) Continue study.
(9) *Strychnine*: Discontinue topic.

Pesticide Formulations: Herbicides

(1) *Acetochlor*: Discontinue topic.
(2) *Alachlor, Butachlor, and Propachlor*: Continue study.
(3) *Alachlor/Atrazine Mixtures*: Continue study.
(4) *Bromacil*: Continue study.
(5) *Bromoxynil*: Continue study.
(6) *Chlorophenoxy Herbicides*: (a) Continue official first action status of the following LC methods: combinations of 2,4-D, dicamba, and MCPP amine salts (**984.07**); 2-methyl-4-chlorophenoxyacetic acid (**980.07**); 2,4,5-trichlorophenoxyacetic acid (**980.08**); 2,4-dichlorophenoxyacetic acid esters and amine salts (**978.05**). (b) Continue study.

(7) *Chlorsulfuron*: Continue study.
(8) *Fomesafen*: Discontinue study.
(9) *Glyphosate*: Continue study.
(10) *Metolachlor*: Discontinue study.
(11) *Pesticides in Fertilizers*: Continue study.
(12) *Propanil*: Continue study.
(13) *Dicamba*: Continue study.
(14) *Ethafluralin and Pendimethalin*: Continue study.
(15) *Fluometuron*: Continue study.
(16) *Methazole*: Continue study.
(17) *Metsulfuron-methyl*: Continue study.
(18) *Sulfometuron-methyl*: Continue study.

Disinfectants

(1) *Quaternary Ammonium Compounds*: Continue study.
(2) *Sporicidal Tests*: Continue study.
(3) *Tuberculocidal Tests*: (a) Continue official first action status of method (**965.12**). (b) Continue study.
*(4) *Use-Dilution Test*: (a) Recommend repeal of Use-Dilution methods (**955.14, 955.15, 964.02**). (b) Discontinue study.
(5) *Hard Surface Carrier Test*: (a) Continue official first action status of method (**991.47, 991.48, 991.49**). (b) Continue study.
(6) *Virucidal Tests*: Continue study.

RECOMMENDATIONS FOR OFFICIAL METHODS

Committee on Drugs and Related Topics

RICHARD M. MONTGOMERY, CHAIRMAN
Unilever Research US, Inc., 45 River Rd, Edgewater, NJ 07020

JOHN O'RANGERS, CHAIRMAN ELECT
U.S. Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857

GAYLORD ANTHONY
Warner Lambert Co., Parke Davis Division, 170 Tabor Rd, Morris Plains, NJ 07950

TERRY J. GILBERTSON
The Upjohn Company, 7000 Portage Rd, Kalamazoo, MI 49001

TED M. HOPES
U.S. Food and Drug Administration, New York Regional Laboratory, 850 Third Ave, Brooklyn, NY 11232

MARK G. TORCHIA
St. Boniface General Hospital, Dept of Surgery, Winnipeg, MB, P2H 2A6, Canada

MARK PRESSER, COMMITTEE STATISTICIAN
Unilever Research US, Inc., 45 River Rd, Edgewater, NJ 07020

WILLIAM W. WRIGHT, SECRETARY
U.S. Pharmacopeial Convention, Inc., 12601 Twinbrook Parkway, Rockville, MD 20852

The Committee met on August 7, 1992, in Arlington, VA. The Committee discussed its membership and terms of reference, its General Referees, the status of new methods and study reports, the methods volunteer memo, the protocol and study review forms, the methods volunteer package, the antitrust and conflict of interest policies, the preparations for the 16th Edition of *Official Methods of Analysis* (OMA), and the General Referee reports.

Actions taken and items discussed by the Committee are presented below:

1. Recognized the appointment of Terry Gilbertson as a member of the Committee for 1992–1994, replacing Kenneth Manning.
2. Recognized that Donna Bush and Thomas Doyle resigned as members of the Committee and replacements would be made.
3. Recognized that the appointment of Ted Hopes as a Committee member would expire this year.
4. Recognized that the Committee Statistician, Mark Presser, resigned and a replacement would be made.
5. Recognized the appointment of Rhonda S. Bayoud as the General Referee on Cosmetics.
6. Voted to recommend the reappointment of Linda Ng, General Referee, Drugs IV, for 3 years.
7. Voted to recommend the reappointment of Thomas G. Alexander, General Referee, Drugs V, for 3 years.
8. With the aid of the General Referee reports and a tabulation of the Committee's actions over the past 7 years prepared by Chairman Montgomery, discussed certain topics under various General Referees, notably those items presented below.

Drugs I

a. In view of a vacancy in this General Refereeship, the Committee considered a report prepared by the Chairman and voted to recommend final action status for the 2 topics for which there were first action methods.
b. The Committee voted to recommend acceptance of the report.

Drugs II

a. The Committee discussed the report of General Referee Edward Smith and recommend that the 5 topics that were not active for several years be discontinued. The Committee agreed that if interest is renewed in these topics, Associate Referees could be appointed.
b. The Committee voted to recommend acceptance of the General Referee's report, as modified.

Drugs III

a. In view of a vacancy in this General Refereeship, the Committee considered a report prepared by the Chairman and agreed that 3 first action methods should be recommended for final action.
b. The Committee voted to recommend acceptance of the report.

Drugs IV

a. The Committee considered the report of General Referee Linda Ng.
b. The chiral LC method for D- and L-amphetamines was recommended for final action.

*An asterisk before a recommendation indicates that official action on that recommendation resulted in its appearance in *Changes in Official Methods.*

The recommendations submitted by the Committee on Drugs and Related Topics were adopted by the Association.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and *Changes in Official Methods of Analysis,* 3rd supplement (1992).

c. The collaborative study of Associate Referee Charles Clark's method for heroin was completed, but a further collaborative study was being planned on the use of capillary columns instead of packed columns for gas chromatography.
d. The Committee voted to recommend acceptance of the General Referee's report.

Drugs V

a. The Committee discussed the report of General Referee Thomas G. Alexander.
b. The Committee considered the status of the colorimetric method for Progestational Steroids, **971.43**, and recommended that an Associate Referee should be sought to develop LC methods, with a view to surplussing the colorimetric method.
c. The Committee recommended that the topic Conjugated Estrogens by LC be discontinued, and an Associate Referee should be sought, with a view toward future reinstatement of the topic.
d. The Committee voted to recommend acceptance of the General Referee's report as modified.

Cosmetics

a. In view of a vacancy in this General Refereeship, the Committee considered a report prepared by the Chairman and agreed that 2 first action methods should be recommended for final action and study on one remaining topic, Eye Irritants in Cosmetic Constituents, should be continued.
b. The Committee voted to recommend acceptance of the report.

Diagnostics and Test Kits

a. In view of a vacancy in this General Refereeship, the Committee considered a report prepared by the Chairman and agreed that study of all 6 topics should be discontinued.
b. The Committee agreed that a new General Referee should be sought, and the person appointed should keep abreast of the activities of the AOAC Research Institute. The Committee agreed that topics to be studies should be of a nonclinical nature and should preferably be in the environmental and agricultural fields. It was suggested that guidelines and recommendations for use of test kits should be considered, and attention should be given to recommended methods for sampling, taking specimens, selecting proper containers, and appropriate storage and shipping procedures.
c. The Committee voted to recommend acceptance of the report.

Drug Residues in Animal Tissues

a. The Committee considered the report of General Referee Charlie Barnes.
b. The Committee agreed with the General Referee's recommendations to initiate studies on Quantitative Determination of β-Lactams in Milk by Competitive Receptor Assay and on Sulfamethazine in Plasma and Serum.
c. The Committee stressed that Associate Referees studying methods for antibiotics in milk should specify the type(s) of milk studied: for example, whole milk, pasteurized milk, frozen milk, or dried milk.
d. The Committee suggested that efforts be renewed to study microbiological methods for antibiotic residues.
e. The Committee voted to recommend acceptance of the General Referee's report as modified.

Forensic Sciences

a. The Committee considered the report of General Referee Stanley Cichowicz.
b. The Committee agreed that the first action method for detection of latent fingerprints on paper by chemical development and the comparison of properties of mineral wool insulation should be proposed for final action.
c. The Committee discussed the difficulty in obtaining collaborators for forensic methods and agreed that efforts should be renewed to obtain Associate Referees.
d. The Committee voted to recommend acceptance of the General Referee's report as modified.

9. The following recommendations for official methods will be reported at the Annual International Meeting on September 3, 1992:

Drugs I

*(1) *Acetaminophen in Drug Mixtures*: (a) Adopt as final action the first action LC method for acetaminophen in tablets, **987.12**. (b) Discontinue topic.
*(2) *Diethylpropion Hydrochloride*: (a) Adopt as final action the first action method for the determination of diethylpropion hydrochloride in drug substance and in tablets, **988.23**. (b) Discontinue topic.

Drugs II

(1) *Aminacrine*: Continue study.
(2) *Antihistamines in Combination with Decongestants by HPLC*: Continue study.
(3) *Belladona Alkaloids*: Continue study.
(4) *Colchicine in Tablets*: Continue study.
(5) *Dicyclomine*: Discontinue topic.
(6) *Ergot Alkaloids*: Discontinue topic.
(7) *Physostigmine and Its Salts*: Discontinue topic.
(8) *Rauwolfia Alkaloids (Reserpine and Rescinnamine)*: Discontinue topic.
(9) *Rauwolfia Serpentina*: Discontinue topic.

*(10) *Santonin*: Surplus UV method **962.23**.

Drugs III

*(1) *Halogenated Hydroxyquinoline Drugs*: (a) Adopt as final action the first action method for the LC determination of clioquinol in creams and ointment, **990.14**. (b) Discontinue topic.
*(2) *Hydralazine*: (a) Adopt as final action the first action method for the ultraviolet spectrophotometric determination of hydralazine hydrochloride in tablets, **989.07**. (b) Discontinue topic.
*(3) *Penicillins*: (a) Adopt as final action the first action method for the LC determination of penicillin V potassium in tablets, **990.15**. (b) Discontinue topic.
*(4) *Xanthine Group Alkaloid Drugs*: (a) Adopt as final action the first action microchemical tests, **960.56**. (b) Discontinue topic.

Drugs IV

*(1) *D- and L-Amphetamines—LC Separation*: (a) Adopt as final action the first action method for the LC determination of the enantiomers of amphetamine in bulk drug, syrup, and capsules, **988.28**. (b) Discontinue topic.
(2) *Benzodiazepines*: Continue study.
(3) *Dicloxacillin*: Continue study.
(4) *Flurazepam*: Continue first action status of the LC method for flurazepam hydrochloride in bulk drug and dosage forms, **991.35**.
(5) *Heroin*: Continue study.
(6) *Miconazole Nitrate*: Continue study.
(7) *Piroxicam*: Continue study.

Drugs V

(1) *Aminobenzoic Acid and Salicylic Acid Salts*: Continue study.
(2) *Anabolic Steroids—LC*: Continue study.
(3) *Conjugated Estrogens by LC*: Discontinue topic.
(4) *Cromolyn Sodium*: Continue study.
(5) *Pentaerythritol Tetranitrate*: (a) Continue first action status of the first action method for LC determination of pentaerythritol tetranitrate in pharmaceuticals. (b) Continue study.
(6) *Progestational Steroids*: Initiate topic and appoint Associate Referee.
(7) *Steroids in Tablets*: Continue study.

Cosmetics

(1) *Eye Irritants in Cosmetic Constituents*: Continue study.
*(2) *Water and Alcohol*: (a) Adopt as final action the first action GC method for water and alcohol in cosmetics, **966.22**. (b) Discontinue topic.
(3) *Zirconium*: (a) Adopt as final action the first action colorimetric method for soluble zirconium in antiperspirant aerosols, **976.24**. (b) Discontinue topic.

Diagnostics and Test Kits

(1) *Analytical Release Rates of Drugs from Transdermal Patches*: Discontinue topic.
(2) *Automated Microbial Identification Systems—VITEK*: Discontinue topic.
(3) *Automated Microbial Identification Systems—HP5898A*: Discontinue topic.
(4) *Immunological and Diagnostic Assay of Peptides, Hormones, and Enzymes*: Discontinue topic.
(5) *Multicomponent Analysis of Clinical Specimens*: Discontinue topic.
(6) *Tuberculosis and Enteric Infections by Gene Probe*: Discontinue topic.

Drug Residues in Animal Tissues

*(1) *Benzimidazoles in Cattle Tissues*: (1) Adopt as final action the first action LC method for fenbendazole in beef liver, **991.17**. (b) Discontinue topic.
*(2) β-*Lactam Antibiotics in Milk* (*DELVO test*): Continue study.
(3) β-*Lactam Antibiotics in Milk* (*ELISA test*): Continue study.
(4) β-*Lactam Antibiotics in Milk* (*Qualitative*): Continue study.
(5) β-*Lactam Antibiotics in Milk* (*Competitive Microbial Receptor Assay*): Continue study.
(6) *Enzyme Immunoassays for Antimicrobial Compounds*: Continue study.
*(7) *Ethoxyquin*: Surplus fluorimetric method **968.48**.
*(8) *Sulfamethazine in Milk* (*Chromatographic Methods*): (a) Adopt as first action the LC method studied collaboratively. (b) Continue study.
(9) *Sulfamethazine in Plasma and Serum*: Continue study.
*(10) *Sulfonamides in Milk* (*Chromatographic Methods*): (a) Adopt as first action the LC method for sulfonamides in milk studied collaboratively. (b) Continue study.
(11) *Tetracyclines in Tissues* (*Chromatographic Methods*): Continue study.

Forensic Sciences

*(1) *Fingerprints*: (a) Adopt as final action the first action chemical development method for detection of latent fingerprints on papers, **976.28**. (b) Discontinue study.
(2) *Gunshot Residues*: Discontinue topic.
*(3) *Mineral Wool Insulation*: (a) Adopt as final action the first action microscopic method for the comparison of properties of mineral wool insulation, **981.23**. (b) Discontinue study.

10. Chairman Montgomery presented for discussion some items from the February and May 1992 Meetings of the Official Methods Board:
    a. All valid data must be included in published reports of collaborative studies.
    b. Statistical software is available through the AOAC, and training will be given at the Annual International Meeting.
    c. Sensitivity rate and selectivity rate must be included in qualitative methods.
    d. Weights and measures terminology was specifically defined and should be used in methods.
    e. Method applicability statements are being studied by a task force. The lowest quantifiable level (LQL) is the lowest level collaboratively studied. The lowest detectable level (LDL) appears to be ambiguous.
    f. User reply card will accompany the 3rd Supplement to OMA requesting comments on method performance and safety concerns.
11. AOAC Antitrust Policy (1989) and AOAC Conflict of Interest Policy (1990) were discussed. It was stressed that AOAC volunteers should avoid not only actual conflict of interest but even the appearance of conflict of interest in any given situation. When there is a possibility of conflict of interest, that conflict must be given in writing.
12. Information on Methods Volunteers Appointment Process from the AOAC Technical Director, dated April 15, 1992, was discussed.
13. Ideas for Methods Committee Meetings from the Methods Editor, dated July 10, 1992, were discussed. Included was a description of the review of protocols and collaborative study reports and the forms used in the processes. It was suggested that in the collaborative study report review form it should be made clear that the Method Applicability section is to be completed by the General Referee. It was pointed out that any first action methods with OMA numbers beginning with 991 or lower are eligible for final action consideration if positive use history has been established.
14. Discussed plans for the 16th Edition of OMA scheduled for publication in 1995. Most of the abbreviations will be spelled out, and there will be fewer cross-references to other methods. General Referees should review the methods in their assigned chapters, with particular attention to the equipment equivalency policy. They should consider whether older methods should be proposed for surplus or repeal. Safety precautions should be reviewed. Asbestos and objectionable solvents should be eliminated. Chairman Montgomery expressed the opinion that more information is needed on the proper handling and cleaning of volumetric glassware.
15. Committee members and General Referees present received the Methods Volunteer Information Package for reference.
16. The Committee discussed its Terms of Reference and agreed that no changes were needed in the document adopted in July 1991.

RECOMMENDATIONS FOR OFFICIAL METHODS

Committee on Foods I

JAMES F. LAWRENCE, CHAIRMAN
Health and Welfare Canada, HPB Banting Research Center, Tunney's Pasture, Ottawa, ON, K1A 0L2, Canada

DOUGLAS L. PARK, SECRETARY
University of Arizona, Department of Nutrition and Food Science, Tucson, AZ 85721

DONALD E. CARPENTER
Kraft General Foods, 801 Waugegan Rd, Glenview, IL 60025

DOUGLAS R. ENGEBRETSEN
Land O'Lakes, Inc., 4001 Lexington Ave N., Minneapolis, MN 55440-0116

MAX L. FOSTER
Kansas Board of Agriculture, Div. of Lab., 2524 W. Sixth St, Topeka, KS 66606

JOHN GILBERT
Ministry of Agriculture, Fisheries and Food, Food Safety Directorate, Food Science Lab, Colney Ln., Norwich, NR4 7UZ, United Kingdom

JON E. MCNEAL
U.S. Department of Agriculture, Agricultural Marketing Service, Box 96456, Washington, DC 20090-6456

HARRIET WALLIN
VTT Technical Research Center of Finland, Food Research Lab, Biologinkuja 1, PO Box 203, SF-02150 Espoo, Finland

MARLEEN M. WEKELL
U.S. Food and Drug Administration, 22201 23rd Dr. SE, PO Box 3012, Bothell, WA 98041-3012

TERRY C. NELSEN, COMMITTEE STATISTICIAN
U.S. Department of Agriculture/NRRC/ARS, 1815 N. University St, Peoria, IL 61604

SANDRA PFEIFFER, SAFETY ADVISOR
Gerber Products Co., 445 State St, Fremont, MI 49412

Color Additives

(1) *Anthocyanin Color Additives Exempt from Certification*: Continue study.
(2) *Arsenic, Barium, and Heavy Metals*: Continue study.
(3) *Carotenoid Color Additives Exempt from Certification*: Appoint an Associate Referee; continue study.
(4) *Colors in Candy and Beverages*: Continue study.
(5) *Color in Cosmetics*: Appoint an Associate Referee; continue study.
(6) *Isolation of Colors from Complex Food Matrixes*: Appoint an Associate Referee; continue study.
(7) *Trace Organic Constituents of Certifiable Color Additives*: Appoint Associate Referee; continue study.
(8) *Uncombined Intermediates and Subsidiary Colors in Certifiable Colors*: Continue study.

Dairy Chemistry

(1) *Alkaline Phosphatase Activity in Dairy Foods*: Continue study.
(2) *Babcock Test and Babcock Glassware*: Continue study.
(3) *Composition of Fluid Milk*: Continue study.
(4) *Cryoscopy of Milk*: Discontinue topic.
(5) *Fat in Milk (Gerber test)*: Continue study.
(6) *Iodine*: Adopted as official first action method for iodine in milk (LC method) (August 1992); continue study.
(7) *Lactose in Dairy Products (Enzymatic Method)*: Discontinue topic.
(8) *Mid-Infrared Instrumentation*: Discontinue topic.
(9) *(Robotic) Mojonnier Method*: Continue study.
(10) *Moisture in Cheese*: Continue study.
(11) *Nitrates in Cheese*: Continue study.
(12) *Raw Milk Sampling*: Adopted official first action sampling of milk from bulk tanks and other storage equipment; modification of automated method **970.26** (January 1992); continue study.
(13) *Tyramine*: Discontinue topic.
(14) *Other Topics*: Initiate new topics Absolute Moisture Content of Dairy Products and FTIR Analysis of Dairy Foods; adopt as official final action sampling technique for cheese (**970.30C**); total solids in milk, direct forced-air oven drying (**990.20**), total solids in milk, direct forced-air oven drying after steam table predry (**990.19**), solids-not-fat in milk (**991.21**), freezing point in milk thermister method (**990.22**); editorial changes for **989.04B**(G) after "Length of delivery tube...100–120 mm" to read "Length of delivery tube (sl longer than bottle neck)...105–125 mm" must not touch milk sample when delivered; in Chapter 33 of *Official Methods of Analysis*, where use of air oven is indicated, substitute forced-draft oven; water added to milk (**961.07**) from –0.406°C and –0.598°C to –0.408°C to –0.600°C; water added to milk (**961.07B**) correct weight of NaCl 0.6889 and 1.0207 g reagent-grade NaCl; reference to method **905.02** (Roese-Gottieb) in Chapter 33 to **989.05**, because only the latter has AOAC/IDF/ISO approved status.

*An asterisk before a recommendation indicates that official action on that recommendation resulted in its appearance in *Changes in Official Methods*.

The recommendations submitted by the Committee on Foods I were adopted by the Association.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and *Changes in Official Methods of Analysis*, 3rd supplement (1992).

Flavors

(1) *Additives in Vanilla*: Continue study.
(2) *C14 in Flavoring Materials*: Continue study.
(3) *Deuterium NMR Analysis of Vanillin*: Continue study.
(4) *Licorice Products*: Continue study.
(5) *Moisture in Vanilla Beans*: Appoint an Associate Referee; continue study.
(6) *Vanillin and Ethyl Vanillin*: Adopt as official final action LC method for vanillin (**990.25**); continue study.
(7) *Other Topics*: Adopt as official final action methods for ginger in ginger extract (**920.146c**), total solids in ginger extract (oven drying) (**920.146B**), benzoic acid in almond extract (**909.02**), benzaldehyde in almond extract (**936.10**), citrus oil in oil-based flavors (**926.11**), methanol in vanilla extract (**920.132**), alcohol in various flavor extracts (**950.44A**, **950.44B**, **920.146A**, **920.147A**, **920.147B**), essential oil content (**925.33C**, **932.11**, **932.10**, **920.147E**, **920.148B**, **920.148C**, **942.08**); editorial correction in method **920.146A**, reference to method **955.180** should be **942.06B**.

Food Additives

(1) *Antioxidants*: Adopted official first action phenol antioxidants in oils, fats, and butter oil: LC method (May 1992); continue study.
(2) *Brominated Vegetable Oils*: Appoint Associate Referee; continue study.
(3) *Identification of Irradiated Foods*: Continue study.
(4) *Indirect Additives from Food Packages*: Continue study.
(5) *Monier-Williams Modification*: Discontinue topic.
(6) *Nitrates and Nitrites*: Discontinue topic.
(7) *Nitrosamines in Foods*: Continue study.
(8) *Nitrosamines in Food Contact Items*: Appoint Associate Referee; continue study.
(9) *Nonvolatile Nitro Compounds*: Appoint Associate Referee; continue study.
(10) *Polycyclic Aromatic Hydrocarbons*: Continue study.
(11) *Poly(dimethylsiloxane) (PDMS)*: Continue study.
(12) *Sulfiting Agents in Foods*: Appoint Associate Referee; continue study.
(13) *Sulfites (Flow Injection Analysis)*: Discontinue topic.
(14) *Sulfites (Ion-Chromatographic Methods)*: Discontinue topic.
(15) *Sulfites (Polarographic Methods)*: Continue study.
(16) *Sulfites in Shrimp (Screening Methods)*: Appoint Associate Referee; continue study.
(17) *Urethane in Foods*: Continue study.
(18) *Other Topics*: Initiate new topic HPLC Methods for Sulfites.

Meat, Poultry, and Meat and Poultry Products

(1) *Gluten in Meats:* Continue study.
(2) *Creatinine in Soups and Bouillons*: Continue study.
(3) *Glutamic Acid and Monosodium Glutamate*: Appoint Associate Referee; continue study.
(4) *Heme, Nonheme, and Total Iron in Meat, Fish, and Poultry Products*: Discontinue topic.
(5) *Ion Chromatographic Methods in Meat and Poultry Products*: Continue study.
(6) *LC Methods for Meat and Poultry Products*: Continue study.
(7) *Microwave Techniques for Meat Analysis*: Continue study.
(8) *Nonmeat Protein in Meat and Poultry Products:* Continue study.
(9) *Proteins in Meat and Meat Products*: Appoint an Associate Referee; continue study.
(10) *Potentiometric Determination of Sodium and Salt:* Continue study.
(11) *Protein in Meat and Meat Products (Combustion Method):* Adopted official first action combustion method for crude protein in meat and meat products (May 1992); continue study.
(12) *Proteins in Meat and Meat Products:* Appoint an Associate Referee; continue study.
(13) *Proximate Analysis of Meat Product by Near Infrared:* Continue study.
(14) *Robotic Methods for Meat and Poultry Products*: Transfer topic to Residues Committee.
(15) *Specific Ion Electrode Applications*: Discontinue topic.
(16) *Temperature, Minimum Processing*: Continue study.
(17) *Total Fat*: Continue study.
(18) *Volatiles in Meat and Poultry, and Meat and Poultry Products*: Continue study.
(19) *Other Topics:* Adopt as official final action method for hydroxyproline in meat (**990.26**); initiate new topics Copper Catalyst in Kjeldahl for Protein, Calcium in Mechanical Separated Poultry and Beef, Comparison of ICP and EDTA Methods for Calcium, Phosphopyruvic Kinase Assay, and Supercritical Fluid Methods.

Mycotoxins

(1) *Aflatoxin M*: Designate as Surplus the method for aflatoxin M_1 in milk (**974.17**); 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) *Fumonisins*: Continue study.
(8) *Immunochemical Methods*: Continue study.
(9) *Ochratoxins*: Continue study.
(10) *Trichothecenes*: Continue study.
(11) *Zearalenone*: Continue study.
(12) *Other Topics:* Initiate new topic Patulin; make editorial change to **986.18G**, extraction solvent should be $CHCl_3$–EtOH (8 + 2).

Plant Toxins

(1) *Cyanogenic Glycosides*: Appoint an Associate Referee; continue study.
(2) *Glucosinolates*: Continue study.
(3) *Hydrazines*: Continue study.
(4) *Hypoglycine in Ackee Fruit*: Appoint Associate Referee; continue study.
(5) *Phytoestrogens*: Continue study.
(6) *Pyrrolizidine Alkaloids*: Continue study.
(7) *Steroidal Alkaloids*: Continue study.

Seafood Products

(1) *Conjugated Dienes as Indicators of Decomposition*: Continue study.
(2) *Decomposition by Gas and Liquid Chromatography*: Appoint Associate Referee; continue study.
(3) *Drained Weight of Block Frozen, Raw, Peeled Shrimp*: Appoint Associate Referee; continue study.
(4) *Flow Injection Analysis for Decomposition in Seafood Products*: Continue study.
(5) *Minced Fish in Fillet Blocks*: Discontinue topic.
(6) *Nitrites in Smoked Fish*: Transfer topic to Food Additives.
(7) *Rancidity in Fishery Products*: Discontinue topic.
(8) *Shellfish Decomposition*: Appoint Associate Referee; continue study.
(9) *Solids (Total) in Seafood*: Appoint an Associate Referee; continue study.
(10) *Volatile Amines—TMA and DMA by GC*: Continue study.
(11) *Ciguatoxins (Biochemical Methods):* Continue study.
(12) *Ciguatoxins (LC Methods)*: Continue study.
(13) *Cyanobacterial Peptide Toxins*: Continue study.
(14) *Diarrhetic Shellfish Poisons (HPLC Method):* Continue study.
(15) *Domoic Acid*: Continue study.
(16) *Neurotoxic Shellfish Poisons*: Continue study.
(17) *Neurotoxic Shellfish Poisons:* Continue study.
(18) *Paralytic Shellfish Poisons (Electrochemical Methods)*: Discontinue topic.
(19) *Paralytic Shellfish Poison–Immunoassay Method*: Appoint Associate Referee; continue study.
(20) *Paralytic Shellfish Poison by HPLC*: Continue study.
(21) *Tetrodotoxins*: Discontinue topic.
(22) *Other Topics*: Initiate new topics Diarrhetic Shellfish Toxins (Immunoassay method) and Cell Bioassays for Detection of Seafood Toxins; add statement of method for domoic acid (**991.26**) "Ensure baseline resolution of L-tryptophan from domoic acid; adjust mobile phase composition accordingly. Determine recoveries of domoic acid at the 20 ppm level."

RECOMMENDATIONS FOR OFFICIAL METHODS

Committee on Foods II

JONATHAN DEVRIES, CHAIRMAN
General Mills, Inc., 9000 Plymouth Ave, Minneapolis, MN 55427

DOUGLAS J. BARK, SECRETARY
Woodson Tenent Laboratories, 345 Adams, Memphis, TN 38103

ROGER WOOD
Ministry of Agriculture, Fisheries, and Food, Food Science Laboratory, Colney Lane, Norwich NR4 7UQ, England

ADELINE A. PEAKE
Agriculture Canada, 102 11th Ave, SE, Calgary, AB, T2G 0X5, Canada

JOSEPH C. BRUCCIANI
U.S. Food and Drug Administration, Director of Science, Atlantic Regional Laboratory, 60 8th St, NE, Atlanta, GA 30309

BENJAMIN KRINITZ
U.S. Food and Drug Administration, New York Regional Laboratory, HFR-2680, 850 3rd Ave, Brooklyn, NY 11232

RANDY SMITH
Hazleton Laboratories of America, 3301 Kinsman Blvd, Madison, WI 53704

WALTER HARGRAVES
U.S. Food and Drug Administration, 6502 S. Archer Rd, Summit-Argo, IL 60501

PAUL BELJARRS
Inspectorate of Health, Protection dir Masstricht, Florijnruwe 111, Maastricht 6218–CA, The Netherlands

DAVID EGELHOFER, SAFETY ADVISOR
1407 N. Kenilworth St, Apt. 3, Arlington, VA 22205

JOHN G. PHILLIPS, COMMITTEE STATISTICIAN
U.S. Department of Agriculture, Eastern Regional Research Center, 600 E. Mermaid Lane, Philadelphia, PA 19118

The performance of 3 General Referees was reviewed by the Committee. The Committee recommends the following:

1. *Chocolate & Cacao Products*: A General Referee directly working in the subject area should be appointed by the newly formed Methods Committee on Commodity Foods and Commodity Products.
2. *Fruits & Fruit Products:* Frederick Boland—Reappoint on an interim basis to allow the newly formed Methods Committee on Commodity Foods and Commodity Products to review status.
3. *Vitamins & Other Nutrients*: Mike Deutsch—Reappoint for 3 years.

Alcoholic Beverages

(1) *Alcohol Content*: Continue study.
(2) *Ascorbic Acid in Wine by HPLC*: Continue study.
(3) *Carbon Dioxide in Wine*: Continue study.
(4) *Ethanol in Wine by GLC*: Continue study.
(5) *Ethyl Carbamate in Alcoholic Beverages*: Continue study.
(6) *Glycerol in Wine*: Continue study.
(7) *Glycerol Monooleate in Wine*: Continue study.
(8) *Malic Acid in Wine*: Continue study.
(9) *Malt Beverages and Brewing Materials*: Continue study.
(10) *Poly(dimethylsiloxane) in Wine*: Continue study.
(11) *Sorbic Acid in Wine*: Continue study.
(12) *Sugars in Wine by Enzymatic Methods*: Continue study.
(13) *Sugars in Wine by HPLC*: Continue study.
(14) *Sulfur Dioxide in Wine by Aeration Oxidation*: Continue study.
(15) *Synthetic Colors*: Continue study.
(16) *Tartrates in Wine*: Continue study.
(17) *Lead in Alcoholic Beverages:* Continue study. Committee recommends this topic to be transferred to the Methods Committee on Pesticide Residues and Related Topics.
*(18) The following recommendations were approved by the committee: method **991.18**, "Total Essential Hops and Hop Pellets," to final action status and the general area name of "Alcoholic Beverages" changed to "Beverage Alcohol."

Cereals and Cereal Products

(1) *β-Glucan Methodology:* Continue study. Discussed the General Referee's recommendation to adopt the following change for *Official Methods of Analysis* (OMA): official first action on "β-D-glucan in Cereal Products."

*An asterisk before a recommendation indicates that official action on that recommendation resulted in its appearance in *Changes in Official Methods.*

The recommendations submitted by the Committee on Foods II were adopted by the Association.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and *Changes in Official Methods of Analysis*, 3rd supplement (1992).

Previously approved by the Official Methods Board (OMB).

(2) *Crude Protein by Combustion Methods*: Continue study. Discussed the General Referee's recommendation to adopt the following change for OMA: official first action on "Crude Protein in Cereal grains and Oilseeds by the Combustion Method." Previously approved by OMB.

(3) *Fat Acidity*: Continue study.

(4) *Gliadin in Gluten-Free Products*: Continue study. Discussed the General Referee's recommendation to adopt the following change for OMA pending response to General Referee comments: official first action on "Gliadin in Gluten-Free Products." This method will be evaluated in the upcoming year.

(5) *Gluten in Foods*: Continue study. Adopt the following change for OMA: method **991.19**, "Gluten in Foods," be retitled to "Gliadin in Foods" to reflect that gliadin, instead of gluten, is the analyte determined by this method. Method is to retain first action status until gliadin activity in heat-treated cereal products is documented, i.e., does denatured gliadin participate in side reactions?

(6) *Iron*: Continue study. Adopt the following change for OMA: method **944.02B**, reduction of points for the concentration curve in the colorimetric analysis of iron in flour. Method will be evaluated in the upcoming year.

(7) *Mineral Analysis*: Continue study.

(8) *Near-Infrared Analysis of Cereal Products*: Continue study. Discussed the General Referee's recommendation to adopt the following change for OMA: official first action on "Near-Infrared Reflectance Method for Protein Determination of Wheat," AACC method 39-10, and "Near-Infrared Reflectance Method for Protein–Wheat Flour," AACC method 39-11. The method and data will be submitted and evaluated in the upcoming year.

(9) *Phytates*: Continue study.

*(10) Other General Referee Recommendations Approved by Committee: Appoint Associate Referee for Moisture in Cereal Products and commence study.

Chocolate and Cacao Products

(1) *Carbohydrates in Chocolate Products*: Continue study.

(2) *Alternate Fats in Chocolate*: Appoint new Associate Referee. Continue study.

(3) *Shell in Cacao Products*: Continue study.

(4) *Total and Solid Fat Content in Chocolate Products by NMR*: Appoint new Associate Referee. Continue study.

Dietary Fiber

(1) *Determination by UED*: Discontinue topic.

(2) *Dietary Fiber—Modified Englyst Method*: Continue study.

(3) *Dietary Fiber—Uppsala Method*: Continue study.

(4) *Enzymatic Gravimetric Methods—MES/TRIS*: Continue study.

Enzymatic Gravimetric Methods—Phosphate: Continue study.

During the past year, the Committee recommended that the OMB adopt the following changes for OMA:
First action on "Determination of Soluble Dietary Fiber-Phosphate Buffer Method." First action on "Non-enzymatic method for TDF."

Other General Referee Recommendations Approved by Committee:

(1) Initiate topic and appoint Associate Referee for "Defatting in the Determination of Dietary Fiber"

(2) Initiate topic and appoint Associate Referee for "Celite Quality in the Determination of Dietary Fiber."

(3) Associate Referee review to substitute copper sulfate or copper sulfate–titanium dioxide for mercury catalyst in the total Kjeldahl nitrogen determination step of dietary fiber.

Fats and Oils

(1) *Emulsifiers*: Continue study.

(2) *Hydrogenated Fats*: Continue study.

(3) *Lower Fatty Acids*: Appoint new Associate Referee. Continue study.

(4) *Marine Oils*: Continue study.

(5) *Olive Oil Adulteration*: Continue study.

(6) *Oxidized Fats*: Continue study.

(7) *Sterols and Tocopherols*: Continue study.

(8) *Pork Fats in Other Fats*: Appoint Associate Referee. Continue study.

Other General Referee Recommendations Approved by Committee:

(1) Adopt the following change for OMA: Revise method **965.33,** "Peroxide Value of Oils and Fats" with a cautionary statement: "This method should be carried out in diffuse daylight or in artificial light shielded from a direct light source."

(2) Method **990.27**, "Butyric Acid in Fats Containing Butterfat," to final action status.

Fruit and Fruit Products

(1) *Apple Juice Adulteration*: Continue study.

(2) *Detection of Added Invert Sugars by HPLC/Pulsed Amperometric Detection:* Continue study.

(3) *Fruit Acids*: Continue study.

(4) *Geographic Origin of Orange Juice*: Continue study.

(5) *Identification and Characterization of Fruit Juices*: Continue study.

(6) *Moisture in Dried Fruits*: Appoint Associate Referee. Continue study.

(7) *Naringin and Neohesperidine in Orange Juice*: Continue study.

(8) *Orange Juice Adulteration with Pulpwash*: Continue study. Committee recommendation: method **986.14**, "Adulteration of Processed Florida Orange Juice," incorporate the technical expert committee summary, parts 2–4, stated in Jon DeVries letter of January 23, 1992, to the Associate Referee. Rewrite to be performed by AOAC methods editor under direction of DeVries and then sent to expert panel members for review and comments. After this process, send to the appropriate committee for review and disposition.
(9) *SNIF NMR:* Continue Study.
(10) *Sodium Benzoate in Orange Juice*: Continue study.
(11) *Stable Carbon Isotope Ratio Analysis*: Continue study.

Nonalcoholic Beverages

(1) *Ash in Instant Tea*: Continue study.
(2) *Caffeine and Methyl Xanthines*: Continue study.
(3) *Coumarin in Vanillin Beverages*: Continue study.
(4) *Cyclamate in Cola:* Continue study.
(5) *Decaffeinated Coffee and Tea Solvent Residues*: Continue study.
(6) *Methyl Xanthines in Coffee and Tea:* Continue study.
(7) *Moisture*: Continue study.
*(8) *Pyrrolizidine Alkaloids in Herbal Tea*: Continue study.
(9) *Safrole in Sassafras Root:* Continue study.
(10) *Quinine*: Continue study.

Processed Vegetable Products

*(1) *Aseptic Processing*: Continue study.
(1) *LC Determination of Sugar*: Continue study.
*(2) *pH Determination*: Appoint new Associate Referee. Continue study.
*(3) *Sodium Chloride*: Discontinue topic
*(4) *Total Solids by Microwave Moisture Analyzer*: Continue study.
(5) *Water Activity in Foods*: Appoint Associate Referee. Continue study.

Spices and Condiments

(1) *Bulk Index Methods*: Continue study.
(2) *Capsicum Spices and Oleo Resins—Extractable Color*: Continue study.
(3) *Capsicum Spices and Oleo Resins—Pungency*: Continue study.
(4) *Curcumin in Turmeric*: Continue study.
(5) *Ether vs Methylene Chloride Extract of Spices*: Continue study.
(6) *Moisture in Dried Spices*: Continue study.
(7) *Steam Volatile Oil in Cassia, Sample Preparation*: Continue study.
(8) *Vinegar*: Continue study.
(9) *Water Activity of Spices*: Continue study.

Other General Referee Recommendations Approved by Committee:

(1) Revise method **987.07**, "Piperine in Pepper Preparations," to indicate 60 mesh instead of 30 mesh screen used in sample grinding preparation.

Sugars and Sugar Products

(1) *Amyloglucosidase Enzyme Activity*: Continue study
(2) *Corn Syrup and Sugar Products*: Continue study.
(3) *Enzymatic Methods*: Continue study.
*(4) *Gas Chromatographic Methods*: Continue study
(5) *Honey*: Continue study. Adopt following changes for OMA: method **978.17**, add the use of a continuous-flow automated ^{15}N ^{13}C analyzer for the determination of stable carbon isotope ratio of honey to the current description of the method.
(6) *Lactose Purity Testing*: Continue study.
(7) *Liquid Chromatographic methods*: Appoint Associate Referee. Continue study.
*(8) *Maple Sap, Maple Syrup, and Maple Syrup Products*: Continue study.
(9) *Methods Standardization*: Continue study.
(10) *NIR Analysis of Sugars:* Continue study.
(10) *Oligosaccharides*: Continue study.
(11) *Polarimetric Methods for Measurement of Sugars*: Continue study.
(12) *Stable Carbon Isotope Ratio Analysis*: Continue study.
(13) *Sugars in Cereals*: Continue study.
(14) *Sugars in Syrups*: Continue study.
(15) *Sulfites—Screening Methods*: Continue study.
(16) *Visual Appearance of Sugar by Color, Turbidity, and Reflectance*: Continue study.
(17) *Weighing, Taring, and Sampling*: Continue study.

Vitamins and Other Nutrients

(1) *Amino Acids*: Continue study. Committee recommendations: Discontinue collaborative study work described under Protocol D12, "Amino Acids in Infant Formula."
(2) *Automated Methods*: Continue study.
(3) *Biotin*: Continue study.
(4) *Carotenoids*: Continue study.
(5) *Cholesterol*: Continue study.
(6) *Folic Acid*: Continue study.
(7) *Infant Formula Nutrient Assay*: Continue study. Study Phase IV (iodide, linoleic acid, and vitamin D_3) was given official first action. Study Phase V (folic acid, pantothenic acid, vitamin E, and vitamin A) was given official first action.
(8) *Iodine*: Continue study.
(9) *Protein in Foods, Quality Evaluation*: Continue study.
(10) *Sodium*: Continue study.
(11) *Thiamine—Enzyme and Column Packing Reagents*: Continue study.

*(12) *Vitamin A*: Continue study.
(13) *Vitamin D*: Continue study.
(14) *Vitamin E in Foods*: Continue study.
(15) *Vitamin E in Pharmaceuticals (Gas Chromatography)*: Continue study.
(16) *Vitamin K*: Continue study.
(17) *Vitamins A, D, E, and K by Gel Permeation and LC*: Continue study.

Other Committee Recommendations

(1) AOAC establish a liaison with CEN-TC 174 for global harmonization.
(2) AOAC seek avenues to incorporate the Juice Technical Committee into the process of developing new methods in juice adulteration or AOAC form a Task Force for this challenge.
(3) OMA editor omit protein conversion factors from the methods determining nitrogen content of a matrix. Protein factors, as governed by International Trade Rules or governing body for commodities, should be listed in table format in an appendix to the OMA.

RECOMMENDATIONS FOR OFFICIAL METHODS

Committee on Residues

PAUL E. CORNELIUSSEN, CHAIRMAN
U.S. Food and Drug Administration, Division of Contaminants Chemistry, Washington, DC 20204

KENNETH W. BOYER
Southern Testing and Research Laboratories, Inc., 3709 Airport Drive, Wilson, NC 27893

HENRY B. CHIN
National Food Processors Association, 6363 Clark Ave, Dublin, CA 94568-3001

RONALD F. COOK
FMC Corp., Agricultural Chemicals Group, PO Box 8, Princeton, NJ 08543

W. GEORGE FONG
Florida Department of Agriculture, Division of Chemistry, 3125 Conner Blvd, Tallahassee, FL 32399-1650

HARVEY HUNDLEY
U.S. Environmental Protection Agency, Benefits and Use Division, BARC E, Building 306, Beltsville, MD 20705

BRIAN D. RIPLEY
Ontario Ministry of Agriculture and Food, Provincial Pesticide Residue Testing Laboratory, University of Guelph, Guelph, ON, N1G 2W1, Canada

KEITH A. MCCULLY, SECRETARY
Health and Welfare Canada, Field Operations Directorate, Tunney's Pasture, Ottawa, ON, K1A 0L2, Canada

RICHARD H. ALBERT, COMMITTEE STATISTICIAN
U.S. Food and Drug Administration, Division of Mathematics, Washington, DC 20204

Metals and Other Elements

(1) *Atomic Absorption Spectrophotometry (AAS)*: Continue effort to consolidate present AOAC official AAS methods for individual elements into a unified AAS scheme for multielement analysis of foods and other biological substrates.

(2) *Fluorine*: Continue study to improve the microdiffusion and fluoride-specific electrode method for determining fluoride in foods (*J. Assoc. Off. Anal. Chem.* [1979] **62**, 1065–1069), as specified in Associate Referee's report on the collaborative study on fluoride in infant foods (*J. Assoc. Off. Anal. Chem.* [1981] **64**, 1021–1026).

(3) *Graphite Furnace/AAS*: Continue study to resolve problems found for levels below 20 ppb lead and 1 ppb cadmium in interlaboratory trial of the coprecipitation GF/AAS method for lead and cadmium in foods (*Can. J. Spectrosc.* [1986] **31**, 44–52); if problems are resolved, prepare protocol for collaborative study of this method for approval by the General Referee, Committee Statistician, and Committee on Residues; conduct interlaboratory trial of an improved version of the coprecipitation GF/AAS method for arsenic in foods (*Can. J. Spectrosc.* [1985] **30**, 154–157).

(4) *Graphite Furnace Atomic Absorption Spectrophotometric Determination of Lead and Cadmium Released from Ceramicware:* Complete interlaboratory trial; if satisfactory, prepare protocol for collaborative study for approval by General Referee, Committee Statistician, and Committee on Residues; initiate collaborative study.

(5) *Lead in Calcium Supplements*: Draft, in AOAC official method format, the method developed by the Associate Referee for the determination of lead in calcium supplements for review and comment by the General Referee. Recovery and method performance data must be included with the proposed method. If satisfactory, perform interlaboratory trial, prepare protocol for collaborative study for approval by the General Referee, Committee Statistician, and Committee on Residues; initiate collaborative study.

(6) *Lead in Wines:* Continue study to develop, evaluate, and collaboratively study method for determination of lead in wines.

(7) *Neutron Activation Analysis*: Carry out interlaboratory trial of method for the determination of sodium in biological materials, and if satisfactory, prepare protocol for collaborative study of the method for approval by the General Referee, Committee Statistician, and Committee on Residues; initiate collaborative study.

(8) *Organometallics in Fish*: Continue official first action status of the LC/atomic absorption spectrophotometric method for methyl mercury in seafood, **990.04**.

(9) *Organotin Compounds*: Draft, in AOAC official method format, the method developed for the determination of tributyltin and its degradation products dibutyltin and monobutyltin in shellfish and finfish tissue. Prepare protocol for collaborative study for approval by the General Referee, Committee Statistician, and Committee on Residues; initiate collaborative study.

(10) *Other Topics*: (a) Declare as surplus the following methods: (i) **964.16**, Antimony in Food, Spectrophotometric Method; (ii) **963.21**, Arsenic in Food, Kjledahl Flask Disgestion; (iii) **942.17**, Arsenic in Food, Molybdenum

*An asterisk before a recommendation indicates that official action on that recommendation resulted in its appearance in *Changes in Official Methods*.

The recommendations submitted by the Committee on Residues were adopted by the Association.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and *Changes in Official Methods of Analysis*, 3rd supplement (1992).

Blue Method; (iv) **952.13**, Arsenic in Food, Silver Diethyldithiocarbonate Method; (v) **973.33**, Arsenic in Meat and Poultry, Molybdenum Blue Method; (vi) **945.58**, Cadmium in Food, Dithizone Method; (vii) **960.40** Copper in Food, Colorimetric Method; (viii) **944.07** Fluorine on Apples and Pears, Colorimetric Method; (ix) **935.51**, Lead on Apples and Pears, Colorimetric Method; (x) **934.07**, Lead in Food, General Dithizone Method; (xi) **952.14**, Mercury in Food, Colorimetric Dithizone Method; and (xii) **944.09**, Zinc in Food, Colorimetric Method. (b) Make editorial change in method **975.05**, Cadmium and Lead in Earthenware. Delete "and **973.33**." (c) Delete method **975.05**, Cadmium and Lead in Earthenware from Chapter 8 of *Official Methods of Analysis*, 15th Ed., 1990. (d) Modify method **973.32**, Cadmium and Lead in Earthenware, as follows: (i) Change second paragraph, first sentence of **973.32D**(a) to read "Dil. samples contg 15 μg Pb/mL with HOAc" and (ii) change second paragraph, last sentence of **973.32D**(a) to read "Det. Pb as above."

Multiresidue Methods

(1) *Comprehensive Multiresidue Methodology*: Continue study of the modified California Department of Food and Agriculture Multiresidue Method to improve recoveries of the *N*-methylcarbamates and the investigation of sample weight concentration relationship with "salting out" procedures. If results are satisfactory to General Referee, perform an interlaboratory trial.

(2) *Fumigants*: Perform interlaboratory trial, and if satisfactory, proceed to collaborative study of the method for multifumigants in grain, milled and intermediate grain products, and citrus fruit.

(3) *Low Moisture–High Fat Samples*: Draft in AOAC official method format the method currently being studied by the Associate Referee, along with recovery data for all pesticides and commodities that were evaluated for review and comment by the General Referee. If satisfactory, perform interlaboratory trial.

(4) *Miniaturized Multiresidue Methods for Fat-Containing Foods*: Continue study to develop the miniaturized method for milk with emphasis on its application to polar pesticides and metabolites and investigate alternatives to the use of standards prepared in sample matrix diluent. If satisfactory, develop additional recovery data and conduct interlaboratory trial.

(5) *Miniaturized Multiresidue Methods for Nonfatty Foods*: Continue study of small-scale extraction and develop additional recovery data for selective *N*-methylcarbamates, pyrethroids, and triazoles. Conduct interlaboratory trial with representative chemicals from all classes of pesticides that can be recovered.

(6) *Supercritical Fluid Extraction of Pesticide Residues in Food*: Continue studies on supercritical fluid extraction of pesticide residues from foods.

(7) *Sweep Codistillation*: Draft in AOAC official method format the method based on sweep codistillation and a protocol for collaborative study for review and comment by the General Referee, Committee Statistician, and Committee on Residues; initiate collaborative study.

(8) *Synthetic Pyrethroids*: Conduct interlaboratory trial of the method using the mixed solvent elution system and the wide-bore capillary EC chromatographic system (*J. Assoc. Off. Anal. Chem.* [1991] **74**, 150–152). If satisfactory, prepare protocol for collaborative study for approval by General Referee, Committee Statistician, and Committee on Residues; initiate collaborative study.

Organohalogen Pesticides

(1) *Chlorinated Dioxins*: (a) Continue study to evaluate methods for determining 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and hexachloro-, heptachloro-, and octachloro-substituted dibenzo-*p*-dioxins and dibenzofurans in foods and environmental samples, with ultimate goal of establishing through AOAC collaborative procedures an official method or methods for determining residues of these compounds at parts-per-trillion levels in fish, milk, and other foods.

(2) *Chlorophenoxy Alkyl Acids and Pentachlorophenol*: Continue as official first action the GC method for pentachlorophenol in gelatin, **985.24**. Discontinue topic.

(3) *Methyl Bromide*: Draft in AOAC Official Method format the method for methyl bromide in use in Associate Referee's laboratory for review and comment by General Referee and Committee on Residues. Principle of the method, alternative means to the closed system GC to analyze headspace, procedure for correcting for recovery, calculation procedures, recovery, and method performance data must be included with the proposed method. If satisfactory, develop procedures for handling and transporting food samples containing methyl bromide, and test reliability of procedures by interlaboratory study. If results are satisfactory, prepare protocol for collaborative study on commodities likeliest to retain methyl bromide from fumigation (e.g., nuts and dried fruits), and submit protocol for approval by General Referee, Committee Statistician, and Committee on Residues; initiate collaborative study.

(4) *Polychlorinated Biphenyl (PCB) Determination by Measurement of Specific Congeners*: Draft in AOAC Official Method format a method for PCB determination by measurement of specific individual congeners, including extraction and cleanup steps for review and comment by the General Referee. Recovery and method performance data must be included with the proposed method. If satisfactory, perform interlaboratory trial, prepare protocol for collaborative study acceptable to both the AOAC and the Nordic Committee on Methods of Food Analysis for approval by General Referee, Committee Statistician, and Committee on Residues; initiate collaborative study.

(5) *Polychlorinated Biphenyls (PCBs) in Blood*: Continue study to make method suitable for both PCBs and pesticides in blood serum, and collaboratively study this application of the method.

Organonitrogen Pesticides

(1) *Anilazine*: Appoint an Associate Referee to evaluate LC method of Lawrence and Panopio (*J. Assoc. Off. Anal. Chem.* [1980] **63**, 1300–1303) and other LC or GC methods for determining anilazine residues in food crops and to collaboratively study method selected.

(2) *Benzimidazole-Type Fungicides*: Appoint an Associate Referee to study method for determination of benomyl, thiophanate methyl, and hydrolysis product methyl 2-benzimidazole carbamate (MBC, also known as the fungicide carbendazim) as MBC in fruits and vegetables.

(3) *Captan and Related Fungicides*: Perform collaborative study of method for captan, captafol, and folpet.

(4) *Carbamate Herbicides*: Appoint an Associate Referee to select and collaboratively study method for determining residues of the carbamate herbicides asulam, desmedipham, and phenmedipham in crops.

(5) *Carbamate Insecticides*: Draft in AOAC official method format the LC Method for carbamate insecticides in liver (*J. Agric. Food Chem.* [1989] **72**, 586–592). Perform interlaboratory trial, and prepare a protocol for collaborative study for approval by the General Referee, Committee Statistician, and Committee on Residues; initiate collaborative study.

(6) *Carbofuran*: Appoint an Associate Referee to investigate and collaboratively study methods for determining carbofuran and its carbamate and phenolic metabolites in milk and meat and for determining 3-hydroxycarbofuran glucoside and phenolic carbofuran metabolites in crops.

(7) *Chlorothalonil*: Appoint an Associate Referee to evaluate existing GC multiresidue methods, such as **976.23**, as well as methods specifically designed for determination of chlorothalonil and its 4-hydroxy metabolite in foods, and subject method selected to collaborative study.

(8) *Daminozide and 1,1-Dimethylhydrazine (UDMH)*: Discontinue topic.

(9) *Diquat and Paraquat*: Continue official first action status of the method for diquat and paraquat residues in potatoes. Continue study to improve applicability of method.

(10) *Dithiocarbamate Fungicides*: Appoint an Associate Referee to develop methods for distinguishing dimethyldithiocarbamates from ethylenebisdithiocarbamates and for determining the parent fungicides and their metabolites in foods.

(11) *Glyphosate*: Appoint an Associate Referee to evaluate and collaboratively study analytical methods for determining residues of glyphosate and its metabolite (aminomethyl)phosphoric acid in foods.

(12) *Maleic Hydrazide*: Appoint an Associate Referee to develop and collaboratively study a GC or LC method for determining maleic hydrazide in crops.

(13) *Organonitro Pesticides*: Continue development of suitable cleanup methods for organonitro pesticides in foods.

(14) *Sodium o-Phenylphenate*: Appoint an Associate Referee to develop and collaboratively study a GC or LC method for determining *o*-phenylphenol in foods.

(15) *Substituted Ureas*: Perform collaborative study of multiresidue LC method (*J. Assoc. Off. Anal. Chem.* [1987] **70**, 740–745) for urea herbicides in onions and potatoes.

(16) *Thiolcarbamate Herbicides*: Appoint an Associate Referee to evaluate and collaboratively study methods for determining residues of thiolcarbamate herbicides in crops.

(17) *s-Triazines*: Continue study to select more efficient cleanup procedures for the determination of residues of atrazine, simazine, and cyanazine in agricultural products and to collaboratively study method selected.

Organophosphorus Pesticides

(1) *Extraction Procedures*: Discontinue topic.

(2) *Organophosphorus Pesticide Residues*: Continue study on the recovery of organophosphorus (parent and metabolite) chemicals through **985.22**, followed by oxidation to total sulfones (*Analyst* [1984] **109**, 483–487). Transfer topic to Multiresidue Methods General Refereeship.

(3) *Phosphine*: Appoint an Associate Referee to evaluate methods for determining residual phosphine in grains including the solvent soaking procedure for the extraction of fumigants in grains, **977.18**. Transfer topic to Organohalogen Pesticides General Refereeship.

Radioactivity

(1) *Cesium-137*: Appoint an Associate Referee to evaluate and collaboratively study radiochemical methods for determining Cs-137 in foods and other biological matrixes at levels lower than determinable with official method, **973.67**.

(2) *Iodine-131*: Complete evaluation of the collaborative study of the method for I-131 in milk; submit report and recommendation to the Official Methods Board for possible interim first action status.

(3) *Plutonium*: Appoint an Associate Referee to study the Department of Energy method for determining plutonium in foods, biological materials, and water (HASL-300-Ed 25, *Energy Monitoring Laboratory Procedures Manual* [1982] pp. E-Pu-01-01) and related procedures. When method is finalized, prepare protocol for collaborative study for approval by the General Referee, Committee Statistician, and Committee on Residues; initiate collaborative study.

(4) *Radium-228*: Complete evaluation of the collaborative study; submit report and recommendation to General

Referee and Committee on Residues for possible interim first action status.

(5) *Strontium-89 and -90*: Appoint an Associate Referee to prepare protocol for collaborative study of method of Baratta and Reavey (*J. Agric. Food Chem.* [1969] **17**, 1337–1339) for determining strontium-89 and -90 in foods for approval by General Referee, Committee Statistician, and Committee on Residues; initiate collaborative study.

(6) *Tritium*: Appoint an Associate Referee to evaluate and collaboratively study methods for determining tritium in foods and biological materials.

RECOMMENDATIONS FOR OFFICIAL METHODS

Committee on Microbiology and Extraneous Materials

GAYLE A. LANCETTE, CHAIRWOMAN
U.S. Food and Drug Administration, 60 8th St NE, Atlanta, GA 30309

J. STANLEY BAILEY, SECRETARY
Russell Research Center, ARS, U.S. Department of Agriculture, PO Box 5677, Athens, GA 30613

JACK L. BOESE
U.S. Food and Drug Administration, Division of Microbiology, 200 C St SW, Washington, DC 20204

RUSSELL S. FLOWERS
Silliker Lab, Inc., 1304 Halstead St, Chicago Heights, IL 60411

ANTHONY N. SHARPE
Health and Welfare Canada, Health Protection Branch, Bureau of Microbial Hazards, Tunney's Pasture, Ottawa, ON, K1A 0L2, Canada

VIRGINIA N. SCOTT
National Food Processors Association, 1401 New York Ave, Suite 400, Washington, DC 20005

C. ROBERT GRAHAM
Lancaster Laboratories, Inc., 2425 New Holland Pike, Lancaster, PA 17601

STERLING S. THOMPSON
Hershey Foods Corp., Technical Center, 1025 Reese Ave, Hershey, PA 17033

FOSTER D. MCCLURE, COMMITTEE STATISTICIAN
U.S. Food and Drug Administration, Division of Mathematics, 200 C St SW, Washington, DC 20204

Analytical Mycology and Microscopy

(1) *Geotrichum Mold in Canned and Frozen Fruits, Vegetables, and Fruit Juices*: Continue study.
(2) *Mold Counts by Compound Microscope*: Continue study.
(3) *Mold Detection by Chemical Methods*: Continue study.
(4) *Mold in Reconditioned Nutmeg*: Continue study.
(5) *Standardization of Plant Tissue Concentrations for Mold Counting*: Continue study.
(6) *Vegetable Substitutes in Horseradish*: Continue study.
(7) *Yeasts and Mold, Mycological Media for Isolation*: Continue study.

Filth and Extraneous Materials in Foods and Drugs

*(1) *Aerator, water*, **945.75B(a).** Replace first 2 sentences with "Modify to produce fine, even spray of H_2O by removing screen(s). If aerator has more than 1 disk, use only the 1 with small (ca 1 mm) holes." The next sentence, "(Available... 00200.)," is unchanged. After it, add "Unit may be securely attached to faucet with heavy rubber tubing (wall thickness ca 3.2 mm) to provide suitable working distance from sieve and allow some movement of sprayer head."
(2) *Alkaline Phosphatase in Foods, Electrophoresis Detection Method*: Continue study.
(3) *Baked Goods with Fruit and Nut Tissues, Light Filth by Flotation Method*: Continue study.
(4) *Basil (Unground), Light Filth by Flotation Method*: Continue study.
(5) *Bean Paste, Light Filth by Flotation Method*: Continue study.
(6) *Cheese, Filth by Sieving Method*: New topic.
(7) *Chocolate and Chocolate Products, Light Filth by Flotation Method*: Continue study.
(8) *Coffee (Ground), Light Filth by Flotation Method*: Continue study.
(9) *Condimental Hot Sauces, Light Filth by Flotation Method*: Continue study.
(10) *Condimental Sauces Containing Soy Sauce, Thickeners, and Spices, Light Filth by Flotation Method*: Continue study.
(11) *Crabmeat, Shrimp, and Tuna (Canned), Light Filth by Brine Flotation Method*: Discontinue topic.
(12) *Fish Paste and Sauces, Light Filth by Flotation Method*: Continue study.
(13) *Grain Products, Fecal Contamination by Gas Chromatography Detection Method*: Continue study.
(14) *Grain Products, Light Filth by Flotation Method*: Continue study.
(15) *Grain Products, Mammalian Feces Detection by Alkaline Phosphatase Method*: Discontinue topic.
(16) *Grains (Whole), Internal Insect Infestation by Cracking Flotation Method*: Continue study.
(17) *Grains (Whole), Internal Infestation by ELISA Method*: Continue study.
(18) *Grains and Seeds (Whole), External Light Filth by Flotation Method*: Continue study.
(19) *Rodent Gnawing of Packaging Materials and Foods, Salivary Amylase Test*: Continue study.
(20) *Soybean Curd, Light Filth by Flotation Method*: Continue study.

*An asterisk before a recommendation indicates that official action on that recommendation resulted in its appearance in "Changes in Official Methods."

The recommendations submitted by the Committee on Microbiology and Extraneous Materials were adopted by the Association.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and *Changes in Official Methods of Analysis*, 3rd supplement (1992).

(21) *Spices, Mammalian Feces by Chemical Detection Method*: Continue study.

*(22) *Urine on Grains, Magnesium Uranyl Acetate Test* (first action), **962.28**, *J. Assoc. Off. Anal. Chem.* (1963) **46,** 685: Recommended for surplus status.

(23) *Urine Stains on Foods and Containers, Chemical Detection Methods*: Continue study.

(24) *Vegetable Products (Dehydrated), Light Filth by Flotation Method*: Continue study.

*(25) Other topics: Add the following publications to the list of General References in Chapter 16, Extraneous Materials: "Key for Identification of Mandibles of Stored-Food Insects" (1985) AOAC, 2200 Wilson Blvd, Arlington, VA 22201-3301; "Ecology and Management of Food Industry Pests" (1991) AOAC, 2200 Wilson Blvd, Arlington, VA 22201-3301; "Insect and Mite Pests in Food: An Illustrated Key" (1991) USDA, Aggricultural Handbook No. 655.

Cosmetic Microbiology

(1) *Microbes, Isolation Method*: Continue study.

(2) *Preservative Efficacy, Cosmetic Toiletry and Fragrance Association Method*: Continue study.

(3) *Preservative Efficacy in Solid Cosmetics*: Continue study.

Dairy Microbiology

(1) *Aerobic Plate Counts, Reflective Colorimetry Method for Automated Microbiology*: Transferred from Food Microbiology.

(2) *Bacterial and Coliform Counts in Dairy Products, Petrifilm Methods*: Continue study.

(3) *Bactoscan Methods*: Continue study.

(4) *Coliform in Milk, Impedance Detection by Vitek Systems*: Continue study.

(5) *Coliform in Milk, Impedance Detection by Radiometer America*: Continue study.

(6) *Coliforms in Dairy Products, Pectin Gel Method*: Continue study.

(7) *Listeria monocytogenes in Dairy Products, Detection by Culture Methods*: Continue study.

(8) *Listeria monocytogenes in Dairy Products, Detection by DNA Probe*: Continue study.

(9) *Raw Milk in Cheese, Alkaline Phosphatase Test*: Continue study.

(10) *Somatic Cells, Automated Optical Methods*: Continue study.

(11) *Somatic Cells, Fossomatic Method*: Continue study.

(12) *Salmonella Detection from Dairy Products by Motility Enrichment on Modified Semisolid Rappaport-Vassiliadis Medium*: Continue study.

Drug and Device Related Microbiology

(1) *Biological Indicators, Testing and Standardization*: Continue study.

(2) *Biological Indicators, Ethylene Oxide*: New topic.

(3) *Chemical Indicators*: Continue study.

(4) *Endotoxins, Limulus Amoebocyte Lysate Test*: Continue study.

(5) *Medical Devices, Packaging Integrity*: Continue study.

(6) *Sporicidal Testing of Disinfectants/Sterilants*: Continue study.

Environmental Sanitation Microbiology

(1) *Total Coliforms and Escherichia coli on Surfaces, Defined Substrate Technology*: Continue study.

(2) *Cleanliness of Surfaces, Using an ATP-Based System*: Continue study.

Food Microbiology (Nondairy)

(1) *Aerobic Plate Counts of Foods, Petrifilm Methods*: Continue study.

(2) *Aerobic Plate Counts, Determination Using Redigel Medium*: Continue study.

(3) *Aerobic Plate Counts, Reflective Colorimetry Method for Automated Microbiology*: Transfer to Dairy Microbiology.

(4) *Bacillus cereus Enterotoxin, Microslide Gel Double Diffusion Test*: Continue study.

(5) *Bacillus cereus, Enzyme Immunoassay for Enterotoxins*: New topic.

(6) *Bactoscan Methods*: Discontinue topic.

(7) *Clostridium perfringens, Recovery from Marine Environment by Iron Milk Test*: Continue study.

(8) *Escherichia coli in Chilled and Frozen Foods, MUG Test*: Discontinue topic.

(9) *Escherichia coli in Shellfish, MUG Test*: Discontinue topic.

(10) *Listeria, DNA Probe*: Discontinue topic.

(11) *Listeria, Listeria-Tek Assay*: Continue study.

(12) *Listeria, MICRO-ID System*: Continue study.

(13) *Listeria, Vitek AutoMicrobic System*: Continue study.

(14) *Listeria, Assurance Enzyme Immunoassay*: Continue study.

(15) *Listeria, GENE-TRAK Colorimetric DNA Hybridization Method*: Continue study.

(16) *Listeria, Identification by Gas Chromatography of Cellular Fatty Acids*: Continue study.

(17) *Listeria, Tecra Enzyme Immunoassay*: Continue study.

(18) *Salmonella, Assurance Enzyme Immunoassay*: Continue study.

(19) *Salmonella, GENE-TRAK DNA Hybridization Screening Method*: Continue study.

(20) *Salmonella, ImmunoBand Screening Method*: Continue study.

(21) *Salmonella, ISO-GRID Screening Method*: Continue study.
(22) *Salmonella, Malthus Automated Conductance Method*: Continue study.
(23) *Salmonella, Modified Semisolid Rappaport-Vassiliadis Method for Cocoa and Chocolate*: Continue study.
(24) *Salmonella, Oxoid Method*: Continue study.
(25) *Salmonella, Q-TROL Enzyme Immunoassay Screening Method*: Continue study.
(26) *Salmonlla, Salmonella-Tek Enzyme Immunoassay*: Continue study.
(27) *Salmonella, TECRA Enzyme Immunoassay Screening Method*: Continue study.
(28) *Salmonella, Escherichia coli, and Other Enterobacteriaceae, Identification by Micro ID Diagnostic Kit*: Continue study.
(29) *Salmonella, Escherichia coli, and Other Enterobacteriaceae, Identification by the Vitek Gram Negative Identification Card*: Continue study.
(30) *Salmonella in Chocolate*: Discontinue topic.
(31) *Staphylococcal Enterotoxin, TECRA Enzyme Immunoassay*: Continue study.
(32) *Staphylococcus aureus, GENE-TRAK Colorimetric DNA Hybridization Method*: Continue study.
(33) *Total Coliforms and Escherichia coli, Petrifilm Methods*: Continue study.
(34) *Total Coliforms and Escherichia coli, ColiComplete Discs*: Continue study.
(35) *Vibrio cholerae, Elevated Temperature Enrichment Method*: Discontinue topic.
(36) *Vibrio vulnificus, Identification by Gas Chromatography of Cellular Fatty Acids*: Continue study.

Water Microbiology

(1) *Coliforms and Escherichia coli in Drinking water, Membrane Filter Method*: New topic.
(2) *Coliforms and Escherichia coli in Marine Surface Waters, Broth Tube Method*: New topic.
(3) *Escherichia coli in Marine Surface Waters, Membrane Filter Method*: New topic.
(4) *Total Coliforms and Escherichia coli, Rapid Specific, Simultaneous Enumeration by Defined Substrate Technology Colilert Method*: Continue study.
(5) *Total Coliforms and Escherichia coli, Hydrophobic Grid Membrane Filter/MUG Method*: Continue study.

RECOMMENDATIONS FOR OFFICIAL METHODS

Committee on Feeds, Fertilizers, and Related Topics

HAROLD C. THOMPSON, JR, CHAIRMAN
U.S. Food and Drug Administration, National Center for Toxicology Research, Jefferson, AR 72079

W. EMMETT BRASELTON, JR, SECRETARY
Michigan State University, Department of Pharmacology and Toxicology, East Lansing, MI 48824

DANIEL H. MOWREY, COMMITTEE STATISTICIAN
Lilly Research Laboratory, Division of Eli Lilly Corp., Greenfield, IN 46140

MAIRE C. WALSH, SAFETY ADVISOR
State Laboratory, Abbotstown, Castleknock, Dublin 15, Ireland (01)217700

LARS M. REIMANN
Woodson Tenent Laboratories, PO Box 2135, Memphis, TN 38101

THOMAS M. PARHAM, JR
Arcadian Corp., PO Box 307, Geismer, LA 70734

LORI L. RHODIG
Smith Kline Animal Health Products, 4444 S 76th St, Omaha, NE 68127

CAROLYN A. GEISLER
U.S. Food and Drug Administration, Denver Federal Center, Bldg 20, PO Box 25087, Denver, CO 80225

GEORGE W. LATIMER
Office of the Texas State Chemist, PO Box 3160, College Station, TX 77841-3160

STANLEY E. KATZ
Rutgers University–Cook College, Department of Biochemistry and Microbiology, New Brunswick, NJ 08903

Antibiotics in Feeds

(1) *Apramycin*: Continue study.
(2) *Bacitracin Methylene Disalicylate in Feeds*: Continue study.
(3) *Chlortetracycline in Feeds*: Continue study.
(4) *Direct-Fed Microbiological Products and Silage Inoculants*: Continue study.
(5) *Lincomycin*: Continue study.
(6) *Monensin Microbiological Method*: Continue study.
(7) *Narasin Microbiological Method*: Continue study.
(8) *Neomycin*: Continue study.
(9) *Oxytetracycline in Feeds by Microbiological Methods*: Continue study.
(10) *Tylosin*: Continue study.
(11) *Virginiamycin*: Continue study.
(12) *Virginiamycin with Other Drugs*: Continue study.

Drugs in Feeds

(1) *Amprolium*: Change current topic name to *Amprolium* to obtain list uniformity. Continue study.
(2) *Carbadox*: Continue study.
(3) *Chlortetracycline (LC Method)*: Continue study.
(4) *Ethopabate*: Continue study.
(5) *Furazolidone and Nitrofurazone*: Discontinue topic.
(6) *Lasalocid (LC Method)*: Continue study.
(7) *Melengestrol Acetate*: Continue study.
(8) *Monensin and Narasin (LC Method)*: Continue study.
(9) *Morantel Tartrate*: Continue study.
(10) *Nifursol*: Continue study.
(11) *Oxytetracycline (LC Method)*: Continue study.
(12) *Pyrantel Tartrate*: Continue study.
(13) *Roxarsone*: Add the following parenthetical note after the second sentence in the section on *Preparation of Sample* (**971.47 D**): (Doubling of the sample weight, the phosphate extractant volume, and the extraction vessel volume is necessary to recover 30.0 mL of extract from some matrices.).
(14) *Sampling*: Appoint new Associate Referee. Continue study.
(15) *Sulfadimethoxine and Ormetoprim*: Continue study.
(16) *Sulfa Drug Residues*: Continue study.
(17) *Sulfamethazine and Sulfathiazole*: Continue study.

Feeds

(1) *Amino Acids*: Continue study.
(2) *Atomic Absorption Spectrophotometry*: Continue study.
(3) *Carotenoids*: Continue study.
(4) *Crude Protein*: Continue study.
(5) *Emission Spectroscopy*: Continue study.
(6) *Fat*: Continue study.
(7) *Fiber*: Continue study.
(8) *Iodine and EDDI in Feeds*: Continue study.
(9) *Microscopy*: Continue study.
(10) *Minerals*: Continue study.
(11) *Mixed Feeds—Infrared Reflectance Techniques*: Continue first action status of the method for fiber (acid detergent) and protein (crude) in forage, near-infrared spectroscopic method (**989.03**).

*An asterisk before a recommendation indicates that official action on that recommendation resulted in its appearance in *Changes in Official Methods.*

The recommendations submitted by the Committee on Feeds, Fertilizers, and Related Topics were adopted by the Association.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and *Changes in Official Methods of Analysis*, 3rd supplement (1992).

(12) *Moisture in Mixed Feeds and Forages*: Continue study and first action status of moisture in forage near-infrared reflectance spectroscopy (**991.01**).
(13) *Moisture in Pet Foods*: Continue first action status of Karl Fisher method for determination of moisture in sort-moist pet foods.
(14) *Sampling*: Continue study.
(15) *Vitamins*: Continue study.

Fertilizers

(1) *Aluminum in Aluminum Sulfate-type Soil Acidifiers*: Change from first action to final action status of the method for aluminum in aluminum sulfate-type soil acidifiers, atomic absorption spectrophotometric method (**983.05**).
(2) *Nitrogen by Combustion*: Continue study.
(3) *Iron*: Continue study.
(4) *Manganese in Feed Ingredients*: Continue study.
(5) *Particle Size Range of Peat*: Change from first action to final action status of the method for particle size range of peat, mechanical analysis (**973.03**).
(6) *Phosphorus*: Continue study.
(7) *Potassium*: Continue study.
(8) *Sample Preparation*: Continue study.
(9) *Sampling*: Continue study.
(10) *Sand in Peat*: Change from first action to final action status of the method for sand in peat (**973.05**).
(11) *Slow-Release Mixed Fertilizers*: Continue study.
(12) *Sodium in Fertilizers*: Change from first action to final action status of the methods for sodium in fertilizers, flame photometric method (**974.01**) and atomic absorption spectrophotometric method (**983.04**).
(13) *Urea and Methyleneureas in the Fertilizer and Agricultural Liming Materials*: Continue study.
(14) *Water in Fertilizers*: Continue study.

Nutrients in Soils

(1) *Soil pH*: Continue study.

Tobacco

(1) *Nicotine in Environmental Tobacco Smoke*: Continue first action.
(2) *Nicotine Alkaloids in Tobacco*: Continue study.
(3) *Polyphenols in Tobacco*: Continue study.

Veterinary Analytical Toxicology

(1) *Antibiotic Screening Methods*: Continue study.
(2) *Cholinesterase Colorimetric Method*: Continue first action status of the colorimetric method for whole blood cholinesterase (**991.10**).
(3) *Cholinesterase pH Method*: Continue first action status of the pH cholinesterase method.
(4) *GC/MS Methods*: Continue study.
(5) *Fluoride in Animal Tissue*: Continue study.
(6) *Lead in Animal Tissue*: Continue study.
(7) *Multielement Analysis by ICP*: Continue study.
(8) *Multiple Anticoagulant Screening*: Continue study.
(9) *Natural Products*: Continue study.
(10) *Nitrates and Nitrites*: Submit protocol for a collaborative study on dip-stick method for forage nitrate.
(11) *Pesticides in Toxicological Samples*: Continue study.
(12) *Selenium in Animal Tissue*: Assign new Associate Referee.
(13) *Sodium Monofluoroacetate*: Continue study.
(14) *Vitamins A and E*: Continue study.
(15) *Vitamins D and K*: Retire topic.
(16) *Zinc in Animal Serum*: Continue first action status of the method for serum zinc (**991.11**)

RECOMMENDATIONS FOR OFFICIAL METHODS

Committee on Environmental Quality

ANN B. STRONG, CHAIRWOMAN
Environmental Chemistry Branch, U.S. Army Corps of Engineers, Waterways Experiment Station, 3909 Halls Ferry Rd, Vicksburg, MS 39180

THOMAS F. JENKINS
U.S. Army Corps of Engineers, Cold Regions Research and Engineering Laboratory, 72 Lyme Rd, Hanover, NH 03755

LARRY G. LANE
Mississippi State Chemical Laboratory, PO Box CR, Mississippi State, MS 39762

GREGORY LEYES
Monsanto Company, 800 N. Lindbergh Blvd, St. Louis, IL 63167

JAMES LONGBOTTOM
U.S. Environmental Protection Agency, Monitoring and Support Laboratory, 26 W. Martin Luther King Dr., Cincinnati, OH 45268

CHARLES SELLERS
U.S. Environmental Protection Agency, Office of Solid Waste, OS-331, Rm 243A, 401 M Street SW, Washington, DC 20460

MARVIN STEPHENS
Wadsworth/ALERT Laboratories, Inc., 4101 Shuffel Dr. NW, North Canton, OH 44720

JOAN K. BARTZ, SECRETARY
SEC Donohue, 1313 West Clark St, Pasco, WA 99301

PAUL BRITTON, COMMITTEE STATISTICIAN
U.S. Environmental Protection Agency, EMSL Cincinnati QARD DEB, 26 W. Martin Luther King Dr., Cincinnati, OH 45268

DIRK SHOEMAKER, SAFETY ADVISOR
Nebraska Department of Agriculture, 3703 S. 14th St, Lincoln, NE 68502

The Committee recommended that the Official Methods Board move to adopt the revised Terms of Reference and the Committee activities for 1992.

The Committee recommends that a member of the Laboratory Quality Assurance Committee be appointed as an advisor to the Environmental Quality methods committee.

The Terms of Reference were revised to address the change in the member composition and to reflect the environmental mission of the Committee.

Quality assurance needs to be given the same status as statistics and safety in the collaborative study process of environmental methods. This will increase the likelihood that AOAC methods will be acceptable for environmental regulatory purposes.

Cooperative Studies

(1) *Nitrogen- and Phosphorus-Containing Pesticides in Groundwater*: Adopt as final action the method for Gas Chromatography with Nitrogen Phosphorus Detection Determination of Nitrogen- and Phosphorus-Containing Pesticides in Finished Drinking Waters.
(2) *Organochlorine Pesticides in Finished Drinking Water*: Continue first action status for Gas Chromatographic Electron Capture Detector Determination of 29 Chlorinated Pesticides in Finished Drinking Water.
(3) *Chlorinated Acids in Groundwater*: Finalize study report.
(4) *Pesticides in Water I*: Adopt as first action the method for HPLC Determination of Pesticides in Finished Drinking Water.
(5) *Pesticides (EDB and DBCP) in Water II*: Finalize study report.
(6) *Carbamate Pesticides*: Adopt as final action the method for Measurement of *N*-Methylcarbomoyloximes and *N*-Methylcarbamates in Water by Direct Aqueous Injection HPLC with Postcolumn Derivatization.
(7) *Ethylene Thiourea in Water*: Finalized study report.
(8) *Soils and Sediments*: Seek an Associate Referee.
(9) *Inorganic Analytes*: Adopt as final action the method for Digestion of Solid Wastes for Determination of 23 Elements by Inductively Coupled Plasma Atomic Emission Spectroscopy.
(10) *Metals in Water, Soil, Sludge, and Fly Ash by Inductively Coupled Plasma/Mass Spectrometry (ICP/MS)*: Collaborative study completed.
(11) *Microwave Digestion of Plant Samples for Inorganic Elemental Analysis*: Associate Referee appointed and protocol in development.

Inorganic Methods

(1) *Inductively Coupled Plasma/MS*: Finalize study report.
(2) *Ion Chromatographic Methods for Groundwater*: Continue topic.

*An asterisk before a recommendation indicates that official action on that recommendation resulted in its appearance in *Changes in Official Methods*.

The recommendations submitted by the Committee on Environmental Quality were adopted by the Association.

Section numbers refer to *Official Methods of Analysis* (1990) 15th Ed. and *Changes in Official Methods of Analysis*, 3rd supplement (1992).

Organic Methods

(1) *Diquat and Paraquat*: Appoint an Associate Referee.

(2) *Herbicide Residues in Environmental Waters*: Adopt as final action the method for Determination of Glyphosate and Aminomethylphosphonic Acid in Environmental Waters by Liquid Chromatography.

(3) *Munitions in Wastewater*: Continue first action status for Determination of Munition Residues in Wastewater. A method modification study is planned.

(4) *Explosive Residues*: Adopt as final action the method for Determination of Munition Residues in Soil by Liquid Chromatography.

(5) *Triazine Herbicides*: Continue topic.

(6) *Carbonyl Compounds in Water*: Collaborative study was completed.

(7) *Carbamates in Water*: Associate Referee completed a minicollaborative study on the calibration of the thermospray LC/MS system for the determination of 3 *N*-methylcarbamates, 3 *N*-methylcarbamoyl oximes, 2 substituted urea pesticides, and 1 ester of a substituted carbamic acid.

(8) *Immunoassay for Atrazine*: This topic was divided into 2 Associate Referee topics: (*1*) Enzyme Immunoassay for Atrazine in Water (co-Associate Referees were appointed), and (*2*) Magnetic-Particle Based Enzyme Immunoassay for Atrazine in Water (an Associate Referee was appointed).

EXECUTIVE, OFFICER, AND COMMITTEE REPORTS

Executive Director's Report

RONALD R. CHRISTENSEN
AOAC International, Suite 400, 2200 Wilson Blvd, Arlington, VA 22201

Preface

My main purpose today is to report on the activities of the AOAC staff during the past year in support of the AOAC volunteers and the AOAC programs. Others reported previously or will report to you today on the financial health of the Association, the status of the AOAC[SM] Official Methods activities, the work of the Editorial Board, and the work of the various AOAC committees. As usual, the breadth of staff work encompasses all areas of the Association and is too numerous to cover in detail. Therefore, I will limit my remarks to a few significant accomplishments in each of the major program areas within each of the headquarters' offices.

Executive Director's Office

Beginning with the Executive Director's office, it has as its major responsibilities the coordination and management of the day-to-day activities of AOAC and its staff in accordance with the Association Bylaws and the policies of the AOAC Board of Directors. The office is headed by me, Ronald R. Christensen, and I am assisted by Nora Petty, my executive assistant.

During the past year, our office arranged and coordinated 5 meetings of the Board of Directors and worked with the President, Board members, and staff to develop the agendas and prepare and disseminate background materials for each meeting.

A major portion of my efforts, late in 1991 and early this year, was devoted to incorporating and overseeing the establishment of the AOAC Research Institute. This is the wholly controlled, nonprofit subsidiary of AOAC charged with implementation of the AOAC[SM] Test Kit Performance Testing Program for certifying performance characteristics of proprietary analytical test kits. Work included preparing and filing the incorporation documents, preparing bylaws, nominating and electing an initial board of directors, planning the first meeting of the board, drafting agreements between AOAC International and the Institute, drafting operating documentation for the test kit program, hiring staff, and drafting documentation to apply for tax-exempt status for the Institute.

Other activities of the office included assisting the AOAC Nominating Committee, overseeing and conducting the 1992 election and balloting process, overseeing and conducting performance appraisals of all staff members in January, working with the Administration office to get a policy approved and implemented on sexual harassment, registering "AOAC" with the U.S. Patent and Trade Mark Office as trade and service marks in various categories, providing counsel on various legal issues, working with the Finance office on the 1993 planning and budgeting schedule, responding to several domestic and international government regulations, and representing AOAC at several domestic and international meetings.

Finance and Data Processing

Moving to the office for Finance and Data Processing, this office is responsible for managing 3 distinct service areas for AOAC: finance and accounting, data processing, and order fulfillment. It is headed by Wilson "Bill" Korpi.

One of the major activities that this office undertook this year was the organization of a staff directors planning retreat in June, to begin preliminary planning work for the 1993 budget. About half of this 1-day retreat was devoted to a discussion on how to implement quality management techniques in the association environment. The other half was spent on discussing the budgeting process, program needs, and preliminary plans for 1993.

Another major effort of Finance and Data Processing, shared with the Membership and Marketing office, is the planning devoted to implementation of a new management system data base to manage our membership and marketing records. This system will be housed on our central local area network computer system. (Currently, the membership and marketing records are housed and managed on a separate DEC mini-computer system within the office. This system has grown very inefficient and is costly to maintain.) After developing selection criteria and visiting several sources, a software system and vendor were identified in June. Preliminary work on the design of the data base has begun, and we anticipate installation of the new system later this fall. When complete, the new design of the data base will enable highly automated and efficient record keeping of membership data, volunteer activities, and marketing lists.

In other data processing activities, the Finance and Data Processing office upgraded several of the office utilities programs on the local area network. Every staff member has a "smart" terminal on his or her desk that is connected to the network. Through the network's E-mail system, we have eliminated virtually all paper notes and telephone messages, and documents are now routinely routed electronically from one staff member to another for review, comment, and editing. Also, late this spring, we installed new office software and an

outgoing FAX communications server that now allows every staff member to transmit FAX messages anywhere in the world right from his or her desk.

Membership and Marketing

Turning to the Membership and Marketing office, this AOAC office has overall responsibility for promoting the Association and its various products and services. Coupled with this work is overall responsibility for managing the individual membership, sustaining membership, U.S. representative, and AOAC section's programs. The office is headed by Marilyn Blakely.

In the membership area, the office maintains member records and oversees recruitment and retention of members. As of August 1, 1992, individual membership was 3,636, slightly above last year at the same time. Sustaining member organization membership was 199 as of August 1, down slightly from 1991. Generally, individual membership growth was stagnant for the past 2 years, while sustaining member organization membership has been decreasing slowly over the past 3 to 4 years. Both trends seem primarily attributable to the weak worldwide economies and the need for a more aggressive AOAC program in membership recruitment and retention.

The "AOAC International Membership Directory," which is sent to all members around late March of each year, was revised substantially in early 1992 to incorporate additional information about AOAC that was previously found in the old "AOAC Handbook." Incorporation of this information in the "Directory" will enable the information to be revised annually and kept current.

In other areas, the Membership and Marketing office assisted in the ongoing work of a new AOAC task force that is reviewing the nominating process. It also oversaw the implementation of new procedures for nominating and evaluating candidates for the Fellows Award; revised and disseminated new AOAC section "Model Bylaws" and "Operational Guidelines"; assisted the Director of Finance in applying for IRS group tax-exemption for the sections; and continued a comprehensive marketing and promotion program for the annual meeting, our educational programs, publications, awards, list rental, and the general promotion of the Association.

Administration and Meetings

In a different area, the Administration and Meetings office is responsible for 2 very dissimilar functions of the Association. The first is office administration, including human resources, incoming mail service, receptionist service, supplies, and general office administration. The other function is meeting services, including the annual meetings and exhibitions, educational programs and symposia, and the proposed analyst training and certification program. This office is directed by Margaret "Marge" Ridgell.

In addition to overseeing the total organization and arrangements for this year's annual meeting, work progressed on planning for the 1993 and 1994 annual meetings. The 1993 annual meeting is scheduled for Washington, DC, July 25–29. Although still in negotiation, the 1994 meeting is tentatively scheduled for Portland, OR, in September 1994.

Attendance at this year's annual meeting, here in Cincinnati, OH, is 751 delegates, 240 exhibitors, and approximately 75 spouses and guests, for a total of 1,066 people.

Technical Services

Switching to the Technical Services office, it has primary responsibility for managing and coordinating the scientific and technical affairs of the Association. This includes administering the AOAC Official Methods study and approval process, recruiting and managing methods volunteers and activities, acquiring and administering technical funding agreements, coordinating cooperation with sister organizations, responding to numerous inquiries about AOAC methods and other technical issues, and harmonizing relations and activities with other international organizations. Technical Services is headed by Nancy Palmer.

Major accomplishments this year in the methods validation and technical areas included assisting the volunteers in tracking and moving collaborative method-study protocols and collaborative method-studies through the AOAC Official Methods program. As of mid-year, there were 9 precollaborative studies, 13 protocols, and 73 collaborative studies in the system. Technical Services also published notices in *The Referee* of studies being considered or underway and methods adopted; edited and submitted collaborative studies for publication in the *Journal of AOAC International* in support of First Action adoption; edited and submitted the third supplement to the 15th edition of *Official Methods of Analysis of the AOAC* for publication; and prepared the ballot for Member voting on the adoption of 69 methods as Final Action methods. This was the first time in the history of AOAC that the entire membership had the opportunity to vote on the adoption of methods by mail ballot; 693 Members (20% of the membership) took advantage of this privilege.

Additional activities of the Technical Services office included assisting in the reorganization and expansion of the foods-related methods committees from 2 to 4 committees; preparing a manual describing and explaining the AOAC Official Methods program; organizing and assisting a new task force that looks at methods' requirements needed to support nutrient labeling regulations; conducting a survey to obtain baseline data on method applicability in the nutrient analysis area; representing AOAC at and coordinating liaison activities with several domestic and international meetings; and negotiating a memorandum of understanding between AOAC, ISO, and IUPAC for sponsorship of the next harmonization activity of these 3 organizations, which will be devoted to internal analytical laboratory quality control schemes. The first meeting of that group will take place in Washington, DC, in 1993, just prior to the AOAC annual meeting.

In other areas, the Technical Services office had the main responsibility, early in 1992, for staffing and managing the ini-

tial development of the AOAC Research Institute, the AOAC International subsidiary incorporated in late 1991 to administer the AOAC Test Kit Performance Testing Program. The office was also responsible for negotiating an extension to our cooperative agreement with FDA and for renewing a grant from the USDA, ARS.

Publications

Lastly, a major contributor to the success of AOAC is the Publications office. This office has responsibility for the coordination of peer review, editorial review, and production of the *Journal of AOAC International* (*JAOAC*); management and production of the monthly newsletter *The Referee*; editorial control and publication of *Official Methods of Analysis of the AOAC* (*OMA*) and the annual *Changes in Official Methods of Analysis of the AOAC*; and acquisition, development, review, and production of miscellaneous books and manuals of the Association. Krystyna McIver heads this office.

With regard to *JAOAC*, the Publications office redesigned the *Journal* cover and the inside format of *JAOAC* beginning with the January/February 1992 issue. All issues are now fully typeset in-house on our Ventura Desktop Publishing system. Approximately 80% of all manuscripts (as compared to 60–70% last year) are now being submitted and edited electronically. A new "Regulatory Methods Section," devoted to internally validated regulatory agency methodology, was implemented with the July/August 1992 issue. The symposium publications policy was revised to ensure critical peer review of symposia-related articles published in *JAOAC*. A new printing company was found for the *Journal*, which will result in saving $6,000–$12,000 per year in printing costs and producing higher resolution output. Also, a 12-member academic advisory group was formed to encourage academics to publish applicable research in *JAOAC*.

Early in 1992, the Publications office, using an outside survey firm, conducted an *OMA* users survey of 5,000 purchasers of the 15th edition. Results of this survey will be used to improve the usability of the 16th edition of *OMA* due for production late in 1994. Patricia Cunniff was hired early in 1992 as the editor of the 16th edition, and she will begin the major task of coordinating the staff and volunteer editorial work that will go into the book. To facilitate electronic editing, the entire 15th edition was converted to electronic format and is now available on WordPerfect 5.1. Preliminary work is also underway to assess the market interest and develop electronic versions of *OMA*, tentatively including a CD-ROM version.

With regard to books and miscellaneous publications, the Publications office produced a new edition of the *U.S. EPA Manual of Chemical Methods for Pesticides and Devices* in early April. The "1991 FDA Pesticides Residues Report" was also published recently. The second edition of *Quality Assurance Principles* is currently being translated into Spanish and should be available in January 1993. Also, the Youden and Steiner *Statistical Manual of the AOAC* is in the process of being completely revised.

Conclusion

In closing, it should be noted this report is only a summary of some significant activities and accomplishments at the staff level of AOAC. Many other significant and day-to-day accomplishments have been left out.

Also, this report says nothing specific about the many, many accomplishments of the volunteers serving AOAC, without whom this staff work would not have been accomplished. For those good works, you should listen or look elsewhere for their reports.

I thank you for your attention and, most of all, your committed and continuing support for AOAC International—the scientific association dedicated to analytical excellence. Thank you.

Secretary/Treasurer and the Finance Committee

ARVID MUNSON, SECRETARY/TREASURER
Phoenix Regulatory Associates, Inc., 21525 Ridgetop Circle, Suite 240, Sterling, VA 22170

Other Members: H.B.S. Conacher; E.R. Elkins; A.R. Hanks; H.M. Wehr

The financial health of AOAC International is very strong, in fact it has never been stronger. We have almost $3,000,000.00 ($2,999,108.59) invested in highly secure, federally insured Certificates of Deposit. These funds help to ensure the continued operation of AOAC International and the continued publication of the *Official Methods of Analysis*.

Our current liabilities are offset by our accounts receivables, inventories of books, and other assets. We are a strong association financially.

This year, our auditing firm reported to the AOAC Board of Directors that there were no major deficiencies in our accounting records or accounting procedures.

Statement of Financial Condition
December 31, 1991
Total All Funds

Assets:

Cash	313,452
Accounts receivable (net of allowance of $4,088)	17,819
Contracts and grants receivable	52,090
Accrued interest receivable	65,576
Prepaid insurance	210
Deferred expenditures	13,841
Inventory	330,640
Advances	3,020
Total Current Assets:	$ 796,648
Investments	2,802,397
Deposits	15,829
Furniture and equipment (net of accumulated depreciation of $394,126)	169,857
Total Assets:	$ 3,784,731
Liabilities:	
Accounts payable	$ 56,853
Accrued leave	42,344
Accrued salaries	22,367
Payroll withholding	1,115
Deferred income	602,363
Lessor incentive—current portion	5,871
Deferred rent obligation—current portion	26,534
Total Current Liabilities:	$ 757,447
Lessor incentive—long-term portion	45,630
Deferred rent obligation—long-term portion	212,125
Total Liabilities:	$1,015,202
Fund Balances:	
Unrestricted fund balance	2,077,275
Reserve—16th Edition	600,000
Harvey Wiley	52,209
FAAM	27,770
ADAM	9,950
MAM	2,325
Total Fund Balances:	$ 2,769,529
Total Liabilities and Fund Balances:	$ 3,784,731

Sustaining Members and Supporting Government Agencies

December 11, 1992 (195)

Advanced Instruments, Inc.
Agriculture Canada
Agway Technical Center
Alberta Agriculture
American Council of Independent Laboratories, Inc.
American Crystal Sugar Company
American Cyanamid Company
American Maize Products
The Andersons
Anheuser-Busch, Inc.
Archer-Daniels-Midland
Armour Swift-Eckrich, Inc.
Arizona State Agriculture Laboratory
Arkansas State Plant Board
Association of American Feed Control Officials, Inc.
Association of American Plant Food Control Officials
Association of Public Analysts (UK)
J.T. Baker, Inc.
BASF Corporation Chemicals Division
Beckman Instruments, Inc.
bioMerieux Vitek, Inc.
Bio-Rad Laboratories
Blue Diamond Growers
Boehringer Mannheim
Borden, Inc.
Bristol-Myers-Squibb, U.S. Pharmaceutical & Nutritional Group
Bristol-Myers-Squibb, New Brunswick, NJ
Burdick & Jackson, Division of Baxter Healthcare Corporation
Cacao De Zaan BV
California Department of Food and Agriculture
CAMCO
Campbell Institute for Research & Technology
Campbell Taggart, Inc.
Cargill, Inc.
Castleton Beverage Corporation
CEM Corporation
Central Science Laboratory of the Ministry of Agriculture, Fisheries, and Food
Chemical Waste Management, Inc.
Chesebrough-Pond's, Inc.
CIBA-GEIGY Corporation, Agricultural Division
The Coca-Cola Company
Colorado Department of Agriculture
ConAgra Consumer Frozen Foods Company
Continental Baking Company
Corn Refiners Association, Inc.
CPC International, Inc.
DCA Food Industries Inc.
Delaware Department of Agriculture
DFA of California
Dionex Corporation
Dow Chemical Company
E.I. du Pont de Nemours & Company
Eastman Chemical Products, Inc.
Eli Lilly and Company, Elanco Products Company Division
EM Science
Federation of Migros Cooperatives Central Laboratory
The Fertilizer Institute
Florida Department of Agriculture and Consumer Services
FMC Corporation, Agricultural Chemical Group
FMC Corporation, Food & Pharmaceutical Products Division
A/S N. Foss Electric
Foss Food Technology Corporation

Galbraith Laboratories, Inc.
E & J Gallo Winery
General Foods Corporation
General Mills, Inc.
GENE-TRAK Systems
Georgia Department of Agriculture
Gerber Products Company
Gujarat State Fertilizers Company Limited
Hawaii Chemtect International
Hawaii Department of Agriculture
Hawaii Department of Health
Hazleton Wisconsin Laboratories America, Inc.
Health and Welfare Canada Health Protection Branch
Heinz USA
Hershey Foods Corporation
Heublein Wines
Hoechst-Roussel Pharmaceuticals, Inc.
Hoffmann-La Roche, Inc.
Holly Sugar Corporation
ICI Americas, Inc.
IDEXX Corporation
Illinois Department of Agriculture
IMC Fertilizer, Inc.
Indiana Office of the State Chemist
Indiana State Board of Health
Instituto di Tecnica e Sperimentazione, Lattiero-Caseari di Thiene
International Bio-Synthetics, Inc.
International Specialty Products
Iowa Department of Agriculture
Iowa State Veterinary Diagnostic Laboratory
JEM Laboratory Services
S.C. Johnson & Son, Inc.
Kansas State Board of Agriculture
Kellogg Company
Kentucky Agricultural Experiment Station,
Division of Regulatory Services
Kraft General Foods
The Kroger Company
Kuwait Institute for Scientific Research
Laboratorio Aycon, SA
Laboratorio di Chimica Analitica Applicata SNC (Italy)
Laboratorio Technologio del Uruguay
Laboratorio Quimico SA
Laboratory of the Government Chemist (UK)
Lancaster Laboratories, Inc.
Land O'Lakes, Inc.
L & F Products Company
Thomas J. Lipton, Inc.
Malthus Instruments Ltd.
Marion Laboratories, Inc.
Maryland Department of Agriculture
McCormick & Company, Inc.
McKee Baking Company
McLaughlin Gormley King Company
McNeil Consumer Products Company
Merck Sharp & Dohme Research Laboratories
Mettler Instrument Corporation
Michigan Department of Agriculture
Miles, Inc.
Ministry of Agriculture, Fisheries and Food (UK)
Minnesota Department of Agriculture
3M Company
Mississippi State Chemical Laboratory
Monsanto Agricultural Company
Montana Department of Agriculture
Moorman Manufacturing Company
Nabisco Brands, Inc.
National Food Processors Association
National Laboratory for Agricultural Chemistry (Sweden)
Nebraska Department of Agriculture
Nestec Limited
New Mexico Department of Agriculture
New York Department of Agriculture and Markets
Nicolet Instrument Corporation
NIRSystems
North Carolina Department of Agriculture
North Dakota State Laboratories Department
Northeast Laboratories, Inc.
Novo Nordisk Biochemicals
Nutricia Research
Ocean Spray Cranberries, Inc.
Ohio Department of Agriculture
Oklahoma Department of Agriculture
O.M. Scott & Sons Company
Ontario Ministry of Agriculture and Food
Oregon Department of Agriculture
Organon Teknika Corporation
Ortho Pharmaceutical Corporation
Overseas Merchandise Inspection Company Limited
Pennsylvania Department of Agriculture
Pfizer Inc.
Pharmacia AB
Philip Morris USA
Primus Group, Inc.
The Procter & Gamble Company
Quaker Oats Company
Quebec Department of Agriculture
Ralston-Purina
R.J. Reynolds Tobacco Company
Rhone-Poulenc Ag Company
Ross Laboratories
Sandoz Argo, Inc.
Saskatchewan Soil Testing Laboratory
Joseph E. Seagram & Sons, Inc.
Shaklee Corporation
ShriRam Institute for Industrial Research
Silliker Laboratories, Inc.
South Carolina Department of Agriculture
South Dakota State Chemical Laboratories
Solvay Duphar BV
Spencer Laboratorios
A.E. Staley Manufacturing Company
State Laboratory (Ireland)
Technological Institute (Denmark)
Tennessee Department of Agriculture
Texas Agriculture Experiment Station
Tyson Foods
UIC, Inc.
Unilever Research Laboratory
Vlaardingen
Unilever Research U.S., Inc.
United States Department of Agriculture
Agricultural Research Service
United States Food and Drug Administration
Unipath (UK)
Utah State Department of Agriculture
Virginia Consolidated Laboratory
W.M. Ward
Warner Lambert Company
Waters Division of Millipore
Webb Technical Group, Inc.
Welch Foods, Inc.
Westreco, Inc.
Wisconsin Department of Agriculture Trade/Consumer Affairs
Wyoming Department of Agriculture
Zoecon Corporation

Editorial Board

CLAIRE FRANKLIN, CHAIRWOMAN
Bureau of Human Prescription Drugs, Place Vanier, 355 River Rd, Second Floor, Vanier, ON, K1A 1B8, Canada

Other Members: D. Arnold; J. Ault; F.E. Barton, II; R.A. Durst; J.R. Gorham; A.V. Jain; J. Lichtenberg; W.H. Newsome; J. Sherma; J.T. Tanner; D.L. Terry

In the past year, the Editorial Board has taken an active role in 3 areas of AOAC publishing activities: preparing the 16th edition of the *Official Methods of Analysis* for publication, increasing article submissions in the *Journal of AOAC International*, and expanding AOAC's role as publisher.

16th Edition of Official Methods of Analysis

In preparing the 16th edition of the *Official Methods of Analysis* for publication, a survey questionnaire was sent to some 5000 customers to determine what changes in format they wanted, how to make the compendium more useable, and if customers would subscribe to supplements on a standing order basis. The results of the questionnaire were presented to the Board of Directors at its August 30, 1992, meeting. Strong user dissatisfaction with the useability of the publication indicates a need to revise and remove a number of features to make it more "user friendly." A part-time editor was hired in March 1992 to begin an overall review and edit of the *Official Methods of Analysis* for consistency, clarity of language, and conformance of style, which will not only improve the quality of the compendium but also make it easier to use. An agreement has been reached with the Official Methods Board that the editor will work closely with the General Referees and initiate, coordinate, and implement editorial changes. After review and approval of changes by the General Referees, edited chapters will be submitted by the editor to the chairmen of the methods committees for review and approval before being sent to the Official Methods Board.

As a result of the survey questionnaire, interest was also expressed in added features and other products related to the *Official Methods of Analysis*. Another questionnaire is planned to help identify user needs for specific repackaged products while retaining the current *Official Methods of Analysis* product.

Journal

For the past 2 years, the Editorial Board has implemented a plan to increase the number of articles submitted to the *Journal of AOAC International*, and reports indicate that submissions have increased each of the past 2 years with 28 more manuscripts received in 1991 and 21 more manuscripts received as of August 1992. Among the efforts implemented are letters of invitation sent to authors in competing journals inviting them to consider the AOAC Journal the next time they publish research. For the second year in a row, poster presenters at the AOAC International Annual Meeting have been sent a letter inviting them to submit their research to the AOAC Journal when completed. Staff and AOAC Journal section editors also personally speak to poster presenters during the meeting.

AOAC is more aggressively seeking to publish proceedings and symposia not only from its annual meeting but also from other organizational meetings. And, finally, AOAC will be targeting the academic community to submit research to AOAC for publication.

The Editorial Board has revised the "Instructions to Authors" to attract more authors by removing the requirements for the rigid "cook book" style that was previously requested.

Finally, the Editorial Board has recommended an increase in AOAC Journal subscription rates for 1993.

Books

As a result of advertising efforts, a number of book proposals have been submitted to AOAC. An "Invitation to Publish with AOAC" brochure has also been prepared. It outlines the types of publications produced by AOAC and lists criteria for submitting a publication proposal to AOAC for consideration. A policy on publication of meeting proceedings is also being prepared in answer to requests from meeting organizers who wish to publish their proceedings with AOAC.

Finally, at the Editorial Board's encouragement, distribution agreements are being arranged with other publishers to advertise and sell a select number of their books on a royalty basis to AOAC. Exchange advertising agreements are also being explored.

Official Methods Board

MICHAEL H. BRODSKY, CHAIRMAN
Ontario Ministry of Health, Laboratory Services Branch, Chief, Environmental Bacteriology and Microbiological Support Services, 81 Resources Rd, Etobicoke, ON, M9P 3T1, Canada

Other Members: R.H. Collier; P.E. Corneliussen; J.W. DeVries; G.A. Lancette; J.F. Lawrence; R.M. Montgomery; A.B. Strong; H.C. Thompson; J.G. Phillips; M.G. Torchia

Meetings

The Official Methods Board (OMB) met 4 times during the year to fulfill its primary responsibility of administering the AOAC collaborative study and approval process: August 1, 1991, at the AOAC International Annual Meeting in Phoenix, AZ; January 23–25, 1992, in San Diego, CA; May 28–30, 1992, in St. John's, Newfoundland; and August 29, 1992, at the

AOAC International Annual Meeting in Cincinnati, OH. Following the January and May meetings, reports were published in *The Referee* to inform the membership of OMB activities and to outline key dates for method volunteers.

Method Actions

All actions have been published in *The Referee*.

1. Two methods, postponed from the August 10, 1991, meeting were adopted First Action at the August 15, 1991, meeting.

2. Fourteen methods were adopted First Action at the January 1992 meeting.

3. Eight methods were adopted First Action at the May 1992 meeting.

3.1 Fifty-one methods were recommended for Final Action at the May 1992 meeting (see *The Referee*, February 1992).

3.2 Nine methods were approved for Surplus at the May 1992 meeting (see *The Referee*, July 1992).

3.3 Three methods were recommended for Repeal at the May 1992 meeting (see *The Referee*, July 1992).

3.4 Ten methods were adopted First Action at the August 1992 meeting (see *The Referee*, September 1992).

Awards

OMB annually coordinates the selection process for recognition of excellence in performance by Associate and General Referees:

A. In 1992, the Official Methods Committee nominees for the General Referee Award were as follows: Jack L. Boese, (Committee on Microbiology and Extraneous Materials); Margaret Clarke (Committee on Foods II); and Kenneth P. Stoub, (Committee for Environmental Quality). During the May 1992 meeting in St. John's, Newfoundland, OMB voted to select Boese, General Referee for Filth and Extraneous Materials, as the winner of the General Referee Award for 1992.

B. The Official Methods Committee nominees for Collaborative Study of the Year for 1992 were as follows: Joseph G. Sebranek for "Crude Protein in Meat and Meat Products" (Committee on Foods I); James T. Tanner for "Analysis of Milk-Based Infant Formula, Phase V" (Committee on Foods II); Leon Prosky for "Insoluble Dietary Fiber in Food and Food Products, Enzymatic-Gravimetric Method" (Committee on Foods II); and Donald A. Higgins and Barbara J. Robison for "*Listeria sp.*, Biochemical Identification Method (Micro-ID Listeria)" (Committee on Microbiology and Extraneous Materials). During the May 1992 meeting, OMB voted to select Joseph G. Sebranek for "Crude Protein in Meat and Meat Products" as the Collaborative Study of the Year for 1992.

C. The recipients of the Methods Committee Associate Referee Awards for 1992 were as follows: Joseph G. Sebranek (Committee on Foods I); James T. Tanner (Committee on Foods II); Brian Worobey (Committee on Residues); Russell S. Flowers (Committee on Microbiology and Extraneous Materials); and James E. Longbottom (Committee on Environmental Quality).

Task Forces

Six Task Forces were working this year to provide solutions/guidance dealing with or managing various problem areas:

A. The Task Force on Methods Adoption was created in 1989 and chaired from 1990–1992 by Michael Brodsky. The adoption process was adopted and recommended by OMB in May 1991 and finally approved by the Board of Directors in February 1992. This Official Methods Adoption Process was presented during the Volunteer Education Workshop at the 1991 AOAC International Annual Meeting, and a summary repetition was included in the Volunteer Education Workshop during the 1992 annual meeting. The Task Force was dissolved with the thanks of OMB at the 1992 annual meeting.

B. The Task Force on Operational Policy Guidelines was instituted at the August 15, 1991, meeting of OMB. Under the chair of Ann Strong and with support of Gayle Lancette and Nancy Palmer, this task force was charged with reviewing present OMB operational policies for currentness, redundancies, and contradictions. Their final report was accepted by OMB at its January 1992 meeting, and the task force was dissolved with the thanks of OMB. The recommendations of this task force were incorporated into the OMB Operational Policy Manual. The operational policies in this manual, which included the methods approval process, was subsequently approved by the Board of Directors at its meeting in February 1992.

C. The Task Force on the Reorganization of Foods I and Foods II was instituted at the OMB meeting of August 15, 1991. Under the chair of John DeVries and with assistance from Ray Bowers, Robert Martin, and James Lawrence, this task force was charged with developing a plan for restructuring the 2 food committees. The task force report was accepted by OMB at its May 1992 meeting and recommended to and approved by the Board of Directors at its June 1992 meeting.

D. The Task Force on Reference Standards, Accuracy, and Quality Control was instituted at the OMB meeting of August 15, 1991. Under the chair of Paul Corneliussen and with assistance from John Phillips and Sallee Anderson, this task force was charged with developing an OMB statement/position regarding the inclusion of reference standards in concert with the determination of accuracy and provision of QC procedures in collaboratively studied methods presented for adoption as AOAC official methods. A preliminary report was accepted by OMB at its January 1992 meeting and a summary of OMB's position regarding accuracy was published in the March 1992 issue of *The Referee*.

OMB encourages the inclusion of accuracy in the development, study, and presentation of AOAC methods. In this regard, OMB will require the percent recovery of each analyte per matrix to be determined and reported as an additional statistical parameter.

In consultation with the Laboratory Quality Assurance Committee, the task force recommended changes to the "Checklist for Protocol Design" to include specific reference to QC requirements.

The OMB and Laboratory Quality Assurance Committee chairs attended reciprocal meetings of the respective committees at the 1992 annual meeting to attempt to further resolve outstanding related issues. It is anticipated that additional recommendations will be forthcoming from OMB to the Board of Directors.

Paul Corneliussen, John Phillips, and Michael Brodsky all completed their terms of office as of the 1992 annual meeting; hence, the task force was dissolved with the thanks of OMB, but may be resurrected by the new OMB chair with a renewed mandate.

E. The Task Force on Training Elements for Volunteers, under the chair of Richard Collier and support from John DeVries and Harold Thompson was struck at the August 15, 1991, meeting of OMB. This task force was charged with evaluating the current training sessions and recommending the development of ongoing training programs.

A report was submitted to and approved by OMB at its May 1992 meeting, and the task force was discharged with the thanks of OMB. The report was presented to the Board of Directors for consideration at its August 30, 1992, meeting.

F. The Task Force on Matrix Applicability Statements, under the chair of James Lawrence and supported by Gayle Lancette and Richard Collier, was instituted at the January 1992 meeting of OMB. This task force was charged with revising operational policy statements 2.10 and 2.11 to make method applicability statements less restrictive and more in keeping with current practice and practicality.

Other Actions, Activities, and Decisions

OMB established certain goals and objectives that it hoped to accomplish during 1992. OMB is pleased to report that greater than 90% of its objectives were completed, and the following recommendations were accordingly submitted to the Board of Directors: (*1*) OMB is developing a policy on the use of standard reference materials for analytical methods, which will be recommended to the Board of Directors for approval. (*2*) OMB developed a policy on the calculation of false-positive and false-negative rates in qualitative methods. (*3*) OMB developed a policy on a proactive approach for moving methods from First to Final Action. (*4*) OMB established a working relationship with the Laboratory Quality Assurance Committee. (*5*) OMB developed a policy on the use of specific proprietary names in the Official Methods of Analysis. (*6*) OMB prepared a summary of operational policies from the OMB Manual for inclusion in the volunteer education package. (*7*) OMB developed specific training elements/programs for methods volunteers. The program for the 1992 annual meeting dealt with AOAC's computerized software package for the statistical determination of method performance parameters. (*8*) OMB proposed topical realignments for the Committees on Foods I and Foods II. (*9*) OMB proposed recommendations for the distribution of statistical software for calculation of collaborative study data as developed by the Statistical Committee and endorsed by OMB. (*10*) OMB recommended replacements for the following members of OMB whose terms expire after the 1992 annual meeting: Michael H. Brodsky, chair, OMB; Richard Montgomery, chair, Committee on Drugs and Related Topics; Paul Corneliussen, chair, Committee on Residues; John Phillips, chair, Statistics Committee; and Mark Torchia, chair, Safety Committee.

In addition to these short-term goals, OMB also targeted some long-term objectives. OMB is pleased to report that it has taken, and will continue to take, action on all of the areas: (*1*) evaluate topic alignments of methods committee; (*2*) develop recommendations to be presented to the Board of Directors to broaden AOAC's service base; (*3*) communicate at least twice a year with methods volunteers to outline their individual responsibilities, remind them of important dates/deadlines, encouraging productivity, and extend thanks for their efforts; (*4*) select recipients for the General Referee Award and the Collaborative Study of the Year Award and coordinate Methods Committee's Associate Referee Awards; (*5*) provide an annual report of OMB activities and achievements to the membership; (*6*) coordinate and provide educational opportunities and materials for method volunteers; (*7*) identify areas where methods are needed; and (*8*) maintain and update a compilation of the Operational Policies and Procedures of OMB.

Archives Committee

CHARLOTTE A. BRUNNER, CHAIRWOMAN
U.S. Food and Drug Administration, HFD-473, 200 C St, SW, Washington, DC 20204

Other Members: T.G. Alexander; W. Landgraf; H.M. Stahr

The meeting was called to order by the chairman. The minutes of the last meeting were approved. The chairman reported that the last records and files obtained by the Committee during the Association's move to its new headquarters are being screened to remove duplicates and irrelevant information. When the screening is completed, material will be shipped to the Parks Library at Iowa State University. A discussion followed on how the Committee will handle future documents received from the AOAC staff. A motion was passed to have the Committee screen all material before it is archived.

Charlotte Brunner discussed her concern that the records of regional sections are not being archived. Several requests for these records have been made to various sections without success. Because all sections submit reports and newsletters to the AOAC headquarters, the Committee decided that Krysytna McIver, Director of Publications, will request that a second copy be submitted for the archives. In addition, an announcement will be made at the meeting of the Regional Sections Committee asking that all sections send their important documents to the Archives Committee.

Thomas Alexander has been working with William Eisenberg on collecting personal histories of all AOAC past presidents. AOAC hopes to publish this collection as a book.

After the 1992 AOAC International Annual Meeting, Alexander is planning to visit Charles Gehrke in hopes of obtaining his oral history and files for the archives.

Wynne Landgraf and Frank Ross have taken 2 boxes of documents pertaining to the AOAC Midwest Section to the Parks Library for archiving. Landgraf reported that Mr. Yates, the archivist, has retired, and his replacement will arrive in October.

The authorization of funds for the restoration of old AOAC photographs was discussed. Landgraf and Henry Stahr will contact the Parks Library to determine if the work has been completed and AOAC has been billed. Presently, all AOAC documents are in temporary storage at the library. The Parks Library will be asked to estimate the cost for placing the documents into permanent storage.

Stahr will attempt to get an oral history from this year's president, Edgar R. Elkins. Alexander will try to get Daniel Banes and Eisenberg to give us oral histories.

The Committee would like to thank Dave Sakai for the instrument manuals he donated to AOAC, some of which were used in this year's exhibit.

Because next year's annual meting will be in Washington, DC, the Committee decided to put larger pieces of equipment on display from the FDA laboratories, which could not normally be shipped elsewhere. An exhibit of the president from 100 years ago, 1893, will be featured.

The following year, the Archives Committee will try to borrow instruments from Oregon State University for display. Michael Wehr will be contacted to help obtain the instrumentation.

The Committee decided to feature a display at future meetings of the president from a 100 years before, if enough material can be obtained.

Bylaws Committee

RICHARD J. RONK, CHAIRMAN
U.S. Food and Drug Administration, HFF-22, Rm 1457, Parklawn Bldg, 5600 Fishers Ln, Rockville, MD 20857

Other Members: R.R. Christensen; C.A. Geisler; J. Hillebrandt; T. Jackson; D. Kassera; S.V.W. Pope; G.L. Roach; D.L. Terry; B. Woodward; L.L. Zaika

Recommendations for the Board of Directors

The Bylaws Committee recommends that it be directed to continue its review of the AOAC International Bylaws, as amended effective September 3, 1992, as follows:

Relative to Article III, Membership: The Bylaws Committee suggests that it be directed to examine exactly what an Emeritus member is within the Association. The Committee will need guidance from the Board of Directors as to the meaning of the phrase "The benefits of Emeritus Members shall be determined by the Board of Directors." Benefits essentially define membership. Once the role and benefits of Emeritus members have been clearly defined, this information should be distributed to the general membership.

Relative to Article IV, Elected Officers, Section 2, President: The Committee would like the opportunity to prepare a paper for the consideration of the Board of Directors relative to the phrase "...and shall be responsible for all business affairs of the Association between meetings of the Board of Directors and in accordance with its policies."

Relative to Article V, Nominations and Elections, Section 3, Vacancies: Office of the President: The Committee will recommend some minor word changes to the Board of Directors for consideration at its December 1992 meeting.

Relative to Article VI, Board of Directors, Section 2, Powers and Duties: The committee would like the opportunity to revisit the powers and duties of the Board of Directors with an eye toward less specificity.

Relative to Article VII, Appointed Officers, Section 1, Appointed Officers: The Committee would like to review this in the light of our recommendation concerning Article IV.

Relative to Article VIII, Editorial Board and Standing Committees: The Committee would like the opportunity to review these 4 sections as to the relevance of listing these specific committees in the Bylaws.

Relative to Article XIV, Regional Sections, Section 3, Membership in Regional Sections: The Committee recommends that the first 2 sentences of the paragraph be revised as follows (material proposed to be added is italicized): *All* Members of the Association residing and working within the geographical boundaries of the section *shall be eligible for membership in the section.* Other individuals interested in the purpose of the regional section and residing and working within the boundaries of the section *and meeting the membership qualifications of the section* shall also be eligible for section membership but shall not be eligible for election to the Executive Committee for the section." The Committee recommends that the last line be deleted. It reads, "No person shall be a member of more than one regional section."

Relative to the other Bylaw articles: Minor changes will be recommended to the Board of Directors at the December 1992 meeting.

Basis for Recommendations

The purpose for which the Emeritus category of membership was established and the role of the Emeritus member are unclear. The Committee feels this should be clarified as soon as possible.

The phrase "...and shall be responsible for all business affairs of the Association between meetings of the Board of Directors and in accordance with its policies" appears not to reflect accurately the actual day-to-day management of the Association. The Committee would make suggestions about how that wording might change in light of present policies.

Article VI, Section 2 is only a partial listing of the powers and duties of the Board of Directors and implies that only the duties and powers listed are applicable.

Article VIII lists only 4 specific committees. Parliamentarians generally advise against listing committees in the Bylaws because of the conflict this creates with the power of the Board to establish, appoint, and dissolve committees.

Neither in intent nor practice are all the Members of the Association automatically members of the section in which they reside and work, which is what the original first sentence implied; however, the intent was for them to be eligible for membership in the section. In addition, it should be noted that model bylaws of sections give the Executive Committees of sections the authority to establish membership qualifications for the sections, and these changes would bring this section into agreement with the model bylaws for sections.

There appears to be no reason to prohibit membership in more than one regional section and Members such as laboratory managers may have valid reasons for wanting to belong to and participate in more than one regional section.

Other Discussion

The Committee discussed at length the reasons for denying voting privileges to Emeritus members. They also felt that there was some doubt as to whether or not retired Members have the option of continuing their regular voting membership by paying full membership dues in spite of the fact that they are no longer actively employed.

In addition, they suggest making the use of a capital M when referring to Members of AOAC International to distinguish them from members of Task Forces, Boards, etc.

The Chairman volunteered to draft language to revise Article VI, Section 2.

AOAC INTERNATIONAL BYLAWS

Revised September 3, 1992

ARTICLE I
Name

The name by which this Association shall be known is "AOAC International" (hereinafter referred to as the "Association").[1]

ARTICLE II
Purpose

The primary purpose of the Association is to promote methods validation and quality measurements in the analytical sciences.

ARTICLE III
Membership

Section 1. Classes of Membership

There shall be four (4) classes of membership in the Association: Individual Members, Honorary Members, Emeritus Members, and Sustaining Member Organizations.

Section 2. Qualifications for Membership

A. *Individual Members*

Qualifications for individual membership shall be a degree in science, or equivalent as approved by the Board of Directors, and interest in the purpose and goals of the Association. Scientists shall be eligible for membership provided they are engaged, or have been engaged, directly or indirectly, in analysis or research with respect to foods, vitamins, food additives, pesticides, drugs, cosmetics, plants, feeds, fertilizers, hazardous substances, air, water, and any other products, substances, or phenomena affecting the public health and safety, the economic protection of the consumer, or the protection of the quality of the environment.

[1] AOAC International was incorporated in the District of Columbia on January 16, 1932, as the Association of Official Agricultural Chemists.

B. *Honorary Members*

Honorary Members shall be persons recognized for their substantial contribution toward the achievement of the objectives of the Association. They shall be nominated by the Board of Directors and may be elected by a two-thirds vote of the Members voting. An Honorary Member shall have all the rights and privileges of a Member.

C. *Emeritus Members*

A Member who is no longer actively engaged, directly or indirectly, in analysis or research with respect to foods, vitamins, food additives, pesticides, drugs, cosmetics, plants, feeds, fertilizers, hazardous substances, air, water, and any other products, substances, or phenomena affecting the public health and safety, the economic protection of the consumer, or the protection of the quality of the environment, and who has served the Association as a Member for ten (10) years or more shall be eligible for Emeritus Member status upon written request and payment of the annual Emeritus membership dues. Emeritus members shall not be eligible to vote, hold office or serve on Boards or Committees of the Association, but may serve as officers of a section. The benefits of Emeritus Members shall be determined by the Board of Directors.

D. *Sustaining Member Organizations*

There shall be one (1) class of sustaining membership. A Sustaining Member Organization shall be any agency of a local, state, provincial, or national government, a college or university, or any firm, business, or organization with an interest in the development and interlaboratory evaluation of analytical methodology, provided it is engaged, directly or indirectly, in analysis or research with respect to foods, vitamins, food additives, pesticides, drugs, cosmetics, plants, feeds, fertilizers, hazardous substances, air, water, and any other products, substances, or phenomena affecting the public health and safety, the economic protection of the consumer, or the protection of the quality of the environment.

Section 3. Application for Membership

Application for Membership shall be submitted to the Association. Membership shall become effective upon approval of the Application for Membership, payment of required annual membership dues, entry on the membership rolls, and assignment of an individual member number.

ARTICLE IV
Elected Officers

Section 1. Officers

The officers of the Association shall be Members and shall consist of a President, President-Elect, Immediate Past President, Secretary-Treasurer, and any other officers designated by the Board of Directors.

Section 2. President

The President shall be the principal executive officer of the Association, and shall be responsible for all the business and affairs of the Association between meetings of the Board of Directors and in accordance with its policies. He shall preside at all meetings of the Members and the Board of Directors. He shall call regular or special meetings or authorize mail ballots by the Board of Directors to handle necessary matters, or instruct the appropriate appointed officer to do so.

The President shall appoint the following for lengths of terms designated by the Board of Directors: the Chairman of the Official Methods Board; Committees on Official Methods; an Editorial Board; a Nominating Committee; a Committee on the Wiley Award; a Committee on Fellows; a Long-Range Planning Committee; any other Committees established by the Board; and Liaison Officers.

Section 3. President-Elect

In the absence of the President, or in the event of his inability or refusal to act, the President-Elect shall perform the duties of the President, and when so acting, shall have all the powers of, and be subject to all the restrictions upon the President. The President-Elect shall perform such other duties as from time to time may be assigned to him by the President or by the Board of Directors.

Section 4. Secretary-Treasurer

The Secretary-Treasurer shall give notice of all meetings of the Association, keep a record of all proceedings, attest documents, be responsible for the funds and securities of the Association, and, in general, perform such other duties as are usual of the offices of Secretary or Treasurer and such other duties as may be assigned by the President or by the Board of Directors.

ARTICLE V
Nominations and Elections

Section 1. Elections and Terms of Office

The President-Elect, the Secretary-Treasurer, and the Directors of the Board of Directors shall be elected by a majority of Members voting, from a slate of nominees recommended annually by the Board of Directors. Terms of office for all Officers and Directors shall begin with the adjournment of the annual meeting following their election and shall end with the adjournment of the annual meeting occurring nearest the expiration of their term. The six (6) Directors shall be elected to staggered three-year terms with two Directors elected to full three-year terms each year. The Secretary-Treasurer shall be elected to a one-year term. The President-Elect shall be elected to a one-year term; whereupon the current President-Elect shall become President and the current President shall become the Immediate Past President, each serving a one-year term.

Section 2. Nominating Committee

The Nominating Committee shall annually recommend to the Board of Directors a slate of Members as potential nominees for elected offices. The Nominating Committee shall consist of three (3) members who shall be the previous three (3) Past Presidents of the Association, provided that, if Past Presidents are not available to serve, other Members shall be appointed to the extent necessary to constitute the three (3) member committee.

Section 3. Vacancies: Office of President

If the office of the President shall become vacant, the President-Elect shall thereupon become President of the Association for the unexpired term. Such service will not affect such person becoming President of the Association upon adjournment of the next annual meeting. In the event that the office of President becomes vacant at the time when the office of President-Elect is also vacant, such vacancy shall be filled for the remainder of the election year by the action of the other members of the Board of Directors. If any office other than that of President shall become vacant, the office shall be filled for the remainder of the election year by action of the Board.

Section 4. Vacancies: Board of Directors

If a vacancy of one (1) of the elected positions of the Board of Directors should occur, any Past President may be named by other members of the Board to temporarily fill such vacancy until the next regularly scheduled election. At the next regularly scheduled election nominations will be presented to fill any vacancy, for the unexpired portion of the term remaining.

ARTICLE VI
Board of Directors

Section 1. Composition

The Board of Directors shall consist of ten (10) Members to include the President, President-Elect, Secretary-Treasurer, Immediate Past President, and six (6) Directors, all of whom shall be Individual Members of the Association. The majority of the Board shall be representatives from one (1) or more of the following: a national, state, provincial, or municipal government, a regulatory agency, or academia. No Director of the Board of Directors may be elected to more than two (2), consecutive, three-year terms; appointment or election to fill an unexpired term shall not affect the eligibility of a person to subsequently be elected to two (2) full terms.

Section 2. Powers and Duties

The Board of Directors shall:

- establish general policies, and shall manage the affairs of the Association between meetings of the membership

- determine all activities of the Association
- fix the annual dues for membership, subject to approval by the members
- determine the membership year and the delinquency date
- approve procedures for election to membership and requests for dues waivers, and may nominate Honorary Members
- act on the subscription rates and prices submitted for approval by the Editorial Board
- be responsible for the employment and appointment of individuals necessary for the efficient operation of the Association, and may assign them appropriate titles
- further define, when necessary, official duties of the employees, officers, and committees of the Association, and may assign additional duties to the President-Elect, Secretary-Treasurer, Directors, or other officers, except that of President, in accordance with these bylaws
- fill vacancies in the offices of President-Elect, Secretary-Treasurer, or other offices, except that of President, in accordance with these bylaws
- determine the number and tenure of members of the Editorial Board, Standing Committees, and Liaison Officers
- establish and terminate committees
- determine the time and place of the annual meeting, and may call a special meeting of the Association membership
- set geographic limits, authorize establishment and dissolution of sections, and approve bylaws adopted by the sections
- consider recommendations of the Standing and Special Committees
- review all proposed amendments to the bylaws which are received by the Board and submit the proposals, with Board recommendations, to the membership in accordance with procedures in these bylaws
- upon dissolution of the Association, distribute remaining assets in accordance with these bylaws and other applicable authorities.

Section 3. Meetings

A. Regular meetings shall be held at the call of the President, or as ordered by the Board.

B. Special meetings of the Board may be called by the President.

C. The President shall preside at all meetings except as otherwise provided in these bylaws.

Section 4. Voting

A mail ballot may be authorized by the President.

ARTICLE VII
Appointed Officers

Section 1. Appointed Officers

The Board of Directors may appoint such individuals as are necessary to carry out the following functions: execute the directives and policies of the Board; supervise the Business Office and perform any duties necessary in the day-to-day management of the Association; keep the minutes of the meetings of the Members and the Board of Directors; see that all notices are duly given in

accordance with the provisions of the bylaws; keep a register of membership of the Association and their addresses; edit and produce the publications of the Association.

An appointed officer with concurrence of the President shall appoint General Referees from the Members of the Association, one (1) for each of the general subjects designated by the Official Methods Board.

An appointed officer with concurrence of the President shall appoint Associate Referees on subjects complementary to the general subjects assigned to the General Referees. In lieu of an Associate Referee, the appointed officers with concurrence of the President may appoint an Associate Referee Committee of two (2) or more persons, designating one (1) as Chairman. Appointment of General Referees, Associate Referees, and members of an Associate Referee Committee must have the approval of each appointee's appropriate supervisor.

Section 2. Liaison Officers

The Liaison Officers shall coordinate the development and adoption of uniform analytical methods between the Association and related interested organizations.

ARTICLE VIII
Editorial Board and Standing Committees

Section 1. Editorial Board

The Editorial Board shall be responsible for developing, editing, and publishing the publications of the Association. The appointed officer responsible for editing and producing the publications of the Association shall be a member *ex officio*.

Section 2. Committee on the Wiley Award

The Committee shall consist of six (6) members with two (2) appointed each year to a three-year term. They shall decide on the winner of the Harvey W. Wiley Award from those nominated for the award. The President shall chair the Committee.

Section 3. Committee on Fellows

The Immediate Past President of the Association shall act as chairman of the Committee.

The Committee on Fellows shall select candidates from those nominated and from the general membership for recommendation to the Board of Directors. Any Member who has rendered ten (10) years or more of meritorious service to the Association as a General Referee, Associate Referee, Committee Member, or in any other official capacity shall be eligible for nomination as a Fellow of the Association.

Section 4. Long-Range Planning Committee

The Committee shall make recommendations to the Board of Directors on improving the functions of the Association.

ARTICLE IX
Official Methods of Analysis

The Board of Directors is empowered to develop written procedures for the study, adoption, and change in status of official methods of analysis in accordance with the following principles:

A. Official methods of the Association shall be adopted as final action by mail ballot of the voting membership.

B. Timely notice of proposed method studies, adoption, or change in status will be published in an Association vehicle of general circulation.

C. Methods submitted to the membership for final adoption shall be thoroughly studied, scientifically reviewed, and available in published form prior to balloting.

D. The Official Methods Board shall be responsible for implementation of, and may recommend changes to, the procedures adopted by the Board of Directors.

E. The Official Methods Board and related committees shall not be dominated by any single interest and shall be composed of Members representing a balance of government, academia, and the private sector appropriate to the scope of the group.

F. Opportunity will be provided for materially interested parties to have input during method study and adoption procedures and to comment on the adoption, use of, or change in status of specific methods.

G. Adequate records of technical data, discussions, and decisions on the study, adoption, and change of status of official methods shall be maintained for a reasonable time.

ARTICLE X
Meetings

Section 1. Annual Meeting

The annual meeting of the Association shall be held at the time and place decided by the Board of Directors. A special meeting of the entire Association may be called by the Board of Directors. Announcement therefore shall be made at least two (2) months prior to the time of said meeting.

Section 2. Quorum

One hundred Members authorized to vote in a meeting who are present in person or by proxy shall constitute a quorum at any meeting of the Association which is duly called pursuant to the provisions of these bylaws.

ARTICLE XI
Voting

Section 1. Voting by Mail Ballot

By direction of the Board of Directors, unless otherwise required by these bylaws or by procedures established under these bylaws, voting on any matter, including the election of officers, the election of Honorary Members, amendment of the bylaws, and the approval of dues, may be conducted by mail ballot of the voting membership, and the question thus presented shall be determined according to the votes received by mail, provided in each case votes of at least five (5) percent of the voting membership shall be received. Any and all action taken in pursuance of a mail vote in each case shall be binding upon the Association in the same manner as would be action taken at a duly called meeting.

Section 2. Voting by Proxy

At any meeting of members, a Member of Record, as determined thirty (30) days prior to any meeting, who is entitled to vote may vote by proxy executed in writing by the member or his duly authorized attorney-in-fact. No proxy shall be valid for more than eleven (11) months after the date of its execution unless otherwise provided in the proxy.

ARTICLE XII
Dues and Subscriptions

Section 1. Membership Dues

A. Annual dues for membership in the Association shall be fixed by the Board of Directors, subject to approval by the majority of the members voting by mail ballot.

B. The membership year and the delinquency date shall be determined by the Board of Directors.

C. The authority to grant waivers of membership dues rests with the Board of Directors.

D. Honorary Members and retired Fellows of the Association shall be exempt from payment of dues and annual meeting registration fees.

Section 2. Publications

Subscription rates and prices for any publication of the Association shall be determined by the Editorial Board, subject to approval of the Board of Directors.

ARTICLE XIII
Earnings and Assets

Section 1. Non-Profit Status

A. Regardless of any provision of the bylaws which may be construed otherwise:

[1] No part of the net earnings of the Association shall under any circumstances inure to the benefit of any member or individual.

[2] The Association shall not be operated for a private profit.

B. On lawful dissolution of the Association and after settlement of all just obligations of the Association, the Board of Directors shall distribute all remaining assets of the Association to one (1) or more organizations selected by the Board of Directors which have been held exempt from Federal Income Tax as organizations described in section 501(c)(3) of the Internal Revenue Code of 1954.

Section 2. Political Activities

A. No substantial part of the Association's activities shall consist of carrying on propaganda or otherwise attempting to influence local, state, or national legislation. All activities of the Association shall be determined by the Board of Directors.

B. The Association shall not participate or intervene in any manner in any campaign on behalf of any candidate for a political office.

ARTICLE XIV
Sections

Section 1. Sections

The Board of Directors shall set geographic limits and grant authority to groups of Members of the Association residing or working in the same geographical areas for the establishment of sections.

Section 2. Purpose of Sections

The purpose of sections shall be to promote and sponsor the purpose of the Association.

Section 3. Membership in Sections

Membership in a section shall consist of Members and Honorary Members of the Association residing and working within the geographical boundaries of the section. Other individuals interested in the purpose of the section and residing and working within the boundaries of the section shall also be eligible for section membership but shall not be eligible for election to the Executive Committee for the section. No person shall be a member of more than one (1) section.

Section 4. Bylaws of Sections

Each section shall adopt for its own government, subject to approval of the Board of Directors, bylaws not inconsistent with these bylaws.

Section 5. Dissolution of Sections

When any section shall cease to function as a section for a period of more than one year, or if its membership shall be less than ten (10) Members of the Association for a period of one (1) year, the Board of Directors may terminate the existence of such section.

Section 6. Actions of Sections

No act of a section or its members shall be considered an act of the Association unless expressly authorized, ratified, or affirmed by the Board of Directors.

ARTICLE XV
Technical Divisions

Section 1. Purpose

Technical divisions shall represent communities of interest within the Association which have the purpose of furthering the mission of the Association through the development of the analytical sciences either in a commodity-based or scientific discipline-based field. Their activities shall not duplicate the organizational structure nor conflict with the policies or procedures for the adoption of official methods of analysis by the Association.

Section 2. Creation, Combination, Discontinuance, or Change

Technical divisions may be created, existing technical divisions may be combined or discontinued, or the name of a technical division may be changed under policies and procedures adopted by the Board of Directors. Each technical division shall adopt bylaws not inconsistent with the Association bylaws. The jurisdiction of each technical division shall be described in its bylaws. No act of any technical division or its members shall be considered an act of the Association unless expressly authorized, ratified, or affirmed by the Board of Directors.

ARTICLE XVI
Indemnification

The Association shall have the power to pay, by indemnity, reimbursement, or otherwise, to or for the use of any person designated by resolution of the Board of Directors who was or is a party or is threatened to be made a party to any threatened, pending, or completed action, suit, or proceeding, whether civil, criminal, administrative, or investigative (other than an action by or on behalf of the Association), by reason of the fact he or she is or was a director, officer, committee member, employee or agent of the Association, or was serving as such for another at the request of the Association, against expenses (including legal, accounting, witness, and other), judgments, fines, and amounts paid in settlement so long as such person was not found by a court of competent jurisdiction to have been willfully negligent of the interests of the Association or such person had reasonable cause to believe that his or her conduct was lawful.

ARTICLE XVII
Parliamentary Authority

The rules contained in the current edition of Robert's Rules of Order Newly Revised shall govern the Association in all cases in which they are applicable and in which they are not inconsistent with these bylaws or any special rules of order the Association may adopt.

ARTICLE XVIII
Amendments to the Bylaws

These bylaws may be amended, repealed, or altered, in whole or in part by a three-fourths vote: (a) at any meeting of the Association, provided notice of any amendment proposed for consideration shall be mailed to the last recorded address of each Member at least thirty (30) days prior to the date of the meeting; or (b) by approval of the Members through mail ballot in accordance with the provisions of Article XI, Voting.

All proposed amendments of these bylaws shall be presented in writing to the Board of Directors. The Board shall present the proposals to the Association membership, with recommendations. All amendments to the bylaws, unless otherwise stated, will become effective at the adjournment of the meeting where action is taken or on the day following the certification of a vote by mail ballot.

International Committee

PAUL R. BELJAARS, CHAIRMAN
Inspectorate for Health Protection, Food Inspection Service, PO Box 2516, 6201 GA Maastricht, The Netherlands

Other Members: A.R. Hanks; W. Horwitz; R.H. Lane; M. Lauwaars; A.E. Pohland; R. Rocco; R.C. Rund; J.T. Sabater; A. Williams; R. Wood

The Committee discussed the fact that various reports are compiled from the designated AOAC liaison officers and distributed to the members of the Committee for their information. It is not clear what action is expected to be taken as a result of the information given in those reports. This could be facilitated if a more standardized report format were to be adopted and guidelines were to be provided on the type of information desired. Specific recommendations would be expected as part of the report. It is recommended that these reports should be sent to appropriate AOAC experts.

A wide–ranging discussion of developments in the international community that affect method and measurement validation issues touched on relationships between AOAC and other organizations. Is a formal relationship required in order for methods developed outside the AOAC program to be submitted for AOAC review and adoption? This seemed to be a particular concern for ISO committees developing fertilizer methods. They do not have AOAC official status. It was agreed that AOAC needs to give higher profile to the fact that methods may be submitted by any person or organization desiring AOAC adoption and that no formal "agreement to cooperate" is required. However, it is a requirement that the validation of the proposed method meets the harmonized protocol for interlaboratory study of method performance.

To further strengthen the communication and input with the 2 CEN committees working in the area of fertilizers, the Committee agreed that AOAC needs to pursue liaisons with those CEN committees more aggressively.

The status of the new harmonization document on proficiency testing schemes was reported: It is undergoing another round of revision. Because AOAC had designated another committee to review and comment on the document, it was unclear what additional review might be necessary before the document could be recommended for AOAC endorsement. When individuals or committees have been designated as liaison officers or representatives for AOAC, it was suggested that better definition is needed of their responsibilities and authority.

This latter point was reiterated for the document being developed as an operational guide for liaison and international operations. These aspects of responsibility and authority need to be laid out more clearly. The Committee was invited to send in comments on this document before November 1, 1992.

In reviewing the Terms of Reference, the Committee felt that the mission could be interpreted to mean that the Committee is expected to recommend the international role of AOAC. However, the responsibilities are geared more to identifying than recommending those activities.

The Committee agreed that it would continue to make recommendations; however, it reserved the option to suggest changes in both the activities and name of the Committee to reflect its role more clearly.

In the interests of international harmonization, the Committee favors AOAC's adoption of the ISO format for method layout. The more narrative and explanatory method description is considered a more logical performance-based description than the very terse AOAC "cookbook" approach, which includes little explanatory detail. It was also pointed out that the EC directives specify the ISO format for approved methods.

The Committee discussed at length a proposal by Alex Williams to strengthen AOAC's position within Europe. The proposal, originally submitted to the Board of Directors, was presented to the International Committee for comment. The proposal is based on the premise that EC is the driving force in Europe today, and many of those setting policy in EC either do not know about AOAC or believe it is an American, rather than international, organization. AOAC, which has significant influence and cooperation in the regulatory and industry arena in North America by tradition, needs to build those same kind of links in the regulatory/industry arena in Europe, or risk becoming irrelevant in that market.

Some Committee members felt that AOAC should participate in the various technical working groups of CEN, which will recommend methods to be used. This will be the best way to assure that AOAC methods, which are already globally recognized and accepted, are fully recognized and accepted in EC regulations.

Others felt that a formal recognition at a higher level in CEN and also in EC itself was equally important. However, the Committee agreed that AOAC may not be clear on what it wants out of a stronger relationship, or what the goals would be in such an approach to EC and CEN administrative levels. For instance, it is not clear what the impact would be on sales of AOAC methods products if AOAC methods are or are not designated in Europe.

Because the written proposal for a formal approach to market AOAC products to EC was not distributed in advance, the Committee agreed that those who wished to comment could do so by November 1992, but the Committee was not prepared to make any comment or recommendation to the Board of Directors.

Laboratory Quality Assurance Committee

JERRY HIRSCH, CHAIRMAN
Health Protection Branch, Food and Drug Laboratory Division, 3155 Willingdon Green, Burnaby, BC, V5G-4P2, Canada

Other Members: I. Borst; P.O. Campbell; B. Cottingham; E. Easterly; E. Koenig; J.E. Longbottom; K.A. McCully; R.J. Noel; J.M. Polywacz; C. Weaver; A. Williams; W. Wolf

Recommendations for Board of Directors

A. Expand the Terms of Reference of the Laboratory Quality Assurance Committee (LQAC) to include a statement regarding liaison between LQAC and the Official Methods Board (OMB), and designate the OMB chairman as an ex officio member of LQAC to facilitate the incorporation of QA principles into AOAC official methods.

B. Request Wayne Wolf to provide a detailed draft proposal for comment by LQAC members regarding a Technical Division within AOAC to initiate and implement activities relating to the use of reference materials.

C. Incorporate a QA/laboratory accreditation poster session into the 1993 AOAC International Annual Meeting.

Basis for Recommendations

A. LQAC believes that the method validation process should emphasize QA more. Michael Brodsky was invited to discuss various ways LQAC can work with OMB to integrate QA principles into AOAC official methods. The report of the OMB task force led by Paul Corneliussen was briefly discussed. More QA awareness is evident on the part of method collaborators and Associate Referees. LQAC has incorporated additional QA parameters into the Checklist for Protocol Design, and this is a useful tool. While one method committee has opted to identify a QA advisor, it is generally believed that this is not necessary as long as Associate Referees emphasize QA requirements. Wolf led a discussion on the use of reference materials in validating and improving the quality of analytical measurements.

B. It is recognized that accuracy and reference materials do not receive the same emphasis in the method validation process as do method precision and variability. Increased emphasis on the use of reference materials in the collaborative study process, in implementing AOAC official methods, and in routine use is desirable. More information is required on the proposed Technical Division and how it would facilitate and coordinate AOAC activities relating to the use of reference materials. LQAC comments on Wolf's draft will be collated by Jerry Hirsch and given to Wolf for inclusion in his report to the Board of Directors.

C. The major organizations that cooperate with AOAC and are involved with laboratory accreditation should be invited to participate in a poster session. This format would provide information on accreditation programs and requirements to meeting attendees.

General Report

The meeting was called to order at 1:30 on August 31, 1992. The agenda was reviewed along with the attachments that had previously been distributed.

The Terms of Reference for the Committee, as updated by the Board of Directors on February 24, 1992, were reviewed and accepted. The change to include a focus on laboratory accreditation was noted as was the need for LQAC members to be willing to provide input during the year on QA issues.

The modified QA checklist prepared by various members of the Committee was accepted. This checklist was intended to serve as a guide to analysts, to laboratory managers, and to outside auditors who want to quickly review the QA status in a laboratory. It was recommended that ISO Guide 25 be appended to this checklist and that laboratories be encouraged to obtain further details for developing their QA program from other sources, such as the AOAC *Quality Assurance Principles for Analytical Laboratories.* Information about the checklist will be summarized by Bruce Cottingham and submitted to *The Referee.* This checklist may also be provided as a handout to QA short course attendees.

During the past year, LQAC reviewed the Checklist for Protocol Design and proposed modifications to reflect more emphasis on quality assurance. Brodsky visited the LQAC meeting to discuss the role of the checklist in developing AOAC methods. The checklist will be one of the QA-related topics submitted during the year to *The Referee.*

The role of LQAC in the next harmonization project, the symposium "Harmonization of Internal QA Schemes for Analytical Laboratories" to be held just prior to the 1993 AOAC International Annual Meeting, was discussed. Roger Wood will send draft documents to Paul Campbell and Hirsch for comment and input, and to the QA short course directors Brodsky and Eugene J. Klesta to keep them informed about the content of the symposium. Jim Ault and Sallee Anderson have also expressed interest in this area. AOAC and LQAC should be represented at the 1993 harmonization symposium.

Wolf reviewed BERM-5 and indicated AOAC's support for this meeting. LQAC strongly supports the enhanced role for reference materials. Wolf also reviewed the need for method verification when methods are first developed, implemented in a different laboratory, or routinely used to generate laboratory results. There was considerable discussion on the need to be proactive in making reference materials available in collaborative studies and whether the study itself should be used to validate an uncertified reference material. Wolf also agreed to update the Certified Reference Material list in the appendix of the AOAC *Official Methods of Analysis.* Jim Longbottom is also available to provide comments.

To further emphasize the need for quality assurance, various options on how to write a QA chapter for the AOAC *Official Methods of Analysis* were discussed. Klesta and Brodsky agreed to write the QA chapter using various sources, including the AOAC *Quality Assurance Principles for Analytical Laboratories* and existing information in the appendix of the AOAC *Official Methods of Analysis*.

In the interests of publicizing quality assurance, and the role of QA in the AOAC method validation process in particular, it was requested that LQAC members regularly contribute QA articles to *The Referee*. LQAC members agreed to submit articles on various QA topics, including the QA Checklist for Small Laboratories and the Checklist for Protocol Design. In addition, Jim Peeler will be asked to prepare a QA article based on APHA information. LQAC members should also be prepared to submit reviews on quality assurance papers.

LQAC invited the following persons to speak about the role of various organizations in laboratory accreditation: Ron Christensen (AOAC and A2LA); Alex Williams (ILAC and NAMAS); and Keith McCully (Standards Council of Canada). There is considerable conflict (NIST, ANSI, A2LA, etc.) about who should accredit the accreditors. Most accrediting bodies have an engineering bias, and there is a need for more of a chemistry and microbiology analytical emphasis, partly because of the difficulty in demonstrating traceability in these areas. AOAC and ILAC should have a role here in working to eliminate inconsistencies between accrediting agencies and in harmonizing guidelines. ISO-25 is the basic international standard. NIST has proposed a Conformity Assessment System Evaluation program (CASE) in the United States, but it may have a conflict because it also runs an accreditation program.

Antonio Silva Mendez described the Mutual Recognition Agreements being developed in Europe. AOAC has participated in harmonization efforts with ISO and IUPAC. AOAC now needs to define its role in the laboratory accreditation area, including the identification of the accreditation benefits. The scope of accreditation (for specific methods, for analytes, ...) is an issue that needs to be addressed as does the definition of areas that need accreditation. One possible role for AOAC is to provide a training course for accreditors; this is a natural extension of AOAC's expertise in quality assurance and our QA courses. AOAC has demonstrated strengths in the provision of training courses, and it could work together with these other agencies in a training and coordination role. A number of LQAC members will be asked to prepare a draft statement proposing a LQAC/AOAC role, which will be circulated to LQAC members for comment.

Keith McCully may be the only LQAC member able to attend the Ottawa ILAC 1992 meeting. AOAC should ideally make proposals or comment on guidelines for ILAC.

Brodsky, Klesta, and Hirsch gave a short update of the QA short courses, listing the 1992 courses and the proposed 1993 courses. All courses are being updated based on input received from course attendees. LQAC members should provide input on the QA short courses and provide suggestions for additional instructors.

Other QA issues that were briefly discussed were (*1*) the need to define the degree of uncertainty associated with the results of a method (it was recommended that the Statistics Committee take the primary role and that LQAC and OMB provide comment), and (*2*) the need to investigate the topic of GALPs as it relates to LQAC (a GALP workshop will be part of the 1993 meeting).

LQAC issues for the next year will include continuing emphasis on QA in the method validation process. Particular attention will be focused on using reference materials, developing a stronger relationship with OMB, developing a position statement on laboratory accreditation, providing members with information on accrediting agencies and programs, and looking at QA implications in GALPs.

Meetings, Symposia, and Educational Programs Committee

SAMUEL W. PAGE, CHAIRMAN
U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, 200 C St, SW, Washington, DC 20204

Other Members: R. Bianchi; K.W. Boyer; M.S. Cerny; T.L. Jensen; E.J. Klesta; D.E. Mayers; D. Osheim; J.G. Phillips; H.C. Thompson; A. Viscido; H.C. Wallin

Recommendation for Board of Directors

The Meetings, Symposia, and Educational Programs Committee recommends that the Board of Directors adopt the following policy for the Association: "The official language of the Association for documentation and the annual international meeting will be English. The language used at section meetings of the Association will be at the discretion of the individual sections. Announcements and technical materials may be prepared in other languages in addition to English."

Basis for Recommendation

To recognize the international representation of Association members, the designation of a single language for all official documentation and for the annual international meeting is desired.

This policy would not restrict the use of languages other than English at section meetings or in announcements or technical publications.

Other Discussion

The AOAC Director of Administration and Meetings, Margaret R. Ridgell, reported on the current AOAC annual meeting,

indicating general favorable comments on facilities and technical programs. The changes in the opening session were generally very favorably accepted. However, Frank J. Johnson, representing the Wiley Award Committee, expressed the concern that the removal of the Wiley Award Address from the opening session had reduced the prestige of that award. The Wiley Award Address, formerly presented at the opening session, was now the featured presentation of the Harvey W. Wiley Award symposium. Following discussions, the Meetings, Symposia, and Educational Programs Committee concluded that the recommendation regarding changes in the opening session as approved by the Board of Directors was sufficiently broad to cover the concerns of the Wiley Award Committee. The following recommendations and clarifications were offered: The intent of the Meetings, Symposia, and Educational Programs Committee was to shorten the opening session and to provide some flexibility in recognizing the Wiley award winner. At the personal discretion of the award winner, he/she may address the opening session in accepting the award. This should not be a technical presentation nor longer than 20-25 min. Although the award winner is desired to give a technical presentation in his/her award symposium, it is not required. Also, the award winner is not required to organize this symposium, although he/she may recommend speakers. If the award winner elects to give a technical presentation in the symposium, it will be scheduled not to conflict with any other activity and, if possible, not on the same day as the opening session. The nominator(s) of the award winner will be requested to assist the Meetings, Symposia, and Educational Programs Committee in organizing the symposium and Wiley Award exhibit booth. A list of suggested materials to include in this booth will be prepared by the Meetings, Symposia, and Educational Programs Committee, with assistance requested from the Wiley Award Committee. The Meetings, Symposia, and Educational Programs Committee also recommends that, in the opening session, the awards be given in the ascending order of importance, resulting in the address of the Wiley Award winner immediately following the receipt of the award.

The 1993 meeting plan was reviewed in detail. The Meetings, Symposia, and Educational Programs Committee agreed to hold one symposium open until January 1, 1993, to give the Wiley Award Committee time to consider the selection of an award winner for 1993. Workshops on Good Automated Laboratory Practices, Antibiotics and Drugs in Feeds, and Quality Assurance of Benchtop Mass Spectrometric Data will be planned. In addition, a special poster session by organizations that carry out laboratory accreditation will be held.

The Committee reviewed the status of the Association short courses, including the Continuing Education credits now being offered. The chairman will appoint a working group to review in detail the current short course efforts. This review will include surveys of section needs and will emphasize the use of these training activities to attract new members. The Committee decided that no additional consideration will be given to developing training courses specifically for analyst certification until the issue of Association involvement in analyst certification is settled.

Progress in increasing the international scope of the Association was reviewed. The Committee requested that its suggestions regarding increasing the international scope of the Association meeting activities be implemented for the 1993 annual international meeting. It unanimously approved the recommendation of English as the official language for the meeting and will prepare a listing of additional recommendations regarding AOAC staff procedures to address international concerns.

J. Benjamin Harnish requested that the Association consider Halifax, NS, Canada, as the location of a future Association annual international meeting. The Committee requested that AOAC staff research vendor participation, possible travel restrictions by state government officials, and costs that would be involved in carrying out a meeting in Canada, including, but not limited to, Halifax. This report would be submitted to the Meetings, Symposia, and Educational Programs Committee and the Board of Directors for consideration. Similar, but less precise information, was requested regarding a future meeting in the United Kingdom or elsewhere in Europe.

The chairman will appoint a working group to redraft the AOAC Instructions for Authors at the annual meeting to include guidelines for commercial data and a request to speakers for special considerations for the international scope of their audience.

The chairman will appoint a working group to assist the Meetings Department with hospitality for the 107th AOAC International Annual Meeting in Washington, DC.

Preliminary plans for the 108th AOAC International Annual Meeting were discussed. If this meeting is held in Portland, OR, as planned, the proposed theme is "The Pacific Basin." Special efforts will be made to increase participation from these countries.

Membership Committee

DAVID OSHEIM, CHAIRMAN
USDA/NVSL, Chemistry Section, Box 844, Ames, IA 50010

Other Members: R.M. Beebe; B. Funk; T.P. Layloff; M. Morrison; J.T. Sabater; N. Thiex

Recommendations for Board of Directors

A. That AOAC International offer the same discounts currently offered to Individual Members of the Association (publications, meetings, courses, etc.) to Sustaining Member (SM) organizations.

B. That a new class of membership, Method Validation Participant (MVP), be established to provide volunteers in the methods development program with training, as well as the eligibility to vote on methods and a subscription to *The Referee*. The membership fee would be sufficient to cover the cost of

training, method adoption balloting, and *The Referee* mailing for 3 to 4 years. Participants could become full members with all benefits by paying the cost differential (costs of servicing members, such as providing the membership directory, etc.) between MVP membership and full membership. The training program should be offered both at section meetings and at the AOAC International Annual Meeting.

C. That AOAC International establish a mentoring program for new (first year) AOAC members. A cadre of volunteers should be recruited among established members to periodically contact new members.

Basis for Recommendations

A. Sustaining membership numbers have decreased from 222 in 1988 to less than 200 in 1992. Money saving benefits would be an attractive selling point. Current and potential SMs feel we do not offer adequate value for the SM fee. SMs currently take discounts on publications through their designated representatives. The proposal would enable AOAC to use the discount as a selling point for gaining SMs. Approval of the proposal would also allow discounts for meeting registrations and short course fees for other than SM representatives. Organizations that do not provide Sustaining Member support take discounts through employees who are Individual Members of AOAC; i.e., the current discount structure provides no preferential discount for those who support AOAC over those who do not.

B. Many government agencies are not able to purchase memberships for their employees because of the membership benefits AOAC offers. However, they can pay for training. This would increase the training of volunteers in the methods development program. It would also allow for full membership at a nominal cost to the individual. Offering these courses at section meetings would reduce travel costs for the employer while introducing potential new members to the AOAC.

C. A mentoring program would instill a sense of belonging for new members. Members would be more quickly assimilated into the volunteer process. This program would provide a way for established members to become familiar with new members and increase new member retention.

To publicize and recruit volunteers for the mentoring program, letters would be sent to members who have been with the Association for more than 2 years. They would be asked to serve as contacts (mentors) for new members in their region; i.e., their role is to contact the new members and answer questions about the Association. Also, Regional Sections would be asked to recruit volunteer mentors, and an article on the mentor program would be published in *The Referee* explaining the program and asking for volunteers. To increase the opportunities for contact, member mentors would be matched to new members geographically where possible. Each member mentor would be sent a list of new members in his/her region and would be asked to contact the new members, introduce him- or herself, welcome the new member to AOAC, explain the benefits of membership in AOAC, and offer to answer any questions the new member might have. An explanation of the mentor program would be included along with the name of the new member's mentor in each new member packet. Member mentors could arrange to meet the new members they are mentoring at the AOAC International Annual Meeting. To maximize benefit for new members, the member mentors would be urged to make this as early in the meeting as possible.

Nominating Committee

FRANK J. JOHNSON, CHAIRMAN
Tennessee Valley Authority, National Fertilizer Development Center, Muscle Shoals, AL 35660

Other Members: N. Babiak; A. Gardner; T.P. Layloff; D. Oshiem; S.L. Pfeiffer; R. Ronk; N. Thiex; H.M. Wehr

The Committee proposed the following changes: (*1*) That the AOAC International Nominating Committee be expanded from 3 to 5 members. (*2*) That the composition of the Committee be changed from the 3 immediate past presidents to the 2 immediate past presidents and 3 members-at-large to be appointed by the President with the approval of the Executive Committee. (*3*) That the Nominating Committee recommend one name per vacancy to the Board of Directors. (*4*) That the Board of Directors develop a petition system by which the general membership can place a candidate directly on the ballot, without going through either the Board of Directors or the Nominating Committee.

The Task Force would recommend that the Board require that the petition contain 5% of the total membership (presently, this is about 150 names).

The Task Force recommends that the above proposed changes to the present nominating system be published in *The Referee* with a request for membership input on the proposed changes. After reviewing any membership input, the Task Force will make its recommendations to the Board of Directors.

Regional Sections Committee

AUDREY GARDNER, CHAIRWOMAN
New York State Agricultural Experiment Station,
Food Research Laboratory, Geneva, NY 14456

Other Members: P.R. Beljaars; P. Bolin; V. Burse; T.C. Cronau; J.B. Harnish; J. Hirsch; E. Ibrahim; G.W. Latimer; K.A. McCully; P.J. Oles; J.M. Singer; H.M. Stahr

Recommendation for Board of Directors

The Regional Sections Committee recommends that the Board of Directors strike the following requirement found in Section 1.c., Appendix J, AOAC International Policies, AOAC International Regional Section Operations Guidelines: "The maximum single sponsorship will be $300 US."

Basis for Recommendation

It was the committee's understanding that the Board of Directors justified this statement because of a concern that regional sections would be competing with AOAC International in obtaining sponsors for their meetings.

The Regional Section Committee felt that companies, from which various regional sections solicited sponsorships, would be from local or regional branches and would not interfere with AOAC International's ability to obtain sponsorship from national or international headquarters of any given company.

It was initially noted that $300 was an unrealistically low amount and further noted this was especially impractical in the international arena, where current sponsorships already run well above the guideline's limit.

Harvey W. Wiley Committee

EDGAR R. ELKINS, CHAIRMAN
National Food Processors Association, 1401 New York Ave, Suite 400, NW, Washington, DC 20005

Other Members: W.P. Cochrane; L.W. Doner; F.J. Johnson; R.C. Rund; P.M. Scott; O.L. Shotwell

Recommendations for Board of Directors

A. That the Harvey W. Wiley Award nomination form be revised.

B. That guidelines be developed on the type of information that should be included on the nomination form.

C. That the cash award be increased by $500 a year for the next 5 years.

D. That the Harvey W. Wiley Award brochure be updated.

E. That calls for nominations for the Wiley Award be publicized outside of AOAC International.

Basis for Recommendations

A. The committee believes that, in its present format, the nomination form keeps people from nominating candidates; therefore, the committee would like staff, with the help of the committee, to revise the nominating form.

B. Presently, applications are not consistent in the type of information they contain on the candidates. This lack of information may keep someone who deserves the award from receiving it; therefore, the committee would like staff, with the help of the committee, to develop guidelines on how to complete the nomination form that would be sent to all nominators.

C. The committee feels that the cash award for the highest honor that AOAC awards should be increased to reflect the award's importance.

D. The present brochure that describes both the Harvey W. Wiley Award and Scholarship is out of date. The committee recommends that this brochure be updated and, once updated, sent to all AOAC members with a cover letter soliciting nominations, giving the qualifications for the award, and stating that the award is not restricted to AOAC members.

The brochure should also be sent to AOAC sections to display at their meetings.

E. Because the award is not restricted to members of AOAC, greater emphasis should be placed on publicizing the award and calls for nominations.

Other Discussion

A. The committee discussed the change of the Harvey W. Wiley Award presentation from the opening session to a symposium. The majority felt that they should have been consulted on the change. Frank J. Johnson will attend the meeting of the Meetings, Symposia, and Educational Programs Committee (the committee responsible for the annual international meeting program and format) to express the opinions of the Harvey W. Wiley Award Committee.

B. The committee asked that they be informed and consulted on any proposed changes to the Wiley Award before proposals are implemented.

C. The committee discussed ways that they can become more active in soliciting nominations. It was agreed that the committee should contact people they know to discuss the award and try to get people to send in nominations. The committee also thought committee members who attend section meetings should arrange to say a few words about the Harvey W. Wiley Award.

Harvey W. Wiley Scholarship Award Committee

MARIETTA SUE BRADY, CHAIRWOMAN
Rutgers University, Cook College, Dept of Biochemistry, Lipman Hall, New Brunswick, NJ 08903

Other Members: P. Bulhack; J.W. Fitzgerald; R.G. Krueger; J. Padmore; A.A. Peake; S.L. Pfeiffer

The AOAC International Harvey W. Wiley Scholarship Award Committee selected Michael J. McCall of Illinois State University as the 1992 winner of the AOAC International Harvey W. Wiley Scholarship Award. McCall has been involved for 3 semesters in undergraduate research, working on the development or modification of analytical methods for environmental sampling.

Joint Mycotoxin Committee

PETER M. SCOTT, CHAIRMAN
Health and Welfare Canada, Health Protection Branch, Ottawa, ON, K1A 0L2, Canada

Other Members: D.J. Bark (AACC); R. Bernetti (AACC); M.S. Cerny (IDF); J.C. Henderson (AOCS); D.L. Park (IUPAC); J.J. Pestka (AOAC); A.E. Pohland (IUPAC); A.H. Spandorf (AOCS); D. Sweigart (AOCS); S.N. Tanner (Secretary) (AACC); M.W. Trucksess (AOAC)

The Committee met in Cincinnati, OH, on September 1, 1992, for its annual meeting. New members Dan Sweigart, Jim Pestka, Aaron Spandorf, and Milan Cerny were welcomed. Peter Scott announced he would no longer be chairman of the Joint Mycotoxin Committee, and Mary Trucksess was elected to replace him. The following reports were presented.

IUPAC

The report of the Commission on Food Chemistry of the International Union of Pure and Applied Chemistry (IUPAC) was presented by Doug Park. Ongoing projects are a survey of data on the incidence and levels of ochratoxin A in food and animal feed worldwide, collaborative study on a liquid chromatographic (LC) method for determining fumonisins B_1 and B_2 in cereals and mixed feeds, collaborative study of an immunoaffinity column method for aflatoxin M_1 in milk, and the check sample program for mycotoxins. Many of the projects in the mycotoxin area are joint projects with AOAC International. These activities have been effectively coordinated with AOAC International through the Joint Mycotoxin Committee. A major continuing activity of the Commission on Food Chemistry has been the organization of a series of highly regarded symposia in the area of mycotoxins and phycotoxins. The next Symposium on Mycotoxins and Phycotoxins will be held in Mexico City, Mexico, on November 8–11, 1992.

AOAC International

Peter Scott reviewed the General Referee report on mycotoxins. His main recommendations were as follows: surplus the official final action method **974.17** for aflatoxin M_1 in dairy products; carry out a collaborative study on an immunoaffinity column method for aflatoxin M_1 in milk after protocol approval by AOAC; adopt as official first action the modified Immuno-Dot (Signal Accucup) ELISA for screening aflatoxins B_1, B_2, and G_1 at ≥20 ng/g in corn (corn should be deleted as a matrix in method **990.34**); carry out a collaborative study of a multifunctional column cleanup method for determining aflatoxins in corn, peanuts, almonds, pistachio nuts, and Brazil nuts; complete the collaborative study on ergot alkaloids in grains; carry out a collaborative study of an LC method for ochratoxin A in pig liver and kidney after protocol approval by AOAC; make an editorial change to **986.18** (the extraction solvent in method **986.18C** should be $CHCl_3$–EtOH [8 + 2]); add patulin as a new topic; and continue study on all topics.

AOCS

The report of the Mycotoxin Committee of the American Oil Chemists' Society (AOCS) was given by Jim Henderson. The Smalley Aflatoxin Check Sample program gave no awards to the top performers in the last test kit series. Because the Smalley program is designed to recognize the top performers, in addition to calibrating methods, reagents, and individual performance, follow-up was recommended. Future samples in the Peanut Aflatoxin Series will be raw peanuts ground into a paste rather than the defatted peanut meal currently used. It was noted that this would give lower and more meaningful levels of aflatoxin, better homogeneity, and a lower coefficient of variation.

Analytical methods using robotics were discussed, and it was decided not to incorporate robotic methods into the AOCS methods book.

There was some discussion about the possibility of certain countries reducing the action limit for aflatoxin M_1 to 0.05 ppb. Concern was raised about the present test kit technology and lower detection limits.

AACC

Steven Tanner highlighted the report of the Mycotoxin Committee of the American Association of Cereal Chemists (AACC), which last met in Seattle, WA, on October 14, 1991. The mycotoxin sampling subcommittee (chair, Mike Hurley) recommended that a sampling guideline be incorporated into the AACC *Approved Methods of Analysis*. The AOAC method for determining aflatoxins in corn and peanut butter by LC has

been rewritten by Doug Bark and approved for incorporation into the AACC methods book.

The Committee felt that there was no need for an AACC method for determining deoxynivalenol by LC at the present time. Fumonisins continue to be an AACC concern.

The AACC Mycotoxin Committee has been apprised of the AOAC Research Institute's program on evaluation of test kits.

IDF

Milan Cerny highlighted the activities of the International Dairy Federation (IDF) Group E33-Mycotoxins, which met on March 11, 1992, in Brnssels, Belgium. Fourteen laboratories reported useful results in an IDF collaborative study on a method for aflatoxin M_1 using an immunoaffinity column and LC with fluorimetric detection. A draft questionnaire was sent to the National Committees, which will provide a basis for selection of a suitable method for the rapid and sensitive determination of aflatoxin M_1 in milk and milk products.

Other Business

Sam Page drew attention to renewed interest in patulin and the need for an AOAC Associate Referee. Imported apple juice is being monitored closely by several companies.

The issue of sampling was discussed by Page, who said the area needs more attention and that AOAC International should become more involved in developing specific methods for sampling of food products. Rafaele Bernetti described studies on analyzing aflatoxin–contaminated corn ground to different particle sizes. Variability of the analyses greatly decreased going from No. 10 mesh sieve to No. 20 mesh sieve grindings.

On the subject of fumonisins, the recent interagency meeting and the Harvey W. Wiley Award symposium at the AOAC International Annual Meeting were noted. Aaron Spandorf discussed test kits. He recommended that a large and stable reference sample of corn containing fumonisins be set aside for companies developing test kits for fumonisin.

Steve Tanner gave the Committee a brief overview of the laws governing the exportation of corn from the United States. Presently, regulations require that all exported corn be tested for aflatoxin unless both the buyer and seller agree not to have it tested. No similar law exists for domestic trade in corn.

Future conferences relating to mycotoxins that were brought to the attention of the Joint Mycotoxin Committee are as follows: Aflatoxin Elimination Workshop, November 1–3, 1992, Fresno, CA; VIII International IUPAC Symposium on Mycotoxins and Phycotoxins, November 8–11, 1992, Mexico City, Mexico; AOAC Midwest Regional Meeting, June 7–9, 1993, Des Moines, IA; Gordon Research Conference on Mycotoxins and Phycotoxins, July 19–23, 1993, Plymouth, NH; 107th AOAC International Annual Meeting, July 26–29, 1993, Washington, DC; and the International Congress on Plant Pathology, July 28–August 6, 1993, Montreal, Canada.

AOAC Research Institute

SCOTT COATES, MANAGER
AOAC Research Institute, Suite 400, 2200 Wilson Blvd, Arlington, VA 22201

Mission

The AOAC Research Institute (the "Institute" hereafter) was incorporated in 1991 as a subsidiary of AOAC International, and is located in the AOAC International offices in Arlington, VA. The Institute is a non-profit (tax-exempt status pending), international scientific organization whose primary objective is to promote and conduct activities related to the development, improvement, and understanding of proprietary analytical practices and procedures affecting public health and welfare.

Test Kit Performance Testing Program

The primary objective of the Institute is to provide a third-party validation of test kit product performance claims. This activity is conducted under the Institute's Test Kit Performance Testing Program. The certification program includes an independent review of manufacturer-generated data and an independent laboratory evaluation of test kit performance characteristics.

The Institute recruits independent volunteer experts (known as "Expert Reviewers") to actually review the performance data of test kits. The Expert Reviewers evaluate the data submitted by manufacturers and the data generated by the independent testing laboratories. Test kits that are determined by the Expert Reviewers to perform as claimed are then licensed to use the Institute's "Performance Tested" certification mark. Certified test kits are reviewed annually by the Institute.

The program is fee-based, and the fee for evaluation of a single test kit is US $7,500. Additional test kits submitted at the same time are evaluated for US $5,000 per kit. In addition, the cost of the independent laboratory testing is passed on to the applicant. Fees for independent testing are expected to be about US $2,500 but may vary with the complexity of a test kit.

The Institute initially focused its attention on test kits in the food chemistry, agricultural, and environmental areas. Memoranda of Understanding (MOU) with several U.S. federal agencies regulating test kits in these areas have been executed, providing official recognition of the "Performance Tested" certification mark and the validation program. MOUs with the Federal Grain Inspection Service (FGIS) of the U.S. Dept of Agriculture and with the U.S. Food and Drug Administration (FDA) were signed in October 1992.

Federal Grain Inspection Service

The Memorandum of Understanding with FGIS recognizes the Institute's program to evaluate test kits for aflatoxin residues in grain and grain products. The Institute, working with manufacturers, test kit users, and FGIS, developed testing protocols for these test kits based on FGIS established standards. Manufacturers who successfully validate test kits under this program are licensed to use the Performance Tested certification mark, and may make the claim "Performance Tested in Accordance with Standards Established by FGIS for Test Kits Used To Detect Aflatoxin Residues in Grain and Grain Products."

U.S. Food and Drug Administration

In 1991, at the recommendation of the National Conference for Interstate Milk Shippers, the Pasteurized Milk Ordinance was appended (Appendix N) to establish safe levels for drug residues in milk and to assure that milk supplies are in compliance with these safe levels. Appendix N also established a monitoring and surveillance sampling program for antibiotic residues in bulk milk pickup tankers. To facilitate the increased surveillance activity, Appendix N stipulates that drug residue detection methods may be submitted to the Institute for evaluation at the safe level or tolerance.

In support of the surveillance and monitoring activity required by Appendix N, the Institute and the Center for Veterinary Medicine at FDA (CVM/FDA) executed an MOU to define a cooperative program to evaluate test kits for antibiotic residues in milk. This Test Kit Performance Testing Program, regarding evaluation of the beta-lactim class of test kits, is administered by the Institute and designed to result in test kits that are acceptable to both the Institute and CVM/FDA. Test kits that are acceptable to CVM/FDA will then be recommended to the Milk Safety Branch, Center for Food Safety and Applied Nutrition, which is, in turn, responsible for approving test kits for use in state-level monitoring programs.

The MOU between the Institute and FDA defines a cooperative program in which FDA, through CVM, has significant input into the development of testing protocols and actively participates in the evaluation of test kit submissions. This is achieved by the FDA appointing one of 5 experts to serve on a series of Institute Expert Reviewer Panels. These panels will review data submitted by the manufacturers and the data generated at the independent testing laboratory. The Expert Reviewers will then make recommendations to grant or deny the Performance Tested status for each test kit.

The Institute, in cooperation with CVM/FDA and test kit manufacturers, codified the current FDA standards and established specific data submission requirements for beta-lactams in milk. This document provides guidelines to test kit manufacturers for both data required in the data submissions and the acceptance criteria for dual recognition from the Institute and the FDA. Separate, but similar, documents are planned for other classes of antibiotic residues in milk—tetracyclines, sulfonamides, and aminoglycosides.

General Programs

The Institute opened the Test Kit Performance Testing Program to all test kits for performance evaluation in September 1992 on a first-come, first-served basis. Evaluations for test kits in the dairy chemistry, agriculture, and environmental monitoring areas are expected to be processed quickly once submitted. The Institute's goal is to complete evaluation of these test kits within 90 days from the date of submission. The evaluation periods for test kits in areas outside of the Institute's current expertise are expected to take a little bit longer.

AOAC INTERNATIONAL

AOAC International Officers and Committees: 1993

Board of Directors

President: Henry B.S. Conacher, Health & Welfare Canada, Health Protection Branch, Food Research Div., Tunney's Pasture, Ottawa, Ontario K1A 0L2, Canada

President-Elect: Arvid Munson, Phoenix Regulatory Associates, Inc., 21525 Ridgetop Circle, Suite 240, Sterling, VA 22170

Secretary/Treasurer: Alan R. Hanks, Dept of Biochemistry, Office of the Indiana State Chemist, Purdue University, West Lafayette, IN 47907, (For Direct Mail), 1154 Biochemistry Bldg, 47907-1154

Immediate Past President: Edgar R. Elkins, National Food Processors Association, 1401 New York Ave, NW, Suite 400, Washington, DC 20005

Directors: **P. Frank Ross**, U.S. Dept of Agriculture, National Veterinary Services, Laboratories, Toxicology Laboratory, PO Box 844, 1800 Dayton Rd, Ames, IA 50010; **Albert E. Pohland**, U.S. Food & Drug Administration, 200 C St, SW, Washington, DC 20204; **George H. Boone**, U.S. Food & Drug Administration, 850 Third Ave, Brooklyn, NY 11232; **Eugene J. Klesta**, Chemical Waste Management, Inc., Quality Assurance Programs, 4300 W. 123rd St, Alsip, IL 60658; **Paul R. Beljaars**, Inspectorate for Health Protection, Food Inspection Service, Florijnruwe 111, 6218 CA Maastricht, The Netherlands; **Raymond Matulis**, Kraft General Foods, Inc., 801 Waukegan Rd, Glenview, IL 60025

Staff

Executive Director and General Counsel: Ronald R. Christensen, AOAC International, Suite 400, 2200 Wilson Blvd, Arlington, VA 22201-3301; *Executive Assistant:* Vernora R. Petty

Director of Technical Services and Development: Nancy Palmer; *Administrative Assistant:* Kelly L. Gambrell; *Methods Editor:* Julie M. Norman; *Technical Coordinator:* George R. Heavner; *AOAC Research Institute Manager:* Scott G. Coates; *AOAC Research Institute Administrative Assistant:* D'Arcy Rogers

Director of Administration and Meetings: Margaret R. Ridgell; *Administrative Assistant:* Carolyn A. Dell; *Meetings and Education Coordinator:* Carol L. Rouse; *Receptionist:* Teresa Walker

Director of Finance and Data Processing: Wilson Korpi; *Administrative Assistant:* Marjorie D. Fuller; *Staff Accountant:* Leon M. Bathini; *Mailroom Clerk:* Troy James; *Computer System Manager:* Connie L. Varner; *Fulfillment Coordinator, Publications:* Catherine F. Shiflett; *Fulfillment Clerk, Journal:* Mark R. Stewart

Director of Marketing and Membership: Marilyn L. Blakely; *Marketing Assistant:* Udine Coletta; *Marketing Coordinator:* Dede Landis; *Membership Coordinator:* Carrie F. Glavin

Director of Publications: Krystyna A. McIver; *Administrative Assistant:* Amy L. Martin; *Managing Editor:* Robert E. Rathbone; *Assistant Editor:* Scott E. Reardon; *Publications Production Coordinator:* Donna V. Weiss; *Official Methods of Analysis Editor:* Patricia Cunniff

Representatives

Howard P. Moore, Rural Route 3, 356B, Hohenwald, TN 38462; **Margreet Lauwaars**, PO Box 153, 6720 AD Bennekom, The Netherlands; **Derek Abbott**, 33 Agates Ln, Ashtead, Surrey KT21 2ND, UK

Boards

Editorial Board: Claire Franklin (Bureau of Human Prescription Drugs, Place Vanier, 355 River Rd, Second Floor, Vanier, Ontario K1A 1B8, Canada), *Chairman*; Dieter Arnold; James Ault; Franklin E. Barton II; Richard A. Durst; J. Richard Gorham; Anant V. Jain; James Lichtenberg; W. Harvey Newsome; Joseph Sherma; James T. Tanner; David L. Terry

Mission: To oversee the development, editing, and publishing of all Association publications; to provide for long and short range publication planning.

Official Methods Board: Thomas L. Jensen (Nebraska Dept of Agriculture, 3703 S. 14th St, Lincoln, NE 68502), *Chairman*; Donald E. Carpenter; Richard H. Collier; Jonathan W. DeVries; Gayle A. Lancette; Keith A. McCully; Jon E. McNeal; John O'Rangers; Peter M. Scott; Ann B. Strong; Harold C. Thompson, Jr

Mission: To establish, promote, and recommend uniform policy for the consideration and adoption of methods; to review and adopt official methods according to policy; to provide general oversight for all official methods processes; to resolve disputes and appeals in the methods area in accordance with es-

tablished policy; and to oversee the activities of the Safety and Statistics Committees.

Safety Committee: Dirk Shoemaker (Nebraska Dept of Agriculture, 3703 S. 14th St, Lincoln, NE 68502), *Chairman*; Sandra Pfeiffer; David Egelhofer; Milan Cerny; Maire C. Walsh; Harry Ostapenko; Carlos Abeyta; Mark G. Torchia

Mission: To promote an awareness of safety and health matters within the AOAC membership and to give guidance in that area with particular emphasis on consideration of safety as part of the methods development process.

Statistics Committee: Daniel Mowrey (Lilly Research Laboratories, PO Box 708, Greenfield, IN 46140), Chairman; Robert C. Rund; Jeffrey L. Cawley; Margaret A. Nemeth; Terry C. Nelsen; Robert J. Condon; John G. Phillips; Dennis King; Sallee M. Anderson; Mark Presser; William Horwitz; Max Feinberg

Mission: To establish statistical guidelines for collaborative studies, and to encourage greater use of statistical techniques.

Standing Committees

Archives Committee: Charlotte A. Brunner (U.S. Food & Drug Administration, HFN-473, 200 C St, SW, Washington, DC 20204), *Chairman*; Susan Barkan; Patricia Bulhack; William V. Eisenberg; Wynne Landgraf; Helen L. Reynolds; Evelyn Sarnoff; Henry M. Stahr; Thomas G. Alexander

Mission: To collect and preserve the history of AOAC.

Bylaws Committee: Richard J. Ronk (U.S. Food & Drug Administration, HFF-22, Rm 1457, Parklawn Bldg, 5600 Fishers Ln, Rockville, MD 20857), *Chairman*; Dean Kassera; Jon McNeal; Terry Jackson; Laura Zaika; Gerald L. Roach; David L. Terry

Mission: To serve as an advisory body to the Board of Directors on the revision, drafting, content, and interpretation of the Association's Articles of Incorporation and Bylaws.

Fellows Committee: Edgar R. Elkins (National Food Processors Association, 1401 New York Ave, NW, Suite 400, Washington, DC 20005), *Chairman*; Thomas Fazio; Edwin Jackson; Jon McNeal; Arthur Waltking; Frederick J. King; Elaine A. Bunch

Mission: To select and recommend to the AOAC Board of Directors candidates for the "Fellow of the AOAC" awards.

Finance Committee: Arvid Munson (10226 Forest Lake Dr, Great Falls, VA 22066), *Chairman*; H. Michael Wehr; Alan R. Hanks; Henry B.S. Conacher

Mission: To assist the Board of Directors in carrying out its fiscal responsibility, by oversight of the budget development process, fiscal procedures, and review of current performance of AOAC relative to budget.

Harvey W. Wiley Award Committee: Henry B.S. Conacher (Health & Welfare Canada, Health Protection Branch, Food Research Div., Tunney's Pasture, Ottawa, Ontario K1A 0L2, Canada), *Chairman*; Frank J. Johnson; Robert C. Rund; Landis W. Doner; Peter M. Scott; Odette L. Shotwell; William P. Cochrane

Mission: To select the recipient of the annual AOAC Harvey W. Wiley Award.

Harvey W. Wiley Scholarship Award Committee: James W. Fitzgerald (U.S. Food & Drug Administration, Winchester Engineering & Analytical Center, HFR-NE400, 109 Holton St, Winchester, MA 01890), *Chairman*; Joel Padmore; Patricia Bulhack; Steven C. Slahck; Adeline Peake; Thomas Romer; Rae Gabrielle Krueger

Mission: To select the recipient of the annual AOAC Harvey W. Wiley Scholarship.

International Committee: Paul Beljaars (Inspectorate of Health Protection Dir Maastricht, Florijnruwe 111, 6218 CA, Maastricht, The Netherlands), *Chairman*; Alan R. Hanks; George R. Heavner; Ralph H. Lane; Margreet Lauwaars; Albert E. Pohland; Richard M. Rocco; Robert C. Rund; Alex Williams; Juan Sabater; Roger Wood; William Horwitz

Mission: To assist the Association in the development of a comprehensive program for international activities.

Laboratory Quality Assurance Committee: Jerry H. Hirsch (Health & Welfare Canada, Health Protection Branch, Food & Drug Laboratory Div., 3155 Willingdon Green, Burnaby, British Columbia V5G 4P2, Canada), *Chairman*; Carlton Weaver; Rodney J. Noel; Bruce Cottingham; Keith A. McCully; Paul O. Campbell; Eugene Easterly; James E. Longbottom; Wayne R. Wolf; Joseph Polywacz; Ian Borst; Ellen Koenig; Alex Williams; David B. MacLean; W.K. Wong; Jess Rajan

Mission: To provide advise and make recommendations for adoption and application of quality assurance principles toward the improvement of analytical laboratory operations.

Meetings, Symposia, and Educational Programs Committee: Samuel W. Page (U.S. Food & Drug Administration, Div. of Natural Products, 200 C St, SW, Washington, DC 20204), *Chairman*; Jack Michelson; Marie Siewierski; Anthony Viscido; David Osheim; Harold C. Thompson; Eugene J. Klesta; Milan S. Cerny; Harriet Wallin; Kenneth W. Boyer; Dale E. Mayers; John Phillips

Mission: To plan programs for the Annual International Meeting, topical conferences, etc.; and to oversee short courses and other educational programs.

Membership Committee: David Osheim (U.S. Dept of Agriculture, NVSL, Chemistry Section, Box 844, Ames, IA 50010), *Chairman*; Raymond H. Bowers; Stanley E. Katz; Roberta Beebe; Bonita Funk; Sue Cannon; MarJeanne Morrison; Juan T. Sabater; Nancy Thiex; Donna Montague; Adeline A. Peake; Thomas P. Layloff

Mission: To make recommendations to the Board of Directors on policies and procedures on all types of AOAC memberships.

Nominating Committee: Thomas P. Layloff (U.S. Food & Drug Administration, Div. of Drug Analysis, 1114 Market St, Rm 1002, St. Louis, MO 63101), *Chairman*; Edgar R. Elkins; H. Michael Wehr

Mission: To select a slate of candidates for election of AOAC Officers and Directors.

Sections Committee: Audrey Gardner (New York State Agricultural Experiment Station, Food Research Laboratory, Geneva, NY 14456), *Chairman*; Juan Sabater; Jeffrey M. Singer; Henry M. Stahr; Virlyn Burse; Keith A. McCully; Tom Nowicki; Paul Bolin; Tom C. Cronau; J. Benjamin Harnish; Jerry Hirsch; George W. Latimer, Jr; Philip J. Oles

Mission: To make recommendations to the Board of Directors on policies and procedures pertaining to Sections.

Joint Committees

Joint AOAC/AACC/AOCS/IUPAC Committee on Mycotoxins: *Chairman*: Peter M. Scott; *Representing AOAC:* Howard H. Casper; Mary W. Trucksess; *Representing AACC:* Odette L. Shotwell; Raphael Bernetti; Steven N. Tanner; *Representing AOCS:* Arthur E. Waltking; Robert D. Stubblefield; James C. Henderson; *Representing IUPAC:* Albert E. Pohland; Douglas L. Park; *Representing IDF:* Milan Cerny

Joint AOAC/FDA-CVM/AAFCO Committee on Medicated Feeds: *Representing AOAC:* Hussein S. Ragheb; Robert L. Smallidge: *Representing FDA-CVM:* J. Dennis McCurdy; Henry Schmaus; John O'Rangers: *Representing AAFCO:* Rodney J. Noel; Lars M. Reimann

Liaison Officers

American Association of Cereal Chemists: Ralph H. Lane (University of Alabama, Dept of Food, Nutrition, & Institutional Management, PO Box 870158, Tuscaloosa, AL 35487-0158)

American Association of Pharmaceutical Science: Edward Smith (14203 Castaway Dr, Rockville, MD 20853)

American Council of Independent Laboratories: Kenneth W. Boyer (Southern Testing & Research Laboratory, Inc., 3709 Airport Dr, Wilson, NC 27893)

American Oil Chemists' Society: David Firestone (U.S. Food & Drug Administration, Office of Plant and Dairy Foods & Beverages, HFF-426, 200 C St, SW, Washington, DC 20204)

American Public Health Association:

Dairy Chemistry: Robert Bradley (University of Wisconsin-Madison, Dept of Food Science, 1605 Linden Dr, Madison, WI 53706)

Dairy Microbiology: J. Russell Bishop (Virginia Polytechnic Institute & State University, Dept of Food Science & Technology, Blacksburg, VA 24060-0418)

American Society of Brewing Chemists: Mark H. Schwiesow (Pfizer, Inc., 4215 N. Port Washington Ave, Milwaukee, WI 53212)

American Society of Enologists: Arthur Caputi, Jr (E & J Gallo Winery. PO Box 1130, Modesto, CA 95353)

American Society for Testing & Materials: F10.11: Test Methods: Cleve B. Denny (National Food Processors Association, 6230 Valley Rd, Bethesda, MD 20817)

American Spice Trade Association: James E. Woodbury (14282 Holt Ave, Santa Ana, CA 92705)

California Dept of Food & Agriculture; Science Advisory Committee: George R. Tichelaar (4341 Jan Dr, Carmichal, CA 95608)

Collaborative International Pesticides Analytical Council: Alan R. Hanks (Office of the Indiana State Chemist, Purdue University, West Lafayette, IN 47907)

European Committee for Standardization (CEN/TC 275): Margreet Lauwaars (PO Box 153, 6720 AD Bennekom, The Netherlands)

Institute of Food Technologists: Ronald E. Wrolstad (Oregon State University, Dept of Food Science & Technology, Corvallis, OR 97331)

International Association for Cereal Science: Ralph H. Lane (University of Alabama, Dept of Food, Nutrition, & Institutional Management, PO Box 870158, Tuscaloosa, AL 35487-0158)

International Committee for Uniform Methods of Sugar Analysis: Margaret Clarke (Sugar Processing Research, Inc., 1100 Robert E. Lee Blvd, New Orleans, LA 70124)

International Dairy Federation: Richard M. Rocco (Idetek, Inc., 1057 Sneath Ln, San Bruno, CA 94066)

IDF/ISO/AOAC Tripartite: Margreet Lauwaars (PO Box 153, 6720 AD Bennekom, The Netherlands)

Water Content (E-5): Robert Bradley (University of Wisconsin-Madison, Dept of Food Science, 1605 Linden Dr, Madison, WI 53706)

Lactose (E-6): Dick H. Kleyn (Rutgers University, Dept of Food Science, PO Box 231, New Brunswick, NJ 08903-0231); Leslie West (Kraft General Foods, 801 Waukegan Rd, Glenview, IL 60025); John W. Sherbon (Cornell University, Dept of Dairy & Food Science, Ithaca, NY 14853)

NO_2, NO_3, P, Ca (E-8): Thomas Fazio (U.S. Food & Drug Administration, Office of Scientific Analysis and Support, 200 C St, SW, Washington, DC 20204); Robert Bradley (University of Wisconsin-Madison, Dept of Food Science, 1605 Linden Dr, Madison, WI 53706); Walter Fiddler (U.S. Dept of Agriculture, Eastern Regional Research Center, 600 E. Mermaid Ln, Philadelphia, PA 19118)

Lactic Acid (E-9): John W. Sherbon (Cornell University, Dept of Dairy & Food Science, Ithaca, NY 14853)

Casein (E-11): Robert Bradley (University of Wisconsin-Madison, Dept of Food Science, 1605 Linden Dr, Madison, WI 53706); Shu-Guang Greg Cheng (Kraft General Foods, Tech Center, 801 Waukegan Rd, Glenview, IL 60025)

Pesticide Residues (E-12): Louis G. Tuinstra (State Institute for Quality Control, PO Box 230, 6700 AE Wageningen, The Netherlands); Larry J. Maturin (U.S. Food & Drug Administration, Laboratory for Quality Assurance Br, 6502 S. Archer Rd, Summit-Argo, IL 60501-1399)

Heavy Metals & Other Elements (E-15): Shu-Guang Greg Cheng (Kraft General Foods, Tech Center, 801 Waukegan Rd, Glenview, IL 60025)

Dried Milk, Heat (E-17): John W. Sherbon (Cornell University, Dept of Dairy & Food Science, Ithaca, NY 14853); Robert Bradley (University of Wisconsin-Madison, Dept of Food Science, 1605 Linden Dr, Madison, WI 53706)

Contaminating Microorganisms (E-21): Wallace H. Andrews (U.S. Food & Drug Administration, Office of Special Research Skills, 200 C St, SW, Washington, DC 20204); Larry J. Maturin (U.S. Food & Drug Administration, Laboratory for Quality Assurance Br, 6502 S. Archer Rd, Summit-Argo, IL 60501-1399)

Nonpathogenic Contaminants (E-22): Larry J. Maturin (U.S. Food & Drug Administration, Laboratory for Quality Assurance Br, 6502 S. Archer Rd, Summit-Argo, IL 60501-1399)

Staphylococcus (E-24): Gayle A. Lancette (U.S. Food & Drug Administration, 60 Eighth St, NE, Atlanta, GA 30309)

Numerical Selection of Samples (E-26): William Horwitz (U.S. Food & Drug Administration, 200 C St, SW, Washington, DC 20204)

Nitrogen Content of Milk and Milk Products (E-27): David M. Barbano (Cornell University, Dept of Food Science, Ithaca, NY 14853); Robert Bradley (University of Wisconsin-Madison, Dept of Food Science, 1605 Linden Dr, Madison, WI 53706)

Automated Analysis for Routine Analysis of Raw Milk (E-29): David M. Barbano (Cornell University, Dept of Food Science, Ithaca, NY 14853); Robert Bradley (University of Wisconsin-Madison. Dept of Food Science, 1605 Linden Dr, Madison, WI 53706)

Statistics of Analytical Data (E-30): William Horwitz (U.S. Food & Drug Administration, 200 C St, SW, Washington, DC 20204); J. Timothy Peeler (U.S. Food & Drug Administration, HFF-1, 200 C St, SW, Washington, DC 20204)

Fat by Gravimetric Methods (E-31): David M. Barbano (Cornell University, Dept of Food Science, Ithaca, NY 14853); Robert Bradley (University of Wisconsin-Madison, Dept of Food Science, 1605 Linden Dr, Madison, WI 53706)

Gram-negative Pathogens (E-32): Wallace H. Andrews (U.S. Food & Drug Administration, Office of Special Research Skills, 200 C St, SW, Washington, DC 20204); Larry J. Maturin (U.S. Food & Drug Administration, Laboratory for Quality Assurance Br, 6502 S. Archer Rd, Summit-Argo, IL 60501-1399)

Mycotoxins (E-33): Peter M. Scott (Health & Welfare Canada, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario K1A 0L2, Canada); Stanley Nesheim (U.S. Food & Drug Administration, Office of Plant and Dairy Foods & Beverages, 200 C St, SW, Washington, DC 20204)

Free Fatty Acids (E-39): Robert Bradley (University of Wisconsin-Madison, Dept of Food Science, 1605 Linden Dr, Madison, WI 53706)

Fat (E-40): Dick H. Kleyn (Rutgers University, Dept of Food Science, PO Box 231, New Brunswick, NJ 08903-0231)

Preservatives (E-43): B. Denis Page (Health & Welfare Canada, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario K1A 0L2, Canada); Robert G. Ackman (Canada Institute of Fisheries Technology, Technical University of Nova Scotia, PO Box 1000, Halifax, Nova Scotia B3J 2X4, Canada)

Vitamin A (E-46): Ellen J. DeVries (Duphar BV, PO Box 900, 1380 DA Weesp, The Netherlands); Shu-Guang Greg Cheng (Kraft General Foods, Tech Center, 801 Waukegan Rd, Glenview, IL 60025)

Antibiotics (E-47): Stanley E. Charm (Charm Sciences, 36 Franklin St, Malden, MA 02148); Larry J. Maturin (U.S. Food & Drug Administration, Laboratory for Quality Assurance Br, 6502 S. Archer Rd, Summit-Argo, IL 60501-1399)

Variable Counts (E-48): J.D. Cunningham (University of Guelph, Environmental Biology, Guelph, Ontario N1G 2W1, Canada)

Milkfat in Mixtures (E-49): Robert Bradley (University of Wisconsin-Madison, Dept of Food Science, 1605 Linden Dr, Madison, WI 53706)

Sampling Techniques (E-50): Wallace H. Andrews & George Jackson (U.S. Food & Drug Administration, Office of Special Research Skills, 200 C St, SW, Washington, DC 20204); Larry J. Maturin (U.S. Food & Drug Administration, Laboratory of Quality Assurance Br, 6502 S. Archer Rd, Summit-Argo, IL 60501-1399)

Phosphatase (E-52): Richard M. Rocco (Idetek, Inc., 1057 Sneath Ln, San Bruno, CA 94066); Dick H. Kleyn (Rutgers University, Dept of Food Science, PO Box 231, New Brunswick, NJ 08903-0231); Gopala Murthy (1615 Northwood Dr, Cincinnati, OH 45237)

Functional Properties of Milk Protein Products (E-55): Robert Bradley (University of Wisconsin-Madison, Dept of Food Science, 1605 Linden Dr, Madison, WI 53706)

Heat Resistant Protein (E-62): Shu-Guang Greg Cheng (Kraft General Foods, Tech Center, 801 Waukegan Rd, Glenview, IL 60025)

Listeria (E-64): Robert M. Twedt (U.S. Food & Drug Administration, 200 C St, SW, Washington, DC 20204); Anthony D. Hitchins (U.S. Food & Drug Administration, Office of Special Research Skills, 200 C St, SW, Washington, DC 20204)

Robotics (E-66): Robert Bradley (University of Wisconsin-Madison, Dept of Food Science, 1605 Linden Dr, Madison, WI 53706)

International Organization for Standardization (ISO)

Advisory Group to ISO/TC 34 Agriculture Food Products: Margreet Lauwaars (PO Box 153, 6720 AD Bennekom, The Netherlands)

Agricultural Food Products (ISO/TC34): George R. Heavner (AOAC International, 2200 Wilson Blvd, Suite 400, Arlington, VA 22201-3301), Liaison Coordinator

Oleaginous Seeds & Fruits (ISO/TC34/SC2): Gary R. List (U.S. Dept of Agriculture, Northern Regional Research Center, 1815 N. University St, Peoria, IL 61604)

Derived Products of Fruits & Vegetables (ISO/TC34/SC3): Edgar R. Elkins (National Food Processors Association, 1401 New York Ave, NW, Suite 400, Washington, DC 20005)

Cereals & Pulses (ISO/TC34/SC4): Ralph H. Lane (University of Alabama, Dept of Food, Nutrition, & Institutional Management, PO Box 870158, Tuscaloosa, AL 35487-0158); Raymond Tarleton (American Association of Cereal Chemists, 3340 Pilot Knob Rd, St. Paul, MN 55121)

Milk & Milk Products (ISO/TC34/SC5): Robert Bradley (University of Wisconsin-Madison, Dept of Food Science, 1605 Linden Dr, Madison, WI 53607)

Meat & Meat Products (ISO/TC34/SC6): David Soderberg (U.S. Dept of Agriculture, Food Safety & Inspection Service, Rm 519, Annex Bldg, 300 12th St, SW, Washington, DC 20250), Chemistry; Charles P. Lattuada (U.S. Dept of Agriculture, Food Safety & Inspection Service, Bldg 302, BARC East, 10300 Baltimore Ave, Beltsville, MD 20705-2350), Microbiology

Spices & Condiments (ISO/TC34/SC7): James E. Woodbury (14282 Holt Ave, Santa Ana, CA 92705)

Microbiology (ISO/TC34/SC9): Wallace H. Andrews (U.S. Food & Drug Administration, Office of Special Research Skills, 200 C St, SW, Washington, DC 20204)

Animal Feeding Stuffs (ISO/TC34/SC10): Franklin E. Barton, II (U.S. Dept of Agriculture, Richard B. Russell Research Center, 950 College Station Rd, Box 5677, Athens, GA 30613)

Animal & Vegetable Fats & Oils (ISO/TC34/SC11): David Firestone (U.S. Food & Drug Administration, Office of Plant and Dairy Foods & Beverages, HFF-426, 200 C St, SW, Washington, DC 20204)

Dried Fruits & Vegetables (ISO/TC34/SC13): Frank A. Mosebar (DFA of California, Box 270-A, Santa Clara, CA 95052)

Fresh Fruits & Vegetables (ISO/TC34/SC14): Clarence E. Harris (U.S. Dept of Agriculture, Marketing Research Branch, PO Box 96456, Washington, DC 20090-6456)

Coffee (ISO/TC34/SC15): Shirley Wright (U.S. Dept of Agriculture, Science Division, PO Box 96456, Washington, DC 20090-6456)

Starch (ISO/TC93): Donald B. Whitehouse (Cerestar SA NV, Havenstraat 84, B1800 Vilvoorde, Belgium)

Fertilizers (ISO/TC134): Robert C. Rund (Purdue University. Dept of Biochemistry, West Lafayette, IN 47907); Peter F. Kane (Purdue University, Dept of Biochemistry, West Lafayette, IN 47907)

Soil Quality (ISO/TC190): Thomas L. Jensen (Nebraska State Dept of Agriculture, 3703 S. 14th St, Lincoln, NE 68502)

International Union of Pure & Applied Chemistry: Albert E. Pohland (U.S. Food & Drug Administration, 200 C St, SW, Washington, DC 20204); Food Chemistry Commission, David Firestone (U.S. Food & Drug Administration, Office of Plant and Dairy Foods & Beverages, HFF-426, 200 C St, SW, Washington, DC 20204)

Association of American Plant Food Control Officials:

Magruder Committee: Ronald R. Christensen (AOAC International, 2200 Wilson Blvd, Suite 400, Arlington, VA 22201-3301)

Organization International du Cacao et du Chocolat: Open

International Organization for Legal Metrology: Eugene H. Klesta (Chemical Waste Management, Inc., 4300 W. 123rd St, Alsip, IL 60658)

Pharmaceutical Manufacturers Association — Vitamin E Committee: Alan J. Sheppard (U.S. Food & Drug Administration, Washington, DC 20204)

U.S. Pharmacopeial Convention: Thomas P. Layloff (U.S. Food & Drug Administration, National Center for Drug Analysis, 1114 Market St, St. Louis, MO 63101)

Committee on Pesticide Formulations and Disinfectants

Richard H. Collier (CERIS, 1231 Cumberland Ave, Suite A, West Lafayette, IN 47906-1317); *Chairman*; **Warren Bontoyan** (Maryland Dept of Agriculture State Chemist, 50 Harry S. Truman Pkwy, Annapolis, MD 21401); **Peter D. Bland** (ICI Americas Inc., Western Res. Center, 1200 S. 47th St, PO Box 4023, Richmond, CA 94804-0023); **Dwight Harder** (Arizona Dept of Agriculture, 1688 W. Adams, Phoenix, AZ 85007-2617); **Jo Gillespie** (Ministry of Agriculture, Fisheries & Food, Central Science Laboratory, Hatching Green, Harpenden, Hartfordshire AL5 2BD, UK); **Walter W. Bond** (Hospital Environment Laboratory Branch, Hospital Infections Program, National Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA 30333); **Arthur H. Hofberg, Jr** (Ciba Geigy Corp., Analytical Section, Production Technology Dept, PO Box 18300, Greensboro, NC 27419), *Secretary*; **Margaret A. Nemeth** (Monsanto Co., T2A, 800 N. Lindbergh Blvd, St. Louis, MO 63167), *Committee Statistician*; **Harry Ostapenko** (Heinz USA, 1062 Progress St, Pittsburgh, PA 15212), *Safety Advisor*

CIPAC Studies

Referee:
Alan R. Hanks, Office of the Indiana State Chemist, Purdue University, West Lafayette, IN 47909

Bentazon (Basagran)
Thomas M. Schmitt, BASF Corp., Manager Analytical Research, 1419 Biddle Ave, Wyandotte, MI 48192-3736

Cyhexatin (CIPAC method)

Cyanazine (CIPAC method)

DDT (CIPAC method)

Deltamethrin (CIPAC method)

Maneb in Fentin Formulations (CIPAC method)

Methamidophos (Monitor) (CIPAC method)

Phosphamidon (CIPAC method)

Pirimiphos-Methyl (CIPAC method)

Disinfectants

Referee:
Aram Beloian, U.S. Environmental Protection Agency, H7503W, 401 M St, SW, Washington, DC 20460

Hard-Surface Carrier Tests
Joseph R. Rubino, Lehn & Fink Product Co., 225 Summit Ave, Montvale, NJ 07645

Tuberculocidal Tests
Donna Suchmann, MicroBioTest, Inc., 14280 Sully Field Circle, Suite 200, Chantilly, VA 22021

Virucidal Tests
Bonita Baskin, ViroMed Laboratories, Inc., 5500 Feltl Rd, Minnetonka, MN 55343

Pesticide Formulations: Fungicides & Rodenticides

Referee: Open

Benomyl
Mikio Chiba, Agriculture Canada, Research Station, Vineland Station, Ontario LOR 2EO, Canada

Carboxin & Oxycarboxin
Milton Parkins, Uniroyal Chemical Co., Crop Protection Analytical Div., Elm St, Maugatuck, CT 06770

Chlorothalonil
Gregory E. Walls, Recerca, Inc., 7528 Auburn Rd, PO Box 1000, Painesville, OH 44077

Dithiocarbamate Fungicides
Warren R. Bontoyan, Maryland Dept of Agriculture, University of Maryland, Chemical Bldg, Rm 0233, College Park, MD 20742

Tebuconazole (Folicur)
James W. Baird, Miles Inc., Hawthorne Rd, Box 4913, Kansas City, MO 64120

Pesticide Formulations: Herbicides

Referee:
David F. Tomkins, Monsanto Co., PO Box 473, Muscatine, IA 52761

Alachlor, Butachlor, & Propachlor
David F. Tomkins, Monsanto Co., PO Box 473, Muscatine, IA 52761

Atrazine/Alachlor Mixtures
David F. Tomkins, Monsanto Co., PO Box 473, Muscatine, IA 52761

Bromoxynil
Laurence J. Helfant, Rhone Poulenc Ag Co., PO Box 12014, Research Triangle Park, NC 27709

Chlorophenoxy Herbicides
Richard D. Larson, South Dakota State University, Chemistry Dept, Shepard Hall, Brookings, SD 57007

Dicamba
Benjamin A. Belkind, Sandoz Crop Protection Corp., 1300 E. Touhy Ave, Des Plaines, IL 60018

Fluometuron
Arthur H. Hofberg, Ciba-Geigy Corp., Analytical Section, PO Box 18300, Greensboro, NC 27419

Glyphosate
Lynn W. Morlier, Monsanto Co., Central Laboratory, PO Box 174, Luling, LA 70070

Methazole
Benjamin A. Belkind, Sandoz Crop Protection Corp., 1300 E. Touhy Ave, Des Plaines, IL 60018

Pesticide Formulations: Other Insecticides, Synergists, & Repellents

Referee:
Stephen C. Slahck, Miles Inc., Hawthorn Rd, PO Box 4913, Kansas City, MO 64120

Allethrin
Dean Kassera, McLaughlin Gormley King Co., 8810 Tenth Ave, N, Minneapolis, MN 55427

Azinphos-Methyl (Guthion)
Karin A. Mede, Rhone Poulenc Ag Co., Box 12014, Research Triangle Park, NC 27709

Cyfluthrin
Stephen C. Slahck, Miles Inc., Hawthorn Rd, PO Box 4913, Kansas City, MO 64120

Cyromazine (Larvadex)
Arthur H. Hofberg, Ciba-Geirgy Corp., Agriculture Div., PO Box 18300, Greensboro, NC 27419

Dipropyl Isocinchomeronate (MGK Repellant 326)
Dave Carlson, McLaughlin Gormley King Co., 8810 Tenth Ave, Minneapolis, MN 55427

Ethoprop
James Smith, Rhone-Poulenc, Inc., Box 352, Mt Pleasant, TN 38474; J.F. Lowder, Rhone-Poulenc, Inc., Box 12014, Research Triangle Park, NC 27709

Fenamiphos (Nemacur)
James E. Conaway, Jr, E.I. du Pont de Nemours & Co., Inc., DuPont Experimental Station, Bldg 402, PO Box 80402, Wilmington, DE 19880-0402

Methomyl
James E. Conaway, Jr, E.I. du Pont de Nemours & Co., Inc., DuPont Experimental Station, Bldg 402, PO Box 80402, Wilmington, DE 19880-0402

Oxydemeton-Methyl (Metasystox-R)
Stephen C. Slahck, Miles Inc., Hawthorn Rd, PO Box 4913, Kansas City, MO 64120

Oxamyl
Glenn A. Sherwood, Jr, E.I. du Pont de Nemours & Co., Inc., DuPont Experimental Station, Bldg 402, PO Box 80402, Wilmington, DE 19880-0402

Committee on Drugs and Related Topics

John O'Rangers (U.S. Food & Drug Administration, Center for Veterinary Medicine, MPN II, HFV-142, 7500 Standish Place, Rockville, MD 20855), *Chairman*; **G.D. Anthony** (Warner-Lambert Co., Parke-Davis Div., 170 Tabor Rd, Morris Plains, NJ 07950); **Mark G. Torchia** (St. Boniface General Hospital, Dept of Surgery, 409 Tache Ave, Winnipeg, Manitoba P2H 2A6, Canada); **Terry Gilbertson** (The Upjohn Co., Animal Health Drug Metabolism, 7000 Portage Rd, Kalamazoo, MI 49001-0199); **Richard M. Montgomery** (Unilever Research US, 45 River Rd, Edgewater, NJ 07020); **William W. Wright** (U.S. Pharmacopeia, 12601 Twinbrook Pkwy, Rockville, MD 20852), *Secretary*; **Robert J. Condon** (U.S. Food & Drug Administration, Center for Veterinary Medicine, MPN II, Rm N344, HFV-120, 7500 Standish Place, Rockville, MD 20855), *Committee Statistician*; **Mark G. Torchia** (St. Boniface General Hospital, Dept of Surgery, 409 Tache Ave, Winnipeg, Manitoba P2H 2A6, Canada), *Safety Advisor*

Drugs I

Referee: Open

Drugs II

Referee:
Edward Smith, 14203 Castaway Dr, Rockville, MD 20853

Aminacrine
Elaine A. Bunch, U.S. Food & Drug Administration, PO Box 3012, 22201 23rd Dr, SE, Bothell, WA 98021

Antihistamines in Combination with Decongestants by HPLC
Raja Achari, Bristol Myers Products, 1350 Liberty Ave, Hillside, NJ 07207

Belladona Alkaloids
Luis W. Levy, PO Box 17-08-8354, Quito, Ecuador

Cloramphenicol in Egg
Henk J. Keukens, RILKILT, PO Box 230, 6700 AE Wageningen, The Netherlands

Colchicine in Tablets
Richard D. Thompson, U.S. Food & Drug Administration, 240 Hennepin Ave, Minneapolis, MN 55401

Drugs III

Referee: Open

Drugs IV

Referee:
Linda Ng, U.S. Food & Drug Administration, HFD-150, 5600 Fishers Ln, Rockville, MD 20857

Alendone in Combination with Clorthalidone
Everett H. Jefferson, U.S. Food & Drug Administration, 1114 Market St, St. Louis, MO 63101

Benzodiazepines
Eileen Bargo, U.S. Food & Drug Administration, 900 Madison Ave, Baltimore, MD 21201

Dicloxacillin Bulk Drug & Its Preparations
Mei Chich Hsu, Food & Drug Bureau, Dept of Health, 161 Kuen Yang St, Nankang, Taipai 11513, Taiwan, ROC

Guaifenesin
Myron Rhodes, U.S. Food & Drug Administration, 1114 Market St, St. Louis, MO 63101

Heroin
Charles C. Clark, Drug Enforcement Administration, 5205 NW 84th Ave, Miami, FL 33166

Miconazole Nitrate
Judith Genzale, Tilloson Rubber Co., 5 Curtis St, Saugus, MA 01906

Piroxican in Pharmaceutical Preparations
Maria Ines Rocha Miritello Santoro, Faculdade de Ciencias Farmaceuticas, Universidade de Sao Paulo, Caixa Postal 66355, Sao Paulo, SP CEP 05389 Brazil

Terbutaline
William E. Jull, U.S. Food & Drug Administration, 1114 Market St, St. Louis, MO 63101

Terfenadine
Lettie J. Travis, U.S. Food & Drug Administration, 1114 Market St, St. Louis, MO 63101

Drugs V

Referee:
Thomas G. Alexander, 16716 Huron St, Accokeek, MD 20607

Aminobenzoic Acid and Salicylic Acid Salts in Pharmaceuticals
Richard Thompson, U.S. Food & Drug Administration, 240 Hennepin Ave, Minneapolis, MN 55401

Cromolyn Sodium in Bulk and Dosage Forms
Linda Ng, U.S. Food & Drug Administration, HFD-150, 5600 Fishers Ln, Rockville, MD 20857

Pentaerythritol Tetranitrate
Marvin Carlson, U.S. Food & Drug Administration, 240 Hennepin Ave, Minneapolis, MN 55401

Steroids in Tablets
Elaine Bunch, U.S. Food & Drug Administration, PO Box 3012, 22201 23rd Dr, SE, Bothell, WA 98021

Diagnostics & Test Kits

Referee: Open

Drug Residues in Animal Tissues

Referee:
Charlie J. Barnes, U.S. Food & Drug Administration, Office of Science, HFV-501, Bldg 328A, BARC East, Beltsville, MD 20705

β-Lactam Antibiotics in Milk by LC (Qualitative)
William A. Moats, U.S. Dept of Agriculture, Meat Science Research Laboratory, ARS, Bldg 201, BARC East, Beltsville, MD 20705

β-Lactam Residues in Milk by Delvotest
Wesley N. Kelly, South Dakota State Laboratory, South Dakota State University, Dairymicro Bldg, PO Box 2104, Brookings, SD 57007-0647

Multi-Residue Chromatographic Procedures for Sulfonamides in Milk
Michael D. Smedley, U.S. Food & Drug Administration, Div. of Veterinary Medical Research, HFV-501, BARC East, Center Rd, Bldg 328A, Beltsville, MD 20705

Quantitative Determination of β-Lactams in Milk by Competitive Microbial Receptor Assay
Stanley E. Charm, Charm Sciences, Inc., 36 Franklin St, Malden, MA 02148

Sulfamethazine in Plasma & Serum
David Allison, Idetek, Inc., 1057 Sneath Ln, San Bruno, CA 94066

Tetracyclines in Tissues by LC
James D. MacNeil, Agriculture Canada, Health of Animals Laboratory, 116 Veterinary Rd, Saskatoon, Saskatchewan S7N 2R3, Canada

Cosmetics

Referee:
Rhonda S. Bayoud, Mary Kay Cosmetics, 8787 Stemmons Freeway, Dallas, TX 75247

Forensic Sciences

Referee:
Stanley M. Cichowicz, U.S. Food & Drug Administration, Div. of Microanalytical Evaluations, 200 C St, SW, Washington, DC 20204

Committee on Additives, Beverages, and Food Process Related Analytes

Donald E. Carpenter (Kraft General Foods, 801 Waukegan Rd, Glenview, IL 60025), *Chairman*; **Elia D. Coppola** (Ocean Spray Cranberries, Inc., Food Research, 1 Ocean Spray Dr, Lakeville Middleboro, MA 02349); **Walter Fiddler** (U.S. Dept of Agriculture, Eastern Regional Research Center, 600 E. Mermaid Ln, Philadelphia, PA 19118); **Laura L. Zaika** (U.S. Dept of Agriculture, Eastern Regional Research Center, 600 E. Mermaid Ln, Philadelphia, PA 19118); **Harriet Wallin** (VTT Technical Research Center of Finland, Food Research Laboratory, Biologinkuja 1, PO Box 203, SF-02150 Espoo, Finland); **B. Denis Page** (Health & Welfare Canada, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario K1A OL2, Canada); **Benjamin Krinitz** (Whitestone Laboratories, 147-27 Seventh Ave, Whitestone, NY 11357-1620), *Secretary*; **John G. Phillips** (U.S. Dept of Agriculture, Eastern Regional Research Center, 600 E. Mermaid Ln, Philadelphia, PA 19118), *Committee Statistician*; **Dirk Shoemaker** (Nebraska Dept of Agriculture, 3703 S. 14th St, Lincoln, NE 68502), *Safety Advisor*

Beverage Alcohol

Referee:
Randolph H. Dyer, Bureau of Alcohol, Tobacco & Firearms, 1401 Research Blvd, Rockville, MD 20850

Alcohol Content
Samuel I. Blittman, Bureau of Alcohol, Tobacco & Firearms, 355 N. Wiget Ln, Walnut Creek, CA 94598

Ascorbic Acid in Wine
Bruno Trombella, E & J Gallo Winery, PO Box 1130, Modesto, CA 95353

Carbon Dioxide in Wine
Arthur Caputi, Jr, E & J Gallo Winery, PO Box 1130, Modesto, CA 95353

Ethanol in Wine by GLC
Arthur Caputi, Jr, E & J Gallo Winery, PO Box 1130, Modesto, CA 95353

Ethyl Carbamate in Alcoholic Beverages
Benjamin J. Canas, U.S. Food & Drug Administration, Div. of Natural Products, 200 C St, SW, Washington, DC 20204

Glycerol Monooleates in Wine
Tony Ribeiro, E & J Gallo Winery, PO Box 1130, Modesto, CA 95353

Malic Acid in Wine
David T. Chia, U.S. Custom Service Laboratory, Rm 1429, 630 Sansome, San Francisco, CA 94111

Malt Beverages & Brewing Materials
Mark H. Schwiesow, Pfizer, Inc., 4215 N. Port Washington Ave, Milwaukee, WI 53212

Polydimethylsiloxane in Wine
Durward Ray Walker, E & J Gallo Winery, PO Box 1130, Modesto, CA 95353

Sorbic Acid in Wine
Arthur Caputi, Jr, E & J Gallo Winery, PO Box 1130, Modesto, CA 95353

Sugars in Wine by Enzymatic Methods
Guenther Henniger, Boehringer Mannheim GmbH, Bahnhofstrasse 9-15, 8132 Tutzing O88 Postfach 120, Germany

Sulfur Dioxide in Wine by Ripper Method
Barry H. Gump, California State University-Fresno, Dept of Chemistry, Fresno, CA 93740-0070

Synthetic Colors
John Steele, 13305 Burkhart St, Silver Spring, MD 20904

Tartrates in Wine
Masao Ueda, E & J Gallo Winery, PO Box 1130, Modesto, CA 95353

Food Additives

Referee:
Thomas Fazio, U.S. Food & Drug Administration, Office of Scientific Analysis & Support, 200 C St, SW, Washington, DC 20204

Antioxidants
B. Denis Page, Health & Welfare Canada, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario K1A 0L2, Canada

Antioxidants in Foods
Charles R. Warner, U.S. Food & Drug Administration, Div. of Product Manufacture & Use, 200 C St, SW, Washington, DC 20204

Identification of Irradiated Foods
Leslie G. Ladomery, FAO/IAEA Div. of Nuclear Techniques in Food & Agriculture, Wagramerstrasse 5, PO Box 200, A-1400 Vienna, Austria

Indirect Additives from Food Packages
Henry Hollifield, U.S. Food & Drug Administration, 200 C St, SW, Washington, DC 20204

Nitrosamines
Nisu P. Sen, Health & Welfare Canada, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario K1A 0L2, Canada

Polycyclic Aromatic Hydrocarbons
Frank L. Joe, U.S. Food & Drug Administration, Div. of Product Manufacture and Use, 200 C St, SW, Washington, DC 20204

Polydimethylsiloxane
R. Firmin, 70 Rue St Georges, 1050 Brussels, Belgium

Urethane in Foods
Benjamin J. Canas, U.S. Food & Drug Administration, Div. of Natural Products, 200 C St, SW, Washington, DC 20204

Flavors

Referee:
Dana A. Krueger, Krueger Food Laboratories, Inc., 24 Blackstone St, Cambridge, MA 02139

Additives in Vanilla
Sidney Kahan, Kahansultants, Inc., 66 Peachtree Ln, Roslyn Heights, NY 11577

C-14 in Flavoring Materials
Dana A. Krueger, Krueger Food Laboratories, Inc., 24 Blackstone St, Cambridge, MA 02139

Licorice Products
Peter S. Vora, McAndrews & Forbes Co., Third St & Jefferson Ave, Camden, NJ 08104

Vanillin & Ethyl Vanillin
Sidney Kahan, Kahansultants, Inc., 66 Peachtree Ln, Roslyn Heights, NY 11577

Vanillin by Deuterium NMR Analysis
Glenn E. Martin, Martin Associates, 4517 N. Carlin Springs Rd, Arlington, VA 22203

Color Additives

Referee: Open

Anthocyanin Color Additives Exempt from Certification
Ronald E. Wrolstad, Oregon State University, Dept of Food Science & Technology, Corvallis, OR 97331

Arsenic, Barium & Heavy Metals
Nancy Hepp, U.S. Food & Drug Administration, Div. of Science Applied Technology, 200 C St, SW, Washington, DC 20204

Colors in Candy & Beverages
Mary Young, U.S. Food & Drug Administration, New York Regional Laboratory, HFF-2660, 850 Third Ave, Brooklyn, NY 11232

Uncombined Intermediates & Subsidiary Colors in Certifiable Colors
Alan Scher, U.S. Food & Drug Administration, 200 C St, SW, Washington, DC 20204

Nonalcoholic Beverages

Referee:
John M. Newton, U.S. Food & Drug Administration, HFR-9160, 50 Fulton St, San Francisco, CA 94102

Ash in Instant Tea
Francis J. Farrell, Thomas J. Lipton, Inc., Analytical Services Dept, 800 Sylvan Ave, Englewood Cliffs, NJ 07632

Coumarin in Vanillin Beverages
Richard D. Thompson, U.S. Food & Drug Administration, 240 Hennepin Ave, Minneapolis, MN 55401

Cyclamate in Cola
Latif Kahn, Royal Crown Cola, 1000 Tenth St, Columbus, GA 31902

Decaffeinated Coffee & Tea—Solvent Residues
B. Denis Page, Health & Welfare Canada, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario K1A 0L2, Canada

Methyl Xanthines in Coffee & Tea
John M. Newton, U.S. Food & Drug Administration, HFR-9160, 50 Fulton St, San Francisco, CA 94102

Safrole in Sassafras Root
Marvin Carlson, U.S. Food & Drug Administration, 240 Hennepin Ave, Minneapolis, MN 55401

Spices & Other Condiments

Referee:
James E. Woodbury, 14282 Holt Ave, Santa Ana, CA 92705

Capsicum Spices & Oleo Resins—Extractable Color
James E. Woodbury, 14282 Holt Ave, Santa Ana, CA 92705

Capsicum Spices & Oleo Resins—Pungency
Mark Parrish, McCormick & Co, 202 Wight Ave, Hunt Valley, MD 21031

Curcumin in Turmeric
Ted Lupina, Kalsec, Inc., 3711 W. Main St, PO Box 511, Kalamazoo, MI 49005

Moisture in Dried Spices
Louis A. Sanna, Santa Maria Chili, Inc., PO Box 6013, Santa Maria, CA 93456

Steam Volatile Oil in Cassia
Philip Guarino, McCormick & Co., Inc., 11350 McCormick Rd, Hunt Valley, MD 21031

Vinegar
Dana A. Krueger, Krueger Food Laboratories, Inc., 24 Blackstone St, Cambridge, MA 02139

Water Activity of Spices
Susan Schena, Cal Compak Foods, PO Box 265, 4906 First St, Santa Ana, CA 92702

Volatile Oil in Spices
Fran S. Ertl, Botanicals International, 2550 El Presido St, Long Beach, CA 90810-1193

Committee on Natural Toxins

Peter M. Scott (Health & Welfare Canada, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario K1A OL2, Canada), *Chairman*; **Glenn A. Bennett** (U.S. Dept of Agriculture, Northern Regional Research Center, 1815 N. University, Peoria, IL 61604); **John Gilbert** (Ministry of Agriculture, Fisheries & Food, Food Safety Directorate, Colney Ln, Norwich, NR4 7UQ, UK); **Hans P. van Egmond** (National Institute of Public Health & Environmental Protection, PO Box 1, 3720 BA Bilthoven, The Netherlands); **Marleen M. Wekell** (U.S. Food & Drug Administration, 22201 23rd Dr SE, PO Box 3012, Bothell, WA 98041-3012); **Michael W. Gilgan** (Dept of Fisheries and Oceans, Scientific and Technical Services Laboratory, PO Box 550, Halifax, Nova Scotia B3J 2S7, Canada); **Samuel W. Page** (U.S. Food & Drug Administration, Div. of Natural Products, 200 C St, SW, Washington, DC 20204), *Secretary*; **Terry Nelsen** (USDA/NRRC/ARS, 1815 University St, Peoria, IL 61604), *Committee Statistician*; **Sandra Pfeiffer** (Gerber Products Co., 445 State St, Fremont, MI 49412), *Safety Advisor*

Mycotoxins

Referee:
Mary W. Trucksess, U.S. Food & Drug Administration, Div. of Natural Products, 200 C St, SW, Washington, DC 20204

Aflatoxin M
Hans van Egmond, Laboratory for Residue Analysis, National Institute of Public Health & Environmental Protection, Antonie van Leeuwenhoeklan 9, PO Box 1, 3720 Biltohoven, The Netherlands

Alternaria Toxins
Angelo Visconti, Istituto Tossine e Micotossine da Parassiti Vegetali, Consiglio Nazionale Delle Ricerche, 197/F Via G. Amendola, 70125 Bari, Italy

Citrinin
David Wilson, University of Georgia, Dept of Plant Pathology, Tifton, GA 31794

Cyclopiazonic Acids
Joseph W. Dorner, U.S. Dept of Agriculture, ARS, National Peanut Research Laboratory, 1011 Forrester Dr, SE, Dawson, GA 31742

Ergot Alkaloids
George Ware, U.S. Food & Dmg Administration, New Orleans District, 4298 Elysian Fields Ave, New Orleans, LA 70128

Fumonisins
Ronald D. Plattner, U.S. Dept of Agriculture, Northern Regional Research Center, 1815 N. University, Peoria, IL 61604

Immunochemical Methods
James J. Pestka, Michigan State University, Dept of Food Science & Nutrition, East Lansing, MI 48824

Ochratoxins
Stanley Nesheim, U.S. Food & Drug Administration, Office of Plant and Dairy Foods & Beverages, 200 C St, SW, Washington, DC 20204

Patulin in Apple Juice
Alan Brause, Analytical Chemical Service of Columbia, Inc., 9151 Ramsey Rd, Suite 190, Columbia, MD 21045

Trichothecenes
Robert M. Eppley, U.S. Food & Drug Administration, Div. of Natural Products, 200 C St, SW, Washington, DC 20204

Zearalenone
Glenn A. Bennett, U.S. Dept of Agriculture, Northern Regional Research Center, 1815 N. University, Peoria, IL 61604

Plant Toxins

Referee:
Joseph M. Betz, U.S. Food & Drug Administration, Div. of Natural Products, 200 C St, SW, Washington, DC 20204

Glucosinolates
Douglas I. McGregor, Agriculture Canada, 107 Science Crescent, Saskatoon, Saskatchewan S7N 0X2, Canada

Hydrazines
Joseph M. Betz, U.S. Food & Drug Administration, Div. of Natural Products, 200 C St, SW, Washington, DC 20204

Phytoestrogens
Shia S. Kuan, U.S. Food & Drug Administration, 4298 Elysian Fields Ave, New Orleans, LA 70122

Pyrrolizidine Alkaloids
Robert M. Eppley, U.S. Food & Drug Administration, Div. of Natural Products, 200 C St, SW, Washington, DC 20204

Steroidal Alkaloids
Allen S. Carman, U.S. Food & Drug Administration, 4298 Elysian Fields Ave, New Orleans, LA 70122

Seafood Toxins

Referee:
James M. Hungerford, U.S. Food & Drug Administration,

Seafood Products Research Center, 22201 23rd Dr, SE, PO Box 3012, Bothell, WA 98041-3012

Cell Bioassays
Ronald Manger, U.S. Food & Drug Administration, Seafood Products Research Center, 22201 23rd Dr, SE, PO Box 3012, Bothell, WA 98041-3012

Ciguatoxins—Biochemical Methods
Douglas Park, University of Arizona, Dept of Nutrition & Food Science, 309 Shantz Bldg, Tucson, AZ 85721

Ciguatera by HPLC
Robert W. Dickey, U.S. Food & Drug Administration, Fishery Research Branch, No. 1 Iberville, PO Box 158, Dauphin Island, AL 36528

Cyanobacterial Peptide Toxins
Judith Pace, U.S. Army Medical Research Institute of Infectious Diseases, Pathophysiology & Pathobiology, Fort Detrick, Frederick, MD 21702

Diarrhetic Shellfish Poisons
Takeshi Yasumoto, Tokoku University, Faculty of Agriculture, Dept of Food Chemistry, Tsutsumidori, Sendai 980, Japan

Domoic Acid
Michael A. Quilliam, Institute for Marine Biosciences, 1411 Oxford St, Halifax, N.S. B3H 3Z1

Flow Injection Analysis
James M. Hungerford, U.S. Food & Drug Administration, Seafood Products Research Center, 22201 23rd Dr, SE, PO Box 3012, Bothell, WA 98041-3012

Neurotoxic Shellfish Poisons
Daniel G. Baden, Rosenthal School of Marine & Atmospheric Sciences, 4600 Rickenbacker Causeway, Miami, FL 33149

Paralytic Shellfish Poison by HPLC
Sherwood Hall, U.S. Food & Drug Administration, HFF-423, 200 C St, SW, Washington, DC 20204

Committee on Food Nutrition

Jonathan W. DeVries (General Mills Inc., 9000 Plymouth Ave, Minneapolis, MN 55427), *Chairman*; **Randy Smith** (Hazleton Laboratories America, Inc., 3301 Kinsman Blvd, PO Box 7545, Madison, WI 53707); **Sungsoo Lee** (Kellogg Co., 235 Porter St, PO Box 3423, Battle Creek, MI 49016-3423); **James T. Tanner** (U.S. Food & Drug Administration, Div. of Programs and Enforcement Policy, 200 C St, SW, Washington, DC 20204); **W.O. Landen** (U.S. Food & Drug Administration, Atlanta Regional Laboratory, 60 Eighth St, NE, Atlanta, GA 30309); **Michele Bono** (Food Laboratory, 1001 St. Lawrence, Longueuil, Quebec J4K 1C7, Canada); **Douglas J. Bark** (Woodson Tenent Laboratories, 345 Adams, Memphis, TN 38103), *Secretary*; **John G. Phillips** (USDA/ERRC, 600 E. Mermaid Ln, Philadelphia, PA 19118), *Committee Statistician*

Dietary Fiber

Referee:
Leon Prosky, 8301 Muirkirk Rd, Laurel, MD 20708

Dietary Fiber in Products with Little or No Starch, Non-Enzymatic Gravimetric Method
Betty Li, U.S. Dept of Agriculture, Rm 105, Bldg 161, BARC East, Beltsville, MD 20705

Dietary Fiber—Uppsala Method
Olof Theander, Swedish University of Agricultural Science, Dept of Chemistry, Box 7015 S, 75007 Uppsala, Sweden

Enzymatic Gravimetric Method
Sung Soo Lee, Kellogg Co., 235 Porter St, PO Box 3423, Battle Creek. MI 49016-3423

Fats & Oils

Referee:
David Firestone, U.S. Food & Drug Administration, Office of Plant and Dairy Foods & Beverages, HFF-426, 200 C St, SW, Washington, DC 20204

Emulsifiers
Theresa W. Lee, Ross Laboratories, Analytical Research & Services, 625 Cleveland Ave, Columbus, OH 43216

Hydrogenated Fats
W.M.N. Ratnayake, Health & Welfare Canada, Health Protection Branch, Banting Research Center, Ottawa, Ontario K1A 0L2, Canada

Marine Oils
Robert G. Ackman, Canadian Institute of Fisheries

Technology, Technical University of Nova Scotia, PO Box 1000, Halifax, Nova Scotia B3J 2X4, Canada

Olive Oil Adulteration
Enzo Fedeli, Experimental Station for Oils, via Giuseppe Colompc 79, 20133 Milano, Italy

Oxidized Fats
Michael M. Blumenthal, Libra Laboratory, Inc., 44 Stelton Rd, Piscataway, NJ 08854

Sterols & Tocopherols
Robert J. Reina, U.S. Food & Drug Administration, 1 Montvale Ave, Stoneham, MA 02180-3542

Infant Formula & Medical Diets

Referee:
Martin Bueno, U.S. Food & Drug Administration, Div. of Technical Evaluation, 200 C St, SW, Washington, DC 20204

Infant Formula Nutrient Assay
James T. Tanner, U.S. Food & Drug Administration, Div. of Programs & Enforcement Policy, 200 C St, SW, Washington, DC 20204

Vitamin D in Infant Formula
Matthew Sliva, Bristol-Myers-Squibb Co., 2400 W. Lloyd Expwy, Evansville, IN 47721

Minerals

Referee: Open

Sugars & Sugar Products

Referee:
Margaret A. Clarke, Sugar Processing Research, Inc., 1100 Robert E. Lee Blvd, New Orleans, LA 70124

Analysis of Oligosaccharides in Honeys As An Indicator of Authenticity
Jill Snowden, SGA Associates, 2236 Washington Ave, Suite 301, Silver Spring, MD 20910

Corn Syrup & Sugar Products
Raffaele Bernetti, CPC International, Inc., Moffett Technical Center, PO Box 345, Argo, IL 60501

Determination of Sugars in Syrups
Mary Ann Brannan, U.S. Customs Laboratory, 423 Canal St, New Orleans, LA 70130

Enzymatic Methods in Sugar Analysis
Guenther Henniger, Boehringer Mannheim GmbH, Bahnhofstrasse 9-15, 8132 Tutzing OBB Postfach 120, Germany

Glucoamylase (Amyloglucosidase Enzyme Activity)
Michael T. Elder, Novo Biochemical Industries, Inc., State Rd 1003, Box 576, Franklinton, NC 27525

Honey
Jonathan W. White, 217 Hillside Dr, Navasota, TX 77868

Lactose Purity Testing
Janice R. Saucerman, Bristol Myers Squibb, USPNG, 2404 Pennsylvania Ave, Evansville, IN 47721

Maple Sap, Maple Syrup, and Maple Products
Lynn Whalen, SMI Consulting, Inc., 1 Pamela Court, Willingston, VT 05945

Maple Syrup, Stable Isotope Ratio Analysis
Real Paquim, Quebec Ministry of Agriculture, Fisheries, & Food, 2700 rue Einstein, Ste. Foy, Quebec G1P 3W8, Canada

Methods Standardization
Mary A. Godshall, Sugar Processing Research, Inc., 1100 Robert E. Lee Blvd, PO Box 19687, New Orleans, LA 70179

NIR Analysis of Sugars
Cynthia McDonald-Lewis, NIR Systems, Inc., 12101 Tech Rd, Silver Spring, MD 20904

Oligosaccharides
George Steinle, Suddeutsche Zucker AG, Obrigheim 5, Postfach 1127, D-6718 Gruenstadt 1, Germany

Polarimetric Methods
Ronald Plews, Tate & Lyle Refineries, Thames Refinery, Silvertown, London E16 2EW, UK

Stable Isotope Ratio Analysis
Landis W. Doner, U.S. Dept of Agriculture, ARS, Eastern Regional Research Center, 600 E. Mermaid Ln, Philadelphia, PA 19118

Sugars in Cereals
Lou C. Zygmunt, Quaker Oats Co., 617 W. Main St, Barrington, IL 60010

Sulfites—Screening Methods
Richard Riffer, C&H Sugar Co., 830 Loring Ave, Crockett, CA 94525

Visual Appearance of Sugar by Color, Turbidity, & Reflectance
Margaret A. Clarke, Sugar Processing Research, Inc., 1100 Robert E. Lee Blvd, New Orleans, LA 70124

Weighing, Taring, & Sampling
Michael Steele, R. Markey & Sons, 5 Hanover Square, New York, NY 10004

Vitamins & Other Nutrients

Referee:
Mike J. Deutsch, U.S. Food & Drug Administration, Div. of Programs & Enforcement Policy, 200 C St, SW, Washington, DC 20204

Amino Acids
Robert Zumwalt, Cancer Research Center, 3501 Berrywood Dr, Columbia, MO 65201

Automated Methods
Jonathan W. DeVries, General Mills, Inc., 9000 Plymouth Ave N., Minneapolis, MN 55427

Carotene in Fruits and Vegetables by LC
Robert G. Stevenson, Campbell Soups Co., 66Z Campbell Place, Camden, NJ 08103

Carotenoids
Forrest W. Quackenbush, 2911 Browning St, West Lafayette, IN 47906

Cholesterol
Lori V. Klatt, Hazleton Laboratories America, Inc., 3301 Kinsman Blvd, PO Box 7545, Madison, WI 53707

Folic Acid
Natalie S. Zellmer, Hazelton Wisconsin, 3301 Kinsman Blvd, PO Box 7545, Madison, WI 53707

Iodine
Walter Holak, U.S. Food & Drug Administration, New York Regional Laboratory, HFF-2660, 850 Third Ave, Brooklyn, NY 11232

Sodium
Edgar R. Elkins, National Food Processors Association, 1401 New York Ave, NW, Suite 400, Washington, DC 20005

Thiamine—Enzyme & Column Packing Reagents
Wayne Ellefson, Hazleton Laboratories America, Inc., 3301 Kinsman Blvd, Box 7545, Madison, WI 53707

Vitamin D
Ellen J. DeVries, Duphar BV, PO Box 900, 1380 DA Weesp, The Netherlands

Vitamin E in Foods
Edward Waysek, Hoffmann-La Roche, Inc., Food & Agricultural Products, 340 Kingsland St, Nutley, NJ 07110

Vitamin E in Pharmaceuticals by GC
Alan J. Sheppard, U.S. Food & Drug Administration, Div. of Programs & Enforcement Policy, 200 C St, SW, Washington, DC 20204

Committee on Commodity Foods and Products

Jon E. McNeal (U.S. Dept of Agriculture, Agricultural Marketing Service, PO Box 96456, Washington, DC 20090-6456). *Chairman*; **Roger Wood** (Ministry of Agriculture, Fisheries & Food, Food Science Laboratory, Colney Ln, Norwich NR4 7UQ, UK); **Walter A. Hargraves** (U.S. Food & Drug Administration, 6502 S. Archer Rd, Summitt-Argo, IL 60501); **Paul R. Beljaars** (Inspectorate of Health Protection Dir Maastricht, Florijnruwe 111, 6218 CA, Maastricht, The Netherlands); **Chris Ellis** (Rhode Island Dept of Health, 50 Orms St, Providence, RI 02904); **Darryl M. Sullivan** (Hazleton Laboratories America, Inc., 3301 Kinsman Blvd, PO Box 7545, Madison, WI 53707); **Max L. Foster** (Kansas State Board of Agriculture, Div. of Laboratory, 2524 W. Sixth St, Topeka, KS 66606), *Secretary*; **Steve Malcolm** (Biostatistics & Computer Applications Div., Food Directorate, Banting Research Center, Tunney's Pasture, Ottawa, Ontario K1A OL2 Canada), *Committee Statistician*; **Sandra Pfeiffer** (Gerber Products Co., 445 State St, Fremont, MI 49412), *Safety Advisor*

Cereals & Cereal Products

Referee:
Ralph H. Lane, University of Alabama, Dept of Food, Nutrition, & Institutional Management, PO Box 870158, Tuscaloosa, AL 35487-0158

Beta-Glucan Methodology
Lou C. Zygmunt, Quaker Oats Co., 617 W. Main St, Barrington, IL 60010

Crude Protein by Combustion Analysis
Ronald C. Bicsak, U.S. Dept of Agriculture, Federal Grain Inspection Service, PO Box 20285, Kansas City, MO 64195-0285

Fat Acidity
Richard L. Erickson, Mississippi State Chemistry Laboratory, PO Box CR, Mississippi State, MS 39762

Gliaden in Gluten-Free Products
W. Hekkens, Duinwetering 27, 2203 HL Noordwijk, The Netherlands

Gluten in Foods
John H. Skerritt, CSIRO Wheat Research Unit, Div. of Plant

Industry, PO Box 7, North Ryde, New South Wales 2113, Australia

Iron
James I. Martin, U.S. Food & Drug Administration, 60 Eighth St, NE, Atlanta, GA 30309

Mineral Analysis
Yasmin H. Neggers, University of Alabama, Dept of Food & Nutrition, PO Box 870158, Tuscaloosa, AL 35487-0158

Near Infrared Reflectance Analysis for Cereal Products
Donald E. Koeltzow, U.S. Dept of Agriculture, Federal Grain Inspection Service, PO Box 20285, Kansas City, MO 64195-0285

Phytates
Barbara F. Harland, Howard University, School of Human Ecology, Dept of Human Nutrition & Food, Washington, DC 20059

Chocolate & Cacao Products

Referee: Open

Carbohydrates in Chocolate Products
W. Jeffrey Hurst & John C. Robbins, Hershey Foods Corp., 1025 Reese Ave, PO Box 805, Hershey, PA 17033

Shell in Cacao Products
W. Jeffrey Hurst & John C. Robbins, Hershey Foods Corp., 1025 Reese Ave, PO Box 805, Hershey, PA 17033

Dairy Chemistry

Referee:
Robert L. Bradley, University of Wisconsin-Madison, Food Science Dept, 1605 Linden Dr, Madison, WI 53706

Absolute Moisture Content of Dairy Foods
Douglas B. Emmons, Agriculture Canada, Centre for Food & Animal Research, Ottawa, Ontario K1A 0C6, Canada

Alkaline Phosphatase Activity in Dairy Foods
Linda Bates, Advanced Instruments, Inc., 100 Highland Ave, Needham Heights, MA 02194

Alkaline Phosphatase (Fluorometric)
Richard M. Rocco, Idetek, Inc., 1057 Sneath Ln, San Bruno, CA 94066

Babcock Test & Babcock Glassware
Robert L. Bradley, University of Wisconsin-Madison, Food Science Dept, 1605 Linden Dr, Madison, WI 53706

Cheese Moisture (Microwave)
Todd Kierstead, CEM Corp., PO Box 200, Matthews, NC 28106

Combustion Method for Protein
Joseph G. Sebranek, Iowa State University, Dept of Animal Science, 215 Meat Laboratory, Ames IA 50011

Components of Fluid Milk
David M. Barbano, Cornell University, Dept of Food Science, 105 Stocking Hall, Ithaca, NY 14853

FTIR Analysis of Dairy Foods
Frederick Van de Voort, McGill University, Dept of Food Science & Agricultural Chemistry, 111 Lakeshore Dr, Ste. Anne de Bellevue, Quebec HPX 1C0, Canada

Gerber Test
Dick H. Kleyn, Rutgers University, Dept of Food Science, PO Box 231, New Brunswick, NJ 08903-0231

Iodine in Milk
David Sertl, Ross Laboratories, 625 Cleveland Ave, Columbus, OH 43215

Moisture in Cheese
Robert L. Bradley, University of Wisconsin-Madison, Food Science Dept, 1605 Linden Dr, Madison, WI 53706

Nitrates in Cheese
James E. Hamilton, U.S. Food & Drug Administration, 5600 Fishers Ln, Rockville, MD 20857

Raw Milk Sampling
Dick T. Metzger, Foss Food Technology, 10355 W. 70th St, Eden Prairie, MN 55344

Robotic Mojonnier Method
Robert L. Bradley, University of Wisconsin-Madison, Food Science Dept, 1605 Linden Dr, Madison, WI 53706

Fruits & Fruit Products

Referee:
Frederick E. Boland, U.S. Food & Drug Administration, Div. of Programs & Enforcement Policy, 200 C St, SW, Washington, DC 20204

Apple Juice Adulteration
Thomas A. Eisele, Tree Top, Inc., PO Box 248, Selah, WA 98942

Detection of Added Invert Sugars by HPLC/Pulsed Amperometric Detection
Nicholas Low, University of Saskatchewan, Dept of Applied Microbiology & Food Science, Agriculture Bldg 3E08, Saskatoon, Saskatchewan S7N 0W0, Canada

Fruit Acids
Elia D. Coppola, Ocean Spray Cranberries, One Ocean Spray Dr, Lakeville, Middleboro, MA 02349

Identification & Characterization of Fruit Juices
Ronald E. Wrolstad, Oregon State University, Dept of Food Science & Technology, Corvallis, OR 97331

Geographic Origin of Orange Juice
Seifollah Nikdel, Florida Dept of Citrus, 700 Experiment Station Rd, Lake Alfred, FL 33850

Orange Juice Adulteration with Pulpwash
Betsy Woodward, Florida Dept of Agriculture & Consumer Services, 3125 Conner Blvd, Tallahassee, FL 32301

SNIF NMR
Gilles Martin, Eurofins, Geraudiere CP 4001, 44073 Nantes, France

Sodium Benzoate in Orange Juice
Hyong S. Lee, Florida Dept of Citrus, AREC, 700 Experiment Station Rd, Lake Alfred, FL 33850

Naringin & Neopheoperidin in Orange Juice
Wilbur Widmer, Florida Dept of Citrus, 700 Experiment Station Rd, Lake Alfred, FL 33050

Stable Carbon Isotope Ratio Analysis
Rae Gabrielle Krueger, Krueger Food Laboratories, Inc., 24 Blackstone St, Cambridge, MA 02139

Meat, Poultry, & Meat & Poultry Products

Referee:
David Soderberg, U.S. Dept of Agriculture, Food Safety & Inspection Service, Rm 519, Annex Bldg, 300 12th St, SW, Washington, DC 20250

Antioxidants in Foods
Charles R. Warner, U.S. Food & Drug Administration, Div. of Product Manufacture and Use, 200 C St, SW, Washington, DC 20204

Calcium in Mechanically Separated Poultry and Beef
Pamela Coleman, Armour Swift-Eckrich, Inc. Research Center, Analytical Chemistry Applications, 3131 Woodcreek Dr, Downers Grove, IL 60515-5429

Cholesterol in Eggs
Daniel G. Lebryk, KGF Technology Center, 801 Waukegan Rd, Glenview, IL 60025

Comparison of ICP and EDTA Methods for Calcium
Mai Huynh, U.S. Dept of Agriculture, Food Safety & Inspection Service, 300 12th St, SW, Washington, DC 20250

Copper Catalyst in Kjeldahl Method for Protein
Aron Yoffe, U.S. Dept of Agriculture, Food Safety & Inspection Service, 300 12th St, SW, Washington, DC 20250

Gluten in Meat
John H. Skerritt, CSIRO Wheat Research Unit, Div. of Plant Industry, PO Box 7, North Ryde, New South Wales 2113, Australia

Ion Chromatography Analysis of Meat & Poultry Products
Mark Paine, Illinois Dept of Agriculture, Animal Disease Laboratory, Shattuc Rd, Centralia, IL 62801

LC Methods for Meat and Poultry Products
Sher Ali, U.S. Dept of Agriculture, FSIS Science Eastern Laboratory, Russell Research Center, PO Box 6085, Athens, GA 30604

LC of Creatinine in Soups and Boullions
Rob W. Maeijer, Nestle Nederland B.V., AIIBP, c/o Walstraat 17, 8011 NR Zwolle, The Netherlands

Near Infrared Proximate Analysis of Meat Products
Larry W. Hand, University of Oklahoma, Rm 104b, Animal Science Bldg, Stillwater, OK 74078

Nitrosamine
Walter Fiddler, U.S. Dept of Agriculture, Eastern Regional Research Center, 600 E. Mermaid Ln, Philadelphia, PA 19118

Microwave Techniques
David L. Fish, CEM Corp., PO Box 200, Matthews, NC 28105

Non-Meat Protein in Meat and Poultry Products
Philip R. Goodwin, Cortecs Diagnostics Ltd, Techbase 1, Newtech Square, Deeside, Clwyd, CHS 2NT, UK

Phosphate Assays in Hams and Picnics
L. Kormendy, OHKI Hungarian Meat Research Institute, PO Box 17, H-1453, Budapest, Hungary

Phosphopyruvic Kinase Assays
Carl Davis, U.S. Dept of Agriculture, Agricultural Research Service, Russell Research Center, PO Box 5677, Athens, GA 30613

Potentiometric Determination of Sodium and Salt
Rob W. Maeijer, Nestle Nederland B.V., c/o Walstraat 17, 8011 NR Zwolle, The Netherlands

Protein Determination in Meat by Combustion Method
Joseph G. Sebranek, Iowa State University, Dept of Animal Science, 215 Meat Laboratory, Ames, IA 50011

Sulfites in Foods
Charles R. Warner, U.S. Food & Drug Administration,

Div. of Product Manufacture and Use, 200 C St, SW, Washington, DC 20204

Supercritical Fluid Methods
Jerry King, U.S. Dept of Agriculture, Agricultural Research Service, Northern Regional Research Center, 1815 N. University, Peoria, IL 61604

Total Fat
Max L. Foster, Kansas State Board of Agriculture, Div. of Laboratories, 2524 W. Sixth St, Topeka, KS 66606

Volatiles in Meat and Poultry, and Meat and Poultry Products
Jeffrey P. Donohue, U.S. Dept of Agriculture, FSIS, S & T, PO Box 5080, St. Louis, MO 63115

Seafoods

Referee:
James M. Hungerford, U.S. Food & Drug Administration, Seafood Products Research Center, 22201 23rd Dr, SE, PO Box 3012, Bothell, WA 98041-3012

Chemometric Methods
Bradley Tenge, U.S. Food & Drug Administration, Seafood Products Research Center, 22201 23rd Dr, SE, PO Box 3012, Bothell, WA 98041-3012

Conjugated Dienes as Indicators of Decomposition
Judith Kryznowek, NMFS NE Fisheries Center, Gloucester Laboratory, 30 Emerson Ave, Gloucester, MA 01930

Drained Weight of Block Frozen Peeled or Deveined Shrimp
William Chauvin, Shrimp World, Inc., 417 Eliza St, New Orleans, LA 70114

Fish Flesh Content in Frozen Coated Fisheries Products
Jane Fox-Dobson, NOAA-NMES-Specification Branch, 1 Blackburn Dr, Gloucester, MA 09130

Oxytetracycline in Farm-Raised Fish
Stephen Hadley, U.S. Food & Drug Administration, Seafood Products Research Center, 22201 23rd Dr, SE, PO Box 3012, Bothell, WA 98041-3012

Volatile Amines—TMA & DMA by Gas Chromatography
Ronald C. Lundstrom, U.S. Dept of Commerce, NOAA, National Marine Fisheries Service, Northeast Region, 1 Blackburn Dr, Gloucester, MA 01930

Processed Vegetable Products

Referee:
Thomas R. Mulvaney, U.S. Food & Drug Administration, Office and Plant and Dairy Foods & Beverages, 200 C St, SW, Washington, DC 20204

LC Determination of Sugar
Peter H. Yu, Diversified Research Laboratories, Ltd, 1047 Yonge St, Toronto, Ontario M4W 2L2, Canada

Total Solids by Microwave Moisture Analyzer
Henry B. Chin, National Food Processors Association, Chemistry Div., Western Research Laboratory, Dublin, CA 94568

Commmittee on Pesticide Residues and Related Topics

Keith A. McCully (Health & Welfare Canada, Field Operations Directorate, Jeanne Mance Bldg, Seventh Floor, Ottawa, Ontario K1A OL2, Canada), *Chairman*; **Henry B. Chin** (National Food Processors Association, Chemistry Div., Western Research Laboratory, Dublin, CA 94568); **Ronald Cook** (FMC Corp., Agricultural Chemicals Group, Box 8, Princeton, NJ 08540); **Brian Ripley** (Ministry of Agriculture & Food, Agricultural Laboratory Services, Bldg 43, McGilvray St, University of Guelph, Guelph, Ontario N1G 2W1, Canada); **Stephen G. Capar** (U.S. Food & Drug Administration, Div. of Pesticides & Industrial Chemicals, 200 C St, SW, Washington, DC 20204); **Allan R.C. Hill** (Ministry of Agriculture, Fisheries, & Food, Central Science Laboratory, Hatching Green, Harpenden, Hertfordshire, AL5 2BD, UK); **Larry Lane** (Mississippi State Chemical Laboratory, PO Box CR, Mississippi State, MS 39762); **Harvey Hundley** (U.S. Environmental Protection Agency, Analytical Chemistry Laboratory, Bldg 306, Center Rd, BARC East, Beltsville, MD 20705), *Secretary*; **Richard H. Albert** (U.S. Food & Drug Administration, Div. of Mathematics, HFF-110, 200 C St, SW, Washington, DC 20204), *Committee Statistician*; **Milan Cerny** (Federation of Migros Coops, Management Laboratories, 152, Limmatstrasse, 8005, Zurich, Switzerland), *Safety Advisor*

Metals & Other Elements

Referee:
Milan Ihnat, Agricultural Canada, Land Resource Research Centre, Ottawa, Ontario K1A 0C6, Canada

Atomic Absorption Spectrophotometry (AAS)
Milan Ihnat, Agriculture Canada, Land Resource Research Centre, Ottawa, Ontario K1A 0C6, Canada

Fluorine
Robert W. Dabeka, Health & Welfare Canada, Health Protection Branch, Banting Research Center, Ottawa, Ontario K1A 0L2, Canada

Graphite Furnace—Atomic Absorption Spectrophotometry
Robert W. Dabeka, Health & Welfare Canada, Health Protection Branch, Banting Research Center, Ottawa, Ontario K1A 0L2, Canada

Graphite Furnace Atomic Absorption Spectrophotometric Determination of Lead and Cadmium Released from Ceramicware
Susan C. Hight, U.S. Food & Drug Administration, Div. of Pesticides & Industrial Chemicals, 200 C St, SW, Washington, DC 20204

Lead in Calcium Supplements
Paul H. Siitonen, U.S. Food & Drug Administration, National Center for Toxicological Research, NCTR Dr, Jefferson, AR 72079-9502

Lead in Wines
Alan L. Reisig, BATF Laboratory, 1401 Research Blvd, Rockville, MD 20850

Neutron Activation Analysis
William C. Cunningham, U.S. Food & Drug Administration, NIST, Bldg 235, Rm B108, Gaithersburg, MD 20899

Organometallics in Fish
Walter Holak, U.S. Food & Drug Administration, New York Regional Laboratory, HFF-2660, 850 Third Ave, Brooklyn, NY 11232

Organotin Compounds
Allen D. Uhler, Battelle Ocean Science, 397 Washington St, Duxbury, MA 02332

Multiresidue Methods

Referee:
Leon D. Sawyer, U.S. Food & Drug Administration, Div. of Pesticides & Industrial Chemicals, 200 C St, SW, Washington, DC 20204

Comprehensive Multiresidue Methodology
S. Mark Lee, California Dept of Food & Agriculture, Div. of Inspection Services, Chemical Laboratory Services, 3292 Meadowview Rd, Sacramento, CA 95832

Fumigants
James L. Daft, U.S. Food & Drug Administration, 1009 Cherry St, Kansas City, MO 64106

Low Moisture-High Fat Samples
Gregory Beard, Hershey Chocolate USA, Laboratory Services, 19 E. Chocolate Ave, Hershey, PA 17033

Miniaturized Multiresidue Methods for Nonfatty Foods
Charles H. Parfitt, U.S. Food & Drug Administration, Div. of Pesticides & Industrial Chemicals, 200 C St, SW, Washington, DC 20204

Miniaturized Multiresidue Methods for Fat-Containing Foods
D. Ronald Erney, U.S. Food & Drug Administration, HFR-5360, 1560 E. Jefferson Ave, Detroit, MI 48207

Organophosphorus Pesticide Residues
Ronald R. Laski, U.S. Food & Drug Administration, HFR-2260, 599 Delaware Ave, Buffalo, NY 14202

Supercritial Fluid Extraction of Pesticide Residues in Foods
Marvin Hopper, U.S. Food & Drug Administration, 1009 Cherry St, Kansas City, MO 64106

Sweep Co-distillation
Barrie Magor, Australian Government, Anayltical Laboratories, 11 Willian St, Melbourne, VIC, 300, Australia

Organohalogen Pesticides

Referee:
Bernadette McMahon, U.S. Food & Drug Administration, 200 C St, SW, Washington, DC 20204

Chlorinated Dioxins
David Firestone, U.S. Food & Drug Administration, Office of Plant and Dairy Foods & Beverages, HFF-426, 200 C St, SW, Washington, DC 20204

Methyl Bromide
Joseph H. Ford, U.S. Dept of Agriculture, APHIS PPQ NMRAL, 3505 25th Ave, PO Box 3209, Gulfport, MS 39503

PCBs by Specific Congeners
Kimmo K. Himberg, National Bureau of Investigation, Crime Laboratory, Suvilahdenkatu 10 A, SF-00580 Helsinki, Finland

Polychlorinated Biphenyls in Blood
Virlyn Burse, Center for Environmental Health, Clinical Chemistry Div., 1600 Clifton Rd, Atlanta, GA 30333

Organonitrogen Pesticides

Referee:
W. Harvey Newsome, Health & Welfare Canada, Health Protection Branch, Food Research Div., Tunney's Pasture, Ottawa, Ontario K1A 0L2, Canada

Captan & Related Fungicides
Dalia M. Gilvydis, U.S. Food & Drug Administration, 1560 E. Jefferson Ave, Detroit, MI 48207

Diquat & Paraguat
Brian L. Worobey, Health & Welfare Canada, Bureau of Chemical Safety, Tunney's Pasture, Ottawa, Ontario K1A 0L2, Canada

Organonitro Compounds
David A. Nortrup, U.S. Food & Drug Administration, Dept of Health & Human Services, 200 C St, SW, Washington, DC 20204

Substituted Ureas
Ronald Luchtefeld, U.S. Food & Drug Administration, 1009 Cherry St, Kansas City, MO 64106

Radioactivity

Referee:
Edmond J. Baratta, U.S. Food & Drug Administration, Winchester Engineering & Analytical Center, 109 Holton St, Winchester, MA 01890

Iodine
D. Gene Easterly, U.S. Environmental Protection Agency, PO Box 93478, Las Vegas, NV 89193-3478

Strontium-90
Marina Silverstone, Dept of Health, Div. of Laboratories/Radiation, 1610 NE 150th St, K17-9, Seattle, WA 98155-7224

Plutonium-239
Pamela Mackill, U.S. Food & Drug Administration, Winchester Engineering & Analytical Center, 109 Holton St, Winchester, MA 01890

Committee on Microbiology and Extraneous Materials

Gayle A. Lancette (U.S. Food & Drug Administration, 60 Eighth St, NE, Atlanta, GA 30309), *Chairman*; **Anthony N. Sharpe** (Health & Welfare Canada, Health Protection Branch, Bureau of Microbial Hazards, Tunney's Pasture, Ottawa, Ontario K1A 0L2, Canada); **Russell S. Flowers** (Silliker Laboratories, Inc., 1304 Halsted St, Chicago Heights, IL 60411); **Jack L. Boese** (U.S. Food & Drug Administration, Div. of Microanalytical Evaluations, 200 C St, SW, Washington, DC 20204); **Virginia Scott** (National Food Processors Association, 1401 New York Ave, NW, Suite 400, Washington, DC 20005); **C. Robert Graham** (Lancaster Laboratories, Inc., 2425 New Holland Pike, Lancaster, PA 17601); **Sterling S. Thompson** (Hershey Foods Corp., Technical Center, 1025 Reese Ave, Hershey, PA 17033); **J. Stanley Bailey** (U.S. Dept of Agriculture, Agricultural Research Service, Russell Research Center, PO Box 5677, Athens, GA 30613), *Secretary*; **Foster D. McClure** (U.S. Food & Drug Administration, Div. of Mathematics, HFF-116, 200 C St, SW, Washington, DC 20204), *Committee Statistician*; **Carlos Abeyta** (U.S. Food & Drug Administration, 22201 23rd Dr, SE, PO Box 3012, Bothell, WA 98041-3012), *Safety Advisor*

Analytical Mycology & Microscopy

Referee: Stanley M. Cichowicz, U.S. Food & Drug Administration, Div. of Microanalytical Evaluations, 200 C St, SW, Washington, DC 20204

Geotrichum Mold in Canned and Frozen Fruits, Vegetables, and Fruit Juices
Stanley M. Cichowicz, U.S. Food & Drug Administration, Div. of Microanalytical Evaluations, 200 C St, SW, Washington, DC 20204

Mold by Chemical Detection
Ruth Bandler & George C. Ziobro, U.S. Food & Drug Administration, Div. of Microanalytical Evaluations, 200 C St, SW, Washington, DC 20204

Standardized Plant Tissue Concentrations for Mold Counting
Raymond Galacci, U.S. Food & Drug Administration, 900 Madison Ave, Baltimore, MD 21201

Vegetable Substitutes in Horseradish
Raymond Galacci, U.S. Food & Drug Administration, 900 Madison Ave, Baltimore, MD 21201

Environmental Sanitation Microbiology

Referee:
Stephen Edberg, Yale University School Medicine, Dept of Laboratory Medicine, Box 3333, New Haven, CT 06510

Cosmetic Microbiology

Referee:
Anthony D. Hitchins, U.S. Food & Drug Administration, Office of Special Research Skills, 200 C St, SW, Washington, DC 20204

Cosmetic Preservative Efficacy Testing
Neal A. Machtiger, Richardson-Vicks Research Center, 51 Byron Place, New Haven, CT 06515

Preservative Efficacy in Solid Cosmetics
Tony T. Tran, U.S. Food & Drug Administration, HFF-234, 200 C St, SW, Washington, DC 20204

Drug- & Device-Related Microbiology

Referee:
Ana M. Placencia, U.S. Food & Drug Administration, Sterility Research Center, 240 Hennepin Ave, Minneapolis, MN 55401

Biological Indicators—Testing & Standardization
Robert Berube, 3M Co., 3M Center, St. Paul, MN 55144

Chemical Indicators
Marvin L. Hart, 3M Co., 3M Center, Bldg 270, Rm 3N04, St. Paul, MN 55144

Endotoxins by Limulus Amebocyte Lysate
Christine W. Twohy, U.S. Food & Drug Administration, MLMI, 240 Hennepin Ave, Minneapolis, MN 55401

Evaluation of Ethylene Oxide Biological Indicators
Christopher A. Demitrius, U.S. Food & Drug Administration, 240 Hennepin Ave, Minneapolis, MN 55401

Packaging Integrity of Medical Devices
Ana M. Placencia, U.S. Food & Drug Administration, Sterility Research Center, 240 Hennepin Ave, Minneapolis, MN 55401

Sporicidal Testing of Disinfectants/Sterilants
James Danielson, U.S. Food & Drug Administration, Sterility Research Center, 240 Hennepin Ave, Minneapolis, MN 55401

Food Microbiology—Dairy

Referee:
J. Russell Bishop, Virginia Polytechnic Institute & State University, Dept of Food Science & Technology, Blacksburg, VA 24060-0418

Bactoscan Methods
J.D. Cunningham, University of Guelph, Dept of Environmental Biology, Guelph, Ontario N1G 2W1, Canada

Petrifilm Methods
Michael S. Curiale, Silliker Laboratories, Inc., 1304 Halsted St, Chicago Heights, IL 60411

Salmonella–Motility Enrichment on Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium
J. De Smedt, Jacobs Suchard, Inc., Corporate Microbiological Services, Montezumalaan 1, B-2200 Herentals, Belgium

Somatic Cells—Automated Optical Methods
Wesley N. Kelley, South Dakota State Laboratory, South Dakota State University, Dairymicro Bldg, PO Box 2104, Brookings, SD 57007-0647

Food Microbiology—Nondairy

Referee:
Wallace H. Andrews, U.S. Food & Drug Administration, Office of Special Research Skills, 200 C St, SW, Washington, DC 20204

Aerobic Plate Counts & Coliforms—Petrifilm-Nondairy Foods
Vernal S. Packard, University of Minnesota, Dept of Food Science & Nutrition, 1334 Eckles Ave, St. Paul, MN 55108; Michael S. Curiale, Silliker Laboratories, Inc., 1304 Halsted St, Chicago Heights, IL 60411

Aerobic Plate Counts in Foods—Redigel Medium
Jonathan Roth, RCR Scientific, Inc., PO Box 340, 206 W. Lincoln Ave, Goshen, IN 46526

Bacillus cereus Enterotoxin—Microslide Gel Double Diffusion
Reginald W. Bennett, U.S. Food & Drug Administration, Div. of Microbiological Studies, 200 C St, SW, Washington, DC 20204

Clostridium perfringens—Iron Milk Test for Recovery from Marine Environment
Carlos Abeyta, U.S. Food & Drug Administration, 22201 23rd Dr, SE, PO Box 3012, Bothell, WA 98041-3012

E. coli and Coliforms—ColiComplete Disc
Philip T. Feldsine, BioControl Systems, Inc., 19805 North Creek Pkwy, Bothell, WA 98011

Listeria–Assurance Enzyme Immunoassay
Philip T. Feldsine, Biocontrol Systems, Inc., 19805 North Creek Pkwy, Bothell, WA 98011

Listeria—Gas Chromatography of Cellular Fatty Acids for Species Identification
Linda English, U.S. Food & Drug Administration, 900 Madison Ave, Baltimore, MD 21201

Listeria-GENE-TRAK Colorimetric DNA Hybridization Method
Michael S. Curiale, Silliker Laboratories, Inc., 1304 Halsted St, Chicago Heights, IL 60411

Listeria—Listeria-Tek Assay
Michael S. Curiale, Silliker Laboratories, Inc., 1304 Halsted St, Chicago Heights, IL 60411

Listeria—Micro-ID
Barbara Robinson, Organon Teknika Corp., 100 Akzo Ave, Durham, NC 27704

Listeria—Vitek Automicrobic System for Species Identification
Loralyn H. Weiss, Kraft General Foods, Inc., Technology Center, 801 Waukegan Rd, Glenview, IL 60025

Salmonella, E. coli, and Other Enterobacteriaceae
Michael T. Knight, Q Laboratory, Inc., 2014 Harrison Ave, Cincinnati, OH 45214

Salmonella—Assurance Enzyme Immunoassay Screening Method
Philip T. Feldsine, BioControl Systems, Inc., 19805 North Creek Pkwy, Bothell, WA 98011

Salmonella—Malthus Automated Conductance Methods
Donald Gibson, Ministry of Agriculture, Fisheries & Food, Torry Research Station, 135 Abbey Rd, PO Box 31, Aberdene ABG BDG, UK

Salmonella, E. coli, & Other Enterobacteriaceae—Micro ID
Russell S. Flowers, Silliker Laboratories, Inc., 1304 Halsted St, Chicago Heights, IL 60411

Salmonella—GENE-TRAK DNA Hybridization Screening Method
Russell S. Flowers, Silliker Laboratories, Inc., 1304 Halsted St, Chicago Heights, IL 60411

Salmonella—HGMF Methods
Phyllis Entis, QA Life Sciences, Inc, 6645 Nancy Ridge Dr, San Diego, CA 92121

Salmonella—Immunoband Screening Method
Russell S. Flowers, Silliker Laboratories, Inc., 1304 Halsted St, Chicago Heights, IL 60411

Salmonella—Modified Semisolid Rappaport-Vassiliadis Medium for Screening Chocolate and Cocoa
John E. Milas, Jacobs Suchard, Inc., 4656 West Kinzie, Chicago, IL 60644

Salmonella—Oxoid Method
Michael S. Curiale, Silliker Laboratories, Inc., 1304 Halsted St, Chicago Heights, IL 60411

Salmonella—Q-TROL Enzyme Immunoassay Screening Method
Russell S. Flowers, Silliker Laboratories, Inc., 1304 Halsted St, Chicago Heights, IL 60411

Salmonella—TECRA Enzyme Immunoassay Screening Method
Russell S. Flowers, Silliker Laboratories, Inc., 1304 Halsted St, Chicago Heights, IL 60411

Staphylococcal Enterotoxin—TECRA Visual Enzyme Immunoassay
Reginald W. Bennett, U.S. Food & Drug Administration, Div. of Microbiological Studies, 200 C St, SW, Washington, DC 20204

Vibrio vulnificus—Identification by GC of Cellular Fatty Acids
Warren Landry, U.S. Food & Drug Administration, 3032 Bryan St, Dallas, TX 75204

Filth & Extraneous Materials in Foods & Drugs

Referee:
Jack L. Boese, U.S. Food & Drug Administration, Div. of Microanalytical Evaluations, 200 C St, SW, Washington, DC 20204

Alkaline Phosphatase in Foods by Electrophoresis
George C. Ziobro, U.S. Food & Drug Administration, Div. of Microanalytical Evaluations, 200 C St, SW, Washington, DC 20204

Fecal Contamination in Grain Products by Chemical Detection Methods
George C. Ziobro, U.S. Food & Drug Administration, Div. of Microanalytical Evaluations, 200 C St, SW, Washington, DC 20204

Grains (Whole), Internal Insect Infestation by ELISA Method
G. Barrie Kitto, University of Texas at Austin, Center for Biotechnology, Welch Hall 4.260C, Austin, TX 78712

Internal Insect Infestation of Whole Grains—Cracking Flotation Methods
Richard Trauba, U.S. Food & Drug Administration, 240 Hennepin Ave, Minneapolis, MN 55401

Light Filth in Baked Goods with Fruit & Nut Tissues—Flotation
Joseph K. Nagy, U.S. Food & Drug Administration, Second & Chestnut Sts, Rm 900, Philadelphia, PA 19106

Light Filth in Bean Paste by Flotation
John R. Bryce, U.S. Food & Drug Administration, Div. of Microanalytical Evaluations, 200 C St, SW, Washington, DC 20204

Light Filth in Dates, Flotation Method
John R. Bryce, U.S. Food & Drug Administration, Div. of Microanalytical Evaluations, 200 C St, SW, Washington, DC 20204; Marvin Nakashima, U.S. Food & Drug Administration, Div. of Microanalytical Evaluations, 200 C St, SW, Washington, DC 20204

Light Filth (External) in Grains and Seeds by Flotation Method
James Gallman, U.S. Food & Drug Administration, 60 Eighth St, NE, Atlanta, GA 30309

Light Filth in Sauces Containing Soy Sauce, Thickeners, and Spices by Flotation Method
Marvin Nakashima, U.S. Food & Drug Administration, Div. of Microanalytical Evaluations, 200 C St, SW, Washington, DC 20204

Light Filth in Unground Basil—Flotation
Beverly Kent, U.S. Food & Drug Administration, Buffalo District, 599 Delaware Ave, Buffalo, NY 14202

Light Filth in Cheese
Marvin Nakashima, U.S. Food & Drug Administration, Div. of Microanalytical Evaluations, 200 C St, SW, Washington, DC 20204

Light Filth in Chocolate & Chocolate Products—Flotation Methods
C. Robert Graham, Lancaster Laboratories, Inc., 2425 New Holland Pike, Lancaster, PA 17601

Light Filth in Condimental Hot Sauces—Flotation Methods
John R. Bryce, U.S. Food & Drug Administration, Div. of Microanalytical Evaluations, 200 C St, SW, Washington, DC 20204

Light Filth in Dehydrated Vegetable Products—Flotation Methods
Francis J. Farrell, Thomas J. Lipton, Inc., Analytical Services Dept, 800 Sylvan Ave, Englewood Cliffs, NJ 07632

Light Filth in Fish Paste & Sauces—Flotation Methods
John R. Bryce, U.S. Food & Drug Administration, Div. of Microanalytical Evaluations, 200 C St, SW, Washington, DC 20204

Light Filth in Grain Products—Flotation Methods
John R. Bryce, U.S. Food & Drug Administration, Div. of Microanalytical Evaluations, 200 C St, SW, Washington, DC 20204

Light Filth in Ground Coffee—Flotation Methods
Gerald E. Russell, U.S. Food & Drug Administration, 1560 E. Jefferson Ave, Detroit, MI 48207

Light Filth in Soybean Curd—Flotation Methods
Marvin Nakashima, U.S. Food & Drug Administration, Div. of Microanalytical Evaluations, 200 C St, SW, Washington, DC 20204

Rodent Gnawing
Jack L. Boese, U.S. Food & Drug Administration, Div. of Microanalytical Evaluations, 200 C St, SW, Washington, DC 20204

Urine Stains—Chemical Methods
Robert S. Ferrera and Patricia A. Valdes, U.S. Food & Drug Administration, Div. of Microanalytical Evaluations, 200 C St, SW, Washington, DC 20204

Urine Stains on Foods—Chemical Methods
Patricia A. Valdes, U.S. Food & Drug Administration, Div. of Microanalytical Evaluations, 200 C St, SW, Washington, DC 20204

Mammalian Feces in Spices—Chemical Detection
Harriet R. Gerber, U.S. Food & Drug Administration, HFC-141, Div. of Field Science, 5600 Fishers Ln, Rockville, MD 20857

Water Microbiology

Referee:
Alfred P. DuFore, U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratories, 26 W. Martin Luther King Dr, Cincinnati, OH 45268

Committee on Feeds, Fertilizers, and Related Topics

Harold C. Thompson, Jr (U.S. Food & Drug Administration, National Center for Toxicological Research, Jefferson, AR 72079), *Chairman*; **Stanley E. Katz** (Rutgers University–Cook College, Dept of Biochemistry and Microbiology, New Brunswick, NJ 08903); **Thomas M. Parham, Jr** (Arcadian Corp., Triazone Div., PO Box 307, Geismer, LA 70734); **Lars Reimann** (Woodson Tenent Laboratories, 345 Adams, PO Box 2135, Memphis, TN 38101); **George W. Latimer, Jr** (Office of the Texas State Chemist, PO Box 3160, College Station, TX 77841-3160); **Dana Perry** (University of Arizona, Veterinary Diagnostic Laboratory, Tucson, AZ 85721); **Toni Rihs** (Swiss Federal Research Station for Animal Production, CH 1725, Posieux, Switzerland); **W. Emmett Braselton Jr** (Michigan State University, Dept of Pharmacology & Toxicology, East Lansing, MI 48824), *Secretary*; **Daniel H. Mowrey** (Lilly Research, Laboratories Div. of Eli Lilly & Co., Greenfield Laboratory, PO Box 708, Greenfield, IN 46140), *Committee Statistician*; **Maire C. Walsh** (State Laboratory, Abbotstown, Castleknock, Dublin 15, Ireland), *Safety Advisor*

Antibiotics in Feeds

Referee:
Hussein S. Ragheb, Purdue University, Dept of Biochemistry, West Lafayette, IN 47907

Apramycin
John W. Lamb, Elanco Products Co., PO Box 1750, Indianapolis, IN 46285

Bacitracin Methylene Disalicylate
Anil D. Desai, A.L. Laboratories, Inc., 400 State St, Chicago Heights, IL 60411

Bambermycin
Mustapha Mustapha, Barrow-Agee Laboratories, PO Box 156, Memphis, TN 38101

Chlortetracycline
Mary Lee Hasselberger, Nebraska Dept of Agriculture, Laboratory Div., 3703 S. 14th St, Lincoln, NE 68502

Direct-Fed Microbiological Products & Silage Inoculants
Steve Waskow, Far-Mor Biochem, 6120 W. Douglas Ave, Milwaukee, WI 53218

Lincomycin
Gerald L. Stahl, The Upjohn Co., Downtown Complex, 9760 209 5, Kalamazoo, MI 49001

Monensin Microbiological Method
Mark R. Coleman, Lilly Research Laboratories, PO Box 708, Greenfield, IN 46140-0708

Narasin Microbiological Method
Mark R. Coleman, Lilly Research Laboratories, PO Box 708, Greenfield, IN 46140-0708

Neomycin
Gerald L. Stahl, The Upjohn Co., Downtown Complex, 9760 209 5, Kalamazoo, MI 49001

Oxytetracycline by Microbiological Methods
Mary Lee Hasselberger, Nebraska Dept of Agriculture, Laboratory Div., 3703 S. 14th St, Lincoln, NE 68502

Tylosin
Mark R. Coleman, Lilly Research Laboratories, PO Box 708, Greenfield, IN 46140-0708

Virginiamycin
James A. Miller, Smithkline Animal Health Products, 1600 Paoli Pike, West Chester, PA 19380

Virginiamycin with Other Drugs
Hussein S. Ragheb, Purdue University, Dept of Biochemistry, West Lafayette, IN 47907

Drugs in Feeds

Referee:
Robert L. Smallidge, Office of the Indiana State Chemist, 1154 BCHM Bldg, Purdue University, West Lafayette, IN 47907-1154

Amprolium
Robert L. Smallidge, Office of the Indiana State Chemist, 1154 BCHM Bldg, Purdue University, West Lafayette, IN 47907-1154

Carbadox
Alicia Henk, Pfizer, Inc., 1107 South Missouri 291, Lee's Summit, MO 64081

Chlortetracycline (LC Method)
Mary Lee Hasselberger, Nebraska Dept of Agriculture, Laboratory Div., 3703 S. 14th St, Lincoln, NE 68502

Ethopabate
Joseph Hillebrandt, Degussa Corp., PO Box 188, School House Ln, Waterford, NY 12188

Lasalocid by HPLC
Alexander MacDonald, Hoffmann-La Roche, Inc., Food & Agricultural Products, 340 Kingsland St, Nutley, NJ 07110

Melengestrol Acetate
Raymond Davis, The Upjohn Co., Henrietta St Laboratories, Kalamazoo, MI 49001

Monensin, Tylosin, & Narasin Chromatographic Method
Mark R. Coleman, Lilly Research Laboratories, PO Box 708, Greenfield, IN 46140-0708

Morantel Tartrate
Linda D. Werner, Pfizer, Inc., 1107 South Missouri 291, Lee's Summit, MO 64081

Nifursol
Ellen Jan De Vries, Solvay Duphar B.V., PO Box 900, 1380 DA Weesp, The Netherlands

Oxytetracycline by HPLC
Mary Lee Hasselberger, Nebraska Dept of Agriculture, Laboratory Div., 3703 S. 14th St, Lincoln, NE 68502

Pyrantel Tartrate
Joyce Konrardy, Pfizer, Inc., 1107 South Missouri 291, Lee's Summit, MO 64081

Sulfadimethoxine and Ormetoprim
Alexander MacDonald, Hoffmann-La Roche, Inc., Food & Agricultural Products, 340 Kingsland St, Nutley, NJ 07110

Sulfa Drug Residues
Valerie Reeves, U.S. Food & Drug Administration, HFV-502, Bldg 328A, BARC East, Beltsville, MD 20705

Sulfamethazine & Sulfathiazole
Dwight M. Lowie, North Carolina State Dept of Agriculture, Constable Laboratory, 4000 Reedy Creek Rd, Raleigh, NC 27607

Feeds

Referee:
William R. Windham, U.S. Dept of Agriculture, ARS, PO Box 5677, Athens, GA 30677

Amino Acids
Cynthia R. Llames, DeGussa Corp., 4 Pearl Ct, Allendale, NJ 07401

Atomic Absorption Spectrophotometry (AAS)
Robert A. Isaac, University of Georgia, Soil Testing & Plant Analysis Laboratory, FPO 8934290, 2400 College Station Rd, Athens, GA 30605

Carotenoids
D.E. McNaughton, Ruakura Animal Research Center, Ministry of Agriculture & Fisheries, Private Bag, Hamilton, New Zealand

Crude Protein
Peter F. Kane, Purdue University, Dept of Biochemistry, West Lafayette, IN 47907

Emission Spectroscopy
Robert A. Isaac, University of Georgia, Soil Testing & Plant Analysis Laboratory, FPO 8934290, 2400 College Station Rd, Athens, GA 30605

Fiber
David R. Mertens, U.S. Dairy Forage Research Center, 1925 Linden Dr, W., Madison, WI 53706

Inorganic Elemental Constituents of Plant Samples, Microwave Digestion Method
Robert Miller, DANR Analytical Laboratory, University of California at Davis, Davis, CA 95616-8627

Iodine & EDDI in Feeds
George W. Latimer, Jr, Office of the Texas State Chemist, PO Box 3160, College Station, TX 77841-3160

Microscopy
Patricia Ramsey, CDFA Chemical Laboratories, 3292 Meadowview Rd, Sacramento, CA 95832

Minerals
Ronald L. Baker, Chemetal Corp., 711 Pittman Rd, Baltimore, MD 21226

Mixed Feeds-Infrared Reflectance Techniques
Franklin E. Barton, II, U.S. Dept of Agriculture, Richard B. Russell Research Center, 950 College Station Rd, Box 5677, Athens, GA 30604

Moisture in Mixed Feeds & Forages
William R. Windham, U.S. Dept of Agriculture, ARS, PO Box 5677, Athens, GA 30604

Moisture in Pet Foods
Roy E. Schulze, Ralston Purina Co., Checkerboard Square, St. Louis, MO 63164

Vitamin A in Feeds by HPLC
John L. MacDonald, Ralston Purina Co., Checkerboard Square, St. Louis, MO 63164

Fertilizers & Agricultural Liming Materials

Referee:
Peter F. Kane, Purdue University, Dept of Biochemistry, West Lafayette, IN 47907

Fertilizer Sulfur
Kim Anderson, Univesity of Idaho, Analytical Laboratory, Holm Center, Muscow, ID 83843

Iron
James Silkey, Oregon Dept of Agriculture, 635 Capitol St, NE, Salem, OR 97310

Manganese in Feed Ingredients
Ronald L. Baker, Chemetal Corp., 711 Pittman Rd, Baltimore, MD 21226

Nitrogen by Combustion Techniques
Donald F. Tate, Illinois Dept of Agriculture, Chemistry Laboratory, PO Box 19281, Springfield, IL 62794

Phosphorus
Joe Gliksman, ICM Fertilizer, Inc. PO Box 1035, Mulberry, FL 33860

Potassium by Flame Photometric Detection
Natalie Newlon, Purdue University, 1154 Biochemistry Bldg, West Lafayette, IN 47907-1154

Sample Preparation
Robert L. Beine, Kentucky Agricultural Experiment Station, Div. of Regulatory Services, Regulatory Services Bldg, University of Kentucky, Lexington, KY 40506

Sampling
Douglas Caine, Vigoro Industries, Inc., PO Box 4139, Fairview Heights, IL 62208

Trace Analysis in Fertilizers
David Averitt, IMC Fertilizer, Inc., PO Box 1035, Mulberry, FL 33860

Urea & Methlyeneureas
Thomas M. Parham, Jr, Arcadian Corp., Triazone Div., PO Box 307, Geismar, LA 70734

Water
James A. Farley, Tennessee Valley Authority, NFE 2J-M, Muscle Shoals, AL 35660

Nutrients in Soils

Referee: Open

pH Measurements in Soils
Yash Kalra, Forestry Canada, Northern Forestry Center, 5320 122nd St, Edmonton, Alberta T6H 3S5, Canada

Tobacco

Referee:
W. Wesley Weeks, North Carolina State University, Crop Science Dept, Box 7620, Raleigh, NC 27695-7620

Nicotine in Environmental Tobacco Smoke
Michael W. Ogden, R.J. Reynolds Tobacco Co., BGTC 611 13 102E, Reynolds Blvd, Winston-Salem, NC 27102

Nicotine Alkaloids in Tobacco
Linda Crumpler, R.J. Reynolds Tobacco Co., Research & Development, PO Box 2959, Winston-Salem, NC 27102

Polyphenols in Tobacco
Maurice E. Snook, U.S. Dept of Agriculture, ARS, Russell Research Center, PO Box 5677, Athens, GA 30613

Veterinary Analytical Toxicology

Referee:
P. Frank Ross, U.S. Dept of Agriculture, National Veterinary Services Laboratory, Toxicology Laboratory, PO Box 844, Ames, IA 50010

Antibiotic Screening Methods
Wynne Landgraf, U.S. Dept of Agriculture, National Veterinary Services Laboratory, PO Box 844, Ames, IA 50010; Stephen C. Ross, Illinois Dept of Agriculture, Animal Disease Laboratory, Shattuc Rd, Centralia, IL 62801

Cholinesterase by Colorimetric Method
Karen S. Harlin, University of Illinois, Dept of Veterinary Biosciences, 2001 S. Lincoln, Urbana, IL 61801

Cholinesterase—pH Method
Paula Martin Imerman, Iowa State University, College of Veterinary Medicine, Veterinary Diagnostic Laboratory, Ames, IA 50010

Fluoride
David Osheim, U.S. Dept of Agriculture, National Veterinary Services Laboratory, Chemistry Section, PO Box 844, Ames, IA 50010

Lead in Tissues
Robert J. Everson, Purdue University, Animal Disease Diagnostic Laboratory, West Lafayette, IN 47907

Multielement Analysis by ICP
W. Emmett Braselton, Jr, Michigan State University, Dept of Pharmacology & Toxicology, East Lansing, MI 48824

Multiple Anticoagulants—Screening Methods
Larry Felice, Dept of Veterinary Diagnostic Investigation, Minnesota Veterinary Diagnostic Laboratory, 1943 Carter Ave, St. Paul, MN 55108

Natural Products
George Rottinghaus, University of Missouri-Columbia, Veterinary Medical Diagnostic Laboratory, PO Box 6023, Columbia, MO 65211

Nitrates & Nitrites
Norman R. Schneider & Michael P. Carlson, Veterinary Diagnostic Center, Dept of Veterinary Science, Fair St & E. Campus Loop, Lincoln, NE 68583-0907

Pesticides in Toxicological Samples
Henry M. Stahr, Iowa State University, College of Veterinary Medicine, Veterinary Diagnostic Laboratory, Ames, IA 50010

Sodium Monofluoroacetate
Henry M. Stahr, Iowa State University, College of Veterinary Medicine, Veterinary Diagnostic Laboratory, Ames, IA 50010

Vitamins A & E
Roy A. Smith, Alberta Agriculture, O.S. Longman Bldg, 6909 116th St, Edmonton, Alberta T6H 4P2, Canada

Zinc
Dana Perry, University of Arizona, Veterinary Diagnostic Laboratory, Dept of Veterinary Science, Tucson, AZ 85721

Committee on Environmental Quality

Ann B. Strong (U.S. Army Corps of Engineers, Waterways Experiment Station, Environmental Chemistry Branch, 3909 Halls Ferry Rd, Vicksburg, MS 39180), *Chairman*; **Charles Sellers** (U.S. Environmental Protection Agency, Office of Solid Waste, OS/331, 401 M St, SW, Washington, DC 20460); **James E. Longbottom** (U.S. Environmental Protection Agency, EMSL Cincinnati QARD PMB, 26 W. Martin Luther King Dr, Cincinnati, OH 45268); **Marvin Stephens** (Wadsworth/Alert Laboratories, Inc., 4101 Shuffel Dr, NW, North Canton, OH 44720); **Gregory Leyes** (Monsanto Co., U4G, 800 N. Lindbergh Blvd, St. Louis, MO 63167); **Thomas F. Jenkins** (U.S. Army CRREL, 72 Lyme Rd, Hanover, NH 03755); **Joan Bartz** (2902 S. Vancouver St, Kennewick, WA 99337), *Secretary*; **Paul Britton** (U.S. Environmental Protection Agency, EMSL, Cincinnati QARD DEB, 26 W. Martin Luther King Dr, Cincinnati, OH 45268), *Committee Statistician*

Cooperative Studies

Referee:
Joseph R. Donnelly, Lockheed Engineering & Sciences Co., Environmental Programs Office, 1050 E. Flamingo Rd, Suite 120, Las Vegas, NV 89119

Carbamate Pesticides in Water
Kenneth W. Edgell, The Bionetics Corp., 16 Triangle Park Dr, Cincinnati, OH 45246

Chlorinated Acids in Groundwater by GC/EC
Kenneth W. Edgell, The Bionetics Corp., 16 Triangle Park Dr, Cincinnati, OH 45246; James E. Longbottom, U.S. Environmental Protection Agency, EMSL Cincinnati QARD PMB, 26 W. Martin Luther King Dr, Cincinnati, OH 45268; Viorica Lopez-Avila, Mid-Pacific Environmental Laboratory, 625B Clyde Ave, Mountain View, CA 94039

Nitrates in Soils
Robert Miller, University of California, DANR Analytical Laboratory, Davis, CA 95616-8627

Nitrogen & Phosphorus Containing Products in Groundwater
Kenneth W. Edgell, The Bionetics Corp., 16 Triangle Park Dr, Cincinnati, OH 45268

Organohalogen Pesticides in Water
Marie Siewierski, Rutgers University-Cook College, McLean Laboratories, Environmental Science, Box 231, New Brunswick, NJ 08903

Inorganic Methods

Referee: Open

ICP & Ion Chromatographic Methods in Groundwater
James E. Longbottom, U.S. Environmental Protection Agency, EMSL Cincinnati QARD PMB, 26 W. Martin Luther King Dr, Cincinnati, OH 45268; Bruce Warden, Waste Management, Inc., Environmental Monitoring Laboratories, Inc., 2100 Cleanwater Dr, Geneva, IL 60134

Inorganic Ions in Water, Ion Chromatographic Method
Kenneth W. Edgell, The Bionetics Corp., 16 Triangle Park Dr, Cincinnati, OH 45246

Organic Methods

Referee:
Viorica Lopez-Avila, Mid-Pacific Environmental Laboratory, 625B Clyde Ave, Mountain View, CA 94039

Determination of Carbonyl Compounds in Waters
Kenneth W. Edgell, Bionetics Corp., 16 Triangle Park Dr, Cincinnati, OH 45246

Explosives Residues in Soil
Thomas F. Jenkins, U.S. Army CRREL, 72 Lyme Rd, Hanover, NH 03755

Herbicides
Mark E. Oppenhuizen, Monsanto Agricultural Co., Unit of Monsanto Co., 700 Chesterfield Pkwy, St. Louis, MO 63198

Immunoassay for Atrazine
Robert Harrison, Immunosystems, Inc., 4 Washington Ave,

Scarborough, ME 04074; Shirley J. Gee, University of California, Dept of Entomology, Davis, CA 95616-8584

Magnetic Particle Based Enzyme Immunoassay for Atrazine
David P. Herzog, Ohmicron Corp., 375 Pheasant Run, Newtown, PA 18940

Munitions in Waste Water
Thomas F. Jenkins, U.S. Army CRREL, 72 Lyme Rd, Hanover, NH 03755

Thermospray LC/MS Analysis of Carbamates
Tammy Jones, U.S. Environmental Protection Agency, 944 E. Harmon Ave, Las Vegas, NV 89109

OFFICIAL METHODS OF ANALYSIS

Changes in Official Methods of Analysis

By vote of the Official Methods Board during meetings January 23–25, May 28–30, August 29, and September 3, 1992, the following recommendations on specific methods were adopted, ie., new methods were adopted first action, and first action methods were revised, repealed, or declared surplus as recommended by the appropriate General Referee and Methods Committee. According to Official Methods Board Policy, adoption of first action methods and changes or revisions in first action methods become official immediately upon vote by the Official Methods Board. Methods are adopted final action by ballot vote of AOAC membership. Sixty-nine first action methods were adopted final action during the year.

Chapter and method numbers refer to *Official Methods of Analysis* (1990) 15th edition and "Changes in Official Methods of Analysis," first supplement (1990), second supplement (1991), and third supplement (1992).

These newly adopted methods and the changes in existing first action methods will be published in their entirety in "Changes in Official Methods of Analysis" fourth supplement (1993), which will be sent to purchasers of the 15th edition of *Official Methods of Analysis* who have returned to AOAC International the card bound into Volume 1 of the 15th edition. The Collaborative studies of AOAC methods, which include the data that support adoption of the methods as well as the method itself and a description and discussion of the study, are published in the bimonthly *Journal of AOAC International.* Methods adopted by AOAC International on the basis of published Collaborative studies by other organizations are supported by a summary of the appropriate study in the *Journal*, with reference to the full publication.

The 15th edition (1990) of *Official Methods of Analysis*, together with its supplements, comprises the methods of the Association.

Ch. 1. Agricultural Liming Materials

No additions, deletions, or other changes.

Ch. 2. Fertilizers

(1) The first action method for particle size range of peat, mechanical analysis was adopted final action, **973.03**.

(2) The first action method for sand in peat was adopted final action, **973.05**.

(3) The first action method for nitrogen (total) in peat was adopted final action, **973.06**.

(4) The first action method for volume, peat, alternative method was adopted final action, **973.07**.

(5) The first action method for volume weight, water-holding capacity, and air capacity of water-saturated peat materials was adopted final action, **973.08**.

(6) The first action method for sodium in fertilizers, flame photometric method was adopted final action, **974.01**.

(7) The first action method for sodium in fertilizers, atomic absorption spectrophotometric method was adopted final action, **983.04**.

(8) The first action method for aluminum in aluminum sulfate-type soil acidifiers, atomic absorption spectrophotometric method was adopted final action, **983.05**.

(9) The revised final action method for sampling of liquid fertilizers, Texas liquid sampler method was adopted first action, **969.01**.

Ch. 3. Plants

No additions, deletions, or other changes.

Ch. 4. Animal Feed

No additions, deletions, or other changes.

Ch. 5. Drugs in Feeds

(1) The final action method for roxarsone in feeds and premixes, spectrophotometric method was revised, **971.47**.

Ch. 6. Disinfectants

No additions, deletions, or other changes.

Ch. 7. Pesticide Formulations

(1) The first action method for methyl parathion in pesticide formulations, gas chromatographic method was adopted final action, **977.04.**

(2) The first action method for parathion in pesticide formulations, gas chromatographic method was adopted final action, **978.06**.

(3) The first action method for fenitrothion technical and pesticide formulations, alternative gas chromatographic method was adopted final action, **989.02**.

(4) The method for methamidophos in technical products and pesticide formulations, liquid chromatographic method was adopted first action, CIPAC-AOAC method, **992.01**.

(5) The method for methazole in technical and pesticide formulations, liquid chromatographic method was adopted first action, **992.02**.

(6) The first action method for methyl parathion in pesticide formulations, liquid chromatographic method was declared surplus, **977.05**.
(7) The first action method for parathion in pesticide formulations, liquid chromatographic method was declared surplus, **978.07**.
(8) The final action method for fenitrothion technical and pesticide formulations, gas chromatographic method was declared surplus, **985.07**.
(9) The first action method for DDVP in pesticide formulations, infrared spectrophotometric method was repealed, **964.04**.
(10) The first action method for DDVP in pesticide formulations, infrared spectrophotometric method was repealed, **966.07**.

Ch. 8. Hazardous Substances

(1) The first action method for *N*-nitrosodibutylamine in latex infant pacifiers, gas chromatographic method was adopted final action, **986.01**.
(2) The method for cadmium and lead in earthenware was editorially revised, **975.05**.

Ch. 9. Metals and Other Elements at Trace Levels in Foods

(1) The first action method for mercury (methyl) in fish and shellfish, rapid gas chromatographic method was adopted final action, **988.11**.
(2) The first action method for copper, iron, and nickel in edible oils and fats, direct graphite furnace, atomic absorption spectrophotometric method was adopted final action, **990.05**.
(3) The final action AOAC-ASTM method for cadmium and lead in earthenware, atomic absorption spectrophotometric method was revised, **973.32.**

Ch. 10. Pesticide and Industrial Chemical Residues

(1) The first action method for organochlorine pesticides in water, gas chromatographic method was adopted final action, **990.06**.
(2) The first action method for polychlorinated biphenyls (as aroclor 1254) in serum, gas chromatographic method was adopted final action, **990.07**.
(3) The method for pesticides in water, liquid chromatographic method with ultraviolet detector (national pesticide survey method 4) was adopted first action, **992.14**.
(4) The method for diquat and paraquat residues in potatoes, liquid chromatographic method was adopted first action, **992.17**.
(5) The method for ethylene thiourea (ETU) residues in finished drinking waters, gas chromatographic method with nitrogen-phosphorus detection was adopted first action, **992.31**.
(6) The method for chlorinated acidic residues in finished drinking waters, gas chromatographic method with electron capture detection was adopted first action, **992.32**.

Ch. 11. Waters and Salt

No additions, deletions, or other changes.

Ch. 12. Microchemical Methods

No additions, deletions, or other changes.

Ch. 13. Radioactivity

No additions, deletions, or other changes.

Ch. 14. Veterinary Analytical Toxicology

(1) The method for cholinesterase in blood, pH method was adopted first action, **992.20**.

Ch. 15. Cosmetics

(1) The first action method for water and ethyl alcohol in cosmetics, gas chromatographic method was adopted final action, **966.22**.
(2) The first action method for zirconium (soluble) in antiperspirant aerosols, colorimetric method was adopted final action, **976.24**.

Ch. 16. Extraneous Materials: Isolation

(1) The method for light filth from fish products containing spice, flotation method was adopted first action, **992.10**.
(2) The method for light filth in sauces containing soy sauce, thickeners, and spices, flotation method was adopted first action, **992.12**.
(3) The method for light filth in tofu, sieving method was adopted first action, **992.13**.

Ch. 17. Microbiological Methods

(1) The first action method for total coliform and *Escherichia coli* counts in foods, hydrophobic grid membrane filter/MUG (ISO-GRID) method was adopted final action, **990.11**.
(2) The method for motile and non-motile *Salmonella* in foods, polyclonal enzyme immunoassay method was adopted first action, **992.11**.
(3) The method for *Listeria sp.*, biochemical identification method (MICRO-ID Listeria) was adopted first action, **992.18**.
(4) The method for *Listeria sp.*, biochemical identification method (Vitek GPI and GNI) was adopted first action, **992.19**.
(5) The method for total coliforms and *E. coli* in foods, confirmed detection (by ColiComplete) substrate supporting disc method was adopted first action, **992.30**.
(6) The method for *Salmonella* in foods, hydrophobic grid membrane filter screening method was repealed, **985.42**.
(7) The method for extraneous materials (foreign matter) in products, isolation techniques was editorially revised, **945.75**.

Ch. 18. Drugs: Part I

(1) The first action method for xanthine group alkaloid drugs, microchemical tests was adopted final action, **960.56**.

(2) The first action method for hydralazine hydrochloride in drug tablets, spectrophotometric method was adopted final action, **989.07**.
(3) The first action method for clioquinol in creams and ointments, liquid chromatographic method was adopted final action, **990.14**.
(4) The first action method for penicillin V potassium in tablets, liquid chromatographic method was adopted final action, **990.15**.

Ch. 19. Drugs: Part II

(1) The first action method for chlorpropamide in drug tablets, liquid chromatographic method was adopted final action, **986.37**.
(2) The first action method for acetaminophen in drug tablets, liquid chromatographic method was adopted final action, **987.12**.
(3) The first action method for diethylpropion hydrochloride in drug substance and tablets, liquid chromatographic method was adopted final action, **988.23**.

Ch. 20. Drugs: Part III

(1) The first action method for morphine sulfate in bulk drug and injections, liquid chromatographic method was adopted final action, **989.08**.
(2) The first action method for santonin in drug mixtures, ultraviolet absorption method was declared surplus, **962.23**.

Ch. 21. Drugs: Part IV

(1) The first action method for cortisone acetate in bulk drug and dosage forms, liquid chromatographic method was adopted final action, **988.25**.
(2) The first action method for dexamethasone acetate in bulk drug and suspensions, liquid chromatographic method was adopted final action, **988.26**.
(3) The first action method for dexamethasone in drug substance and elixirs, quantitative and identification methods was adopted final action, **988.27**.

Ch. 22. Drugs: Part V

(1) The first action method for enantiomers of amphetamine in bulk drugs, syrups, and capsules, liquid chromatographic method was adopted final action, **988.28**.

Ch. 23. Drugs and Feed Additives in Animal Tissues

(1) The method for sulfamethazine residues in milk, liquid chromatographic method was adopted first action, **992.21**.
(2) The first action method for ethoxyquin residues in animal tissues, photofluorometric method was declared surplus, **968.48**.

Ch. 24. Forensic Sciences

(1) The first action method for detection of fingerprints (latent) on papers, chemical development method was adopted final action, **976.28**.
(2) The first action method for mineral wool insulation, comparison of properties was adopted final action, **981.23**.

Ch. 25. Baking Powders and Baking Chemicals

No additions, deletions, or other changes.

Ch. 26. Distilled Liquors

No additions, deletions, or other changes.

Ch. 27. Malt Beverages and Brewing Materials

(1) The first action method for *N*-nitrosodimethylamine in beer, gas chromatographic method, method II was adopted final action, **982.12**.
(2) The method for ethanol and original gravity content in beer, SCABA method was adopted first action, **992.29**.

Ch. 28. Wines

No additions, deletions, or other changes.

Ch. 29. Nonalcoholic Beverages and Concentrates

No additions, deletions, or other changes.

Ch. 30. Coffee and Tea

No additions, deletions, or other changes.

Ch. 31. Cacao Bean and its Products

No additions, deletions, or other changes.

Ch. 32. Cereal Foods

(1) The method for total dietary fiber, enzymatic-gravimetric method was adopted first action, **992.16**.
(2) The method for crude protein in cereal grains and oilseeds, generic combustion method was adopted first action, **992.23**.
(3) The method for (1 → 3)(1 → 4)-beta-D-glucan in grains & cereals, enzymatic-spectrophotometric method was adopted first action, **992.28**.
(4) The first action method for gluten in foods, colorimetric monoclonal antibody enzyme immunoassay method was editorially revised, **991.19**.

Ch. 33. Dairy Products

(1) The first action method for *N*-nitrosodimethylamine in nonfat dry milk, gas chromatographic method was adopted final action, **984.16**.
(2) The first action method for fat in milk, modified Mojonnier ether extraction method was adopted final action, **989.05**.
(3) The method for iodine (as iodide) in pasteurized liquid milk and skim milk powder, liquid chromatographic method was adopted first action, **992.22**.
(4) The modified method for sampling of milk from bulk tanks and other storage equipment, automated method, was adopted first action **970.26**.
(5) The final action IDF-ISO-AOAC method for fat in cream was revised, **920.111**.
(6) The revised first action method for fat in raw milk was revised, **989.04**.

(7) The final action IDF-ISO-AOAC method for water (added) in milk, thermistor method was revised, **961.07**.

Ch. 34. Eggs and Egg Products

No additions, deletions, or other changes.

Ch. 35. Fish and Other Marine Products

No additions, deletions, or other changes.

Ch. 36. Flavors

(1) The first action method for color (insoluble in amyl alcohol) in vanilla extract, colorimetric method was adopted final action, **920.133**.

(2) The first action method for benzoic acid in almond extract was adopted final action, **909.02**.

(3) The first action method for methanol in vanilla extract, titrimetric method was adopted final action, **920.132**.

(4) The first action method for ginger extract, spectrophotometric (430 mm) method was adopted final action, **920.146A**.

(5) The first action method for ginger extract, solids was adopted final action, **920.146B**.

(6) The first action method for ginger extract, ginger (qualitative test) was adopted final action, **920.146C**.

(7) The first action method for peppermint, spearmint, and wintergreen extracts was adopted final action, **920.147A**.

(8) The first action method for peppermint, spearmint, and wintergreen extracts, alcohol-method II was adopted final action, **920.147B**.

(9) The first action method for peppermint, spearmint, and wintergreen extracts, alcohol-method III (gas chromatographic method) was adopted final action, **920.147C**.

(10) The first action method for peppermint, spearmint, and wintergreen extracts, Babcock method was adopted final action, **920.147E**.

(11) The first action method for anise and nutmeg extracts, alcohol, gas chromatographic method was adopted final action, **920.148A**.

(12) The first action method for anise and nutmeg extracts, oil-method I was adopted final action, **920.148B**.

(13) The first action method for anise and nutmeg extracts, Babcock method was adopted final action, **920.148C**.

(14) The first action method for oils of lemon and orange in extracts, by precipitation in presence of mineral oil, Babcock method was adopted final action, **925.33C**.

(15) The first action method for oils of lemon, orange, or lime in oil-base flavors, polarization method was adopted final action, **926.11**.

(16) The first action method for oil in cassia, cinnamon, and clove extracts was adopted final action, **932.10**.

(17) The first action method for essential oil in flavor extracts and toilet preparations, Babcock method was adopted final action, **932.11**.

(18) The first action method for benzaldehyde in almond extract, gravimetric method was adopted final action, **936.10**.

(19) The first action method for essential oil in emulsion was adopted final action, **942.08**.

(20) The first action method for alcohol in almond extract was adopted final action, **950.44A**.

(21) The first action method for alcohol in almond extract was adopted final action, **950.44B**.

(22) The first action method for alcohol in almond extract, method III (gas chromatographic method) was adopted final action, **950.44C**.

(23) The first action method for alcohol in cassia, cinnamon, and clove extracts, method III (gas chromatographic method) was adopted final action, **950.45C**.

(24) The first action method for vanilla resins in vanilla extract, paper chromatographic qualitative test was adopted final action, **960.36**.

(25) The first action method for alcohol in flavors, gas chromatographic method was adopted final action, **973.23**.

(26) The first action method for alcohol in lemon, orange, and lime extracts, gas chromatographic method was adopted final action, **975.24**.

(27) The first action method for alcohol in flavor extracts and toilet preparations, gas chromatographic method was adopted final action, **975.25**.

(28) The first action method for glycyrrhizic acid or glycyrrhizic acid salts in licorice products, liquid chromatographic method was adopted final action, **982.19**.

(29) The first action method for sugars in licorice extracts, liquid chromatographic method was adopted final action, **984.17**.

(30) The first action method for vanillin, vanillic acid, *p*-Hydroxybenzaldehyde, and *p*-Hydroxybenzoic acid in vanilla extract, liquid chromatographic method was adopted final action, **990.25**.

(31) The final action method for plant material (foreign) in vanilla extract, paper chromatographic method, preparation of authentic vanilla extract, FEMA method was declared surplus, **960.37C(b)**.

(32) The first action method for ginger extract was editorially revised, **920.146A**.

Ch. 37. Fruits and Fruit Products

(1) The method for corn-derived acetic acid in apple cider vinegar, detection by carbon stable isotope ratio analysis was adopted first action, **992.08**.

(2) The method for sugar beet-derived syrups in frozen concentrated orange juice, stable isotope ratio mass spectrometric method was adopted first action, **992.09**.

(3) The final action method for anthocyanins in fruit juices, paper chromatographic method was declared surplus, **967.17**.

(4) The final action method for malvidin glucosides in grape juice, paper chromatographic method was declared surplus, **968.21**.

(5) The final action method for organic acids (foreign) in fruit juices, paper chromatographic method was declared surplus, **969.30**.

Ch. 38. Gelatin, Dessert Preparations, and Mixes

No additions, deletions, or other changes.

Ch. 39. Meat and Meat Products

(1) The method for crude protein in meat and meat products, combustion method was adopted first action, **992.15**.

Ch. 40. Nuts and Nut Products

No additions, deletions, or other changes.

Ch. 41. Oils and Fats

(1) The first action method for erucic acid in oils and fats, thin layer and gas chromatographic method was adopted final action, **985.20**.

(2) The first action method for total *trans* fatty acid isomers in margarines, gas chromatographic method was adopted final action, **985.21**.

(3) The first action method for triglycerides in fats and oils, gas chromatographic method was adopted final action, **986.19**.

(4) The final action AOCS-AOAC method for peroxide value of oils and fats was editorially revised, **965.33**.

Ch. 42. Vegetable Products: Processed

No additions, deletions, or other changes.

Ch. 43. Spices and Other Condiments

(1) The first action ASTA-AOAC method for piperine in pepper preparations, spectrophotometric method was revised, **987.07**.

Ch. 44. Sugars and Sugar Products

No additions, deletions, or other changes.

Ch. 45. Vitamins and Other Nutrients

(1) The method for vitamin E activity (All-rac-α-tocopherol) in milk-based infant formula, liquid chromatography method was adopted first action, **992.03**.

(2) The method for vitamin A (retinol isomers) in milk and milk based infant formula, liquid chromatography method was adopted first action, **992.04**.

(3) The method for folic acid (pteroylglutamic acid) in infant formula, microbiological method was adopted first action, **992.05**

(4) The method for vitamin A (retinol) in milk-based infant formula, liquid chromatographic method was adopted first action, **992.06**.

(5) The method for pantothenic acid in milk-based infant formula, microbiological turbidimetric method was adopted first action, **992.07**.

(6) The method for iodide in ready-to-feed milk-based infant formula, ion-selective electrode method was adopted first action, **992.24**.

(7) The method for linoleic acid in ready-to-feed milk-based infant formula, gas chromatography method was adopted first action, **992.25**.

(8) The method for vitamin D_3 (cholecalciferol) in ready-to-feed milk-based infant formula, liquid chromatographic method was adopted first action, **992.26**.

(9) The method for trans-vitamin K_1 (phylloquinone) in ready-to-feed milk-based infant formula, liquid chromatographic method was adopted first action, **992.27**.

Ch. 46. Color Additives

No additions, deletions, or other changes.

Ch. 47. Food Additives: Direct

(1) The modified final action method for phenolic antioxidants in oils, fats, and butter oil, liquid chromatographic method was adopted first action, **983.15**.

Ch. 48. Food Additives: Indirect

No additions, deletions, or other changes.

Ch. 49. Natural Poisons

(1) The first action method for deoxynivalenol in wheat, gas chromatographic method was revised, **986.18C**.

Instructions to Authors

Scope of Articles and Review Process

The *Journal of AOAC International* publishes articles that present, within the fields of interest of the Association: unpublished original research; new methods; further studies of previously published methods; background work leading to development of methods; compilations of authentic data of composition; monitoring data on pesticide, metal, and industrial chemical contaminants in food, tissues, and the environment; technical communications, cautionary notes, and comments on techniques, apparatus, and reagents; invited reviews and features. Emphasis is on research and development of precise, accurate, sensitive methods for analysis of foods, food additives, supplements and contaminants, cosmetics, drugs, toxins, hazardous substances, pesticides, feeds, fertilizers, and the environment. The usual review process is as follows: *(1)* AOAC editorial office transmits each submitted paper to appropriate subject matter editor, who solicits peer reviews; *(2)* editor returns paper to author for revision in response to reviewers' comments; editor accepts or rejects revision and returns paper to AOAC editorial office; *(3)* AOAC editorial staff edits accepted papers, returns them to authors for approval, and transmits approved manuscripts to desktop publisher; *(4)* desktop publisher sends page proofs to author for final approval.

General Information

Follow these instructions closely; doing so will save time and revision. For all questions of format and style not addressed in these instructions, consult recent issue of *Journal* or current edition of *Council of Biology Editors Style Manual*. For describing collaborative studies, contact AOAC Technical Services for guidelines.

1. Write in clear, grammatical English.

2. To Managing Editor, AOAC International, Suite 400, 2200 Wilson Blvd, Arlington, VA 22201-3301 USA, submit typewritten original plus 3 photocopies (1 side only, white bond, 8½ × 11 in. [21½ × 28 cm] of complete manuscript in order as follows—1. Title page; 2. Abstract; 3. Text (introduction, method or experimental, results and/or discussion, acknowledgements, references); 4.Figure captions; 5. Footnotes; 6. Tables with captions, one per page; 7. Figures.

3. Suggest in a covering letter the names of at least 4 qualified reviewers, i.e., individuals engaged in or versed in research of the type reported.

4. **DOUBLE SPACE all typed material.** Manuscripts not double spaced will be returned for retyping. Do not right justify or use proportional spacing; avoid hyphenation.

5. Use letter quality printer for word-processed manuscripts; manuscripts prepared on dot matrix printers of less than letter quality may be refused. Once a manuscript has been accepted for publication, authors will receive instructions for submitting the final version of their accepted manuscript to AOAC on diskette. AOAC accepts MS-DOS-based files from most word processing packages or ASCII text files on MS-DOS-formatted diskettes. (DO NOT SEND DISKETTE WITH ORIGINAL MANUSCRIPT SUBMISSION.)

Format and Style

1. **Title page** (separate sheet, **double spaced**): title of article, authors' names (full first, middle initial if any, full last), authors' addresses including mail codes.

2. **Abstract** (separate sheet, **double spaced**): ≤ 200 words. Provide specific information, not generalized statements.

3. **Text** (consecutive sheets, **double spaced**): *Introduction.* Include information on why work was done, previous work done, use of compound or process being studied.

Method or Experimental. Consult recent issue of *Journal* for proper format. Separate special reagents/apparatus from details of procedure and list in sections with appropriate headings; avoid use of brand names. (Common reagents/apparatus or those which require no special treatment need not be listed separately.) Place detailed operations in separate sections with appropriate headings (e.g., Preparation of Sample, Extraction and Cleanup). Include necessary calculations; number of significant figures must reflect accuracy of method. Use metric units for measurements of quantities wherever possible. Note hazardous and/or carcinogenic chemicals.

Results/Discussion. Cite tables and figures consecutively in text with Arabic numerals. *Do not* intersperse tables and figures in text.

Acknowledgments. Give brief thanks (no social or academic titles) or acknowledge financial aid in this section.

References. Submitted papers or unpublished oral presentations may not be listed as references; cite them in text as unpublished data or personal communications. Cite all references to previously published papers or papers in press in numerical order in text with number in parentheses on line (*not* superscript). List references numerically in "References" in *exactly* (arrangement, punctuation, capitalization, use of ampersand, etc.) styles of examples shown below or see recent issue of *Journal* for less often used types of entries. Follow *Chemical Abstracts* for abbreviations of journal titles.

JOURNAL ARTICLE REFERENCE

(1) Engstrom, G.W., Richard, J.L., & Cysewski, S.J. (1977) *J. Agric. Food Chem.* **25,** 833–836

BOOK CHAPTER REFERENCE

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

BOOK REFERENCE

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

OFFICIAL METHODS REFERENCE

(1) *Official Methods of Analysis* (1990) 15th Ed., AOAC, Arlington, VA, secs **29.070–29.072**

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

5. **Footnotes** (separate sheet(s), **double spaced**): Avoid use of footnotes to text. Include "Received...Accepted..." line; location/date of presentation, if appropriate; present address(es) of author(s); identification of corresponding author, if not senior author, proprietary disclaimers; institution journal series numbers.

6. **Tables** (one per page, **double spaced**): Refer to recent issue of *Journal* for proper layout and style, especially use of horizontal lines. Do not draw in vertical lines. Include descriptive title sufficient that table stands alone without reference to text. Provide heading for *every* vertical column. Abbreviate freely; if necessary, explain in footnotes. Indicate footnotes by lower case superscript letters in alphabetical order. Do not use one-column tables; rather, incorporate data in text.

7. **Figures:** The *Journal* does not publish straight line calibration curves; state such information in text. Do not duplicate data in tables and figures. Submit original drawings or black/white glossy photographs with original manuscript; photocopies are acceptable only for review. Prepare drawings with black India ink or with drafting tape on white tracing or graph paper printed with nonreproducible green ink. Lines, letters, numbers, and symbols must be sharp and unbroken. Use type at least 2 mm high to allow reduction to page or column size. Identify ordinate and abscissa and give value in *Journal* style (e.g., "Wavelength, nm," "Time, min"). Label curves with letters or numbers; avoid all other lettering/numbering on face of figure (see **Figure captions**). Identify each figure on back with number and authors' names.

8. **Miscellaneous** Abbreviation for liter is L; abbreviation for micron is μm. Do not italicize common Latin expressions such as et al. and in vitro; for nomenclature of spectrophotometry, gas chromatography, and liquid chromatography, follow practice of American Society for Testing and Materials (in particular, do not use "high performance," "high pressure," or the abbreviation "HP" with "liquid chromatography").

JOIN AN AOAC REGIONAL SECTION OR START ONE IN YOUR AREA!!

CURRENT AOAC REGIONAL SECTIONS

Central Regional Section

President: Marjeanne Morrison
O.M. Scott & Sons Co
1411 Scottslawn Road
Marysville, OH 43041
(513) 644-0011

Eastern Ontario-Quebec Regional Section

Chairman: Michele G. Bono
Health & Welfare Canada
Health Protection Branch
1001 St Laurent O
Longueuil PQ J4K 1C7
Canada
(514) 646-1353

Europe Regional Section

President: Juan Sabater
Laboratory Dr. Sabater S A
Calle De Londres 6
08029 Barcelona
Spain
+34 (3) 3228806

MidAtlantic USA Regional Section

President: David MacLean
6422 Alloway Court
Springfield, VA 22152
(703) 451-1578

Mid-Canada Regional Section

President: Tom Nowicki
Canadian Grain Commission
1401-303 Main Street
Winnipeg, Manitoba, R3C 3G1
Canada
(204) 983-3345

Midwest Regional Section

Chairman: P. Frank Ross
US Department of Agriculture
National Vet Service Lab
PO Box 844
1800 Dayton Rd
Ames, IA 50010
(515) 239-8542

New York-New Jersey Regional Section

President: Jeff Singer
DuPont Merck
1000 Stewart Avenue
Garden City, NY 11530
(516) 832-2171

Northeast Regional Section

President: J. Benjamin Harnish
Nova Scotia Department
of Agriculture and Marketing
PO Box 550
Truro NS, B2N 5E3
Canada
(902) 893-6562

Pacific Northwest Regional Section

Chairman: Andrew Held
MEI Charlton, Inc
2233 SW Canyon Rd
Portland, OR 97201
(503) 228-9663

Pacific Southwest Regional Section

President: Paul Bolin
DFA of California
1855 S Van Ness
Fresno, CA 93721
(209) 233-7249

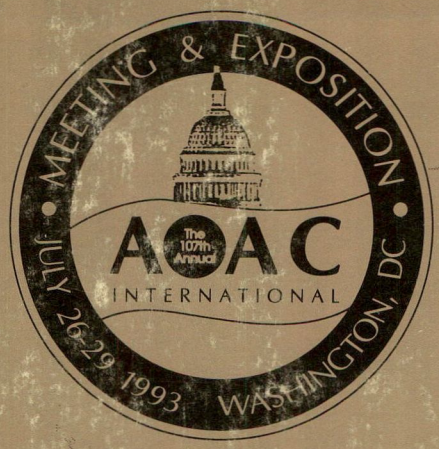
Southeast-USA Regional Section

President: Virlyn Burse
Cen for Env Health
Clinical Chem Div
1600 Clifton Rd
Atlanta, GA 30333
(404) 488-4176

Southwest-USA Regional Section

President: Tom Wilson
Colonial Sugars, Inc
PO Box 3360
Gramercy, LA 70052-3360
(504) 869-5521

For information on starting a regional section, contact; Regional Section Coordinator, AOAC International, 2200 Wilson Blvd, Arlington, VA 22201-3301, USA, +1 703 522 3032, fax +1 703 522-5468.



The 107th Annual AOAC International Meeting & Exposition

A Focus on Methods Needs for Chemical and Biological Analysis

Symposia

- Laboratory Management: OSHA Compliance
- Technology of the Future: Neural Networks/Artificial Intelligence
- Pesticide Residues in Humans
- Capillary Electrophoresis
- Analytical Methods for Seafood Toxins
- Steroids—How are They Abused and Analyzed?
- Immunoassays for Pesticide Residues and Chemical Contaminants
- Oil Spill Contaminants Methodology
- Microbiology: Disinfectants
- **The Fifth International Symposium on the Harmonization of Internal Quality Assurance Schemes for Analytical Laboratories***

* Sponsored by AOAC International, ISO and IUPAC

Plus . . .

More than 200 poster presentations

Workshops: **Good Laboratory Practices, including Automated Laboratory Practices**
Preparing for Laboratory Inspections
Antibiotics and Drugs in Feeds

Regulatory Roundtable: **Labeling Issues in the International Arena—What Are the Methods Requirements?**

Open Forum, Laboratory Equipment Exposition, Modern Laboratory Workshop, Short Courses

For more information, contact the AOAC International Meetings Department:
+1 (703) 522-3032 or FAX +1 (703) 522-5468