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# JOURNAL OF AOAC INTERNATIONAL

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AOAC INTERNATIONAL

JANCA 75(1)1–226 (1992)
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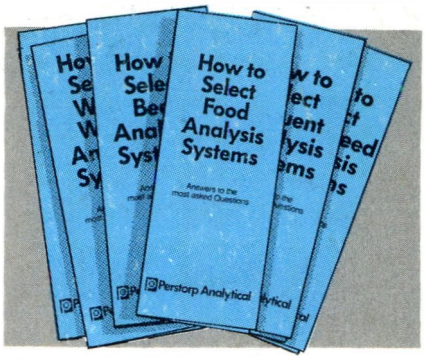


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## RECOMMENDATIONS FOR OFFICIAL METHODS

## EXECUTIVE, OFFICER, AND COMMITTEE REPORTS



## AOAC INTERNATIONAL



## OFFICIAL METHODS OF ANALYSIS

# Books in Brief

**Food and Packaging Interactions II.** Edited by Sara J. Risch and Joseph H. Hotchkiss. Published by the American Chemical Society, 1155 16th St, NW, Washington, DC 20036, 1991. 265 pp. Price: U.S. & Export: $59.95. ISBN 0-8412-2122-7.

Once simply a convenient container to hold and carry food, packaging has now become an integral part of the food chain. This new volume provides the most comprehensive and current information available on food packaging interactions, including presentations on migration, scalping, and permeability. A significant portion of the volume covers research on microwave susceptor packaging, the materials that promote browning and crisping of products. This is a valuable resource for food product development scientists and engineers who need to understand what can happen to foods in different types of packages. Regulatory agencies can also use this book to keep pace with new developments and to maintain appropriate regulations. It includes information on regulation of packaging for the United States, United Kingdom, and European Community.

**Analytical Methods in Toxicology.** By H.M. Stahr. Published by John Wiley & Sons, Inc., 1 Wiley Dr., Somerset, NJ 08875-1272, 1991. 328 pp. Price: $69.95. ISBN 0471-85136-1.

The analytical chemist is ordinarily taught with inanimate samples. Yet, the real world of the analytical toxicologist is fraught—from ceiling to floor—with biological hazards. How to sensibly arm one's self with a clearcut, hazard-reducing laboratory regimen and at the same time conduct thorough analyses of toxic substances is the subject of this indispensable guide. *Analytical Methods in Toxicology* is a manual of procedures used and compiled by the Chemistry Laboratory, Veterinary Diagnostic Laboratory at Iowa State University. In explicit steps, the book outlines how to handle and store specimen samples, the length of an analysis, how to use equipment sensibly, what to wear, what sort of cleanup regimen to maintain—in short, how to skillfully negotiate the potential hazards of laboratory work.

**Stationary Phases in Gas Chromatography.** By H. Rotzsche. Published by Elsevier Science Publishers, PO Box 211, 1000 AE Amsterdam, The Netherlands, 1991. 424 pp. Price: U.S. $166.50/Dfl. 325.00. ISBN 0-444-98733-9.

The primary aim of this volume is to make the chemist familiar with the numerous stationary phases and column types, with their advantages and disadvantages, to help in the selection of the most suitable phase for the type of analytes under study. The book also provides detailed information on the chemical structure, physico-chemical behavior, experimental applicability, physical data of liquid and solid stationary phases and solid supports. Such data were previously scattered throughout the literature. To understand the processes occurring in the separation column and to offer a manual both to the beginner and to the experienced chromatographer, one chapter is devoted to the basic theoretical aspects. Further, as the effectiveness of the stationary phase can only be considered in relation to the column type, a chapter on different column types and the arrangement of the stationary phase within the column is included. The secondary aim of this book is to stimulate the development of new and improved standardized stationary phases and columns, in order to improve the reproducibility of separations, as well as the range of applications.

**CTFA International Cosmetic Ingredient Dictionary.** Published by the Cos-

metic, Toiletry, and Fragrance Association, 1101 17th St, NW, Suite 300, Washington, DC 20036, 1991. $395.00.

CTFA recently published the new 4th edition of the *CTFA International Cosmetic Ingredient Dictionary.* It is a reference book that many analytical chemists will find very useful to have in their laboratories. Although the dictionary's focus is personal care products, many of the same ingredients are used in a wide range of household products, some food and drugs, and other products of interest to laboratory personnel. The new edition of CTFA Dictionary contains the latest information on more than 5300 cosmetic, toiletry, and personal care product ingredients cross referenced to over 23000 chemical synonyms and trade names. It also includes the names of 450 domestic and overseas suppliers of these ingredients.

**Immunoassays for Trace Chemical Analysis: Monitoring Toxic Chemicals in Humans, Food, and the Environment.** Edited by Martin Vanderlaan, Larry H. Shankar, Bruce E. Watkins, and Dean W. Roberts. Published by the American Chemical Society, 1155 16th St, NW, Washington, DC 20036, 1991. 354 pp. Price: U.S. & Export $79.95. ISBN 0-8412-1905-2.

Increasing public concern over chemical exposures and the quality of the food supply has resulted in increased pressure on regulatory agencies to do more sampling for more chemicals. This new volume explores the use of immunoassays as alternative methods for conducting this needed sampling. It brings together a broad range of the applications of analytical immunochemistry, including immunoassays for chemical residues in food and the environment, for natural toxins, and for monitoring human exposure to toxic chemicals. Each section begins with an informative review highlighting the major issues in that section. Also included are 3 appendixes that provide up-to-date compilations of references organized according to applications: environmental monitoring, mycotoxin analysis, and DNA- and protein-adduct analyses.

# NEW RELEASE FROM AOAC INTERNATIONAL


*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.
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AOAC

# New Products

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### Teflon Tanks Molded in Many Sizes

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### Bio-Sil Reversed Phase Silica

Bio-Sil bonded phase silicas are a complete line of silica gel based products for use in reversed phase and normal phase chromatography applications. The line includes C18, C8, TMS, phenyl, cyano, and amino bonded phases. This silica is

manufactured in large batches and is derivatized under computer controlled conditions to insure reproducibility. The silicas are available in 15–35 μm and 40–63 μm particle sizes, for use in medium or low pressure applications. Bio-Rad Laboratories.
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### Gas Chromatograph with New Discharge Ionization Detector

The Series 590 isothermal gas chromatograph has been engineered for trace gas analysis, with sensitivity at the low part per billion level, for applications in the electronic and specialty gases marketplace. Highlighted applications include detecting trace impurities in $H_2$, He, and $0_2$, as well as $SiH_4$ and $GeH_4$ in $AsH_3$. It contains a single discharge ionization detector, which is non-radioactive, universal, and concentration dependent. Unique sample isolation valves have been designed to provide faster and easier start-up than radioactive, ionization-type detectors. GOW-MAC Instrument Co., Inc.
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### Turn HPLC System Into Carbamate Analyzer

To analyze for carbamate pesticides according to EPA Method 531.1, a post-column analysis module can be easily connected to an existing HPLC system. The post-column carbamate analysis module from Pickering Laboratories comes complete with everything required, including dual reagent pumps for hydrolysis and OPA reagents, a column temperature controller, a heated reactor for hydrolysis, an ambient-temperature reactor for the fluorescent isoindole derivatization, pulse dampeners, flow conditioners, and safety interlock devices to prevent backflow of reagent into the column. Also included are 2 columns—packed with specially selected silica, serialized and pre-run for repeatable separation of the 12 common carbamates—plus reagents and standards. Pickering Laboratories.
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### Benchtop Microprocessor pH Meter with Audible Alarms

A programmable, PC-compatible benchtop microprocessor pH/mV/°C meter is designed for use in checking the pH of samples and experiments in a laboratory. The HI 9017 features 6 internal programs to enter buffers and to set audible alarms to alert users when selected pH, millivolt, and temperature values have been reached. All data collected can be input into a personal computer through the meter's RS232 interface. Hanna Instruments.
Circle No. 306 on reader service card.

### Petroleum Hydrocarbon Analyzers for Analysis of Oil Contamination in Water or Soil

Two new portable petroleum hydrocarbon analyzers have been introduced: the TPH analyzer measures the total petroleum hydrocarbons in soil or water samples while the TPH plus discriminates between the aromatic and aliphatic hydrocarbon levels in these samples. Digital displays provide readouts directly in mg/L with sensitivities in the ppm range. Both analyzers are durable and waterproof and are designed for use either directly on site or in a laboratory environment. General Analysis Corp.
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### Convenient and Economical LiChroCART Cartridge Design with manu-CART End Fittings

An easy to use, economical HPLC column cartridge system features uniquely designed, reusable manu-CART end fittings that require simple finger tightening even for high pressure operation. Available for 4 mm and 10 mm ID column cartridges, the manu-CART end fittings are supplied complete with a set of collets, which, when reversed, permit the use of an integrally designed guard column that creates no dead space. EM Separations.
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### Inlet System For EPA Method 8275, A New Field Screening Method for Thermal Extraction GC-MS

The ThermEx all quartz inlet system, interfaced to a gas chromatograph/mass spectrometer (GC-MS), is ideally suited for EPA Method 8275, a recently released method for detecting the presence of semi-volatile organic compounds in solid wastes and soils. This method uses thermal chromatography/mass spectrometry for the field screening of semi-volatile organic compounds. Compounds identifiable by Method 8275 include aniline, chlorinated phenols and benzenes, benzothiophenes, carbazole and aldrin, as well as 2- through 6-ring PNAs (PAHs), and PCBs. The ThermEx inlet thermally desorbs and extracts organics from solid or semisolid samples into GC-MS systems for applications where fast, reliable results are desired. Ruska Instrument Corp.
Circle No. 309 on reader service card.

### Multi-Purpose Laboratory Cutting Mills

The Gilson Fritsch Cutting and Precutting Mills provide laboratories with unprecedented capability for cutting and preparing virtually any type of test samples of soft to medium hard materials as well as fibrous and bulky substances. Models LC-124 and LC-125 use 4 rotating and 3 stationary knives that cut and shear sample material in the food-safe aluminum grinding chamber. The rotating cutting chambers act as an air pump

to carry product pieces through replaceable sieve inserts as soon as the desired size is reached. Gilson Co., Inc.
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### Detecting Diethylene Glycol in Wine

Diethylene glycol, a harmful substance sometimes used illegally to enhance the body and sweetness of low quality wine products, can be easily monitored to levels as low as 20 ppm with the Aminex HPX-87H column and the HRLC isocratic refractive index analyzer. Conventional methods for the detection of diethylene glycol are sometimes difficult, but the combination of a high resolution HPLC column and a high sensitivity refractive index detector provides a rapid, reliable method for screening wine samples for adulteration. Bio-Rad Laboratories.
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### Chemistry Graphics Software for Windows

ChemWindow v2.0 is a chemistry drawing program designed to create typeset quality graphics for chemical reports and papers. Version 2.0 is a major upgrade of the original ChemWindow; more than 40 new features were added to ChemWindow v2.0 including multiple drawing styles, a built-in scrapbook, style-sensitive templates, improved text handling, and the ability to read and edit Macintosh documents created by ChemWindow's sister product, ChemIntosh. ChemWindow templates are made by the user, saved in a document, and loaded into a pop-up palette. SoftShell International Ltd.
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### Save Time and Money, Achieve More Accurate Analysis, and Improve Your Laboratory's Reputation

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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.

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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!

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# For Your Information

## Meetings

*February 2–4, 1992:* Southwest USA Regional Section Meeting, Dallas, TX. Contact: Donna Montague, 211 S. Johnson St, Little Rock, AR 72205, telephone 501/686-5140.

*February 4–6, 1992:* Southeast USA Regional Section Meeting, Atlanta, GA. Contact: James Hess, USDA AMS CSSD, Eastern Laboratory, 645 Cox Rd, Gastonia, NC 28054, telephone 704/867-3873.

*May 11–12, 1992:* Northeast Regional Section Meeting, Nova Scotia, Canada. Contact: Ben Harnish, Nova Scotia Dept of Agriculture and Marketing, Box 550, Truro, NS, B2N 5E3, Canada, telephone 902/893-6562.

*June 8–10, 1992:* Midwest Regional Section Meeting, Champaign, IL. Contact: Karen Harlin, University of Illinois, Department of Veterinary Bioscience, 2001 S. Lincoln, Urbana, IL 61801, telephone 217/244-1569.

*June 24–26, 1992:* Pacific Northwest Regional Section Meeting, Olympia, WA. Contact: Norma J. Corristan, Oregon Dept of Agriculture, 635 Capitol St NE, Salem, OR 97310-0110, telephone 503/378-3793.

*August 30–September 3, 1992:* 106th AOAC Annual International Meeting and Exposition. Cincinnati, OH. Contact: AOAC Meetings Department, Suite 400, 2200 Wilson Blvd, Arlington, VA 22201-3301, telephone 703/522-3032.

*July 26–29, 1993:* 107th AOAC Annual International Meeting and Exposition, Washington, DC. Contact: AOAC Meetings Department, Suite 400, 2200 Wilson Blvd, Arlington, VA 22201-3301, telephone 703/522-3032.

## AOAC Prepares to Expand Methods Validation Programs

AOAC is moving forward with plans to launch 2 new methods validation programs, in addition to the interlaboratory collaborative study, review, and approval process leading to AOAC official methods. The 2 new programs are the test kit "Reviewed and Recognized ($R^2$)" evaluation of proprietary kit performance, and the "Peer Verified" status for methods that have not undergone the full collaborative study process.

*Background.*—Impetus for the new programs came from focus group sessions conducted for AOAC during 1990. The limited but in-depth survey of constituency attitudes and needs had been promoted by the AOAC Long Range Planning Committee to seek direction in areas of methods validation, international cooperation, and member services.

Focus group participants were asked to comment on the importance of validated methods, the suitability of the interlaboratory collaborative study process, and the acceptability of alternative method validation approaches. Results showed that fully collaborated methods are critical for fulfilling governmental, intergovernmental, and international regulatory requirements, but by their nature, require considerable effort, time, and expense. Participants pointed to the need for more efficient (both in cost and time) mechanisms for identifying good methods, subjecting them to limited testing, and making them available to the analytical community. Participants indicated that, even with minimum confirmation, such methods are needed and useful.

They also pointed to the need for AOAC to respond to the fast developing area of proprietary test kit-based methods and to provide at least minimum assurance to the user that kits performed as advertised.

*Reviewed and Recognized ($R^2$) Status for Test Kits.*—The purpose of this program is to provide third party evaluation of kit performance, as claimed by the manufacturer. The program is intended to give manufacturers an alternative to the traditional AOAC interlaboratory collabora-

tive study process for multiple, rapidly changing proprietary kit-based methods, to provide regulatory agencies with a useful measure of kit performance, and to provide the user with assurance that kits tested by the third party met performance as advertised.

A task force, headed by Board Director P. Frank Ross, outlined a proposed program and developed procedures for the applicant, reviewers, and program administrator. In developing the program, the task force consulted members, the intended participants, and other product certification organizations, and reviewed internationally accepted documents on third party certification systems, validation systems, proprietary techniques, and performance evaluation.

In summary, the proposed program will be fee-based and operated through a nonprofit subsidiary. The applicant will supply a data package including a completed application form, signed indemnification agreement, description of manufacturing quality assurance program, test kit and insert, information on intended use and users, suggested evaluation protocol for independent laboratory, and data on performance specifications. Test kit performance will undergo an independent laboratory assessment. Data from the test kit applicant and the independent laboratory will be evaluated by the designated test kit reviewers. Under the current proposal, successful applicants would be licensed to use the AOAC "Reviewed and Recognized ($R^2$)" logo on their kits, inserts, and advertising. Processes will also be in place for renewals and revisions.

*Peer Verified Methods Status.*—AOAC members have consistently voiced a need for access to a greater number of methods than are granted official status by AOAC. The fully collaborated, strenuously reviewed, first action methods of the association are seen as the ultimate, high class, solid methods depended on for over 100 years for critical analyses. But laboratories have many analytical needs that can be satisfactorily addressed by much less rigorously validated methods.

In addressing this area, the Board of Directors considered 3 aspects:

First, most focus group participants believed that AOAC should publish lesser validated methods. There are ways, other than the collaborative study, to validate methods with reasonable certainty.

Second, many regulatory organizations currently use test methods for reg-

ulatory purposes that are validated through processes less rigorous than the AOAC collaborative study.

And third, advances in technology, both in the products to be tested and the methodology of test methods, make it difficult for AOAC to keep abreast of such developments with a slow-moving, deliberate process like interlaboratory validation and review.

For these reasons, the Board decided to pursue a new, additional program for validated methods, with these goals:

- The quality of the methods should still be high, even though the verification process would be less scientifically rigorous;
- This process should be separate and distinct from the collaborative study process, but could be a conduit to that process;
- The verification process should be fast, and a means for rapid dissemination of accepted methods should be developed.

A program outline was developed by the task force, headed by Board Director Alan R. Hanks. In the proposal, the method developer will be responsible for generating a data package consisting of in-house test data plus verification data from a second laboratory. The in-house data will cover the following:

- method development data, where available and applicable;
- method principle, applicability, scope, and limitations;
- standard curves for all specified analytes, matrixes, and concentration ranges;
- ruggedness testing of all critical variables;
- performance characteristics including day-to-day precision plus accuracy for critical steps and overall;
- interferences and their characterization;
- false positive/false negative rates;
- quality assurance requirements including use of reference materials and system suitability tests;
- sampling requirements, sample acceptability, and storage requirements.

Under the current proposal, the second laboratory will be recruited by the method developer, will have experience in the type of method, and will receive the method write-up as the developer proposes, and test samples. The test samples will preferably include duplicate samples analyzed by the originating laboratory, reference standard materials, and, where appropriate, incurred residue samples. The laboratory will follow a protocol to verify the performance characteristics of the method, and comment on ease of use, special requirements, and presentation.

Results will be reviewed by at least 2 expert reviewers, designated by AOAC, according to predetermined procedures and criteria for acceptance. Accepted methods will be granted "Peer Verified" status, and will be published, possibly in loose-leaf format on a subscription basis, and/or possibly in electronic format on a bulletin board. The bulletin board option is attractive to the task force because it affords the capability to generate a dialogue on critical points, applicability (or nonapplicability) to analytes or matrixes beyond the original test, improvements in technology of the methods, and the like.

Similar to the test kit confirmation program, the peer verified process is expected to be timely, with the goal to complete the review and verification as rapidly as possible after the application is received.

*Alternative Programs and the Collaborative Study Process.*—The "Reviewed and Recognized ($R^2$)" program will provide the user of the test kit confirmation that, for specifications stated by the manufacturer, the kit performed as stated when evluated by an independent laboratory. The "Peer Verified" program will provide the user with methods that have been tested for performance and repeatability in at least one other laboratory. In each instance, the users will have to decide whether the minimum levels of confidence provided by either program fill the particular need.

A full collaborative study will be required in those instances where the user requires a higher degree of confidence in the test results than is afforded by either the $R^2$ or the "Peer Verified" program. AOAC will continue to encourage rigorous collaborative study of test kits and lesser validated methods and their adoption as official methods when the user requires that degree of confidence in the measurements.

Therefore, both task forces and the Official Methods Board will be working together to harmonize the intralaboratory and ruggedness testing protocols required in all 3 processes. This will enable method applicants to continue more easily from the kit review or peer verification programs into the collaborative study process without duplication or regeneration of preliminary data.

It is to be emphasized that details of new AOAC programs are still being developed by AOAC staff and the task forces. The draft programs, as they develop, are available. Comments on the programs are encouraged from all interested persons. For further information, or to obtain a copy of the program documents, contact: AOAC Technical Services, AOAC, 2200 Wilson Blvd, Suite 400, Arlington, VA 22201-3301, telephone 703/522-3032, fax 703/522-5468.

## AOAC to Launch Two New Short Courses in 1992

Two new short courses, both with wide audience appeal, will expand AOAC's educational program beginning in 1992. "Improving Your Technical Writing

Skills" and "How to Testify as an Expert Witness" are both 2-day programs designed to develop and improve the skills and to complement the scientific expertise of attendees.

"Improving Your Technical Writing Skills" will be taught by Helen L. - Reynolds, former Technical Editor of the U.S. Food and Drug Administration. The course will offer insight on how to get your paper published by the journal of your choice and how to minimize the need for revisions. It will emphasize organizing your materials, which Reynolds describes as "the key to the process." Attendees will leave with helpful hints, checklists, and memory aids.

Participants will be encouraged to send samples of their writing to the course director in advance of the course, and will find a written critique waiting for them in the classroom. Students will separate into working groups for practical exercises and individual feedback. Another unique feature of this course is a follow-up from the course director at no additional cost.

Reynolds taught this course during her years at FDA and suggests that it might be subtitled "Easing the Agony of Writing a Scientific Paper."

She has also made an AOAC videotape, "How to Write Your Collaborative Study Report."

"How to Testify as an Expert Witness" will be taught by Carol Henderson- Garcia, Associate Professor of Law at Nova University Law Center in Fort Lauderdale, FL. Garcia's program includes both lecture and role-playing with immediate critique and feedback. All participants are videotaped and receive a copy of their tape as part of the course materials.

She discusses such issues as responding to questions credibly, courtroom demeanor—how to present yourself as a credible expert witness—and how to avoid getting yourself into trouble when you are testifying. The combination of lecture and practical exercises provides not only the basic information needed, but useful tips on how to use it effectively.

AOAC member Robert Bianchi, Drug Enforcement Administration, says, "Dr. Garcia has presented this course to the forensic science community, with overwhelming success and standing room only. She is very professional, knowledgeable, and has good practical experience."

Garcia's extensive criminal litigation experience includes 4 years on the staff of the United States Attorney in Washington, DC. In addition to her courtroom responsibilities, she was also responsible for the training of new Assistant United States Attorneys.

AOAC Director of Administration and Meetings Margaret R. Ridgell says, "We are very enthused about both of these new courses. They will offer valuable information and good practical experience for scientists, which will save them time and permit them to function more effectively. That is worth a great deal these days."

## COMING IN THE NEXT ISSUE

### DRUGS IN FEEDS

■ Determination of Monensin in Raw Material, Premix, and Animal Feeds by Liquid Chromatography with Correlation to Microbiological Assay—J.M. Rodewald, J.W. Moran, A.L. Donoho, and M.R. Coleman

### DRUG RESIDUES IN ANIMAL TISSUES

■ Analytical Strategy for Regulatory Control of Residues of Chloramphenicol in Meat: Preliminary Studies in Milk—H.J. Keukens, M.M.L. Aerts, W.A. Traag, J.F.M. Nouws, W.G. de Ruig, W.M.J. Beek, and J.M.P. den Hartog

### MYCOTOXINS

■ Bioassay, Extraction, and Purification Procedures for Wortmannin, the Hemorrhagic Factor Produced by *Fusarium oxysporum* N17B Grown on Rice—H.K. Abbas, C.J. Mirocha, W.T. Shier, and R. Gunther

### VITAMINS AND OTHER NUTRIENTS

■ Fluorometric Determination of Thiamin Vitamers in Chicken—J.B. Fox, Jr, S.A. Ackerman, and D.W. Thayer

# Conference Report

## Analytical Methods Validation: Bioavailability, Bioequivalence, and Pharmacokinetic Studies

VINOD P. SHAH, KAMAL K. MIDHA[1], SHRIKANT DIGHE, IAIN J. MCGILVERAY, JEROME P. SKELLY, AVRAHAM YACOBI, THOMAS LAYLOFF, C.T. VISWANATHAN, C. EDGAR COOK, R.D. MCDOWALL, KENNETH A. PITTMAN, and SIDNEY SPECTOR

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This is a summary report of the conference on "Analytical Methods Validation: Bioavailability, Bioequivalence, and Pharmacokinetic Studies." The conference was held December 3–5, 1990, in the Washington, DC, area and was sponsored by the American Association of Pharmaceutical Scientists, U.S. Food and Drug Administration, Federation International Pharmaceutique, Health Protection Branch (Canada), and AOAC International. The purpose of this report is to represent our assessment of the major agreements and issues discussed at the conference. The report is also intended to provide guiding principles for validation of analytical methods employed in bioavailability, bioequivalence, and pharmacokinetic studies in humans and animals.

The objectives of the conference were as follows: (*1*) to reach a consensus on what should be required in analytical methods validation and the procedures to establish validation; (*2*) to determine processes of application of the validation procedures in the bioavailability, bioequivalence, and pharmacokinetic studies; and (*3*) to develop a report on analytical methods validation, which may be referred to in developing future formal guidelines.

Acceptable standards for documenting and validating analytical methods with regard to processes, parameters, or data treatments were discussed because of their importance in assessment of pharmacokinetic, bioavailability, and bioequivalence studies. Other topics that were considered essential in the conduct of pharmacokinetic studies or in establishing bioequivalency criteria, including measurement of drug metabolites and stereoselective determinations, were also deliberated.

### Introduction

Analytical methods employed for the quantitative determination of drugs and their metabolites in biological samples play a significant role in evaluation and interpretation of bioavailability, bioequivalence, and pharmacokinetic data. Well-characterized and fully validated analytical methods must be used to yield reliable results that can be satisfactorily interpreted. Analytical methods and techniques are constantly being changed and improved, and, in many instances, they are at the cutting edge of the technology. Each analytical technique has its own characteristics that will vary from drug to drug. Moreover, the appropriateness of the technique may also be influenced by the ultimate objective of the study. Specific validation criteria are needed for methods intended for analysis of each analyte (drug and/or metabolite).

While validation of each method will stand on its own, there may be situations where comparison of the methods will be necessary, e.g., when more than one method has been employed in a long-term study. When sample analysis is conducted at more than one site, it is necessary to validate the analytical method(s) at each site and provide appropriate validation information for different sites to establish inter-laboratory reliability. Unless a method is used on an on-going basis, thus providing confidence in its continued validity, the method must be documented as currently valid before analysis of samples in the study. Adequate validation for the above purpose often consists of running a standard curve with new quality control samples to show that the responses, relationship, and general characteristics of the method are similar to previous validation results.

### Analytical Method Validation

Method validation includes all of the procedures required to demonstrate that a particular method for the quantitative determination of the concentration of an

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analyte (or series of analytes) in a particular biological matrix is reliable for the intended application. Some of the more commonly employed bio-analytical techniques include physico-chemical methods (*1*), such as gas and liquid chromatography (GC and LC), a variety of procedures using mass spectrometry (MS) (direct MS, tandem MS, and combination techniques such as GC/MS, LC/MS), and biological methods (*2*), such as those based on immunoassay procedures (RIA, EMIT, ELISA) and microbiological methods. Many of the principles, procedures, and requirements are common to all types of analytical methodologies.

The parameters essential to ensure the acceptability of the performance of an analytical method are storage conditions, accuracy, precision, sensitivity, specificity (selectivity), response function, reproducibility, and stability of the drug in the matrix under study. Although there are various stages in development and validation of an analytical procedure, the analytical method validation can be envisioned to consist of 2 distinct phases: (*1*) the analytical method development phase in which the assay is defined, and (*2*) application to actual analysis of samples from pharmacokinetic, bioavailability, and bioequivalence studies.

### *Analytical Methods Development Methods Establishment: (Chemical Assays)*

The following principles of analytical method validation provide steps for the development of a new method or establishing an existing method in a particular laboratory for the first time. Any modification of an analytical method would require revalidation of the procedures. Analytical method validation should be performed to support pharmacokinetic, bioequivalence, and related studies in a new drug application or an abbreviated new drug application. Full methods validation may not be necessary in conducting exploratory pharmacokinetic studies. It is suggested that validation include investigation of samples from dosed subjects.

### *Principles of Method Validation-Method Establishment*

(*1*) A specific, detailed description and protocol of the method should be written (standard operating procedure).

(*2*) Each step in the method should be investigated to determine the extent to which environmental, matrix, material, or procedural variables from the time of collection of the material up to analysis and including the time of analysis, may affect the estimation of analyte in the matrix. Variability of matrix due to physiological state may need to be considered.

(*3*) A method should be validated for the intended use, employing an acceptable protocol. All experiments used to make claims or draw conclusions about the validity of the method should be presented in a report (method validation report).

(*4*) Whenever possible, the same biological matrix as that in the intended samples should be used for validation purposes. (For tissues of limited availability, such as bone marrow, physiologically appropriate proxy matrixes may suffice.) The stability of the analyte (drug and/or metabolite) in the matrix during the collection process and the sample storage period should be assessed, preferably before sample analysis. It is recommended that stability of analyte in matrix from dosed subjects be confirmed. Accuracy, precision, reproducibility, response function, and the specificity of the method with respect to endogenous substances, metabolites and known degradation products should be established with reference to the biological matrix. In regard to specificity, there should be evidence that the substance being quantitated is the intended analyte.

(*5*) The concentration range over which the analyte will be determined must be defined in the method, based on evaluation of actual standard samples over the range, including their statistical variation. This defines the *Standard Curve.*

(*6*) A sufficient number of standards must be used to adequately define the relationship between concentration and response. The relationship between response and concentration must be demonstrated to be continuous and reproducible. The number of standards to be used will be a function of the dynamic range and nature of the concentration response relationship. In many cases, 5–8 concentrations (excluding blank values) may define the standard curve. More standard concentrations may be necessary for nonlinear than for linear relationships.

(*7*) The accuracy and precision with which known concentrations of analyte in biological matrix can be determined must be demonstrated. Within and between run accuracy and precision should be calculated using commonly accepted statistical procedures. This can be accomplished by analysis of replicate sets of analyte samples of known concentrations from an equivalent biological matrix. At a minimum, 3 concentrations representing the entire range of the calibration curve should be studied: one near the lower limit of quantitation (LOQ), one near the center, and one near the upper boundary of the standard curve. For a method to be considered valid, specific criteria must be set for accuracy and precision over the range of the standard curve.

(*8*) The limit of quantitation (LOQ) is the lowest concentration on the standard curve that can be measured with acceptable accuracy, precision, and variability. The LOQ should be determined using at least 5 samples independent of standards

and determining the coefficient of variation and/or appropriate confidence interval. The LOQ should serve as the lowest concentration on the standard curve and should not be confused with the limit of detection (LOD, see *Glossary*).

### *Specific Recommendations for Method Validation*

(*1*) The stability of the analyte in biological matrix at intended storage temperature(s) should be established. In addition, the influence of freeze/thaw cycles (a minimum of 2 cycles at 2 concentrations in duplicate) should be studied.

(*2*) The specificity of the assay methodology should be established using 6 independent sources of the same matrix.

(*3*) The accuracy and precision should be determined using a minimum of 5 (excluding blank sample) determinations per concentration. The mean value should be within ±15% of the actual value, except at LOQ, where it should not deviate by more than ±20%. The precision around the mean value should not exceed 15% coefficient of variation (CV), except for LOQ, where it should not exceed 20% CV. Other methods of determining accuracy and precision that meet these limits may be equally acceptable.

(*4*) The standard curve should consist of 5–8 standard points, excluding blank, using single or replicate samples. The standard curve should cover the entire range of expected concentrations.

(*5*) For the response function, the simplest relationship for response vs concentration should be determined and the fit should be statistically tested. The function should be represented using an appropriate algorithm or graphical technique.

### *Analytical Validation: Application to Routine Drug Analysis*

Many of the above principles under method establishment are relevant to prestudy validation. This section will emphasize the validation parameters that should be performed during routine application of a method to a particular study.

With acceptable variability defined by validation data, analysis of biological samples can be done by single determination without a need for duplicate or replicate analysis. The need for duplicate analysis should be assessed on a case-by-case basis. For a robust procedure of low variability with accuracy and precision routinely well within tolerances, single analysis would suffice. For a difficult procedure with a labile analyte when the precision and accuracy tolerances are difficult to achieve, duplicates may be essential. A procedure should be developed that documents the reasons for re-analysis.

A standard curve should be generated for each analytical run for each analyte and should be used to calculate the concentration of analyte in the unknown samples assayed with that run. A standard curve that will cover the entire range of concentrations in the unknown samples must be used. Estimation of unknowns by extrapolations of a standard curve below the low standard or above the high standard is not recommended. Instead, it is suggested that the standard curve be redetermined or samples be reassayed after dilution. Quality control (QC) samples should be used to accept or reject the run. These QC samples are matrix spiked with analyte.

(*1*) A standard curve should consist of 5–8 standard points, excluding blank (either single or replicate) covering the entire range.

(*2*) Response function is determined by appropriate statistical tests based on the actual standard points during each run in the validation.

(*3*) For system suitability, a specific procedure (or sample) can be identified to assure the optimum operation of the system employed, based on the analyte and technique.

### *Acceptance Criteria for the Run*

(*1*) *Accuracy and Precision.*—The acceptance criteria are not more than 15% CV for precision and not more than 15% deviation from the nominal value for accuracy. At LOQ, however, 20% is acceptable for both precision and accuracy. It is desirable that these tolerances be provided both for intra-day and inter-day or inter-run experiments.

(*2*) *Quality Control Samples.*—QC samples in duplicate at 3 concentrations (one near the LOQ, one in midrange and one approaching the high end of the range) should be incorporated into each run. Results of the QC samples provide the basis of accepting or rejecting the run.

At least 4 of the 6 QC samples must be within 20% of their respective nominal values; 2 of the 6 QC samples (not both at the same concentration) may be outside the ±20% respective nominal value. A confidence interval approach yielding comparable accuracy and precision is an acceptable alternative.

(*3*) *Repeat analysis.*—The protocol for repeat analysis should be established a priori. Some aberrant values can be identified that can be attributed to processing errors, equipment failure, poor chromatography, or QC samples outside predefined tolerance. Cautious use of "pharmacokinetic fit" such as a double peak may call for repeat analysis of some samples in the study, but the reasoning should be clearly documented.

## Analytical Method Validation-Immuno and Microbiological Assays

Many of the analytical validation parameters and principles discussed above are also applicable to immuno-and microbiological methods, but there are some specific differences. In immuno- and microbiological assays, the response must be shown to relate to the concentration of the analyte in question.

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***The Referee*** is the monthly newsletter of AOAC International, received by association members as part of their membership benefits. With more than 3,500 members of AOAC, readers include analytical chemists, microbiologists and other biologists, biochemists, toxicologists, spectroscopists, forensic and other scientists in laboratory, administrative and top management positions. ***The Referee*** is their source for information regarding AOAC's worldwide methods development program, collaborative studies, meetings, symposia, regional sections, methods concerns, publications, and employment needs and opportunities.

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### Selectivity Issues

As with chromatographic methods, the bioassay must be demonstrated to be selective for the analyte. An alternative method, if rigorously established, may be used to compare the results of the bioassay.

For bioassay, an appropriate combination of other techniques may be used to show selectivity, including: (*1*) comparison of standards in biological fluids with standards in buffer to detect matrix effects; (*2*) parallelism of diluted clinical samples with diluted standards to detect presence of closely related compounds; (*3*) serial separation techniques (e.g., extraction and chromatography), with the bio-assay as detector, to demonstrate that the response is only due to the analyte in question; and (*4*) metabolite (or endogenous compound) cross-reaction may be initially assessed by comparison of displacement curves, but in critical cases it should also be assessed by addition of metabolite to analyte. Similar criteria will be applicable when the drug is concomitantly administered with other drugs.

### Quantitation Issues

Criteria for precision and accuracy of immuno- and microbiological assays should be based on the requirements of the study and should match those of chromatographic methods. Any decision to run the sample analysis in single/duplicate/triplicate should be based on variability.

Immunoassay standard curves are essentially nonlinear, and generally require more concentration points to define the fit over the range claimed.

It should be established that an acceptable curve fitting model is being used by examining statistics for goodness of fit, back-calculation of standards and control sample results.

Both upper as well as lower limit of quantitation must be defined by acceptable accuracy, precision, or confidence interval criteria based on the study requirements.

For all assays, the accuracy of the *reported results* is the key factor. This accuracy may be improved by using replicate samples. If replicate samples must be measured during the validation to improve accuracy, then the same procedure must be followed for unknown samples.

If there are intermediate steps between the plasma (or other biological matrixes) and the final assay (such as extraction of biological sample followed by immunoassay), and if parallel processed standards in the biological matrix are not being used, recovery must be established and used in determining results. Possible approaches to assess efficiency and reproducibility of recovery are: (*1*) use of radiolabeled tracer analyte (quantity too small to affect the assay), (*2*) advance establishment of reproducible recovery, (*3*) use of an internal standard which is not recognized by the antibody, but can be measured by another technique.

For correction for nonspecific matrix effects, separation techniques may be used to remove the effect or the matrix may be used in defining the standard curve in controls and samples. The use of standards in matrix is recommended. This approach will obviate many of the above concerns.

### Other Issues

*Commercial kits.*—These are available for both immuno- and microbiological assays and the analytical methods based on such kits should be validated. The validation assures that the bioassay kit is applicable to the study problem and for assuring that subsequent batches or lots of kits have performance characteristics similar to the original validated kit or the test. Any modifications and extensions of assays from one kit (or test) to another must be validated.

*Measurement of metabolites.*—The complex area of determination of drug metabolites in bioavailability studies to support drug submissions was discussed. The questions differed somewhat according to the objective of the application of the bioanalytical measurement, e.g., bioequivalence vs pharmacokinetic profiling.

Some situations exist in bioavailability/bioequivalence studies where (*1*) the parent drug can not be measured in biological samples and only metabolite can be measured, (*2*) the parent drug along with active and/or inactive major metabolites can be measured, (*3*) more than one metabolite is present, and (4) the accumulation of metabolite is augmented, e.g., in the case of renal impairment. Under such situations should one measure the metabolites? Can decision criteria be developed for measuring the metabolite in such situations? From the discussions, the following were suggested: (*1*) All methods applied for measuring drug and metabolites should be validated for that particular study matrix, with the same general parameters listed above (accuracy, precision, specificity, recovery, and reproducibility); and (*2*) pharmacokinetic, bioavailability, and bioequivalence studies should be based upon the moieties that contribute significantly to the *pharmacologic* or therapeutic effect.

*Stereoisomer assays.*—The need for stereoselective determination in bioavailability/bioequivalence studies was another issue that was discussed. There are many drugs that are administered as racemic mixtures, and they may undergo stereoselective metabolism and/or elimination. One isomer may be more active than the other. Under what circumstances should one measure individual drug isomers and/or metabolites isomers from biological matrix? The following were suggested: (*1*) All methods used for measurement of stereoisomer should be validated (with emphasis

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

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on stereospecificity); (*2*) for bioequivalence studies of an existing racemic product, a stereospecific assay is not required if the rate and extent profiles are superimposable (within usual statistical boundaries); and (*3*) for new chemical entities, the pharmacokinetic profiles for stereoisomer should be characterized in normal subjects.

*Pharmacodynamic measurements.*—The final difficult issue identified was the area of pharmacodynamic measurements. The suggestions were as follows: (*1*) All pharmacodynamic procedures used for definitive bioequivalence or related studies must be fully validated under controlled conditions and should include a placebo; and (*2*) the pharmacodynamic effect measured for bioequivalence studies should be related to the actual pharmacologic (therapeutic) end point of the drug's activity.

## Glossary

*Accuracy.*—Closeness of determined value to the true value. Generally, recovery of added analyte over an appropriate range of concentrations is taken as an indication of accuracy. Whenever possible, the concentration range chosen should bracket the concentration of interest.

*Analyte.*—An analyte is a specific, unique chemical moiety in the form(s) it would be found in a biologic matrix.

*Biological matrix.*—A biological matrix is a unique material of biological origin that can be prepared in a reproducible manner. Examples are blood, serum, plasma, urine, feces, saliva, sputum, and various discrete tissues.

*Limit of detection.*—The lowest concentration of an analyte that the analytical process can reliably differentiate from background levels.

*Limit of quantitation.*—The lowest concentration of an analyte that can be measured with a stated level of confidence.

*Linear range.*—Generally taken as the range over which the procedure has been demonstrated to give a linear detector response. A reproducible nonlinear response curve, however, can also be acceptable. Nonlinearity is certainly the case with immunological procedures.

*Method.*—A method is a set of all of the procedures involved in the collection, processing, storage, and analysis of a biological matrix for an analyte.

*Precision.*—This describes the closeness of replicate determinations of an analyte by an assay. Precision can be further sub-divided into within-day precision or intra-assay precision and between-day precision or inter-assay precision.

*Specificity.*—Ability of method to measure only what it is intended to measure

*Standard curve.*—The relationship between the experimental response value and analytical concentration is commonly referred to as a "standard curve" or a calibration curve.

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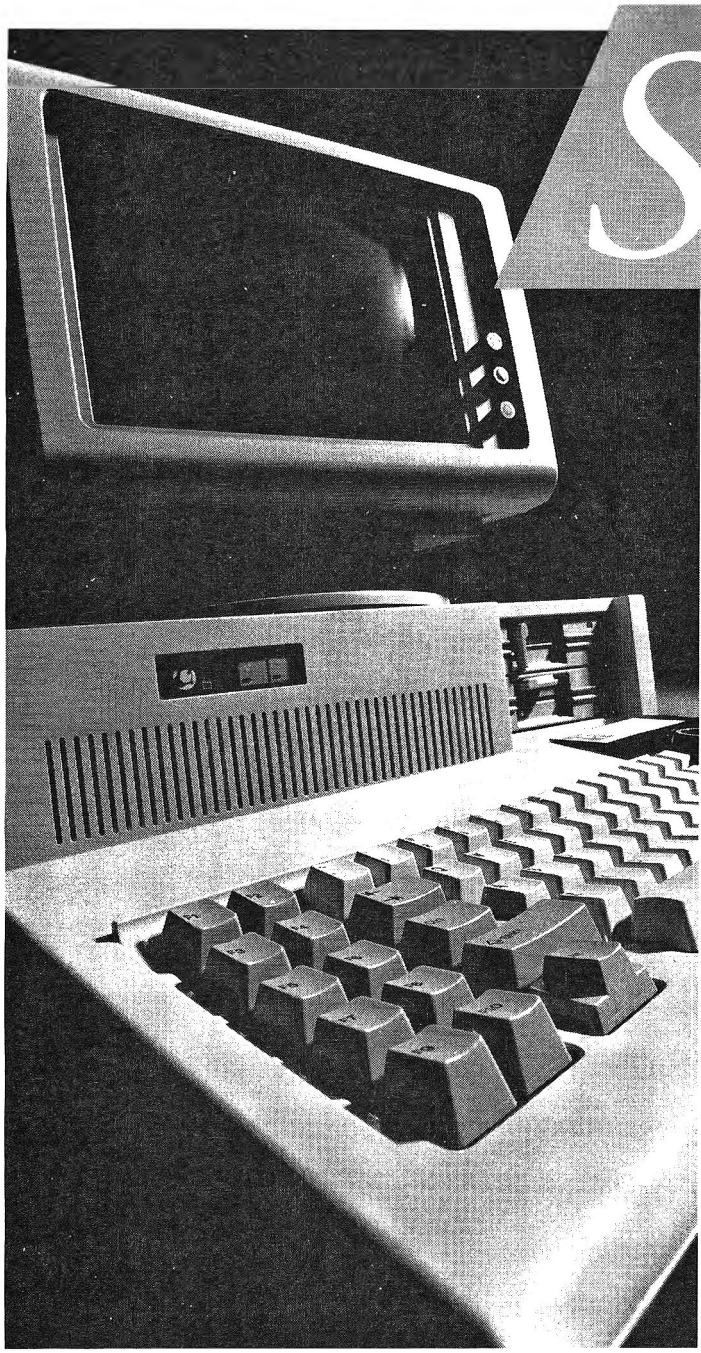

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JOURNAL ARTICLE REFERENCE

(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**

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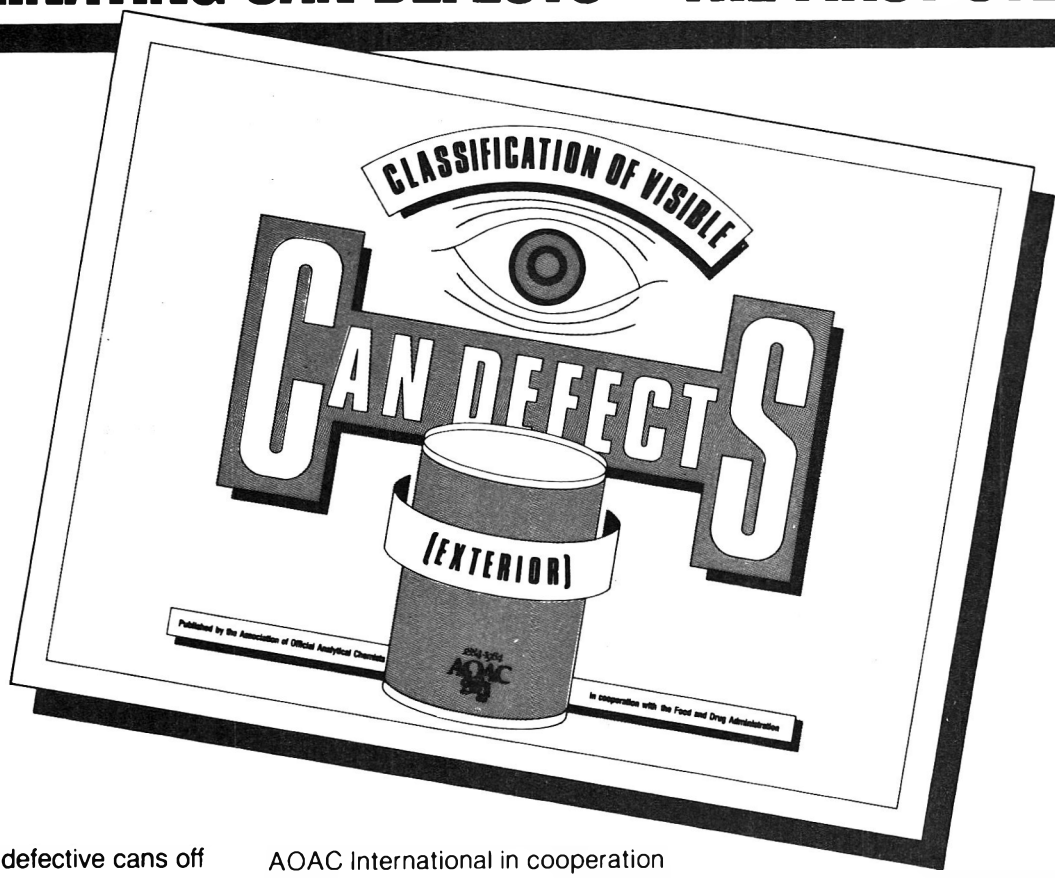
# ELIMINATING CAN DEFECTS—THE FIRST STEP




How can we keep defective cans off the market?

Botulism and other forms of food poisoning can sometimes be traced to defective cans that have leaked and thereby allowed micro-organisms to enter food. Recognizing a can defect, doing something to correct the cause, and removing the defective cans from commerce will help prevent food poisoning outbreaks. The first step is to ensure that responsible personnel know how to identify defective cans.

AOAC International in cooperation with the Food and Drug Administration, has published a pamphlet that unfolds to a 24″ × 36″ chart, suitable for wall display, to help food industry personnel learn to identify can defects quickly. The chart uses a combination of photographs, easy-to-follow explanations, and color coding to illustrate can defects, classify them according to their degree of potential hazard, and show what to look for in routine inspection of the finished product.

The chart is a valuable reference resource for food processors, salvage operators, retail food personnel, wholesalers and state and local government sanitarians.

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KEYNOTE ADDRESS

# Pollution, Pesticides, and Cancer

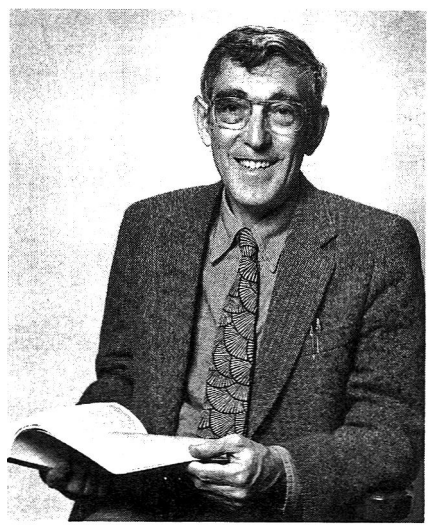
BRUCE N. AMES
University of California, Division of Biochemistry and Molecular Biology, Barker Hall, Berkeley, CA 94720

In the last several decades, there has been a persistent widespread belief among many groups in this country that nature is benign and that man-made things—i.e., modern technology—have destroyed our benevolent relationship with nature. This yearning for a time when man was happily in harmony with nature is a yearning for a time that never existed: In reality, life before the modern industrial era was for most people, even in Thomas Hobbes' time, "nasty, brutish, and short." Disease and malnutrition ensured a very short average life expectancy, an early end to the misery of life in a natural world.

The history of agriculture is one of a nonending contest with pests such as insects and fungi. Fields of crops, which often have been bred to have low levels of natural plant defensive chemicals in order to be more edible for human consumption, are easy sources of food for thousands of species of insects and fungi. Infestation of crops by pests can have dramatic impacts on human life: Last century, the potato fungus *Phytophthora infestans* wiped out the potato crop of Ireland, which led to the deaths of over a million people due to malnutrition (which made people susceptible to disease) and starvation. The relationship between pesticides and disease is significant. DDT, the first synthetic pesticide, eradicated malaria from many parts of the world, including the United States. It was so effective against many diseases because (*1*) it was lethal to many vectors of disease, e.g., mosquitos, tsetse flies, lice, ticks, and fleas; and (*2*) it was lethal to many crop pests, and so significantly increased the supply of food and lowered the cost of food, making fresh nutritious foods accessible even to relatively poor people. In Ceylon, for example, in less than 20 years of DDT use, the number of cases of malaria decreased from 2 800 000 per year to 17. (After Ceylon stopped using DDT, the number of malaria cases increased again.)

Some ideologists have twisted the story of pesticides: Instead of pesticides freeing us from disease, they assert, pesticides are bringing us disease. There are many misconceptions about the relationship between environmental pollution and human disease, particularly cancer, and these can lead to errors in risk perception, which in turn can lead to counterproductive regulatory policies. Accurate science is crucial for assessing public risk from environmental hazards. As scientific information about a subject increases, public risks often need to be reassessed and public policy refined.

Presented at the 105th AOAC Annual International Meeting, August 12–15, 1991, at Phoenix, AZ.



The attempt to prevent cancer by regulating low levels of synthetic chemicals by "risk assessment," using worst-case, one-in-a-million risk scenarios, is not scientifically justified. Testing chemicals for carcinogenicity at near-toxic doses in rodents does not provide enough information to predict the excess numbers of human cancers that might occur at low-dose exposures. In addition, this cancer prevention strategy is enormously costly; it is counterproductive because it diverts resources from much more important risks, and, in the case of synthetic pesticides, makes fruits and vegetables more expensive, thus serving to decrease consumption of foods that help to prevent cancer.

The regulatory process does not take into account: (*1*) that the natural world of chemicals makes up the vast bulk of chemicals humans are exposed to; (*2*) that the toxicology of synthetic and natural toxins is not fundamentally different; (*3*) that about half of the *natural* chemicals tested chronically in rats and mice at the maximum tolerated dose are carcinogens; (*4*) that testing at the maximum tolerated dose frequently can cause chronic cell killing and consequent cell replacement (a risk factor for cancer that can be limited to high doses), and that ignoring this greatly exaggerates risks; and (*5*) that an extrapolation from high to low doses should be based on an understanding of the mechanisms of carcinogenesis.

## The Causes of Cancer

The main causes of cancer appear to be mutagenesis (DNA damage) and mitogenesis (cell division). Normal rates of mutagenesis in mammals are high. Mutagens (chemicals that

damage DNA) cause cancer by mutating the DNA of cells in ways that cause them to proliferate in an uncontrollable fashion. It is generally agreed that several mutations are necessary to convert a normal cell to a cancer cell capable of uncontrolled growth. Mutagens are often assumed to be exogenous agents (coming from outside the body), e.g., synthetic chemicals; however, many endogenous mutagens (produced inside the body) are formed naturally during normal metabolic processes, such as oxygen utilization, which produces DNA-damaging oxidants. Thus, in a sense, breathing oxygen is equivalent to irradiating the body. Studies in our laboratory have shown that normal metabolism causes chronic massive oxidative DNA damage: we estimate that the number of oxidative hits to DNA per cell per day is about 100 000 in rats and 10 000 in humans. All mammals have numerous defenses to counter this damage, such as enzymes that repair damaged DNA, but this repair is imperfect. DNA damage in somatic cells accumulates with time because a considerable proportion of an animal's resources is devoted to reproduction at a cost to maintenance. Proteins can become oxidized as well, and other laboratories have shown that normal protein oxidation is extensive and that oxidized proteins accumulate with age, contributing to brain dysfunction. Thus, oxidative damage appears to be a major contributor to many of the degenerative diseases of aging, including cancer, because not all the DNA damage is repaired.

Mitogenesis (cell division) increases mutagenesis and carcinogenesis because DNA adducts are converted to mutations when a cell divides. Dividing cells are much more at risk than are nondividing, quiescent cells. Agents that cause chronic cell division are, therefore, indirectly mutagenic (and commonly carcinogenic). Saccharin, for example, is not itself a mutagen, but high doses of saccharin given to rodents cause sufficient cell division to be carcinogenic. Low doses, however, would be expected to have no carcinogenic effect. Agents that cause chronic cell division (e.g., by irritation and inflammation of tissues) appear to be important in many of the known causes of human cancer: estrogen, for example, which causes cell proliferation in breast tissue, is a risk factor for breast cancer; hepatitis B and C viruses and alcohol, which induce cell wounding and subsequent cell proliferation in the liver, are risk factors for liver cancer; high salt intake and *Helicobacter* bacterial infection, which induce chronic irritation of the stomach lining, are risk factors for stomach cancer; papilloma virus, which can cause chronic infection and proliferation of cells of the cervix, is a risk factor for cervical cancer; asbestos and tobacco smoke, which irritate the lungs, are risk factors for lung cancer. For the chemicals associated with occupational cancer, worker exposures usually have been at near-toxic doses that would be likely to cause cell proliferation.

A marked decrease in age-specific cancer rates has accompanied the marked increase in life span that has occurred in the last 60 million years of mammalian evolution. For example, cancer rates are high in 2-year-old rodents, but extremely low in 2-year-old humans. Cancer incidence increases with approximately the fifth power of age, both in short-lived species such as rats and mice and in long-lived species such as humans. Thus, cancer is one of the degenerative diseases of old age, although exogenous factors can substantially increase it (e.g., cigarette smoking in humans) or decrease it (e.g., calorie restriction in rodents). One important factor in longevity appears to be basal metabolic rate, which is much lower in humans than in rodents and could markedly affect the level of endogenous mutagens produced by normal metabolism.

According to the National Cancer Institute's 1987 statistics review, "The age adjusted mortality rate for all cancers combined except lung cancer has been declining since 1950 for all individual age groups except 85 and above." Although incidence rates for some cancers have been rising, trends in recorded incidence rates may be biased by improved registration and diagnosis. Even though mortality rates for cancers at particular sites can be shown to be increasing (for example, non-Hodgkin's lymphoma, melanoma) or decreasing (for example, stomach, cervical, rectal), establishing causes remains difficult because of the many changing aspects of our life-style. Life expectancy continues to increase every year.

Cancer clusters in small geographical areas are expected to occur by chance alone, and epidemiology lacks the power to establish causality in these cases. It is important to show that a pollution exposure that purportedly causes a cancer cluster is significantly greater than the background of exposures to naturally occurring rodent carcinogens.

### *Causes of Cancer in Animal Tests*

Animal cancer tests are conducted at near toxic doses—the maximum tolerated dose (MTD) of the test chemical—for long periods of time, which can cause chronic mitogenesis. Chronic dosing at the MTD can be thought of as chronic wounding, which is known to be both a promoter of carcinogenesis in animals and a risk factor for cancer in humans. Thus, a high percentage of all chemicals might be expected to be carcinogenic at chronic, near-toxic doses and this is exactly what is found. About half of all chemicals tested chronically at the MTD are carcinogens.

Synthetic chemicals account for 82% of the 427 chemicals adequately tested for carcinogenicity in both rats and mice. Although humans eat vastly more natural than synthetic chemicals, natural chemicals have never been tested systematically. Of the natural chemicals that have been tested, about half are carcinogens, which is about the same as found for synthetic chemicals. It is unlikely that the high proportion of chemicals found to be carcinogens in rodent studies is due simply to selection of suspicious chemical structures. Most chemicals were selected because of their use as industrial compounds, pesticides, drugs, or food additives.

### *Dietary Pesticides: 99.99% Natural*

Daniel H. Janzen of the University of Pennsylvania wrote, "Plants are not just food for animals...The world is not green. It is colored lectin, tannin, cyanide, caffeine, aflatoxin, and canavanine."

Nature's pesticides are one important subset of natural chemicals. Plants produce toxins to protect themselves against fungi, insects, and animal predators. Tens of thousands of these natural pesticides have been discovered, and every species of

plant analyzed contains its own set of perhaps a few dozen toxins. When plants are stressed or damaged, such as during a pest attack, they may greatly increase their natural pesticide levels, occasionally to levels that can be acutely toxic to humans. We estimate that Americans eat about 1.5 g of natural pesticides per person per day, which is about 10 000 times more than they eat of synthetic pesticide residues.

Concentrations of natural pesticides in plants are usually measured in parts per thousand or per million rather than parts per billion, the usual concentration of synthetic pesticide residues or of pollutants in water. We estimate that the human diet contains roughly 5000 to 10 000 different natural pesticides and their breakdown products. For example, 49 natural pesticides (and metabolites) are ingested when cabbage is eaten. Only 2 have been tested for carcinogenicity. Lima beans contain a completely different array of 23 natural toxins that, in stressed plants, range in concentration from 0.2 to 33 parts per thousand fresh weight. None appears to have been tested yet for carcinogenicity or teratogenicity. Many leguminous plants contain canavanine, a toxic arginine analog that, after being eaten by animals, is incorporated into protein in place of arginine. Feeding alfalfa sprouts (1.5% canavanine dry weight) or canavanine itself to monkeys causes a lupus erythematosus-like syndrome. Lupus in humans is characterized by a defect in the immune system that is associated with autoimmunity, antinuclear antibodies, chromosome breaks, and various types of pathology. The toxicity of nonfood plants is well known. Plants are among the most commonly ingested poisonous substances for children under 5 years of age.

Surprisingly few plant toxins have been tested for carcinogenicity. Among 1052 chemicals tested in at least one species in chronic cancer tests, only 52 are naturally occurring plant pesticides. Among these, 27 are carcinogenic. Even though only a tiny proportion of the plant toxins in our diet has been tested so far, the 27 natural pesticides that are rodent carcinogens are present at levels above 10 ppm in the following foods: anise, apple, basil, Brussels sprouts, cabbage, caraway, carrot, cauliflower, celery, cherries, cloves, coffee (brewed), comfrey herb tea, dill, eggplant, endive, fennel, grapefruit juice, grapes, honey, horseradish, lettuce, mango, mushrooms, mustard (brown), nutmeg, orange juice, parsley, parsnip, pear, pepper (black), plum, potato, rosemary, sage, sesame seeds (heated), tarragon, and thyme In addition, the following foods contain these 27 natural pesticides at levels below 10 ppm: apricot, banana, broccoli, cantaloupe, cinnamon, cloves, cocoa, collard greens, currants, guava, honeydew melon, kale, lentils, peach, peas, pineapple, radish, raspberries, tea, tomato, and turnip.

Thus, it is probable that almost every fruit and vegetable contains natural plant pesticides that are rodent carcinogens. The levels of these 27 rodent carcinogens in the above plants are commonly thousands of times higher than the levels of synthetic pesticides.

Caution is necessary in interpreting the implications of ingesting natural pesticides that are rodent carcinogens. It is not argued here that these dietary exposures are necessarily of much relevance to human cancer. What is important in our analysis is that exposures to natural rodent carcinogens may cast doubt on the relevance of far lower levels of exposures to synthetic rodent carcinogens. Particular natural pesticides that are carcinogenic in rodents can be bred out of crops if studies of mechanism indicate that they may be significant hazards to humans.

### *Residues of Pesticides*

The U.S. Food and Drug Administration (FDA) has assayed food for 200 chemicals, including the synthetic pesticide residues thought to be of greatest importance, and the residues of some industrial chemicals, such as polychlorinated biphenyls. FDA found residues for 105 of these chemicals. The United States intake of the sum of these 105 chemicals averages about 0.09 mg per person per day, which we compare with an intake of 1500 mg of natural pesticides. Thus, the average intake of pesticides is 99.99% natural. Other analyses of synthetic pesticide residues are similar.

About half (0.04 mg) of this daily intake of synthetic pesticides is composed of 4 chemicals that are not carcinogenic in rodent tests: ethylhexyl diphenyl phosphate, chlorpropham, malathion, and dicloran. Thus, the intake of known or potential rodent carcinogens from synthetic residues is only about 0.05 mg a day.

The cooking of food is also a major dietary source of potential rodent carcinogens. Cooking produces about 2000 mg per person per day of mostly untested burnt material that contains many rodent carcinogens—for example, polycyclic hydrocarbons, heterocyclic amines, furfural, nitrosamines—as well as a plethora of mutagens.

Thus, the number and amount of total synthetic pesticide residues, including those that are carcinogenic, appear to be minimal compared to the background of naturally-occurring chemicals in the diet. Roasted coffee, for example, is known to contain 826 volatile chemicals; 21 have been tested chronically and 16 are rodent carcinogens. Caffeic acid, a nonvolatile rodent carcinogen, is also present. A typical cup of coffee contains at least 10 mg (40 ppm) of rodent carcinogens (mostly caffeic acid, catechol, furfural, hydro- quinone, and hydrogen peroxide). Thus, the 10 mg of known natural rodent carcinogens in a cup of coffee (only a few percent of the chemicals have been tested) would be equivalent in amount ingested to a year's worth of synthetic pesticide residues (assuming half of the untested synthetic residue weight turns out to be carcinogenic in rodents).

The evidence on coffee and human health has been recently reviewed, and to date it is insufficient to show that coffee is a risk factor for cancer in humans. The same caution discussed above about the implications for humans of natural rodent carcinogens in the diet apply to coffee and the products of cooked food.

### *Similar Toxicology*

It is often assumed that because plants are part of human evolutionary history, while synthetic chemicals are recent, the mechanisms that animals have evolved to cope with the toxicity of natural chemicals will fail to protect us against synthetic chemicals. An example of this view is the statement of Rachel

Carson: "For the first time in the history of the world, every human being is now subjected to contact with dangerous chemicals, from the moment of conception until death." We find this assumption flawed for several reasons.

Defenses that animals have evolved are mostly of a general type, as might be expected, because the number of natural chemicals that might have toxic effects is so large. General defenses offer protection, not only against natural but also against synthetic chemicals, making humans well buffered against toxins. These defenses include the following: (*1*) The continuous shedding of cells exposed to toxins—the surface layers of the mouth, esophagus, stomach, intestine, colon, skin, and lungs are discarded every few days. (*2*) The induction of a wide variety of general detoxifying mechanisms, such as antioxidant defenses or the Phase II electrophile-detoxifying systems. Cells that are exposed to small doses of an oxidant, such as radiation or hydrogen peroxide, induce antioxidant defenses and become more resistant to higher doses of oxidants, whether synthetic or natural. Natural or synthetic electrophiles induce Phase II detoxifying enzymes that are effective against both. (*3*) The active excretion of planar hydrophobic molecules (natural or synthetic) out of liver and intestinal cells. (*4*) DNA repair, which is effective against DNA adducts formed from both synthetic and natural chemicals, and is inducible in response to DNA damage.

Anticarcinogenic chemicals in the diet, such as antioxidants, help to protect humans against carcinogens but do not distinguish between synthetic and natural carcinogens. It has been argued that synergism between synthetic carcinogens could multiply hazards, but this is equally true of natural carcinogens.

The fact that defenses are usually general, rather than specific, for each chemical makes good evolutionary sense. The reason that predators of plants evolved general defenses against toxins is presumably to be prepared to counter a diverse and ever-changing array of plant toxins in an evolving world. If a herbivore had defenses against only a set of specific toxins, it would be at a great disadvantage in obtaining new foods when favored foods became scarce or evolved new toxins.

Various natural toxins, some of which have been present throughout vertebrate evolutionary history, nevertheless cause cancer in vertebrates. Mold aflatoxins, for example, have been shown to cause cancer in trout, rats, mice, monkeys, and possibly in humans. Eleven mold toxins out of 16 tested have been reported to be carcinogenic. Many of the common elements, such as salts of lead, cadmium, beryllium, nickel, chromium, selenium, and arsenic, are carcinogenic or clastogenic (agents that break chromosomes) at high doses, despite their presence throughout evolution. Selenium and chromium, nevertheless, are essential trace elements in animal nutrition.

Humans have not had time to evolve into a "toxic harmony" with all of the plants in their diet. Indeed, very few of the plants that humans eat would have been present in an African hunter-gatherer's diet. The human diet has changed drastically in the past few thousand years, and most humans are eating many recently introduced plants that their ancestors did not—for example, cocoa, tea, potatoes, tomatoes, corn, avocados, mangoes, olives, and kiwi fruit. In addition, cruciferous vegetables, such as cabbage, broccoli, kale, cauliflower, and mustard were used in ancient times primarily for medicinal purposes and spread as foods across Europe only in the Middle Ages. Natural selection works far too slowly for humans to have evolved specific resistance to the food toxins in these newly introduced plants.

DDT bioconcentrates in the food chain as a result of its unusual lipid solubility. However, natural toxins can also bioconcentrate. DDT is often viewed as the typically dangerous synthetic pesticide because it persists for years. It is representative of a class of chlorinated pesticides. Natural pesticides bioconcentrate if lipophilic. For example, the teratogens from potato, solanine (and its aglycone solanidine), and chaconine are found in the tissues of potato eaters. Although DDT is unusual with respect to bioconcentration, it is remarkably nontoxic to mammals, has saved millions of lives, and has not been shown to cause harm to humans.

To a large extent DDT, the first major synthetic insecticide, replaced lead arsenate, a major pesticide used before the modern era. Lead arsenate is even more persistent than DDT, and although natural, both lead and arsenic are carcinogenic.

These arguments undermine many assumptions of current regulatory policy and necessitate a rethinking of policy designed to reduce human cancer. Minimizing pollution is a separate issue and is clearly desirable for reasons other than effects on public health. There is a sizeable literature on why focussing on worst case, one-in-a-million risks, rather than major risks, impedes intelligent risk reduction.

It is by no means clear that many significant risk factors for human cancer will be discovered by screening assays. Dietary imbalances, such as antioxidant and folate deficiencies, are likely to be major contributors to human cancer, and understanding these should be, but is not, a major priority of research. Understanding why caloric restriction dramatically lowers cancer and mitogenesis rates and extends life span in experimental animals should also be a major research priority. More studies on mechanisms of carcinogenesis should also be of high priority.

Synthetic pesticides have markedly lowered the cost of vegetables and fruit, thus increasing consumption. Other than giving up smoking (causing 30% of cancer and 25% of heart disease) eating more fruits and vegetables and less fat may be the best way to lower risks of cancer and heart disease.

In conclusion, the attempt to prevent cancer by regulating low levels of synthetic chemicals by traditional "risk assessment," using worst-case, one-in-a-million risk scenarios, is not scientifically justified. This does not mean that chemical regulation per se is undesirable. The question is how best to regulate pollution, such that tradeoffs are efficiently factored into regulatory policy. One way is by putting pollution control in the realm of the free market, for example, by auctioning off pollution licenses or taxing polluters depending on the amount of pollution produced. According to A.S. Blinder (*Hard Heads, Soft Hearts*, Addison-Wesley, Reading, MA, 1987), "The secret [of the market's success] is the market's unique ability to accommodate individual differences—in this case, differences among polluters... the profit motive will automatically assign

the task of pollution abatement to the low-cost firms—something no regulators can do." This solution would partition economic tradeoffs most efficiently. Firms that can relatively inexpensively reduce their pollution will have a strong incentive to do so to avoid paying the pollution tax. Even if the risks of a particular type of pollution are initially overestimated, it is the firms that can cost-effectively change their pollution habits that have the incentive to do so, inflicting the lowest overall cost on the consumer. As new scientific information leads to the reassessment of these risks, or as the values of a society change, the tax on different types of pollution can be raised or lowered.

It is the inexorable progress of modern technology and scientific research that is likely to lead to a decrease in cancer death rates, a decrease in birth defects, a decrease in pollution, and an increase in the average human life span.

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WILEY AWARD ADDRESS

# The Biomolecular Temperament of Staphylococcal Enterotoxin in Thermally Processed Foods

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To have been chosen as the recipient of the Association of Official Analytical Chemists' Harvey W. Wiley Award for 1991 is an honor for me. It is also an honor to belong to an organization, the AOAC, that shares my convictions about the role played by analytical methods in the decision-making process. When analytical methods are reliable and efficient, they contribute more to science than highly theoretical regulatory and quality control speculations. I hope that this honor will not mark an endpoint in my career, because I plan to continue to work on the development of tests that ultimately serve to protect the public's health.

Enterotoxin produced by some strains of *Staphylococcus aureus* is still an important cause of foodborne illness. This microbial toxin is formed in foods that are contaminated by staphylococci, which then proliferate in the food matrix. The bacterial populations grow profusely when the food is abused by time or temperature. Subsequently, these organisms synthesize a metabolite that can cause illness when the food is consumed. The predominant symptoms of the disease, i.e., nausea, acute vomiting, diarrhea, and sometimes prostration, generally occur within 2 to 6 h after consumption. Recovery normally takes place within 1 or 2 days. Fatalities are rare, but discomfort and loss of valuable time are not insignificant.

Most outbreaks of staphylococcal intoxication are caused by foods that do not receive a high thermal treatment; staphylococci survive in sufficient numbers in these foods to form the toxin before the food is consumed. This toxin is referred to as preformed toxin. However, some heated foods have also been incriminated in illnesses that display the typical symptoms of this disease. Foods that receive enough heat to render the bacterium nonviable and yet cause food poisoning have included boiled goat's milk (1), spray-dried milk (2, 3), cooked sausage (4), and canned lobster bisque (5). In the last-named instance, 2 people became ill. The incriminated lobster bisque reportedly had been heated to 118°C for 86 min and yet was considered underprocessed (6). The toxin was identified serologically; the food received no special treatment that would interfere with its serological identification.

In 2 instances, the U.S. Food and Drug Administration (FDA) has taken regulatory action on staphylococcal-contaminated thermally processed foods. In 1982, thermally processed

infant formula was incriminated in foodborne illness (7), and more recently, in 1989, mushrooms that received a higher than normal thermal treatment as a means of product preservation were implicated in staphylococcal foodborne illnesses (8).

Numerous studies have been conducted on the thermal stability of staphylococcal toxin in foods (4, 9–12). It was concluded from these studies that the heat process normally used in the canning industry is adequate to destroy any enterotoxin in a food matrix (6). This concept of an adequate safeguard has become an article of faith in the canning industry. As a consequence, the thermal food processor sometimes pays little attention to preventing time-temperature abuse of food products that may have become contaminated with staphylococci before the canning process.

In this presentation, I would like to share with you the mounting evidence that staphylococcal enterotoxins in thermally processed foods are a potential health hazard. I will discuss what I have called the temperamental behavior of the toxin as dictated by both thermal denaturation and renaturation conditions. I will give some perspectives on the mechanisms of the toxin molecule's conformational changes and describe a concept about the effects of thermal processing on the serological and biological fate of enterotoxin that is at variance with traditional ideas. Finally, I will mention an application of this concept to solve a regulatory problem, namely, how enterotoxin is being identified in the canned mushrooms that were incriminated in recent food poisoning outbreaks.

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**Table 1. Symptomatic response of human volunteers to orally administered staphylococcal enterotoxin type A (SEA) unheated and heated (100°C, 25.4 min)[a]**

| Dose, ng/kg | Response | |
|---|---|---|
| | Unheated No. ill/no. fed | Heated No. ill/no. fed |
| 5 | NA[b] | 1/6[c] |
| 10 | 0/4 | 1/6[c] |
| 50 | 1/4 | 1/6 |
| 400 | 3/3 | 6/6 |

[a] Modified from Dangerfield (13).
[b] NA, not administered.
[c] Anorexia and nausea only.

Over the years, investigators had theorized that when toxin is heated, as it is in thermally processed foods, its biological activity (toxicity) is reduced, and it is less readily identifiable by serological assay (4). With respect to biological activity, the epidemiology of foodborne illness provides the best evidence of the thermal stability of staphylococcal toxins. That is, the foodborne illness itself verifies that the toxin has retained its toxicity. However, there may not be a direct correlation between the biological and serological activities of the toxin. Serological evidence does not always support the epidemiological findings. In other words, the toxin that is apparently causing the illness is not always found by in vitro serological assay.

To test this unusual behavior, FDA conducted a study (13) using human volunteers in 1972. For this study, staphylococcal enterotoxins produced by serotypes A and B (SEA and SEB) were heated at 100°C in saline containing 2% gelatin and 0.3% proteose peptone, pH 7.0. Various amounts of heated or unheated enterotoxin were mixed into milk to be ingested by healthy human males after breakfast. The dose level was determined by the number of people who became ill after ingesting the unheated toxin. On the basis of the established dose of unheated toxins, further studies were then conducted with heated toxins. It was found that as the time of heating the toxin increased, the number of people displaying the symptoms of staphylococcal poisoning decreased, and as the time of heating decreased, more people became ill. No reduction was noted in the serological detectability after 8 min of heating at 100°C; however, the titer was greatly reduced after 25 min of heating.

The FDA study, reported by Dangerfield (13), also showed that when volunteers ingested heated toxin, they became more severely ill than those who ingested the same dose of unheated toxin. In fact, 3 of 6 individuals in this trial who consumed SEB required replacement of parenteral fluid and electrolytes. Fever occurred in 10 of the 16 ill individuals. Results with SEA were even more dramatic. Persons ingesting SEA heated at 100°C for 25.4 min displayed symptoms typical of the illness. Moreover, their clinical condition was more severe than that of persons receiving similar doses of unheated enterotoxin. Of the 6 persons who consumed the heated enterotoxin, one suffered a rapid onset of extreme generalized abdominal pain in addition to vomiting and diarrhea. These symptoms persisted for 12 h. When smaller doses were ingested, one person at each dose level (0.005 and 0.01 μg/kg) displayed the anorexia and nausea that are consistent with staphylococcal foodborne illness. Comparative results with heated and unheated preparations of SEA are shown in Table 1. Results with heated SEA provided further evidence that the toxin's biological activity increased after heat treatment. These findings piqued our curiosity regarding the effect of heat on the serological and biological activities of the toxin.

During the 1960s and 1970s, my laboratory received many reports and samples of various canned foods that had been incriminated in food poisoning outbreaks. Examination of the epidemiological findings, including symptoms, strongly suggested that the illnesses had been caused by staphylococci. However, serological tests of these incriminated products gave no positive results for the presence of toxin (14). Our inability to find toxin in these suspect canned foods was frustrating and puzzling; finding a solution to the problem grew into an obsession. Plans were made to reevaluate earlier findings on the thermal stability of staphylococcal enterotoxins.

To restudy the potential hazard of these metabolites in thermally processed foods, SEA and SED were added to infant formula and cream of celery soup; the persistence of serological and biological activities in the toxins was determined during simulated commercial canning. When both products were retorted at 123.9°C with $F_0$ values of approximately 3 and 8, the enterotoxins were serologically reduced or inactivated; however, they remained biologically active when injected into kittens (15).

Test samples of toxin containing unheated and heated infant formula and celery soup were extracted, and the toxins (SEA and SED) were separated from food proteins by ion exchange chromatography (16, 17); the chromatographed eluates were then studied serologically by the microslide gel double diffusion test (18). Lyophilized and chromatographed eluates of the retorted products containing SEA and SED were rehydrated in physiological saline and injected intravenously into kittens. The kittens were observed for emesis to determine if the heated toxins retained their biological activities. Table 2 summarizes the biological response in kittens to SEA in retorted infant formula. The SEA in the product retained some serological activity after being heated to an $F_0$ of 3, and produced emetic responses in 4 of 6 dosed kittens. Yet, in infant formula heated to an $F_0$ value of 8, SEA was serologically inactivated, although this toxin preparation also produced emesis in 4 of the 6 kittens injected.

The biological activity of SED in retorted infant formula is summarized in Table 2. SED was serologically inactivated at the minimum and maximum $F_0$ values of 3 and 8. Of the 6 kittens injected with chromatographed infant formula containing SED, 4 and 3, respectively, produced emetic responses from preparations with $F_0$ values of 3 and 8.

The biological activity of SEA in retorted condensed cream of celery soup received intended $F_0$ values of 3 and 8 for the 123.9°C process. Of 6 kittens tested, 5 produced emetic responses to SEA in soup heated to an $F_0$ value of 3, whereas only

**Table 2. Biological activity in kittens of staphylococcal enterotoxins A (SEA) and D (SED) in retorted infant formula and condensed cream of celery soup[a]**

| Product-toxin serotype | $F_0$ value (equiv. min at 121.1°C) for 123.9°C steritort process | Serological activity[b] | Biological activity: Emetic response/no. Injected |
|---|---|---|---|
| Infant formula | | | |
| SEA | 3 | + | 4/6 |
| SEA | 8 | – | 4/6 |
| Infant formula | | | |
| SED | 3 | – | 4/6 |
| SED | 8 | – | 3/6 |
| Infant formula | | | |
| control[c] | 3 | ND[d] | 0/6 |
| control | 8 | ND | 0/6 |
| Celery soup | | | |
| SEA | 3 | – | 5/6 |
| SEA | 8 | – | 3/6 |
| Celery soup | | | |
| SED | 3 | – | 1/6 |
| SED | 8 | – | 4/6 |
| Celery soup | | | |
| control | 3 | ND | 0/6 |
| control | 8 | ND | 0/6 |

[a] Source: Bennett and Berry (15).
[b] +, Serological activity; –, no serological activity.
[c] Product contained no SEA or SED.
[d] ND, not done.

3 demonstrated emetic activity after receiving enterotoxin in soup heated to an $F_0$ value of 8.

Table 2 summarizes the response with SED contained in retorted cream of celery soup. Although only 1 of 6 kittens produced an emetic response to SED heated to an $F_0$ of 3, SED heated for a longer time ($F_0$ of 8) caused 4 kittens to become ill. These data suggest that fewer biological determinants were exposed with the $F_0$ of 3 than with the $F_0$ of 8 process, and that there was less biological activity in the product treated at the $F_0$ of 3.

Kittens gave no emetic response to infant formula or soup prepared without toxin. These studies were highly significant in providing a better understanding of the toxin as a molecular entity, but, more important, we learned that toxin that was serologically inactivated during thermal processing could remain biologically active. This work answered baffling and frustrating questions about why incriminated canned foods did not show the presence of enterotoxin when examined serologically in the laboratory.

The staphylococcal enterotoxins are relatively simple, fairly low (26 000–29 000) molecular weight (MW) proteins, easily soluble in water and in saline. They are single polypeptide chains that contain relatively large amounts of lysine, aspartic and glutamic acids, and tyrosine (6). Substitutions of various amino acid residues in the enterotoxin molecule have been made in efforts to identify the site of action.

Heat causes change in the form of globular proteins. The architecture of the molecule may become altered in a stepwise

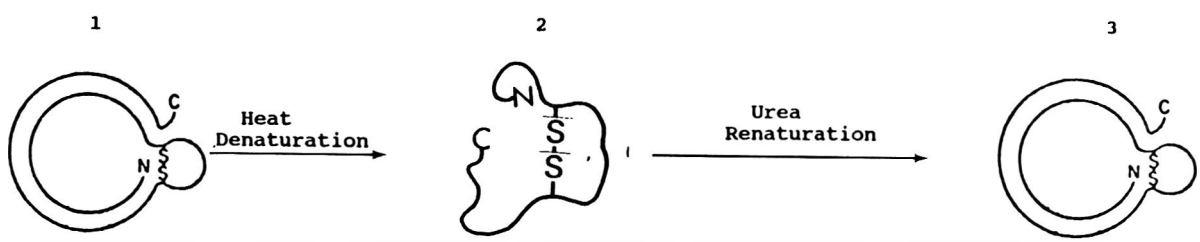


**Figure 1. Schematic representation of (1) native structure of staphylococcal enterotoxin (21); (2) proposed effect of retort temperatures or other severe heat conditions on structural configuration of toxin molecule; and (3) effect of urea on reconformation of toxin to quasi-original molecular form.**

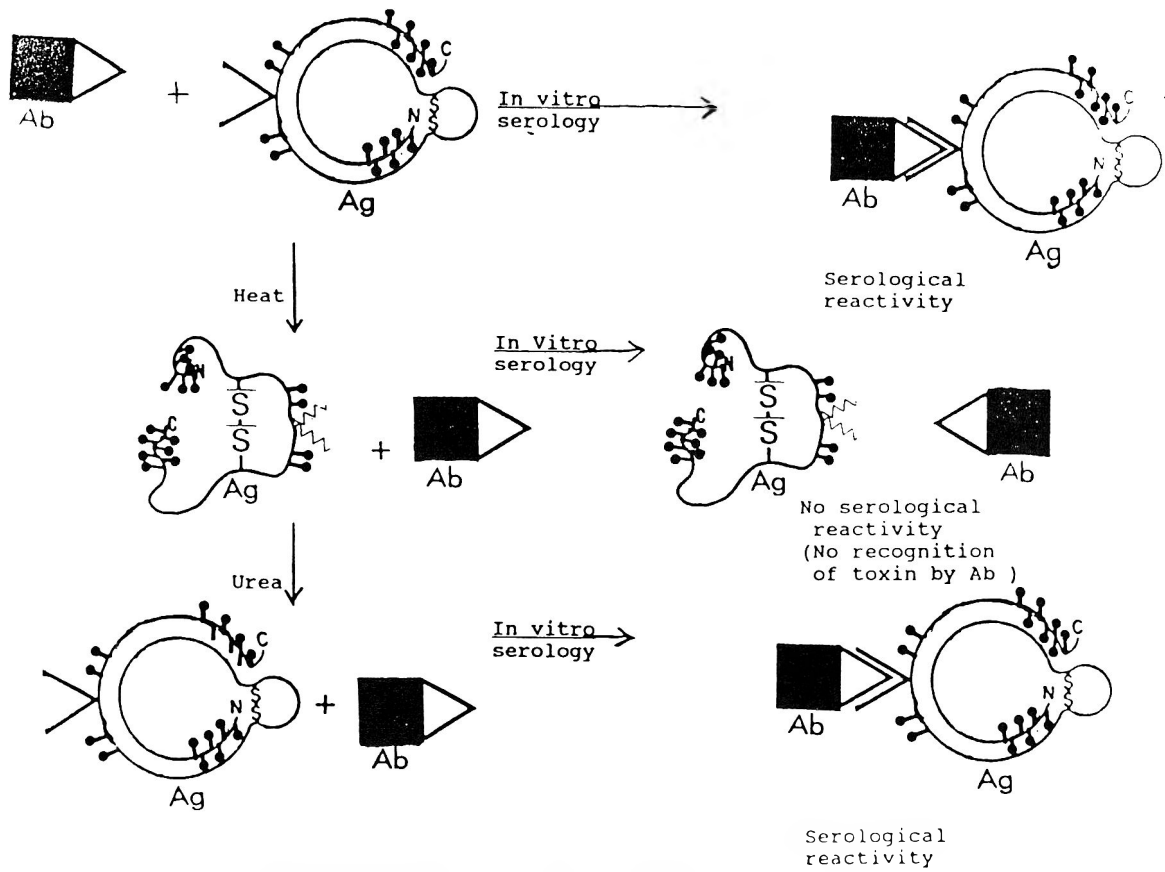


**Figure 2. Schematic representation of serological reactivity of specific antibody (Ab), with native structure of staphylococcal enterotoxin (SET; the antigen [Ag]) with biological (⋔) and serological (>) sites [top row]; lack of serological reactivity of heat-altered SET ( ⩙ ) to specific Ab, although retaining biological activity [middle row]; and serological reactivity of the specific Ab with quasi-original of SET after exposure to urea [bottom row].**

fashion, starting with the native form through partially denatured intermediates, to a fully denatured type. The intermediate forms may be stable or unstable and may revert back to the native form under certain conditions. A hypothetical scheme based on that of Jaenicke (19) for the heat aggregation of globular proteins and that of Westhead (20) for heat-denatured yeast enolase was proposed to explain the contrary effects of heat and urea on staphylococcal enterotoxin (11). The influence of heat and urea on the denaturation and renaturation of staphylococcal enterotoxin structure (21) is shown in Figure 1. Although it was suggested that the toxin molecule was split as a result of extraction, renaturation clearly shows this was not the case.

That not all staphylococcal enterotoxins behave similarly is an old observation. SEA and SED do not react serologically like SEB and SEC (22). Under certain heat conditions, partially denatured or unfolded forms of SEB and SEC spontaneously revert back to their native forms during storage. SEA has more leucine than does SEB (23, 24). Possibly, during heating, these leucine groups are exposed and their hydrophobic nature causes them to interact. This new formation restrains the toxin from spontaneously refolding to a native or quasi-native form, thus masking the serologically reactive sites on the molecule (11). Urea treatment of a heated preparation in which this phenomenon has occurred seems to relieve molecular constraint by breaking hydrophobic bonds; the molecule refolds, and previously masked serologically active sites reappear, making identification of the enterotoxin again possible. Although this explanation appears plausible, it is a hypothesis yet to be proved. A simple diagram of typical antigen (toxin)-antibody reactivity and the effect of denaturation and renaturation is shown in Figure 2.

Early in 1989, staphylococcal foodborne illnesses were associated with the consumption of thermally processed mushrooms from the People's Republic of China. These outbreaks prompted multistate recalls of mushrooms produced by certain canneries in China. The FDA refused entry into this country of all shipments of mushroom cans of institutional size from China (8). Previously unopened cans of the product from all of these outbreaks contained staphylococcal enterotoxin, which was found serologically in either untreated or in urea-treated, (i.e., toxin-renaturated) mushroom extracts. The process of identifying toxin in thermally processed mushrooms involves extraction of the toxin from the food, urea treatment of a portion of the extract to renature any toxin that may have been

preformed (i.e., formed before thermal processing) and then denatured, and serological identification of the enterotoxin by an enzyme-linked immunosorbent assay (ELISA) or other acceptable serological tool.

In the extraction of processed mushrooms, 100 mL 0.25M Tris, pH 8.0, was added to each 100 g of product and homogenized in a Waring blender at high speed for 3 min. The mushroom slurries were transferred to centrifuge bottles (stainless steel or equivalent), and then centrifuged at 27 300 × *g* for 20 min at 5°C. The resulting extracts were decanted into suitable containers (bottles or equivalents), and 100 mL of each extract was reserved for urea treatment. Approximately 5 mL was removed from each extract and filtered through a cotton-plugged disposable syringe. The cotton-plugged syringe was prepared by inserting a piece of cotton about 0.5 cm thick into a syringe; it was wetted by passing 5.0 mL of distilled water through the syringe to ensure tight packing of the cotton. For filtration, the plunger was removed and a 5.0 mL aliquot of extract was placed in the syringe; the plunger was then reinserted and the extract was carefully pumped through the cotton plug into a plastic vial.

The 5.0 mL cotton-filtered extracts were adjusted, when necessary, to between pH 7.0 and 8.0 with NaOH or HCl. Then, 100 µL of "sample additive" contained in the ELISA kit was added and the preparation was mixed thoroughly. A 200 µL aliquot of each extract was tested, as required by the kit's instructions.

The extracts (100 mL) reserved for urea treatment were placed in 250 mL beakers to which 36.0 g urea (MW 60.06) was added to obtain a 6M solution. The solutions were mixed on a magnetic stirrer for 4–5 h at 25°C, after which each extract was placed in dialysis tubing (cellulose casing of 2–3 cm flat width with 12 000–14 000 MW exclusion; the tubing had been previously checked for leaks by filling it with distilled water). Dialysis took place in 30% polyethylene glycol (PEG; MW 20 000) and continued until a volume of 5 mL or less was reached. After dialysis, the tubing's exterior was washed thoroughly with tap water to remove residual PEG. The tubing was then soaked in 0.25M Tris, pH 8.0, until its total volume did not exceed 5 mL of the urea-treated extract. Extracts were removed from the dialysis tubing, clarified by centrifugation, and filtered through absorbent cotton. When necessary, the pH of the 5.0 mL extracts was adjusted to between 7.0 and 8.0 with NaOH or HCl, then 100 µL of sample additive (from the ELISA kit) was mixed into each extract.

An ELISA using polyvalent antisera (antibody to toxin serotypes A, B, C, D, and E) in a double polyclonal antibody "sandwich" configuration was used to screen test samples for the presence of staphylococcal enterotoxin in both untreated and urea-treated extracts of the canned mushrooms (25). In fact, a commercial kit, the TECRA™ Staphylococcal Enterotoxin Visual Immunoassay (26) was used because of its previous success with a wide variety of enterotoxin contaminated foods (27).

The protocol consisted of adding 200 µL aliquots of the 5.0 mL additive-treated extracts (pH adjusted to 7.0–8.0) to prewashed (10 min at 25°C) anti-SET (serotypes A–E)-coated

**Table 3. Serological[a] reactivation[b] of thermally inactivated *Staphylococcus aureus* enterotoxin in canned mushrooms[c]**

| | Treatment | |
|---|---|---|
| Product designation | No urea | Urea |
| 317, sub 8 | - | + |
| 563, sub 16 | - | + |
| 880, subs 4, 6, 11 | - | + |

[a] Determined by enzyme-linked immunosorbent assay (ELISA) and other methods.
[b] Exposure to 6M urea.
[c] Product processed for 30 min at 250°F.

**Table 4. Identification of *Staphylococcus aureus* enterotoxin in native form[a] by ELISA or other serological method, and effect of 6M urea on serological inactivation**

| | Treatment | |
|---|---|---|
| Product designation | No urea | Urea |
| 563, sub 7 | + | - |
| 411 | + | - |

[a] Normal structural conformation of molecule.

**Table 5. Identification of multiple structural forms of staphylococcal enterotoxin in canned mushrooms**

| | Extract treatment | |
|---|---|---|
| Product designation | No urea | Urea |
| 448 | +(1.727)[a] | +(>3.00) |
| 377 | +(>3.00)[b] | +(0.618) |
| 344–B.C. | +(>3.00) | +(>3.00) |
| | +(1:320,[c] 0.234[d]) | +(1:1,280,[c] 0.220[d]) |

[a] Absorbance measurement on microtiter plate reader.
[b] Absorbance limit, 3.00.
[c] Dilution of extract in positive (>0.200) absorbance range.
[d] Absorbance reading at specified extract dilution; absorbance reading ≤0.200 negative for toxin.

**Table 6. Spontaneous serological reactivation of *Staphylococcus aureus* enterotoxin in canned mushrooms during storage (5°C)**

| | | ELISA results | |
|---|---|---|---|
| Sample designation | Treatment | Initial analysis | Later analysis (1–4 wk) |
| 563 (7) | Untreated | +(0.618)[a,b] | +(1.178) |
| 563 (10) | Untreated | -(0.200)[c] | +(0.509) |
| 146 (4d) | Urea | ±(0.203) | +(0.235) |
| 492 (4) | Urea | +(0.264) | +(0.327) |

[a] Absorbance measurement on microtiter plate reader; $A_{405}$–$A_{490}$ (dual wavelength).
[b] Absorbance values >0.200 indicate presence of toxin.
[c] Absorbance values ≤0.200 indicate absence of toxin.

microtiter wells and incubating for 2.0 h at 35°C. The wells were washed (3 times) and treated with 200 μL of polyvalent antisera (A–E) enzyme conjugate and incubated for 1 h at 25°C. The wells were then washed (5 times) and the substrate was treated (200 μL, 30–45 min at 25°C) until 20 μL stop solution was added. A positive test (green) indicated enzyme action on the colorless substrate. For visual determinations, the tray was placed on a white background and individual test wells were compared to a color standard (color comparator) supplied in the kit. Absorbance (A) of the test solutions was determined in a microtiter tray reader (V Max Kinetics Microplate Reader, Molecular Devices, Menlo Park, CA 94025-9994), using dual wavelengths ($A_{405}$–$A_{490}$). Test solutions that gave an absorbance of >0.200 were considered positive for enterotoxin. Those that gave an absorbance of ≤0.200 were considered negative for staphylococcal enterotoxin.

If confirmation by the AOAC method was necessary, the 5 mL (200 μL) urea-treated preparation was extracted with $CHCl_3$, diluted with 40 volumes of 0.005M phosphate buffer, pH 5.7, chromatographed as described in the AOAC International *Official Methods of Analysis*, 15th Ed., **980.32**, and tested by microslide gel double diffusion, **976.31** (28). When necessary, untreated mushroom extracts were processed by the AOAC method (16); some were also assayed by the reversed passive latex agglutination (RPLA) test (29). However, in some cases RPLA tests were variable and appeared to lack sensitivity (30).

What appeared to be different forms of enterotoxin (native, denatured, or partially denatured) were identified in the canned mushrooms by serological tests. Enterotoxin in its native form was found in mushrooms that did not require exposure to urea. Mushrooms containing bacterial biomolecules that had undergone a conformational change because of the thermal process required exposure to urea before serological identification of the toxin. Some mushrooms also contained multiple forms of the toxin. In consequence, toxin presence was identified in both the untreated (no urea) and the urea-treated mushrooms. Test results for mushrooms containing identifiable enterotoxin in its serologically heat-altered form, toxin in its apparent native form, and toxin in multiple forms are shown in Tables 3, 4, and 5, respectively.

The evidence reviewed here shows that preformed staphylococcal enterotoxin can be altered serologically in foods that have received high heat treatment. This change of configuration of the biomolecule prevents it from reacting with antibody to the native form of the antigen, probably because the specific antibody cannot serologically recognize the structurally altered toxin. Nevertheless, the toxin retains biological activity (15) and can produce symptoms of staphylococcal intoxication (8). Outbreaks of foodborne illness caused by staphylococcal enterotoxin in thermally processed mushrooms have often necessitated urea renaturation of the denatured toxin before it can be identified with specific antibody. With some mushrooms, toxin was identified without urea renaturation of the biomolecule. Because it is not possible to predict the structure of the enterotoxin (native or quasi-original form), it is always necessary to assay both urea-treated and untreated preparations suspected

**Table 7. Serological degradation of *Staphylococcus aureus* enterotoxin in canned mushrooms after storage (5°C)**

| Sample designation | Treatment | ELISA results: Initial analysis | ELISA results: Later analysis (1–4 wk) |
|---|---|---|---|
| 563 | Untreated | +(0.254)[a,b] | +(0.228) |
| 212 | Urea | +(0.278) | −(0.147)[c] |
| 491 | Urea | +(0.243) | −(0.161) |
| 880 (4) | Urea | +(0.443) | −(0.154) |
| 880 (6) | Urea | +(1.839) | ±(0.209) |
| 880 (11) | Urea | +(0.971) | +(0.326) |
| 212 (2) | Urea | +(0.278) | −(0.147) |

[a] Absorbance measurement with microtiter plate reader; $A_{405}$–$A_{490}$ (dual wavelength).
[b] Absorbance values >0.200 indicate presence of toxin.
[c] Absorbance values ≤0.200 indicate absence of toxin.

of containing the toxin. Urea changes the natural form of the enterotoxin just as heat does. Tatini (4) showed that when milk inoculated with toxins SEA and SED was pasteurized and then exposed to urea, the serological titer of the toxins increased in the milk. Sometimes, the native form of the toxin was present in the product without apparent can defects. This suggests that spontaneous serological reactivation of denatured SEA occurs under certain specific thermal conditions. Observations in my laboratory support the existence of this phenomenon (22). Table 6 shows examples of spontaneous serological reactivation of the toxin. Another distinct behavioral temperament of the toxin is serological degradation after the protein is extracted from the food; this phenomenon is presented in Table 7. Both events await further study.

The outbreaks of foodborne illnesses caused by contaminated mushrooms (8) and lobster bisque (5) serve as models for outbreaks that might occur with other thermally processed foods. It is urgent that food processors use good manufacturing practices to harvest, transport (31), and process foods, and that they insist that employees practice good hygiene to prevent the food intended for canning from becoming a hazard to health.

In conclusion, the problem of Chinese canned mushroom contamination with staphylococcal enterotoxin required something more innovative than standard testing. The approaches applied arose from a concept that was somewhat at variance with traditional thinking about the microbiological safety of canned foods, enterotoxigenic staphylococci, and time-temperature abuse. As we approach the 21st century, other challenging problems will face us in a world in which food is produced, prepared, preserved, and transported on a global scale.

We must always attempt to solve problems in which human health is at risk. Generally, the only limitations to innovation are the size of our ideas and the resources available for implementation. Protection of the public health is basic to all other endeavors in life. To paraphrase Rustum Ray (a material scientist, professor at Pennsylvania State University and member of the National Academy of Engineering): "That which is con-

cerned with life will be remembered for life." Although concern for the protection of the public health is the direct responsibility of the food manufacturer, it is also the special responsibility of scientists, including microbiologists, chemists, and sanitarians, to ensure the safety of our food supply.

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PRESIDENT'S ADDRESS

# Risking for Success

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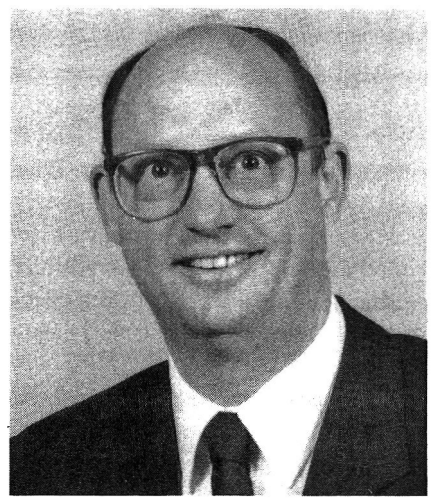
Over the past few months, I have thought frequently about what should be said in this president's address—and what kind of thoughts I would like to leave with you in regard to AOAC's future. Although titles may or may not have meaning, I am going to title today's address "Risking for Success" because it accurately reflects, in my judgment, what AOAC needs to do in the next few years.

I have often thought that there is a subtle irony to president's messages of this type. One of the best opportunities to communicate with the membership comes at the end of the president's term, when what has been done, has been done. You hope that you have done things right because you know that there will be little opportunity in the future to have as significant an impact. But, in the final analysis, it is probably best to have the world turn in this manner.

At this time, let me express my thanks to all of you whom the Board of Directors and I have had the pleasure of serving over the past year—and to all the methods volunteers, committee members, and other that make the process work. All associations are dependent on their volunteers. But, AOAC relies on this aspect much more than most because our methods process, the why and wherefore of AOAC, relies almost exclusively on volunteers. Let me express my special thanks to the Board—they are an excellent group to work with—and a particular thanks also to our headquarter's staff. They, more than most of you realize, keep this Association alive and well.

Those of you who have been reading the series of president's messages that have appeared in *The Referee* over the past year may remember my first installment. I wrote that association presidents have a narrow window of time to influence the course and direction of an association. While association leaders spend many years learning and gaining a leadership position, their opportunity to impact an association is fleeting at best. This is probably beneficial. As I have noted in the past, I do not see a president's role and term in office necessarily as one that accomplishes a large number of concrete items, but rather one that affects the vision, the dynamics, and future directions of the association for the benefit of its membership and the organizations that we serve. I hope over the past year that I have succeeded to some extent in this regard.

While over 100 years old for some aspects of its operation, AOAC is, in many other ways, an Association that is only 10



or 15 years old. This greatly influences where we are today and what we need to do in the next few years. We spent the first 90 years establishing the Association as an internationally recognized source of exceedingly sound collaboratively studied methods—*Official Methods of Analysis*. This was all done essentially in the rather protective environment of U.S. federal agencies—first the U.S. Department of Agriculture and later the U.S. Food and Drug Administration. We have spent the past dozen years adjusting not only to a new operational environment of an independent scientific society, but also to a world of rapidly changing analytical science.

Operationally and financially, the Association is sound. Our executive director and mangers are very competent. The internal operations of our Association have essentially undergone a complete revision in the past 3 years, and I believe the Board is satisfied that AOAC is well managed and efficiently run.

Many Directors and I felt it important to evaluate the Association's effectiveness in serving our clients: We wanted to know your needs in the changing scientific environment and to adjust the course of AOAC to ensure that we meet these needs. Many of us have heard certain questions repeated over the past several years: Is AOAC responsive in methods validation? What role should AOAC play internationally? And, apart from the methods process, how can we better serve our membership? My intent as president was to validate the thinking of our membership and clients in these areas and to develop some dynamic courses of action that would make AOAC an organi-

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zation responsive to the needs. Of particular importance to me was the methods area. As a vision statement, I would like AOAC to be perceived as the methods organization internationally.

As many of you know, our long-range planning committee outlined key areas of inquiry and established focus groups to obtain a summary of your thinking. The Board then took the results of these focus groups, combined with their individual knowledge and experience, and recommended some courses of action. Let me summarize what has come out of this process, what the Board's recommendations are, and what can be the future influences on the Association. These recommendations can be exceedingly significant and far-reaching for the Association, and they can change the dynamics and operation of the Association for the better. They are not, however, without risk. To a degree, they will be a change from the past. Some people may question the wisdom of some of the changes. Some will say that changes are coming too slowly. The Board and I have tried to devise a program that is responsive to the changes that, in our judgement and on the basis of what we have heard, are needed to keep the Association competitive, while, at the same time, retaining those things, particularly the collaborative study process, that are identified as fundamental and important to the integrity of AOAC.

First, we recommended, and you approved, a name change for our Association to AOAC International. This change reflects not only the broadening membership base of the Association beyond chemists, but also the increasing role of the Association internationally, and the globalization of what we do.

Second, our mission statement was revised to read, "The primary purpose of AOAC International is to promote methods validation and quality measurements in the analytical sciences." This rewording significantly broadens the mission of AOAC beyond methods validation by incorporating such areas as training and publications in which we are already significantly involved, and potential future areas such as laboratory accreditation or reference standards.

Third, we have established protocols and an approval mechanism for test kits, currently termed $R^2$ for "Reviewed and Recognized Methods." These protocols allow AOAC to be responsible to the needs of users of these test kits. They are also a means by which test kit manufacturers can validate the kits to assure their scientific soundness in a manner that is compatible with their intended use and the rapidly changing technology that exists in this area. The usefulness of this concept has already been documented, with FDA agreeing to use this approach to validate test kits for use in drug residue analysis in the dairy product area.

Fourth, we have established a task force, chaired by Director Alan Hanks, to develop protocols for peer verified methods that are validated through processes less vigorous than the AOAC collaborative study process. Methods submitted for approval under this class may first be published in the *Journal of AOAC International*, then republished as compendia in loose leaf form. The intent behind the test kit and peer verified methods initiatives is to be responsive to rapid changes in scientific methods development, responsive to those who want AOAC's involvement in this type of activity, without diminishing the status or extent of our official methods.

In short, we are trying to develop a responsiveness to the methods area that is sufficiently separated form the collaborative study process, that enhances rather than threatens our historical methods base, while, at the same time, providing a linkage to this process that would allow these lesser validated methods to enter into the collaborative study process should there be a desire and a need to do so.

Fifth, the Board has created the position of international technical coordinator on staff in line with stated policy of enhancing the Association's international activity and visibility. The position will be responsible for enhancing our activities with international standard organizations, coordinating our international liaisons, expediting the incorporation of collaborative studies done outside of AOAC into the Association, and generally increasing our activities and visibility in the international area.

Sixth, we are proposing significant enhancements to our membership services area. These enhancements will focus on several areas, but three are worthy of mention at the moment.

### *Training and Short Courses*

Emphasis will be placed on the development of basic "how-to" courses for analysts in both functional analytical areas such as gas chromatography and liquid chromatography, and in discipline-based areas such as pesticide residue analysis and drug analysis. The current thought is to design these programs around a mentor approach.

### *Analyst Certification Program*

These programs would be linked but go significantly beyond the AOAC method development/validation processes. The intent would be to develop a program that would certify an analyst's expertise in a given analytical discipline.

An education/certification task force headed by Past President Thomas Layloff has been established in these two areas.

### *Information Technology Area*

The Board has authorized a task force that will provide recommendations on information technology programs, including the concepts of publishing AOAC methods electronically and establishing an electronic bulletin board for analysts.

It has, indeed, been quite a year.

Let me close by thanking you again for the opportunity to serve. I hope that I, as your president, and your Board, along with the assistance of capable staff, have steered the Association along a course that will fulfill our vision of AOAC as *the* methods organization. It will now be your responsibility and opportunity to see that the process is completed. Risking for success will be your challenge to assure the future of AOAC International.

Thank you.

DISCUSSIONS IN ANALYTICAL CHEMISTRY

# Modern Thin-Layer Chromatographic Pesticide Analysis Using Multiple Development

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In an earlier editorial (1), thin-layer chromatography (TLC) and high performance thin-layer chromatography (HPTLC) were compared to the more popular analytical method, high performance column liquid chromatography (LC). It was suggested that one reason why these planar chromatography methods are not more widely used in the United States is the lack of education in the methods received by scientists in their undergraduate and graduate studies. This is especially true of modern, instrumentalized HPTLC, in which area the greatest current advances and future promise reside.

In a typical undergraduate chemistry curriculum, students may separate inks or food dyes by paper chromatography in general chemistry laboratory. They may also do an experiment in organic chemistry laboratory involving characterization of the purity of a reaction product on a silica gel-coated microscope slide or sheet spotted with solution from a crude micropipet and developed in a beaker covered with a watch glass or aluminum foil. The detection of the spots may be done by exposure to iodine vapors or inspection under UV light. Most quantitative analysis and instrumental analysis texts and laboratory manuals have chapters and experiments on gas chromatography (GC) and column LC, but very little material on TLC, and less or none on instrumental HPTLC.

The paucity of training in and understanding of TLC has led to a low level of use in most American analytical laboratories. This is clearly illustrated by the relatively few TLC methods contained in the AOAC International *Official Methods of Analysis* and the small number of TLC papers published annually in the *Journal of AOAC International*, especially outside the mycotoxin area. Pesticide analysis is a typical example of a field in which little application of TLC occurs in the United States. The latest edition of the FDA *Pesticide Analytical Manual*, Volume I, contains a 5-page section (No. 610) on TLC confirmation, dated January 1, 1968, and a chapter (No. 4) on the qualitative determination of organochlorine and organophosphorus residues, dated July 1, 1969. The TLC pesticide methods contained in the AOAC International *Official Methods of Analysis* (**970.52**) are correspondingly old. As might be imagined, considerable advances have taken place in the techniques and applications of pesticide TLC in the more than 20 years since these methods were originally published (2–6).

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For example, 2 recent papers (7) reported procedures for the identification and quantitation of over 100 pesticides, predominantly insecticides and fungicides, by HPTLC employing an automated spotting device and computer-assisted densitometric evaluation. $R_f$ values and detection limits with 3 detection reagents were tabulated.

A modern HPTLC quantitative analysis differs markedly from the type of experiment encountered by students in college laboratory courses. In a typical HPTLC analysis, sample and standard solutions are applied to a 10 × 10 cm high performance silica gel or chemically bonded silica gel layer of 200 μm thickness using a mechanical or automated spot or band applicator. The mobile phase is chosen by use of optimization methods such as simplex or prisma, and chromatograms are produced by horizontal, multiple, or overpressured development. The natural visible absorbance, UV absorbance, or fluorescence of the separated zones is measured by automated densitometric scanning at an optimal wavelength. If necessary to aid detection of analytes, prechromatographic derivatization is performed or a postchromatographic detection reagent is applied to the layer by spraying or dipping to enhance absorption or fluorescence of zones before scanning. Sample preparation for HPTLC is accomplished using procedures similar to those for GC and column LC; recently, solid phase extraction (SPE) has been widely applied for water analysis (8).

Multiple development of TLC plates is known to provide increased resolution of complex mixtures. The traditional multiple development process involves repeated manual development of a plate in the same direction with the same solvent over the same distance. Each subsequent development moves the trailing edges of the zones closer to the fronts, resulting in narrower bands and improved resolution and detection sensitivity. Automated multiple development (AMD), which is a variation of the classical multiple development method, is one of the most promising instrumental TLC methods for pesticide analysis. A number of papers have been published recently describing the principles, techniques, and applications of AMD for determination of pesticide residues in water samples. Perhaps the earliest was the report by Burger (9). The purpose of this editorial is to acquaint readers with a selection of these papers so that the potential of the AMD method will be appreciated.

AMD involves plate development with a solvent gradient. The plate is developed 10–25 times in one direction with solvents of decreasing strength (i.e., decreasing polarity for nor-

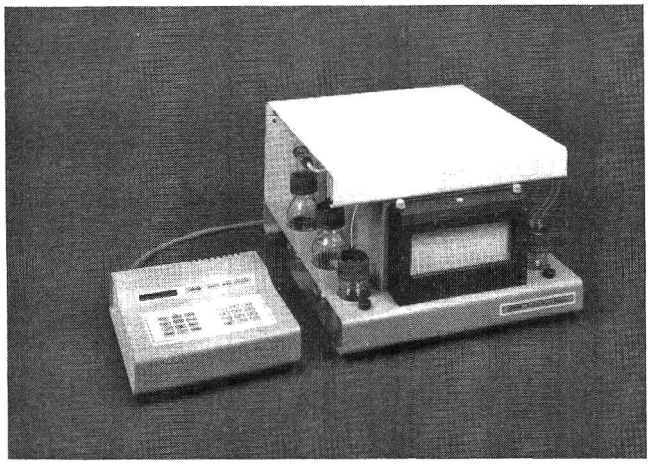

**Figure 1. Camag fully-programmable automated multiple development system.**

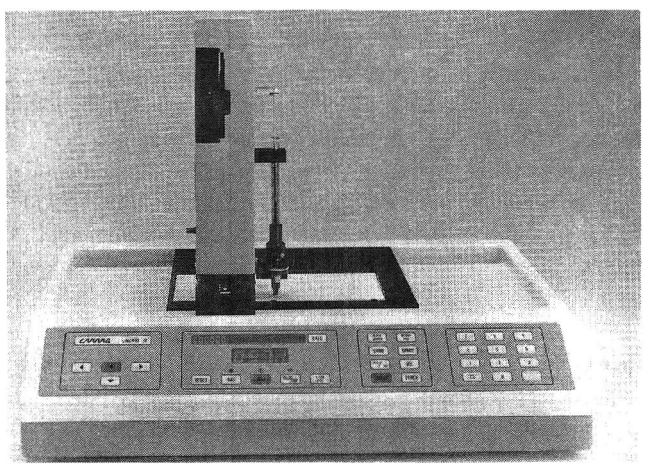

**Figure 2. Camag Linomat IV narrow band spray-on sample applicator.**

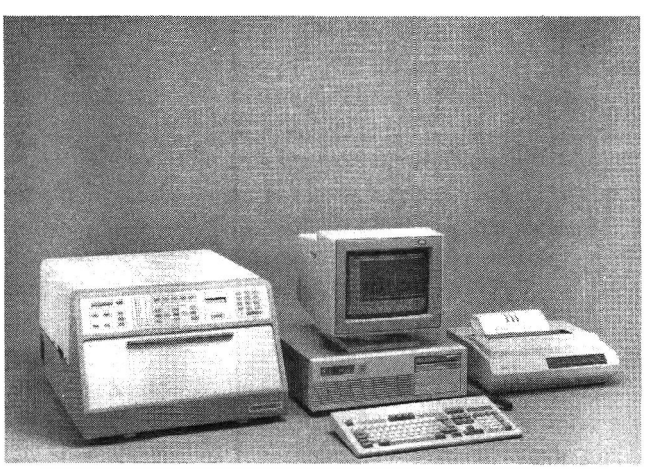

**Figure 3. Camag computer-controlled TLC Scanner II [Photographs and permission for publication were obtained from Helga Rehmert (for Dieter Janchen), Camag, Muttenz, Switzerland].**

mal phase TLC) over migration distances that increase by ca 3–5 mm each. The previous solvent is removed from the developing chamber, the plate is dried under vacuum, and the layer is equilibrated with a controlled atmosphere between developments. The commercial instrument available for AMD (Figure 1) is under complete microprocessor control and is extremely reproducible. Zones are produced in the form of narrow bands, and migration distances are independent of the sample matrix for a given solute. Optimization of separation conditions for unknown samples is convenient.

The fundamentals and methodology of the AMD technique for pesticide determination were reviewed (10). It is shown in this review that each step of the analysis leads to an increase in the probability of identification by comparison with a standard substance. Because migration distances are highly reproducible (on the same HPTLC plate and different plates), a narrow window of $R_f$ values is adequate for pesticide identification. Identification is preliminarily obtained and then confirmed by running consecutive screening and confirmation chromatograms, coupled with multiwavelength scanning and selective postchromatographic detection reagents. For water analysis, SPE cleanup and enrichment by a factor of 5000 precedes TLC, so that samples containing as little as 20 ng pesticide/L can be quantitated. At least 100 substances can be screened on an HPTLC plate, and the AMD technique uses only a few milliliters of mobile phase, avoiding waste disposal problems.

HPTLC/AMD was applied to the simultaneous monitoring of phenylureas, carbamates, and triazines according to European Economic Community drinking water regulations. Pesticides were extracted using a C18 SPE column and recovered by elution with acetonitrile. Standards and column eluate samples were sprayed onto silica gel high performance plates in the form of bands using the microprocessor-controlled Camag Linomat IV (Figure 2). Plates were developed with a 30-step methanol–dichloromethane gradient, and zones on developed plates were measured with the UV reflectance mode of a densitometric scanner (Figure 3) at 190–300 nm (11).

The HPTLC/AMD procedure was optimized for the determination of 24 pesticides in water by using spray-on application of samples; a 100 μm silica gel layer thickness instead of the usual 200 μm; two 20-step universal elution gradients, one based on dichloromethane for screening and one based on *tert*-butyl methyl ether for confirmation, with 1 mm distance increments instead of 3 mm; and densitometric evaluation of plates. The changes in layer thickness and gradients increased the sensitivity and speed of the analysis. A C18 cartridge was employed for extraction, and a silica gel cartridge for additional cleanup when the C18 cartridge eluate was colored (12).

The principles of AMD can be applied manually if the automated instrument is not available. This was illustrated by the separation of 8 organochlorine insecticides on a silica gel HPTLC plate developed 5 times over distances rising from 28 to 70 mm, with dichloromethane containing increasing percentages of *n*-hexane. Samples and standards were applied as 10 mm bands with a Linomat IV, zones were detected by dipping the layer in silver nitrate or 3,5,3′,5′-tetramethylbenzidine

reagent, and quantitation was performed by reflectance scanning at 550 nm (13).

It is hoped that this and the earlier (1) editorial will encourage readers to study the cited references on quantitative, instrumental HPTLC and pesticide analysis by TLC, and that they will then be convinced of the benefits of introducing the use of AMD and the other described modern procedures in their laboratories for the analysis of pesticides and other analytes in all types of sample matrixes.

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DAIRY PRODUCTS

# Quantitative Analysis of Agricultural and Food Products: A New Instrumental and Computerized Approach

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**A new software package, ANAQUANT, was specifically designed for a long-term approach to the quantitation of compounds in biological products. Its functionality and validity were tested by measuring fat and protein contents in liquid cow's milk using Fourier transform infrared spectrometry and a handcrafted transmission flow cell. Calibration and validation standard deviations were 0.2484 and 0.3987 g/kg, respectively, for the prediction of proteins, and 0.3163 and 0.4222 g/L, respectively, for the prediction of butterfat. One month elapsed between calibration of the instrument and the validation study. Results are consistent with those proposed in the literature.**

Quantitative analysis of food and agricultural product compounds is a daily and critical responsibility for the quality control (QC) management of manufactured products. Quantitative values obtained for specific materials are isolated, even if the results are for components related to the same process. Typically, there are no real links between different analyses; they are simply a succession and an accumulation of data and results. This may lead to a misunderstanding of the integral quantitative management of biological product quality, which involves not only the calibration of the analytical device used, but also its ability to provide repeatable and reproducible answers.

These scattered and short-term quality management decisions caused us to look for a new approach to QC performed by analytical instrumentation. The field of Fourier transform infrared (FTIR) spectrometry applied to milk and dairy product analysis was the example chosen for this study. A Nicolet FTIR spectrometer coupled with 2 computers—a Nicolet 1280 for data acquisition and first treatments, and an IBM 286 PC compatible equipped with a numeric co-processor for all other data treatments—was proposed for the analysis of fat and protein in liquid milk. Statistical calculations of the experimental data were performed using a new software package, ANAQUANT, specifically designed for a long-term approach to the quantitation of compounds in biological products. The details of this analytical software environment are to be applied in the near future to on-line and real time QC in food manufacturing plants.

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Agricultural and food products are biological products. Consequently, they are subject to numerous compositional modifications according to time, geographical origin, and/or genetic variation (1). These factors have to be carefully considered in a quantitation process applied to the QC management of manufactured products. This specific point is indeed the most difficult aspect of the quantitative analysis problem; however, it is possible to include these sources of variation using appropriate tools: such as a judicious sampling methodology and a simple algorithm able to provide results with the requested accuracy.

The philosophy of quantitation process management applied to indirect measurement methods already exists. Its steps may be described as follows (2): (*1*) evaluation of the repeatability of the measurement, i.e., comparison of duplicates acquired short term; (*2*) generation of the calibration equations; (*3*) validation of these calibration equations (This consists of the determination of the slope and/or bias errors that typically effect an instrument during a long-term interval); and (*4*) calculation, if needed, of slope and bias error corrective equations.

Actually, a lot of quantitative analysis software available on the market can handle one or another of these steps, but none of them can accomplish all 4 steps simultaneously. The most unique aspect of this software package is its ability to correct slope and bias errors. Usually, this correction is accomplished by fine-tuning through hardware manipulations. This ability may result in automation of the validation and corrective equation generation processes.

The purpose of this study is to propose the use of ANAQUANT, a software package that meets these criteria. The validity of this approach has been established through the quantitation study of fat and protein in liquid raw milk using a Nicolet 740 FTIR spectrometer equipped with a temperature controlled transmission flow cell in the mid-infrared (MIR) region.

This particular analysis was chosen because of the existing scientific literature describing the IR analysis of milk compounds using a multilinear regression (MLR) method (3–6).

## Experimental

### *Apparatus*

(a) *Spectrometer.*—No. 740 FTIR used in the MIR region, equipped with a global MIR source, a KBR beamsplitter, a

transmission flow cell (see (b)), and a triglyceride sulfate detector (Nicolet Instrument Corp., Madison, WI 53711).

(b) *Temperature control and homogenization external device.*—Includes a temperature controlled transmission cell with $CaF_2$ windows and a pathlength of 25 μm. Specifically designed for these experiments (Delta Instruments, The Netherlands).

(c) *Computer.*—Model 1280. Used for data acquisition and storage, and to perform the Fourier transform on the acquired interferograms (Nicolet). This computer was used to provide height and/or area (i.e., integrated value of the spectral region) after baseline correction of each of the selected peaks.

(d) *Computer.*—IBM 286 PC compatible equipped with a numeric co-processor and connected to Nicolet 1280 through a RS 232 C. Data communications were performed under ASCII format using KERMIT facilities. Communication was 19200 baud. Developed quantitation software package, ANAQUANT, was written under TurboBasic Borland 1.0, compiled as an executable file, and installed on this computer.

These experiments were chosen after a survey of milk and dairy product plants and laboratories in France showed a need for a program developed on an IBM compatible PC.

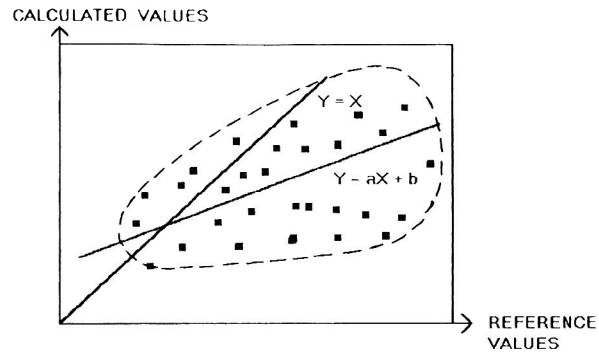


**Figure 1. Chart of "true" values vs "raw" predicted values. Generation of slope and bias corrective equation.**

## ANAQUANT Features

ANAQUANT software was able to handle the 4 major steps of a whole quantitation process (repeatability, calibration, validation, and corrective equations) in a sequential manner using the experimental data provided by the FTIR instrument. To test

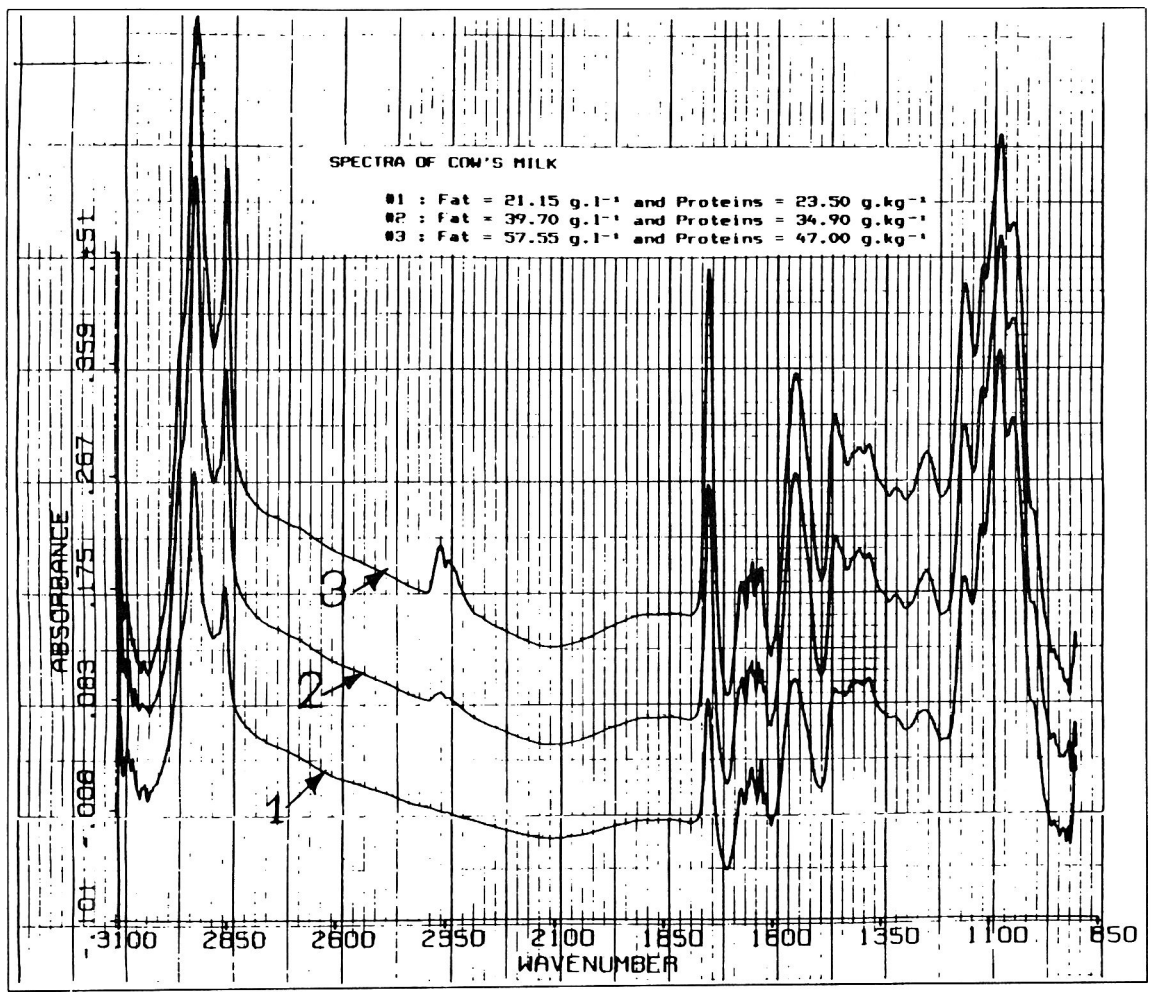


**Figure 2. Spectra of milk obtained after chemical stabilization, homogenization, and temperature control. Measurement performed in the MIR region (3100–400 $cm^{-1}$) using transmission accessory.**

**Table 1. Multilinear regression method: calibration and validation results on fat contents in milk**

| Equation | Calibration correlation coefficient | Calibration standard deviation, g/L | Validation correlation coefficient | Validation standard deviation, g/L |
|---|---|---|---|---|
| 1[a] | 0.9998 | 0.2660 | 0.9946 | 0.7075 |
| 2[b] | 0.9997 | 0.3163 | 0.9980 | 0.4222 |
| 3[c] | 0.9984 | 0.7627 | 0.9932 | 0.7900 |
| 4[d] | 0.9996 | 0.3877 | 0.9982 | 0.3748 |

[a] Fat = 326.17 × Fat B + 19.181 × Prot. + 5.7256; Fat B peak height, 2854 $cm^{-1}$ (reference, 2800 $cm^{-1}$); Prot. peak height, 1548 $cm^{-1}$ (reference, 1489 $cm^{-1}$).
[b] Fat = 195.82 × Fat A + 6.2613 × Prot. + 0.7893; Fat A peak height, 1746 $cm^{-1}$ (reference, 1801 $cm^{-1}$).
[c] Fat = 326.62 × Fat B + 8.454.
[d] Fat = 196.03 × Fat A + 1.6539.

the repeatability of the acquired signal, corrected peak heights and/or areas under the peak after baseline correction of duplicate spectra were compared. In other words, the repeatability of the signal was estimated by the calculation of the residual standard deviation of duplicates about the average height or area of a peak. The algorithm used to generate the calibration curve was the MLR procedure. The variation of a compound's concentration in food, within the biological boundaries, was usually described by a multilinear model. This was particularly true with the measurements of milk compounds using MIR data. Calibration samples that were analyzed were specifically manufactured to set up independent variables.

A validation study of the calibration equations was performed after the analysis of a new set of validation samples. Samples were selected according to the same criteria of independence of the variables. This allowed the estimation of the ability of the instrument to provide long-term, stable results. Meanwhile, an estimation of the error of slope and/or bias was provided by the same calculations. Thereafter, it was simple to calculate slope and bias error corrective equations that mirror the stability of the measurement.

The prediction of values was, and still is, the ultimate goal of quantitative analytical instrumentation. The predicted values were calculated according to the calibration and slope and/or bias corrective equations (Figure 1).

The steps of the algorithms and matrix calculation that were used in ANAQUANT (7, 8) are detailed in Appendices 1 and 2.

### *Application of ANAQUANT to Experimental Data*

This software package was used to set up calibration equations adjusted to the prediction of butterfat and protein content in liquid milk using an FTIR spectrometer. After a judicious choice of standards (9) and sampling protocol, consisting of chemical stabilization by addition of bronopol (2-bromo 2-nitro 2,3-propanediol), homogenization, and temperature control (10, 11), the liquid milk samples were analyzed in transmission in the MIR region (12). Four characteristic spectral regions were chosen with a maximum absorbance value at the following wavenumbers: Fat B, 2854 $cm^{-1}$; Fat A, 1746 $cm^{-1}$; proteins, 1548 $cm^{-1}$; and lactose, 1047 $cm^{-1}$. These 4 peaks are used for routine measurements of butterfat and protein content in milk using filter spectrometers (Figure 2).

MLR calibration equations were processed after the analysis of 25 calibration samples, manufactured according to Leray (9), to preserve the independence of the studied variables, butterfat and protein content. Lactose was not considered as a studied variable; lactose contents in these 25 standards were dependent on the fat and protein contents, and were characterized by a weak variance. As evidenced later in the text, such characteristics do not favor statistical use of this variable.

For the study of butterfat and proteins, 58 and 42 validation samples of herd milk were used, respectively. Concentrations were distributed uniformly between 20.0 and 64.0 g/L for the butterfat content, and between 21.0 and 54.0 g/kg for the protein content. Samples were analyzed with the same spectrometer that was used for the calibration samples, but analyses were performed 1 month later to test the shift of the instrumental response as a function of time. For the determination of butterfat and protein concentrations, the Gerber and Amido Black methods were used, respectively, as chemical reference methods. The MLR tests that were performed on these data are characterized by the following: (*1*) The peak characteristic of

**Table 2. Multilinear regression method: calibration and validation results on proteins contents in milk**

| Equation | Calibration correlation coefficient | Calibration standard deviation, g/kg | Validation correlation coefficient | Validation standard deviation, g/kg |
|---|---|---|---|---|
| 1[a] | 0.9995 | 0.2486 | 0.9954 | 0.3993 |
| 2[b] | 0.9995 | 0.2484 | 0.9955 | 0.3987 |
| 3[c] | 0.9966 | 0.6241 | 0.9956 | 0.4041 |

[a] Protein = 13.384 × Fat B + 202.73 × Protein + 4.4655; Fat B peak height, 2854 $cm^{-1}$ (reference, 2884 $cm^{-1}$); Prot. peak height, 1548 $cm^{-1}$ (reference, 1489 $cm^{-1}$).
[b] Protein = 8.3069 × Fat A + 202.18 × Protein + 4.2559; Fat A peak height, 1746 $cm^{-1}$ (reference, 1801 $cm^{-1}$).
[c] Protein = 203.13 × Protein + 5.7162.

lactose was not included in the best equations. However, several authors described this peak as very useful for the improvement of the quality of the calibration equations. The lactose content was not a variable considered in the manufacture of the calibration milk samples. The instrument was only calibrated for fat and protein content, and this was done according to a previously experimented method (9). Consequently, the lactose peak had a very weak variance, which was not significant enough to be included into the calibration equation. (*2*) The best combination for the prediction of butterfat seemed to be the spectral information loaded by the peaks Fat A (ester carbonyl in mono-, di-, and triglycerides) and proteins (Amide II) (Table 1). Calibration and validation correlation coefficients were very high (>99%), and the calibration and validation standard deviations, 0.3163 and 0.4222 g/L, respectively, were quite close to those described in the literature (13–16). (*3*) The best combination for the prediction of protein seemed to be either the spectral information loaded by the peaks for Fat B (C–H stretch in $CH_2$ and $CH_3$) and proteins (Amide II), or the spectral information loaded by the peaks for Fat A (ester carbonyl in mono-, di-, and triglycerides) and proteins (Amide II). Calibration and validation correlation coefficients were very high (>99%) for both combinations, and the calibration and validation standard deviations were 0.2486 and 0.3993 g/kg, respectively, for the combination Fat B/proteins, and 0.2484 and 0.3987 g/kg, respectively, for the combination Fat A/proteins (Table 2). These values are also consistent with those proposed in the literature.

These results do not indicate any improvement in quantitative results as compared with the current knowledge in the field. The real goal of this study was to set up a software package that is able to handle the whole analytical process in a single software entity. On the basis of this restriction and the obtained results, ANAQUANT appeared well suited to the calculations for which it was written. In addition, it can be automated easily; the only difficulty would be the generation of data files by the analytical device in a format that would be compatible with the format required by ANAQUANT.

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## Appendix 1: Algorithms and Formats

### *Multilinear Regression: Principles*

This method's purpose is the description of the studied phenomenon by a linear combination of independent variables, i.e., the plot of the concentration as a linear function of $n$ supposedly independent variables. Also, this assumes that the studied phenomenon is adequately described by a linear model. Fortunately, in most food products, the variation of the concentration for one compound can be described as a linear combination of variables issued from indirect measurement methods.

This quantitation software package was able to handle the 4 major steps of a quantitation process (repeatability, calibration, validation, and corrective equations) in a sequential manner, using the experimental data provided by the FTIR instrument, as described in *ANAQUANT Features*.

### *Software Package*

*Data and file manipulations.*—The program allows creation, correction, and editing of data and files. A 2-dimensional (observations × variables) matrix format was used. Variables were either the wavenumbers (option of peak heights' measurements), or spectral regions (option of peak areas' measurements). Observations were the spectra (each sample). This matrix format was consistently maintained in the individual sub-programs of ANAQUANT.

### *Repeatability Study*

The calculated parameters for this evaluation were as follows:

The mean of the instrumental value ($x_m$) issued from duplicates for each selected instrumental variable ($x_i$) was determined from the equation:

$$x_m = \sum_{i=1}^{n} \left( \frac{x_i}{n} \right)$$

This value had no real interest in itself, but was useful for the following steps.

Sampling standard deviation ($s$) for each of the mean values previously calculated was determined from the equation:

$$s = \left( \frac{\sum_{i=1}^{n} (x_i - x_m)^2}{n-1} \right)^{1/2}$$

The sampling standard deviation value is reported as a relative standard deviation ($s\%$) for one mean instrumental value:

$$s\% = \frac{(s \times 100)}{x_m}$$

Sampling standard deviation of the instrumental data for each of the selected variables (S%), now taking into consideration all the observations together, was calculated from:

$$S\% = \sum_{i=1}^{m} \left[ \frac{s_i\%}{m} \right]$$

This value might be considered as an estimation of the repeatability of the signal.

### *Generation of Calibration Equations*

Provided the studied concentrations were kept within the biological boundaries, the variations of one of the compound's contents could be described as a multilinear model of the acquired physical signal. This was the main reason for the choice of the MLR model as the calibration algorithm. The algorithm was defined as the following:

$$Y = a_0 + (a_1)(X_1) + (a_2)(X_2) + \ldots + (a_n)(X_n)$$

where Y = predicted value; $a_0$ = constant value; $X_j$ = independent variables; and $a_1,\ldots,a_n$ = regression coefficients. The algorithm used went through the following 9 steps to perform the entire analysis: (*1*) determination of a square matrix for the spectral data; (*2*) inversion of the matrix; (*3*) calculation of covariance ($S^2_{jk}$) and variance ($s^2_j$) for each variable.

$$S^2_{jk} = \frac{\frac{1}{N-1} \sum \left[ \frac{1}{\sigma_i^2} (X_j - Y)(X_k - Y) \right]}{\frac{1}{N} \sum \frac{1}{\sigma_i^2}}$$

with: $$X_j = \frac{\sum \left[ \frac{1}{\sigma_i^2} X_j (x_i) \right]}{\sum \frac{1}{\sigma_i^2}} \quad \text{and} \quad Y = \frac{\sum \left[ \frac{1}{\sigma_i^2} y_i \right]}{\sum \frac{1}{\sigma_i^2}}$$

The variance is calculated from:

$$s_j^2 = s_{jj}^2$$

(*4*) Calculation of the correlation coefficient ($r_{jk}$) is as follows:

$$r_{jk} = \frac{s^2_{jk}}{s_j \times s_k}$$

(*5*) Calculations of the regression coefficients ($a_o$ and $a_j$) are as follows:

$$a_0 = y - \sum_{j=1}^{n} [\, a_j X_j \,]$$

and: $$a_j = \frac{s_y}{s_j} \sum_{k=1}^{n} \left[ \frac{r_{ky}}{r_{jk}} \right]$$

with: $$y = \frac{\sum \left[ \frac{1}{\sigma_i^2} y_i \right]}{\sum \frac{1}{\sigma_i^2}}$$

(*6*) Calculations of the standard deviations $\sigma^2_{aj}$ and $\sigma^2_{a_0}$ of the regression coefficients are as follows:

$$\sigma^2_{aj} = \frac{\frac{1}{N-1} \frac{1}{s_j^2} r_{jj}^{-1}}{\frac{1}{N} \sum \frac{1}{\sigma_i^2}}$$

and:

$$\sigma^2_{a_0} = \frac{\left( \frac{1}{N} + \frac{1}{N-1} \right) \sum_{j=1}^{n} \left[ X_j^2 \frac{1}{s_j^2} r_{jj}^{-1} + \sum_{k=1}^{n} \left( X_j X_k \frac{1}{s_j s_k} r_{jk}^{-1} \right) \right]}{\frac{1}{N} \sum \frac{1}{\sigma_i^2}}$$

(*7*) For the calculation of the residual standard deviation, also called calibration standard deviation, see variance.

(*8*) Calculation of the multiple correlation coefficient ($R^2$) is as follows:

$$R^2 = \sum_{j=1}^{n} \left( b_i \frac{s^2_{jy}}{s_y^2} \right) = \sum_{j=1}^{n} \left( b_j \frac{s_j}{s_y} r_{jy} \right)$$

(*9*) The provided MLR equation, therefore, is as follows:

$$y(x) = a_0 + \sum_{j=1}^{n} [\, a_j X_j (x) \,]$$

The following results are displayed on screen and can be printed out. (*1*) Regression coefficient for each of the explaining (experimental) variables and the constant value of the regression. These are the terms of the calibration equation. (*2*) Standard deviation for each regression coefficient. They allow an estimation of the accuracy of each term of the equation. (*3*) Residual standard deviation (also called calibration standard deviation) of the calibration curve. This value provides an estimation of the dispersion of the points around the calibration curve. (*4*) Multiple correlation coefficient for the multilinear

regression. This last parameter is characteristic of the homogeneity of the points along the calibration curve.

The regression coefficients are stored in a new, 2-dimensional matrix file (regression coefficient × equations). The regression coefficients of several equations can be stored in the same file. Thereafter, this new file can either be displayed on the monitor and printed out, or manipulated for corrections.

### *Prediction of "Raw" Values*

The first step in the prediction of unknown samples can also be used as a step for validation. This part of the program applies the MLR equations previously generated to new instrumental values. Consequently, this step of the software can be used to calculate predicted values for unknown (prediction) or known (validation) samples. Regardless of the kind of data (prediction or validation), this step consists of the following 5 subsections: (*1*) determination of a square matrix whose dimensions are the number of variables requested to explain the phenomenon + 1; (*2*) inversion of the matrix; (*3*) calculation of the regression coefficient; (*4*) calculation of the residual standard deviation; and (*5*) calculation of the standard deviation for each of the regression coefficients.

The regression coefficients and their standard deviations, the residual standard deviations, and the multiple correlation coefficients are displayed and can be printed out and stored in the same manner as in *Generation of Calibration Equations*.

### *Prediction of Corrected Values*

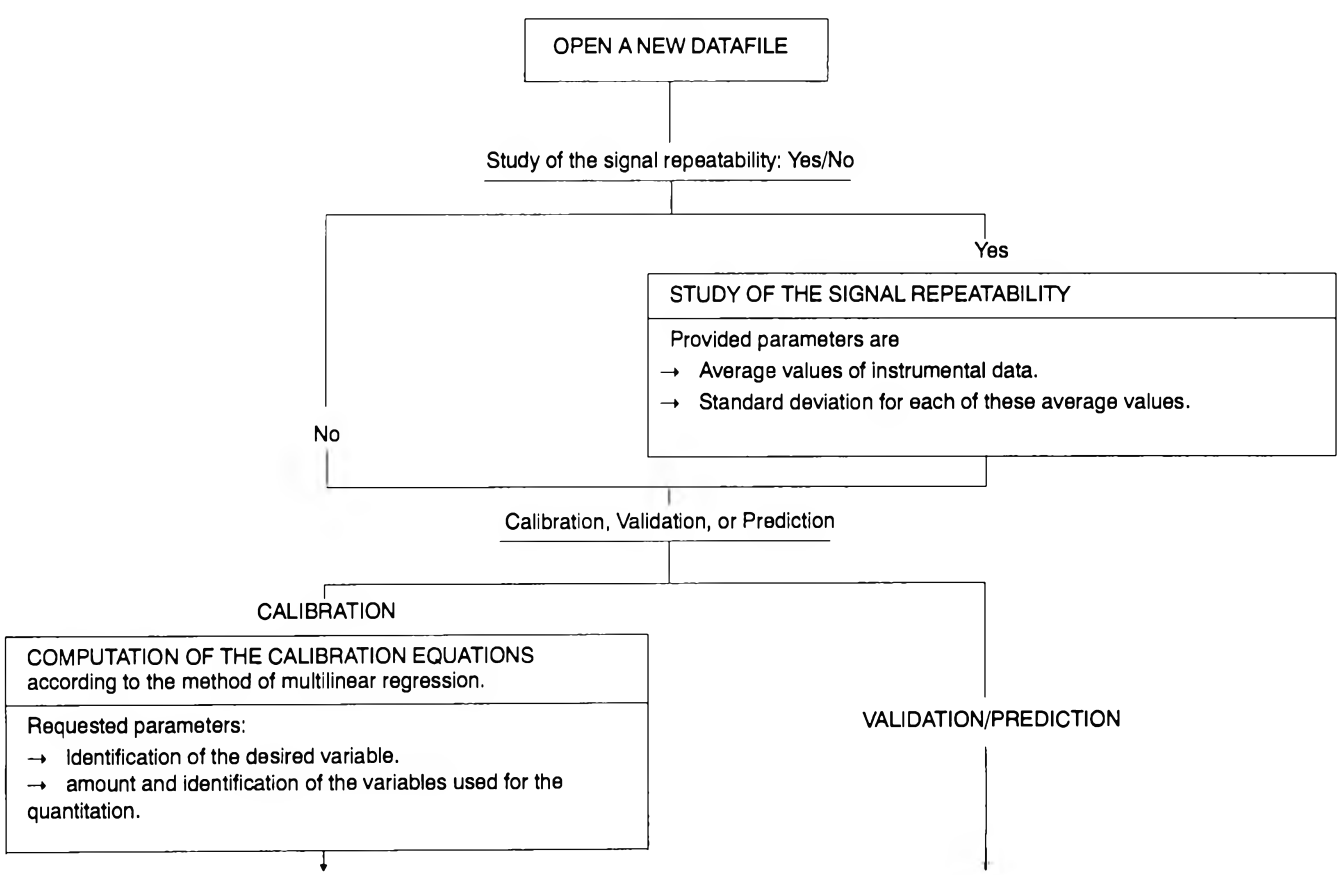
This is the ultimate step of the whole analysis, and it consists of applying the corrective equation, generated after the validation study, to the "raw" predicted values. This involves a readjustment of the predictions according to the exact shift of slope and bias of the instrument. The obtained predicted values are supposedly equal to the "true" chemical values, or at least equal within the allowed standard deviation.

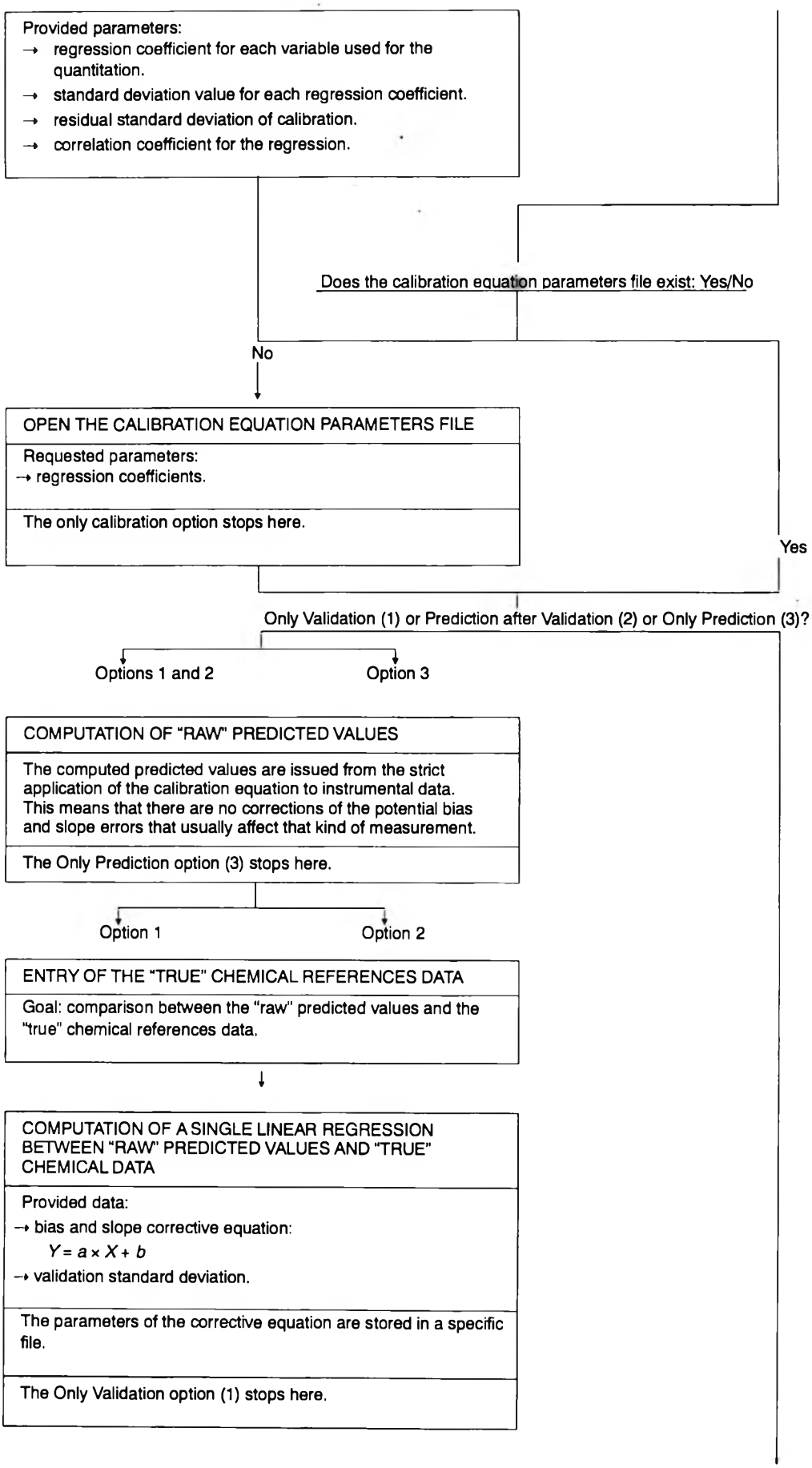
## MLR—ANAQUANT: Practical Aspects

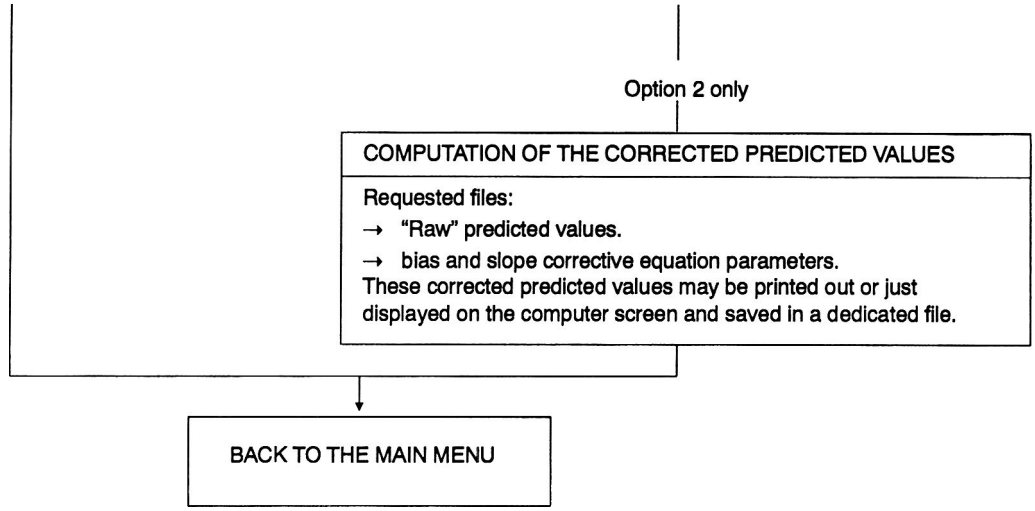
The information required for ANAQUANT analysis is as follows: (*1*) component information; (*2*) peak height information; (*3*) calibration and validation samples; and (*4*) determination of the number of experimental variables used to explain the studied phenomenon.

The information provided by ANAQUANT analysis is as follows: (*1*) prediction of the concentrations in unknown samples; (*2*) calibration diagnostics; (*3*) actual vs calculated concentration diagnostics; (*4*) test of the repeatability and of the general stability of the instrumental method; and (*5*) estimation of the error of slope and bias if needed.

## Appendix 2. ANAQUANT Program Patterns




Provided parameters:
→ regression coefficient for each variable used for the quantitation.
→ standard deviation value for each regression coefficient.
→ residual standard deviation of calibration.
→ correlation coefficient for the regression.
Does the calibration equation parameters file exist: Yes/No
No
Yes
OPEN THE CALIBRATION EQUATION PARAMETERS FILE
Requested parameters:
→ regression coefficients.
The only calibration option stops here.
Only Validation (1) or Prediction after Validation (2) or Only Prediction (3)?
Options 1 and 2
Option 3
COMPUTATION OF "RAW" PREDICTED VALUES
The computed predicted values are issued from the strict application of the calibration equation to instrumental data. This means that there are no corrections of the potential bias and slope errors that usually affect that kind of measurement.
The Only Prediction option (3) stops here.
Option 1
Option 2
ENTRY OF THE "TRUE" CHEMICAL REFERENCES DATA
Goal: comparison between the "raw" predicted values and the "true" chemical references data.
COMPUTATION OF A SINGLE LINEAR REGRESSION BETWEEN "RAW" PREDICTED VALUES AND "TRUE" CHEMICAL DATA
Provided data:
→ bias and slope corrective equation:
Y = a × X + b
→ validation standard deviation.
The parameters of the corrective equation are stored in a specific file.
The Only Validation option (1) stops here.


Option 2 only
COMPUTATION OF THE CORRECTED PREDICTED VALUES
Requested files:
→ "Raw" predicted values.
→ bias and slope corrective equation parameters.
These corrected predicted values may be printed out or just displayed on the computer screen and saved in a dedicated file.
BACK TO THE MAIN MENU

DRUG FORMULATIONS

# Determination of Dicloxacillin Preparations by Liquid Chromatography

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**A reversed-phase liquid chromatographic method was developed for the assay of dicloxacillin in bulk drugs and pharmaceutical preparations. The samples were analyzed on a μBondapak (C18) column with a mobile phase of methanol–4% acetic acid (60 + 40) at a flow rate of 1.5 mL/min, with UV absorbance detection at 254 nm. An equation was derived showing a linear relationship between peak area ratios of dicloxacillin to dimethylphthalate (internal standard) and the dicloxacillin concentration over a range of 2–30 μg (r = 0.9999). Standard addition recoveries ranged from 98.65 to 100.74% (mean 99.70%, n = 6). The coefficient of variation was less than 0.24%. The assay results were compared with those obtained by the official microbiological method, which indicated that the proposed method is a suitable substitute for the microbiological method for potency assays and stability studies of dicloxacillin preparations.**

Liquid chromatographic (LC) determinations of dicloxacillin in biological fluids have been reported (1–4). The present official assay method of the Minimum Requirements for Antibiotic Products of Japan (5) for analysis of dicloxacillin in oral dosage forms involves microbiological agar diffusion. The U.S. *Code of Federal Regulations* (6) describes 2 official methods for potency assay of dicloxacillin: microbiological agar diffusion and iodometric titration. The regulations state that the results obtained from the microbiological assay shall be conclusive.

The microbiological method is lengthy and inferior to the chemical methods in both accuracy and precision (7). The proposed method offers advantages in speed, simplicity, and reliability.

## METHOD

### Reagents and Materials

(a) *Solvents.*—Dimethylphthalate and glacial acetic acid, both reagent grade (E. Merck, Darmstadt, Germany); methanol, LC grade (ALPS Chemical Co.); potassium phosphate, monobasic and dibasic, reagent grade (Wako Pure Chemical Industries, Ltd, Japan).

(b) *Mobile phase.*—Methanol–4% acetic acid (60 + 40, v/v).

(c) *Internal standard stock solution.*—Dissolve 30 g dimethylphthalate in 1 L acetonitrile–water (1 + 1, v/v) to obtain a concentration of ca 30 mg/mL.

(d) *Standard.*—Dicloxacillin sodium was a National Laboratories of Foods and Drugs house standard (National Laboratories of Foods and Drugs, Taiwan, R.O.C.)

(e) *Dicloxacillin standard solution.*—Accurately weigh equivalent to ca 50 mg potency of dicloxacillin standard into a 50 mL volumetric flask, add 0.5 mL internal standard stock solution, dilute to volume with water, and mix.

(f) *Samples.*—Different lot numbers of dicloxacillin sodium bulk drugs were supplied by either Bristol Industries, Ltd, or China Biological & Chemical Laboratories. Capsules of dicloxacillin (125 and 250 mg/capsule) were obtained from commercial sources.

(g) *Sample preparations.*—Transfer quantity of bulk drug substance or homogeneous capsule contents equivalent to ca 50 mg potency of dicloxacillin, to 50 mL volumetric flask, add 0.5 mL internal standard stock solution, dilute to volume with water, and mix.

(h) *Solution for linearity response.*—Prepare 7 solutions of dicloxacillin sodium standard that range in concentration from 0.2 to 3.0 mg/mL, and chromatograph each concentration 6 times.

(i) *Solutions for recovery study.*—Transfer sample composites of commercial preparations of ca 50 mg potency, accurately weighed, to a 50 mL volumetric flask. Add 10, 15, or 20 mg dicloxacillin standard and 0.5 mL internal standard stock solution, respectively. Dilute to volume with water and mix. Chromatograph each solution 3 times.

### Apparatus

(a) *Liquid chromatograph.*—Model 600E consisting of 2 solvent pumps, a Model 490E variable wavelength detector, and a Model 745 data module. Introduce samples through a Model U6K injection valve (Waters Chromatography Div., Milford, MA 01757). Operating conditions: mobile phase flow rate, 1.5 mL/min; detector wavelength, 254 nm; attenuation,

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**Table 1. Recovery of dicloxacillin from commercial capsule composites**

| Added, mg | Found, mg | Rec., % | Av. rec., % |
|---|---|---|---|
| Capsule, 250 mg | | | |
| 10.0 | 9.940 | 99.40 | — |
| 15.0 | 14.798 | 98.65 | 99.60 |
| 20.0 | 20.148 | 100.74 | — |
| Capsule, 125 mg | | | |
| 10.0 | 10.008 | 100.08 | — |
| 15.0 | 14.928 | 99.52 | 99.79 |
| 20.0 | 19.956 | 99.78 | — |

128; 1.0 AUFS; recorder output, 1 V maximum; chart speed, 0.5 cm/min; temperature, ambient. Inject 10 µL each of standard and sample preparations into LC system.

(b) *Chromatographic column.*—Stainless steel, 300 × 3.9 mm id, packed with µBondapak C18, 5 µm particle size (Waters).

### *Microbiological Assay*

Use *Bacillus subtilis* (Culture Collection and Research Center, Taiwan) in the microbiological assay. The medium was purchased from Difco Co. According to the cup plate method (5), prepare the standards and test drugs as 1.0 mg/mL (potency) concentrated solutions with distilled water. Dilute solutions to 10.0 and 2.5 µg/mL with 1% phosphate buffer (pH 6.0) on the day of analysis. Use 5 Petri dishes, 9.0 cm id, for each sample. After incubation for 16–18 h, measure the zone diameter with a zone analyzer (Model ZA-F; Toyo Co., Tokyo, Japan).

**Table 2. Comparison of percent potency of dicloxacillin in capsule formulations as determined by microbiological and LC methods**

| % of label claim | | | |
|---|---|---|---|
| Capsule, 250 mg | | Capsule, 125 mg | |
| Microbiological method[a] | LC method[b] | Microbiological method[a] | LC method[b] |
| 104.3 | 104.1 | 121.0 | 121.7 |
| 101.9 | 102.8 | 112.2 | 110.7 |
| 101.6 | 100.5 | 110.2 | 110.8 |
| 103.9 | 105.0 | 117.2 | 118.7 |
| 103.5 | 101.4 | 95.4 | 94.3 |
| 105.1 | 106.2 | 95.1 | 95.2 |
| 105.0 | 105.9 | 87.5 | 87.3 |
| 99.8 | 102.0 | 86.2 | 84.4 |
| 98.2 | 99.1 | 95.3 | 92.4 |
| 71.4 | 69.1 | 78.8 | 79.3 |
| 63.1 | 61.0 | 77.9 | 78.2 |
| 86.2 | 84.8 | 74.0 | 72.5 |
| 70.2 | 68.7 | 70.6 | 67.8 |
| 83.5 | 83.4 | 55.7 | 56.7 |
| 73.5 | 75.0 | 29.6 | 28.3 |
| 51.5 | 51.2 | 22.7 | 20.9 |
| 48.5 | 46.1 | — | — |
| 43.6 | 44.6 | — | — |

[a] Av. of 5 determinations.
[b] Av. of 3 determinations.

**Table 3. Microbiological and LC assays of dicloxacillin bulk drug substance**

| Sample | | Potency, µg/mg | |
|---|---|---|---|
| | | Microbiological[a] | LC[b] |
| House standard | | 923.6 | 923.6 |
| USP standard | | 972.6 | 971.3 |
| Brand A | 1 | 917.2 | 920.7 |
| | 2 | 941.4 | 940.7 |
| | 3 | 873.0 | 884.2 |
| | 4 | 828.0 | 805.2 |
| Brand D | 1 | 938.0 | 934.2 |
| | 2 | 935.0 | 926.0 |
| | 3 | 940.0 | 949.6 |

[a] Av. of 5 determinations.
[b] Av. of 3 determinations.

## Results and Discussion

The linearity of the peak-area ratio (dicloxacillin vs internal standard) was verified by injection of 7 solutions containing 2–30 µg dicloxacillin and 3 µg dimethylphthalate. A straight line with a correlation coefficient of 0.9999 ($y = 0.0235 + 0.1097x$), was obtained when the ratios of the area counts of the dicloxacillin divided by the area counts of the internal standard were plotted against concentration of dicloxacillin.

The reproducibility (CV) of the proposed method was 0.13%, based on peak-area ratios for 10 replicate injections. The standard deviation was 0.24%.

Results of studies of standard addition recovery of dicloxacillin from sample composites of commercial preparations are shown in Table 1. The average recovery was greater

**Table 4. Microbiological and LC assays of dicloxacillin capsules**

| Sample | | Potency, % | |
|---|---|---|---|
| | | Microbiological[a] | LC[b] |
| A | 1 | 106.0 | 105.9 |
| | 2 | 103.7 | 103.5 |
| B | 1 | 113.8 | 113.7 |
| | 2 | 95.2 | 96.1 |
| C | 1 | 108.5 | 111.7 |
| | 2 | 115.0 | 114.6 |
| D | 1 | 105.5 | 107.4 |
| | 2 | 98.7 | 101.7 |

[a] Av. of 5 determinations.
[b] Av. of 3 determinations.

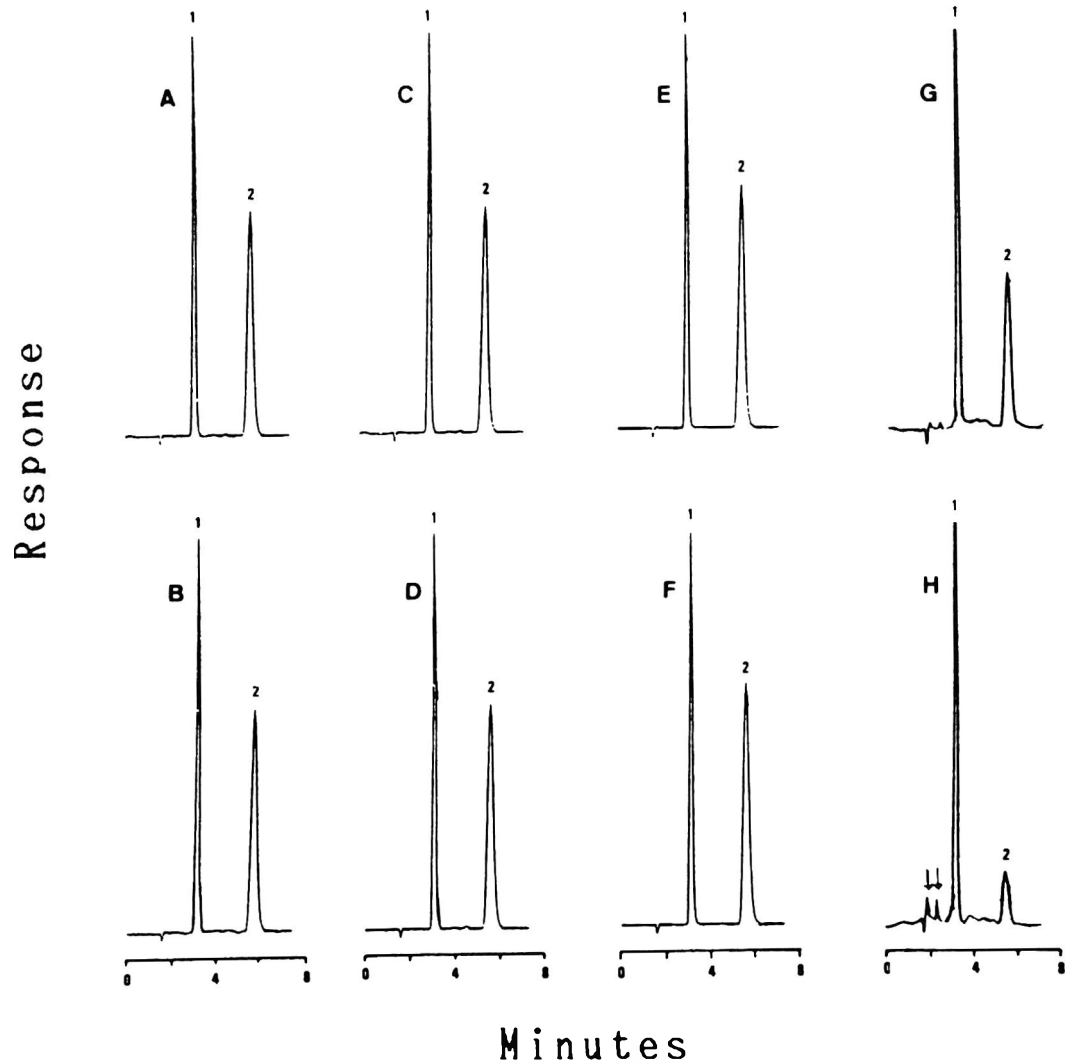


**Figure 1. Typical chromatograms of dicloxacillin preparations: (A) house standard; (B) bulk drug substance; (C) brand A capsule; (D) brand B capsule; (E) brand C capsule; (F) brand D capsule; (G) degradation of brand A capsule at 150°C for 1 day; and (H) degradation of brand A capsule at 150°C for 2 days. Peaks: 1, dimethylphthalate; 2, dicloxacillin. Arrows indicate degradates.**

than 99.60%. These data indicate that the proposed LC method is relatively unaffected by the sample matrix.

Typical chromatograms of the dicloxacillin commercial dosage forms are shown in Figure 1. The retention time was about 3.7 min for the internal standard and 6 min for dicloxacillin. Excipients from capsule formulations did not interfere.

A study was initiated to ascertain the suitability of the proposed method for stability studies. Samples were stored in temperature-controlled cabinets (ambient or 55–150°C). Samples were taken from the cabinets periodically for microbiological and LC assays. The assay values, expressed as a percentage of the label claim, are given in Table 2 for the 2 strengths of capsules dosage forms. The 34 paired values in Table 2 have a correlation coefficient of 0.999. This value indicates that no significant difference was found in the assay values obtained by the 2 analytical methods for degraded or nondegraded samples.

When dicloxacillin capsules were heat degraded, the resulting mixtures yielded chromatograms containing additional peaks, none of which interfered with the interpretation and measurement of the chromatographic peaks for dicloxacillin and dimethylphthalate, as shown in Figure 1. In addition, a decrease in peak height (and/or peak area) with an increase in temperature and time can be observed.

A number of samples of bulk drug substance and commercial formulations of 4 brands were analyzed for dicloxacillin content by LC. These samples were also assayed by the microbiological method. Results are shown in Tables 3 and 4. The Student's *t*-test was applied to the data: analysis showed no significant difference at the 99% confidence level for any of the preparations when assayed by the microbiological or LC meth-

**Table 5. Absorbances and concentrations of solutions for linearity response[a]**

| Sample | Absorbance[b] | Concentration, μg/mL |
|---|---|---|
| 1 | 0.006 | 2.0 |
| 2 | 0.015 | 5.0 |
| 3 | 0.026 | 10.0 |
| 4 | 0.040 | 15.0 |
| 5 | 0.054 | 20.0 |
| 6 | 0.066 | 25.0 |
| 7 | 0.083 | 30.0 |

[a] Correlation coefficient is 0.9986.
[b] Absorbance measured at 254 nm.

ods. Table 5 shows absorbances and concentrations of solutions for linearity response.

This study demonstrates the applicability of the proposed LC method for the potency determination of dicloxacillin in bulk drug and commercial capsules. The method can be successfully used for routine quality control and stability assays, and it offers advantages in speed, simplicity, and reliability.

## Acknowledgments

The authors thank Bristol Industries, Ltd, and China Biological & Chemical Laboratories for the supply of dicloxacillin sodium bulk drugs.

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DRUG RESIDUES IN HUMAN TISSUES

# Determination of Ticarcillin and Clavulanic Acid in Serum by Liquid Chromatography

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**A liquid chromatographic (LC) method using solid-phase extraction for the determination of clavulanic acid and ticarcillin in human serum has been developed. Clavulanic acid and ticarcillin were extracted separately from 1 mL serum using C18 solid-phase extraction cartridges. Eluates were analyzed by the same reversed-phase LC system. The overall mean recovery of clavulanic acid from serum was 99.7 ±2.3%; the lowest level validated in serum was 0.5 μg/mL. The overall mean recovery of ticarcillin from serum was 101.5 ±3%; the lowest level validated in serum was 12.5 μg/mL. Detection limits for clavulanic acid and ticarcillin were 0.117 and 0.749 μg/mL, respectively.**

Timentin is an intravenously administered, broad spectrum antibiotic composed of a combination of potassium clavulanate and disodium ticarcillin in 1:10 ratio. Clavulanic acid is an inhibitor of microbial β-lactamases, and ticarcillin is a β-lactamase labile penicillin (1). In combination, clavulanic acid protects ticarcillin from hydrolysis by β-lactamase (produced by penicillin-resistant bacteria). Previously, clavulanic acid and ticarcillin levels in serum were analyzed in this laboratory on separate liquid chromatographic (LC) systems by 2 different methods. The Infectious Diseases/Immunology Division of Arkansas Children's Hospital, Little Rock, AR, conducted some earlier pharmacokinetics studies on Timentin by the separate methods. Methodologies using separate LC systems for these analyses are common in the literature (2, 3). Favorable analytical conditions for clavulanic acid stability are detrimental to ticarcillin stability and vice versa. Because of the difficulty of obtaining a 2 mL serum sample (required for determination of both analytes by separate in-house assays), an alternative method was sought for analysis of both drugs in a smaller total sample volume.

We have developed an assay for clavulanic acid and ticarcillin that uses a single LC system, decreases the total sample volume required to 1 mL, and promotes the stability of both analytes. Each drug is paired with an internal standard to offset experimental inconsistencies (Figures 1 and 2). Degradation is insignificant if the analysis is completed within 12 h. Depending upon the detector and integrator used, simultaneous chromatographic analysis is possible; however, the sequence of the sample preparation permits the analysis of one component without jeopardizing the stability of the second component. The simultaneous solid-phase preparation of the eluates from the individual samples eliminates repetitive freeze/thaw of clinical samples.

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## METHODS

### *Reagents and Apparatus*

(a) *Solvents.*—Acetonitrile (ACN), methanol (MeOH), chloroform, and water, all LC grade.

(b) *Potassium phosphate buffer, 1%.*—pH 6. Dissolve 2 g potassium dibasic phosphate and 8 g potassium monobasic phosphate in 1 L water.

(c) *Imidazole solution.*—pH 6.8. Weigh 8.25 g imidazole (99+% fluorescence blank), and dissolve in 24 mL water. Add 2 mL 6N HCl, and adjust to pH 6.8 with 6N HCl. Dilute to 40 mL with water and mix well.

(d) Sodium monobasic phosphate buffers.—0.07M, pH 5 (9.6 g/L); and 0.01M, pH 4.

(e) Mobile phase.—Add 270 mL methanol to 730 mL 0.07M sodium monobasic phosphate buffer, filter, and degas under vacuum. Prepare every 2 days.

(f) *Clavulanic acid standard solutions.*—Weigh 50 mg equivalent of lithium clavulanate in 100 mL volumetric flask, dissolve in distilled water, and dilute to volume (stock solution). Dilute 5 mL stock solution to 100 mL with distilled water (Solution A). Dilute 20 mL Solution A to 100 mL with distilled water (Solution B). Prepare daily.

(g) *Ticarcillin standard solutions.*—Weigh 75 mg equivalent of disodium ticarcillin in 100 mL volumetric flask, dissolve in potassium phosphate buffer, and dilute to volume with buffer (Solution A). Dilute 10 mL Solution A to 30 mL with buffer (Solution B). Prepare daily.

(h) *Carbenicillin internal standard solution.*—Weigh 100 mg equivalent of carbenicillin in 50 mL volumetric flask and dilute to volume with potassium phosphate buffer (stock solution, 2 mg/mL; prepare daily). Dilute 5 mL of this stock to 25 mL with buffer (0.40 mg/mL; prepare daily).

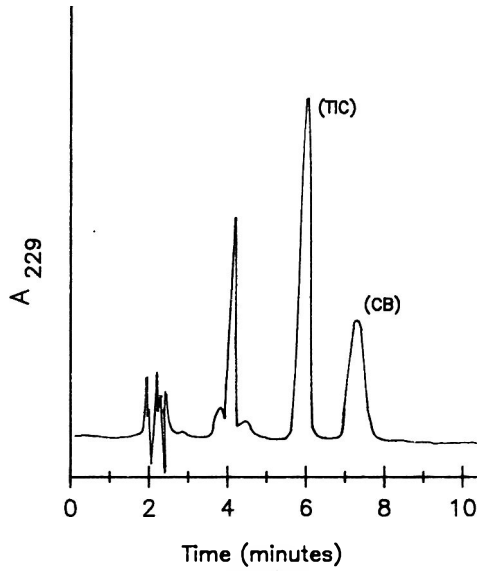


**Figure 1. Chromatogram of human serum spiked with ticarcillin (TIC) and carbenicillin (CB).**

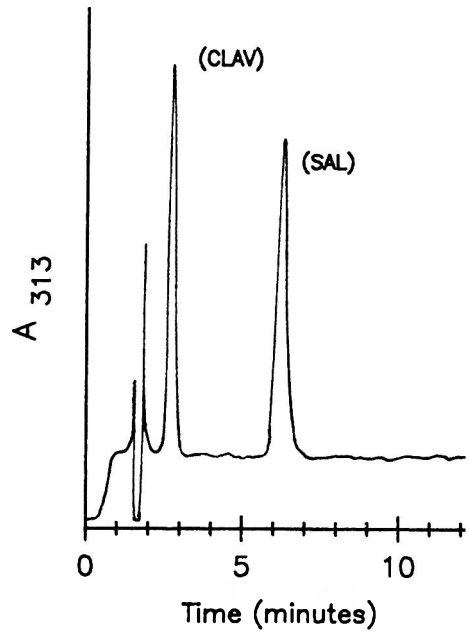


**Figure 2. Chromatogram of human serum spiked with clavulanic acid (CLAV) and salicylic acid (SAL).**

(i) *Salicylic acid internal standard solution.*—Weigh 20 mg salicylic acid in 100 mL volumetric flask and dilute to volume with potassium phosphate buffer.

(j) *LC system.*—Model 510 delivery system and Lambda-Max Model 481 UV detector (Waters Associates, Inc., Milford, MA 01757); μBondapak C18 analytical column (Waters), 10 μm, 30 cm × 4.5 mm, and C18 precolumn filter. Operating conditions: Flow rate, 1.5 mL/min; injection volume, 50 μL; clavulanic acid wavelength, 313 nm (attenuation, 16); and ticarcillin wavelength, 229 nm (attenuation, 128).

### *Sample Preparation and Extraction*

To 1 mL serum sample, add 3 mL potassium phosphate buffer and 0.25 mL carbenicillin internal standard solution, and mix (Figure 3, Steps 1 and 2). Transfer 2.0 mL of this spiked sample to test tube containing 0.5 mL imidazole solution, and let stand 1 h to develop the clavulanic acid–imidazole reaction product (Figure 3, Step 3).

*Ticarcillin extraction (Figure 3, Steps 4–9).*—Load remaining carbenicillin-spiked sample (i.e., without imidazole) onto C18 solid-phase extraction cartridge prepared for ticarcillin by sequential rinse with 3 mL ACN, 2 mL water, and 3 mL pH 5.0 sodium phosphate buffer. After sample is loaded, rinse cartridge with 3 mL pH 5.0 sodium phosphate buffer. Elute ticarcillin and internal standard with 3 mL ACN–pH 5.0 sodium phosphate buffer (6 + 4) into test tube containing 5 mL chloroform. Mix solution well and centrifuge 5 min at 2000 rpm. Transfer aqueous phase containing ticarcillin and internal standard to a sampling vial, and assay for ticarcillin in 50 μL injection volume. Monitor UV response at 229 nm.

*Clavulanic acid extraction (Figure 3, Steps 7, 10–13).*—After 1 h incubation of imidazole–clavulanic acid sample, add 0.1 mL salicylic acid internal standard and mix. Load sample onto C18 solid-phase extraction cartridge prepared for clavulanic acid by sequential rinse with 3 mL MeOH, 2 mL water, and 3 mL pH 5.0 sodium phosphate buffer. After sample is loaded, rinse with 2 mL 0.01M sodium phosphate buffer, pH 4. Elute imidazole–clavulanic acid reaction product and internal standard with 2.5 mL MeOH–0.07M, pH 7.0 sodium monobasic

**Table 1. Accuracy data for clavulanic acid and ticarcillin in human serum**

| | Added, μg/mL | Av. rec., μg/mL[a] | Av. rec., % | RSD, % |
|---|---|---|---|---|
| Clavulanic acid | 5.00 | 4.90 | 98.0 | 5.0 |
| | 2.48 | 2.48 | 99.2 | 2.7 |
| | 0.50 | 0.48 | 96.7 | 8.0 |
| Ticarcillin | 150 | 150.9 | 100.6 | 1.5 |
| | 75 | 75.1 | 100.1 | 2.5 |
| | 25 | 24.7 | 98.6 | 2.8 |

[a] n = 6.

**Table 2. Precision data for clavulanic acid and ticarcillin in human serum**

| | Added, μg/mL | Av. rec., μg/mL[a] | RSD, % |
|---|---|---|---|
| Clavulanic acid | 5.0 | 5.08 | 3.9 |
| | 3.5 | 3.48 | 2.6 |
| | 2.5 | 2.44 | 4.5 |
| | 1.0 | 1.02 | 3.9 |
| | 0.5 | 0.52 | 3.3 |
| Ticarcillin | 150.0 | 149.1 | 2.7 |
| | 75.0 | 74.8 | 2.7 |
| | 50.0 | 52.0 | 2.6 |
| | 25.0 | 24.5 | 2.6 |
| | 12.5 | 12.9 | 5.3 |

[a] n = 6.

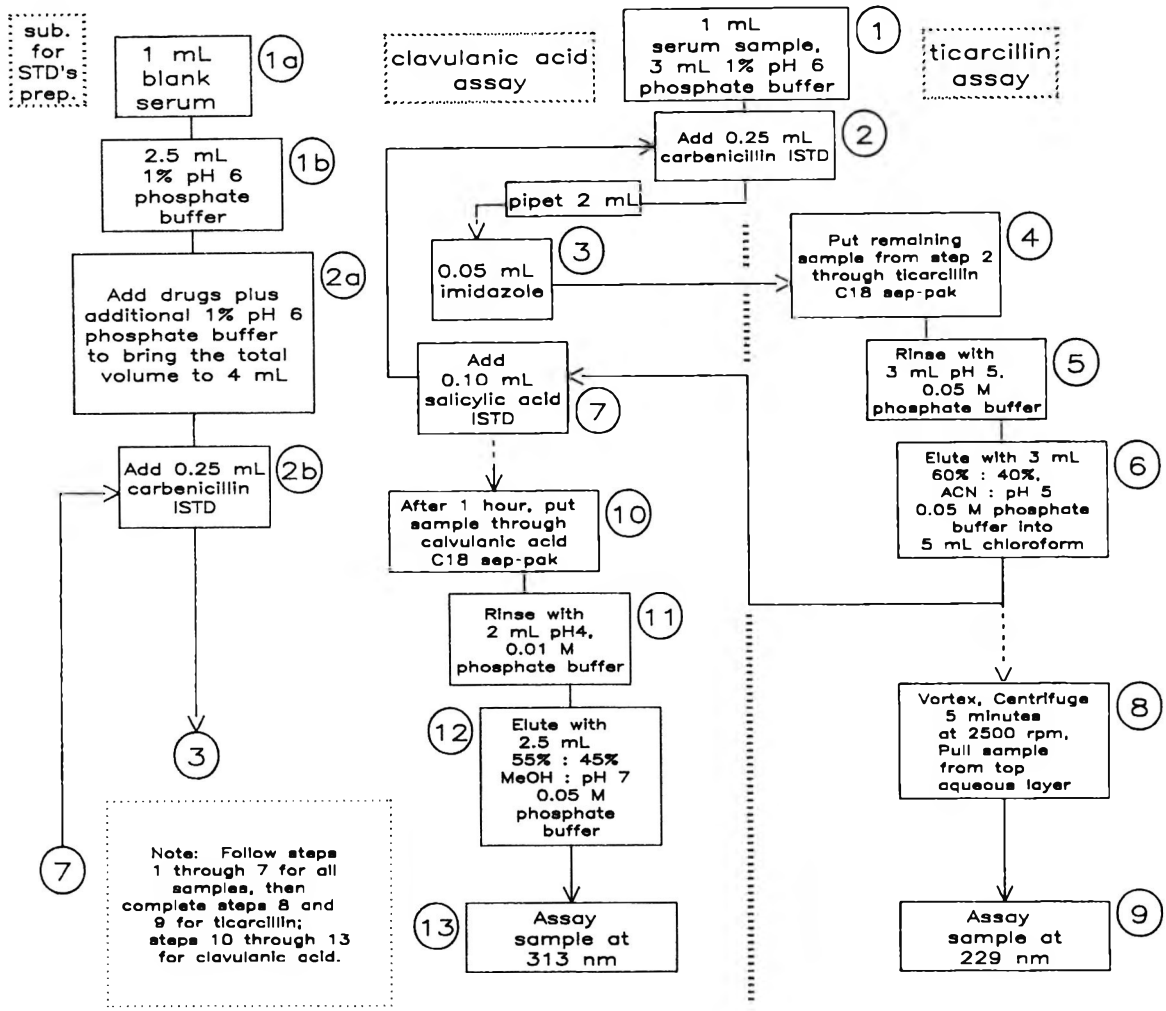


**Figure 3. Flow chart for sample preparation and extraction. The subsection, to the left of the main body (labeled 1a, 1b, 2a, 2b), is substituted for Steps 1 and 2 in the main body when preparing standards. Arrows follow the actual experimental path of the sample preparation. The dotted line down the center designates the division of the 2 determinations, which are executed simultaneously. Step 1 is completed for all samples initially. Steps 2–7 are completed for each sample individually. At this point, clavulanic acid samples are in imidazole with salicylic acid developing color for 1+ h. Ticarcillin samples are in chloroform ready to be centrifuged. Steps 8 and 9 complete the ticarcillin assay. Steps 10–13 complete the clavulanic acid assay. The nature of the LC equipment determines the time lapse between Steps 9 and 10. If the detector is a fixed wavelength, it is advantageous to delay Step 10 until all ticarcillin samples have been assayed.**

phosphate buffer (55 + 45). Assay for clavulanic acid–imidazole complex in 50 µL injection volume. Monitor UV response at 313 nm. Calculate peak data, using peak areas:

$$\text{Analyte } (\mu g/mL) = (y - a)/b$$

where $y$ = peak area ratios (STD/ISTD), $a$ = $y$-intercept of appropriate standard curve, and $b$ = slope of appropriate standard curve.

## Results and Discussion

This LC method has been developed for determination of clavulanic acid and ticarcillin in serum. Average retention times are 6.2 ±0.07 min for ticarcillin and 7.6 ±0.15 min for carbenicillin (Figure 1). Average retention times are 3.0 ±0.05 min for clavulanic acid, and 5.8 ±0.06 min for salicylic acid (Figure 2). Common antibiotics offering possible chromatographic interference were tested on the LC system and found to be noninterfering (Figure 4).

A typical standard curve of clavulanic acid concentrations from 0.25 to 5.0 µg/mL provided a correlation coefficient of 0.99968, and ticarcillin concentrations from 12.5 to 150 µg/mL provided a correlation coefficient of 0.99953, indicating significant linear correlation between concentration and area ratios for both analytes.

Accuracy data were generated at 3 concentrations for both analytes (Table 1). The mean percent recovery was 98

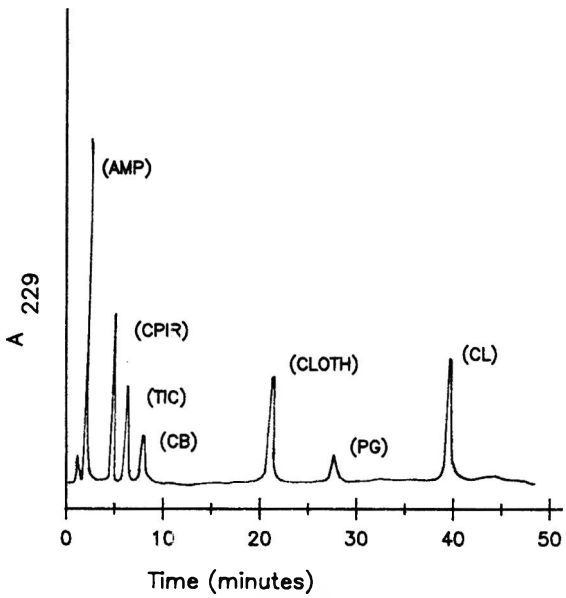


**Figure 4. Isocratic separation of ampicillin (AMP), cephapirin (CPIR), ticarcillin (TIC), and carbenicillin (CB) completed in 10 min. Gradient solvent program is incorporated after 10 min to reduce separation time for cephalothin (CLOTH), Pen G (PG), and cloxacillin (CL).**

±5% for clavulanic acid, and 100 ±2.3% for ticarcillin. Precision data were generated at 5 concentrations for each analyte (Table 2). The mean relative standard deviations (RSD) were 3.6 ±0.1% for clavulanic acid, and 3.2 ±1.2% for ticarcillin. The low RSD values for all data sets demonstrate an excellent fit of calibration points to the linear regression line.

The means of replicate clavulanic acid and ticarcillin determinations in serum were plotted vs their respective standard deviations. The detection limit for the method was assigned to be 2 × SD at zero concentration ($y$-intercept). This approach estimated the minimum measurable concentration that could be distinguished from a blank with 95% confidence. The detection limit for clavulanic acid and ticarcillin in serum were calculated (2 × SD of the system response at zero analyte concentration) to be 0.014 and 0.791 μg/mL, respectively. A statistically defined limit of quantitation (LOQ) for each standard curve (at 95% confidence) was estimated as 2 × SD (in concentration units) of the overall regression line as follows:

$$SD = \frac{\sqrt{d_i^2/(n-2)}}{b}$$

where $d_i^2$ = sum of squares of response ($y$) from the regression line, and $b$ = slope.

If $y$-intercept ≥ 0 then:

$$LOQ = 2 \times SD \text{ (in concentration units)}$$

If $y$-intercept < 0 then:

$$LOQ = (-a/b) + 2 \times SD \text{ (in concentration units)}$$

Typical LOQs for the 2 analytes were 1.9 and 4.8 μg for clavulanic acid and ticarcillin, respectively.

The data support the validity and reliability of this analytical method. In routine practice, the participation of other analysts has confirmed the method's reproducibility. This assay has greatly improved analytical efficiency in our laboratory, first, by permitting analysis of both ticarcillin and clavulanic acid in a much smaller total sample volume; and second, in contrast to the time and instrumental requirements of independent ticarcillin and clavulanic acid analyses, by making it possible to determine both analytes essentially simultaneously and with the same LC system (with appropriate UV wavelength changes).

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DRUGS

# CI–906 and CI–925 Cyclization in Rodent Chow Using Liquid Chromatography for Detection and Assay

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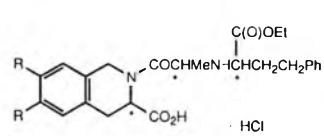
**Two ACE inhibitors, CI–906 and CI–925, cyclize rapidly to the corresponding diketopiperazines PD 109 488 and PD 114 009, respectively, in admixture with 5002 rodent chow. After 2 weeks storage at ambient temperature, 90% of the admixed CI–925 and 68% of the admixed CI–906 cyclized. Liquid chromatography (LC) was used to quantitate both parent drugs and cyclized products. Absence of fat and fish meal from the chow affects the reaction. With CI–925 as the test compound, 90% cyclized after only 3 days in fat-free chow, and 80% cyclized after 3 days in chow free of both fat and fish meal. Chow pre-extracted with methanol has less effect than does whole chow. After 2 weeks, 87% CI–925 remained. Cyclization of CI–925 in methanol solution is also enhanced by the presence of chow extractables. After 2 weeks, 21–25% PD 114 009 was produced in fat-free diet extract, fat- and fish meal-free diet extract, and whole 5002 diet extract, compared to 4–5% produced in methanol alone. We concluded that 5002 chow contains one or more extractable components that promote cyclization of CI–906 and CI–925.**

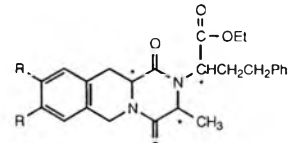
Before newly discovered drugs can be administered to humans as efficacious pharmaceutical agents, short- and long-term toxicity studies must be performed. Such studies often involve the use of mice and rats as test subjects, and admixture of drug with rodent chow is the most economical and least traumatic means of dosing these animals. One of the basic requirements of such studies is that the compound to be tested must remain stable in the diet to which it has been added. Assays to quantitate the drug in diet admixture have been widely reported in the scientific literature, both before and after publication of recommended U.S. Food and Drug Administration guidelines in 1973 (1). Most of the studies confirmed stability of the compound in the dosing medium. For example, Hucker and Stauffer determined that halofenate, cyclobenzaprine, and sulindac were stable for at least a week in rodent diet, using a number of methods for drug detection (2), and Schieffer developed a stability-indicating assay for diacetolol in feed (3). Nonstability of compounds when mixed into diets has also been reported, including experiments with a number of therapeutic agents, antibiotics, coccidiostats, and growth stimulants incorporated into chicken mash and pelleted (4, 5); 3 lactobacillus products in both nonmedicated feed and in feed medicated with lincomycin (6); vitamin C, when processed in compressed diets (7, 8); and clofibrate and tigric acid in short- and long-term storage of drug/diet admixtures (9).

Liquid chromatography (LC), a powerful tool in determining compound purity, has rapidly become popular in quantitating a drug incorporated into a variety of matrixes, including animal chows. LC systems can be readily modified to separate and identify components in a mixture, and adaptation to determination of compound stability follows naturally. Diet extractables, which are removed from admixtures together with the fortifying drug by a solvent such as methanol, are easily separated from the compound of interest by chromatography, making extract cleanup unnecessary. Time is saved by direct injection of a diet extract, and, more important, the possible loss of a stability by-product during cleanup is minimized.

CI–906 (1a), {3S-{2{R*(R*)}3R*}-2-{2-{{1-(ethoxy carbonyl)-3-phenylpropyl}amino}-1-oxypropyl}-1,2,3,4-tetra hydro-3-isoquinoline carboxylic acid hydrochloride (10, 11), and CI–925 (1b), its dimethoxy analog (12), are angiotensin-converting enzyme inhibitors.




1a R = -H
1b R = -$OCH_3$




2a R = -H
2b R = -$OCH_3$

* SSS configuration

Soon after CI–906 and CI–925 were found to be active in the treatment of hypertension and congestive heart failure, toxicity studies were projected, and preliminary drug-in-diet stability information was required. LC, using UV spectrophotometry for detection and methanol as extracting solvent, was selected as the means of separating each of the 2 compounds from diet extractables. In the early stages of assay development, a quite remarkable lack of stability was observed for both CI–906 and CI–925 in diet admixture, with the formation of

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**Table 1. Comparison of cyclization of CI–906 and CI–925 in chow admixtures**

| Day of assay | Rec., mg drug/g diet (RSD) | | | |
|---|---|---|---|---|
| | CI–906 | PD 109 488 | CI–925 | PD 114 009 |
| 0 | 0.970 (3.7) | — | 0.952 (1.0) | — |
| 1 | 0.708 (7.9) | — | 0.538 (2.0) | — |
| 2 | 0.612 (3.9) | 0.388 (1.5) | 0.400 (0.0) | 0.632 (1.0) |
| 3 | — | — | 0.322 (1.4) | 0.718 (0.8) |
| 6 | — | — | 0.196 (2.8) | 0.816 (1.3) |
| 7 | 0.478 (4.5) | 0.477 (1.0) | — | — |
| 14 | 0.322 (7.4) | 0.546 (0.9) | 0.098 (4.6) | 0.934 (1.7) |

cyclized analogs as the result of drug/chow interaction. The products are identified as PD 109 488, 2a for CI–906; and PD 114 009, 2b for CI–925, each representing a diketopiperazine (DKP) analogue.

The purpose of this work was to compare the cyclization rates of CI–906 and CI–925 in diet admixtures, using LC to quantitate, and, if possible, to determine whether a major component of chow contributes to the rearrangement. Stability of the compounds in the methanol used for extraction was considered necessary to the experiments, and is also reported.

## Experimental

### *Apparatus*

(a) *Liquid chromatograph.*—Model 6000A solvent delivery system, Model 450 variable wavelength detector (Waters Chromatography Div., Millipore Corp., Milford, MA 01757). Model 725 autoinjector with 50 μL sample loop (Micromeritics Instrument Corp., Norcross, GA 30093). Omniscribe recorder at 0.25 cm/min chart speed (Houston Instrument, Austin, TX 78753).

(b) *Analytical column.*—Alltech C8 (10 μm, 25 cm × 4.6 mm id) preceded by stainless steel precolumn (10 cm × 4.6 mm id) packed with 30–38 CO:PELL (Whatman Inc., Clifton, NJ 07014).

(c) *Mixer.*—Rotating wheel, constructed in-house, tumbling action, 16 rpm.

(d) *Centrifuge.*—International portable refrigerated centrifuge, set for 10 min at 1500 rpm.

### *Reagents*

(a) *Solvents.*—LC grade acetonitrile and water; spectrophotometric grade methanol.

(b) *Ion sources.*—Reagent grade $(NH_4)H_2PO_4$, 85% syrupy $H_3PO_4$.

(c) *Mobile phase.*—Acetonitrile–0.05M $(NH_4)H_2PO_4$ adjusted to pH 3 with $H_3PO_4$ (3 + 7), degassed for 5 min before use.

(d) *CI–906; CI–925; PD 109 488; PD 114 009.*—Obtained in-house as analytically pure compounds (12).

(e) *Standard solutions.*—Dissolve parent drug or cyclized product in methanol to give a concentration of 20 mg drug/mL. Dilute each solution with methanol to prepare working solutions at 1.6, 1.8, 2.0, 2.2, and 2.4 mg drug/mL.

(f) *Rodent chows.*—Certified 5002 rodent chow, rodent chow without soya oil, and rodent chow without soya oil or protein (fish meal) were supplied by Ralston Purina Co., Richmond, IN; 5002 rodent chow pre-extracted with methanol was prepared in-house by Soxhlet extraction with methanol for 3 × 8 h, followed by chloroform wash and air drying 48 h.

(g) *Soya oil.*—Supplied by Ralston Purina Co.

(h) *Drug/diet admixtures.*—Prepared in-house, using procedures described in Standard Operating Procedures for the Department of Pathology and Experimental Toxicology, Warner-Lambert/Parke-Davis Ann Arbor Laboratories. All admixtures were prepared at 1.0 mg drug/g diet, in 500 g lots, stored in open containers, and stirred daily with a spatula. Ambient temperature for diet storage was 24.2–24.7°C; temperature was taken daily with Model 501 pH meter temperature probe (Orion Research Inc., Cambridge, MA 02142).

(i) *Methanol diet extracts.*—The 4 chows described in (f) were extracted with methanol (4.0 g/40.0 mL, 1 h shaking) followed by centrifuging and transfer of the extract to a clean container.

### *Sample Preparation*

*Drug/diet admixture.*— (*1*) Standards. Weigh 2.0 g 5002 chow into each of five 50 mL round-bottom screw-capped centrifuge tubes. Add 20.0 mL absolute methanol to each tube. Add 1.0 mL of the appropriate standard working solution to each tube to make a series of standards with a final extract concentration of 0.08–0.12 mg drug/mL solution (0.8–1.2 mg drug/g diet). Cap tubes, extract on rotating wheel for 1 h, and centrifuge 10 min. Remove portion of each extract for injection into the LC system. For CI–906 and PD 109 488, detect at 220 nm with a mobile phase flow rate of 2.0 mL/min. For CI–925 and PD 114 009, detect at 284 nm with a mobile phase flow rate of 1.2 mL/min. Prepare fresh standards at each assay interval. (*2*) Stored diets. Weigh 2.0 g diet admixture into each of 5 centrifuge tubes (5 replicate assays). Add 21.0 mL absolute methanol to each tube. Continue as in *Standards*, starting with "Cap tubes...."

*Methanol solutions.*—(*1*) Standards. Accurately weigh CI–906 or CI–925 to a series of five 10 mL volumetric flasks to make working standards with concentrations of 0.8–1.2 mg

drug/mL methanol. Inject an aliquot of each standard into the LC system. Prepare fresh standards at each assay interval. (2) Stored solutions. Weigh 25.0 mg CI–925 into each of a series of 25 mL volumetric flasks. Fill flask to volume with methanol or with an appropriate chow extract (see (i)), and shake well to dissolve. Prepare a similar solution of CI–925 in methanol containing 0.1% soya oil. Remove an aliquot of each solution for initial assay, and for subsequent assays periodically over 2 weeks.

### *Calculations*

*Diet admixtures.*—Plot chromatographic response as the height in millimeters of each standard solution vs the corresponding concentration of drug in mg/mL for each of the compounds to be quantitated. From the resulting linear plot, determine the concentration of drug in the extract ($c$) from the stored diet admixtures as follows:

$$(c = Y - A)/B$$

where $Y$ is the peak height in millimeters of drug or by-product response, $A$ is the $y$-intercept of the standard curve, and $B$ is the slope of the standard curve.

*Solutions.*—Plot chromatographic response as the height in millimeters of each standard solution vs corresponding concentration of CI–925 in mg/mL. From the resulting linear plot, determine the concentration of CI–925 in each stored solution, using the formula given for *Calculations* under *Diet admixtures*. To determine percentage of parent drug remaining and percentage of by-product present, add peak heights of CI–925 and PD 114 009 and calculate each by dividing individual peak heights by total height.

## Results

Cyclization of CI–906 was observed when the solubility and stability of the hydrochloride salt in water at ambient temperature were determined. A solution of the salt was prepared at 100 mg/mL water, with a resulting pH ≈2. After several days, a white precipitate appeared that, when isolated, was identified as the free base of the DKP analogue PD 109488. Similar results were found with CI–925; the product was the DKP PD 114 009. It came as no surprise that both CI–906 and CI–925 cyclized in admixture with rodent chow.

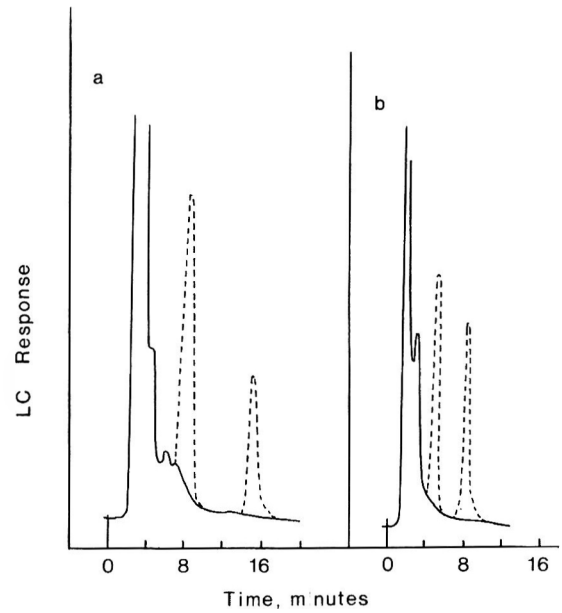


**Figure 1. Chromatograms of: a. Methanol extract of blank chow, 1 g/10 mL, 220 nm; – – – methanol extract of 3-day-old chow admixture with CI–906/PD 109 488, at 220 nm; CI–906 at ca 7 min, PD 109 488 at ca 15 min. b. Methanol extract of blank 5002 chow, 1 g/10 mL, 284 nm; – – – methanol extract of 1-day-old chow admixture with CI–925/PD 114 009 at 284 nm; CI–925 at ca 6 min, PD 114 009 at ca 9.5 min.**

Standard in-house procedure for determining drug stability in diet admixture is room-temperature storage in an open container for a minimum of 2 weeks. Results of 2-week storage of CI–906 and CI–925 diet admixtures are given in Table 1. The chromatograms of CI–906, CI–925, and their respective cyclized products are shown in Figure 1. Concentrations of each of the parent drugs were determined initially and after 1 day. At subsequent intervals, concentrations of both parent compound and cyclized product were determined individually from the appropriate standard curve. Results given in Table 1 for day 0 are averages of 6 replicate samples taken at random from the mix spread on a large sheet of glassine paper. At subsequent intervals, 4 replicate samples were taken from the mix in the storage container. Relative standard deviation (RSD) is given in parentheses after each average. Plots of peak height response

**Table 2. Comparison of CI–925 recovery from 5002 chow and from modified 5002 chow**

| | Rec., mg drug/g diet (RSD) | | | |
|---|---|---|---|---|
| Day of assay | 5002 | Pre-extract 5002 | 5002, no soya oil | 5002, no soya oil, no fish meal |
| 0 | 0.952 (1.0) | 1.07 (2.0) | 0.86 (1.6) | 0.93 (1.6) |
| 1 | 0.538 (2.0) | 0.97 (1.5) | 0.18 (4.5) | 0.33 (4.0) |
| 3 | 0.322 (1.4) | 0.99 (2.1) | 0.10 (29.0) | 0.19 (2.7) |
| 6 | 0.196 (2.8) | — | — | — |
| 7 | — | 0.93 (1.4) | 0.01 (—) | — |
| 14 | 0.098 (4.6) | 0.87 (2.0) | — | 0.11 (10.5) |

**Table 3. Comparison of CI–925 stability in methanol solutions**

| | CI–925 found, mg drug/mL solution | | | | | |
|---|---|---|---|---|---|---|
| Day of assay | MeOH | MeOH[a] | 5002 extract | 5002 extract[b] | Chow extract[c] | Chow extract[d] |
| 0 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| 2 | 0.99 | 0.99 | 0.97 | 0.98 | 0.98 | 0.98 |
| 4 | 0.98 | 0.99 | 0.93 | 0.96 | 0.94 | 0.94 |
| 8 | 0.98 | 0.97 | 0.85 | 0.91 | 0.88 | 0.88 |
| 14 | 0.96 | 0.95 | 0.75 | 0.85 | 0.78 | 0.79 |

[a] 0.1% soya oil added.
[b] Second extract of pre-extracted chow.
[c] Extract of chow without soya oil.
[d] Extract of chow without soya oil or fish meal.

vs concentration were linear for the 5 standards of each compound. RSDs, which were obtained by dividing height by concentration for each standard and averaging the 5 results, were in the 0.5–2.0% range for all compounds. Adding the concentrations of parent drug remaining to the concentrations of the cyclized product formed gives approximately the intended total concentration of 1 mg drug/g diet. CI–925 is by far the less stable of the 2 compounds, with only 10% remaining after 2 weeks, compared to about 32% remaining for CI–906.

The next step in the experiments was to determine if a major component of the chow could affect the rate of cyclization. Ralston Purina Certified 5002 rodent diet is a constant nutrient formulation containing natural grains and nutrient supplements. Among the supplements are fat (soya oil), protein (fish meal), some amino acids, and vitamins and minerals. A complete analysis is given in the Lab Chow Specifications, obtainable from the supplier. CI–925 was selected for these experiments. Its UV detection maximum at 284 nm is more intense than that of CI–906, and chow extract components have less interference at the longer wavelength. Although checking the effect of each component in the complex chow mixture would be implausible, it seemed feasible to test at least the 2 major supplements, soya oil and fish meal, which are added to the chow formulation last.

Four diet admixtures were prepared for 2-week storage and assay, using CI–925 at 1 mg drug/g diet for the mixes: (*1*) Certified 5002 chow, as a control; (*2*) chow pre-extracted with methanol; (*3*) chow without soya oil; and (*4*) chow without soya oil or fish meal. Table 2 compares the concentrations of CI–925 remaining in each of the 4 admixtures at intervals over 2-week storage. The pre-extracted chow has the least effect in promoting CI–925 cyclization, suggesting the presence of an extractable component that influences the reaction.

Because methanol may be involved in CI–925 cyclization, solutions of the compound in methanol were prepared for 2-week observation of cyclization rates. Solutions were prepared in: (*1*) methanol; (*2*) methanol containing 0.1% soya oil; (*3*) methanol containing extracts of 5002 chow; (*4*) methanol containing second extract of 5002 chow (pre-extracted 5002 chow); (*5*) methanol extract of 5002 chow containing no soya oil; and (*6*) methanol extract of 5002 chow containing no soya oil or fish meal.

Results of the stability study of CI–925 in these solutions are given in Table 3. Chromatograms of the solutions containing diet extract were compared to chromatograms of drug in methanol alone for the presence of an unexpected component response, but none was found. Methanol extractables from 5002 chow have essentially the same cyclization rate effects independent of the presence of soya oil or fish meal. Second extracts from pre-extracted chow have a lesser effect, although perceptibly greater than in methanol alone or in methanol with 0.1% soya oil added.

## Discussion

Gu and Strickley (13) studied pH effects on reaction rate constants for cyclization of CI–925 to its corresponding DKP in aqueous solution. They noted a high propensity for DKP formation in acidic media (pH 1–3), with a decrease in rate as pH was increased. Their reaction rate constants give a half-life for CI–925 on the order of at least 12 days. The results of our work indicate a half-life for this compound of fewer than 24 h, but in a significantly different medium, i.e., rodent chow.

Potentially, any of the chow components may interact with an admixed or fortifying compound (14, 15), and examples are provided in the literature (see the introduction). Effects of chow on the natural tendency of CI–906, CI–925, and related compounds to cyclize must be considered. In this semidry medium, much longer diffusion times and more restrictions on molecular motion would be expected. At least 2 conditions are necessary to DKP formation in the diet. First, the diet has to present an environment in which the amine function is neutralized of any protonation, thus allowing it to interact with the carboxyl group. The diet matrix contains an abundance of protein, which facilitates neutralization. On several occasions in our experience with drug/diet admixtures, the salt form of an investigational drug has been recovered as the free base form. Second, binding sites must be available on the chow that accommodate the amine and carboxyl functions in a conformation favorable to DKP formation. Manipulation of Dreiding models of the CI–906 and CI–925 molecules reveals such a conformation, although there is no reason to assign a higher probability to this conformation over other possibilities without knowing more about the corresponding diet binding sites. Absence of the soya

oil component of the diet enhances DKP formation, suggesting better access to the diet binding sites.

The amino acid side chain is the same for both CI–906 and CI–925, and both are configured *S,S,S* in the optical centers involving cyclization. The only difference between the 2 compounds is the substitution of 2 methoxy groups for 2 hydrogens on the isoquinoline ring. The added methoxy groups must improve the binding of the CI–925 molecule to the diet, enhancing the DKP formation rate of CI–925 relative to that of CI–906.

We were curious as to whether optical configuration in CI–925 would affect cyclization. An admixture of the *R,S,S* isomer, in which the optical center adjacent to the nitrogen of the isoquinoline ring is configured *R*, was made with rodent chow, stored for 2 weeks, and assayed. At the end of the 2 weeks, 60% of the *R,S,S* isomer was recovered, compared to 10% recovery for the *S,S,S* isomer. The change in configuration of the isoquinoline steric center substantially reduced the DKP formation rate. No further work was done with the *R,S,S* isomer.

CI–925 DKP formation was also enhanced in methanol extracts of various diets over that in methanol alone. This suggests that other (extractable) factors in chow might be considered when dealing with the stability of these compounds in diet.

## Conclusions

Certified 5002 Rodent Chow accelerates the cyclization of CI–906, CI–925, and related compounds to the corresponding DKP. Neither soya oil nor fish meal is the sole operant component in the diet, and no further experiments with individual components of the complex diet were done. One or more components of chow that increase DKP formation can be extracted with methanol.

## Acknowledgments

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METHOD QUALITY CONTROL

# Specification-Based Modified Control Limits in Quality Control of Trace Chemical Analyses

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**Shewhart $\bar{X}$ and $R$ charts were used to maintain and validate data quality of percent recovery estimates for 8 analytes determined by 4 procedures used routinely in 4 commercial laboratories over a 2-year period. However, because range ($R$) estimates of uncertainty did not include lot-to-lot calibration variability, approximately 24% of the lots were "out-of-control." We extracted pooled standard deviations for $S_O$ (repeatability within lot), $S_L$ (calibration variability), and $S_R$ (reproducibility), which represents the total variability. Values of $S_O$ and $S_L$ were generally similar in size although there were some substantial differences between analytes and between laboratories for a given analyte. When control limits were based on reproducibility rather than repeatability, only about 6% of the lots were "out-of-control." However, these limits are less convenient to compute at the bench, within-lot precision estimates are still required, and there is still no information on data acceptability. Capability estimates from the grand mean ±3 $S_R$ were surprisingly consistent for the 8 analytes. These values coupled with data quality objectives suggested the 82–115% range as the specifications for acceptable individual recoveries. A combination of repeatability limits plus modified limits anchored to specifications retains the simplicity of range computations while offering substantial administrative advantages. Examples are given to illustrate these points.**

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Analytical chemical measurements frequently form the basis for decision-making; therefore, their accuracy and precision must be known to be adequate for the intended use. Adequacy is usually specified by data quality objectives (DQOs) given in each project plan. Although it may seem desirable to reduce measurement uncertainty to a minimum, this approach is often unnecessarily wasteful of both time and money. For example, environmental studies may require analysis of very large numbers of samples to overcome sampling errors that are often much larger than laboratory analysis errors.

For uncertainty estimates to be valid over an extended time frame, the measurement process must remain in a state of statistical control. The information necessary for evaluation is normally acquired by including one or more quality control (QC) samples in each analysis lot. Various QC charting procedures are available to display the results of such analyses (1–6), but the well-known Shewhart $\bar{X}$ and $R$ charts (5, 6) are still the most widely favored. Charts should be maintained and examined in as close to real time as possible so that systematic trends and/or various out-of-control conditions may be detected promptly and appropriate corrective action taken before conducting more analyses.

A critical step in the effective application of QC charts is the choice of experimental protocol. In practice, ranges of many small sets of replicate results are often used to produce an average range ($\bar{R}$) to represent uncertainty in the measurement process. Ranges rather than standard deviations are still commonly used at the bench because very simple arithmetic is required. Because the distribution of sample means ($\bar{X}$) usually approaches normal, the probabilities associated with standard deviations are closely approximated when $R$ is used to set control limits. With an established chart, an analyst need only compute the mean and range for each new set of replicate QC results and plot the points for an instantaneous visualization of the state of control of the process.

However, many analytical procedures require the establishment of a new calibration curve each time a lot of samples is analyzed. Because instrument responses for all of the QC samples in a lot are normally translated into concentrations using a

**Table 1. Summary of analytical methods used for analysis of soils**

| Target analyte | Sample preparation | Determination | High spike concn, μg/g |
|---|---|---|---|
| Mercury | Acid digestion | Cold vapor AA[a] | 0.5 |
| Arsenic | Acid digestion | Furnace AA | 25 |
| Cadmium | Acid digestion | ICP[b] | 25 |
| Chromium | Acid digestion | ICP | 100 |
| Lead | Acid digestion | ICP | 250 |
| Copper | Acid digestion | ICP | 100 |
| Zinc | Acid digestion | ICP | 250 |
| Dibromochloropropane | Solvent extraction | GC/ECD[c] | 0.05 |

[a] Atomic absorption spectrometry.
[b] Inductively coupled argon plasma spectrometry.
[c] Electron capture gas chromatography.

daily calibration function, experimental uncertainties associated with lot-by-lot calibration variations are excluded from the range estimates. This leads to very tight control limits. When calibration variations are substantial, many QC lots will fall outside of control limits.

This paper describes the results of a long-term data quality study of 8 analytes determined by 4 analytical methods used routinely in 4 commercial laboratories over a period of 2 years. QC results from the analysis of over 100 sample lots were available for some of these methods (7). This large amount of data afforded an excellent opportunity to compare the performance (capability) of the 4 laboratories and to examine the effectiveness of $\bar{X}$ and $R$ charts for procedures based on calibration curves, where the calibration error was excluded from estimates used to set control limits. We suggest that $\bar{X}$ and $R$ charts supplemented by specification-based modified control limits offer a simple and effective means of coupling statistical control to DQOs.

## Experimental

*Analytical methods.*—The analytical methods consisted of 4 procedures to estimate the concentrations of 7 metals and dibromochloropropane in soil (Table 1).

*QC methods.*—Control of these methods was achieved in each laboratory via analysis of duplicate soil samples spiked at high concentration near the upper end of the calibration curve, a single low concentration spike, and an unspiked soil sample for each lot of samples analyzed (8). Spike recoveries were estimated from the differences between total and unspiked blank concentrations. For all analytes, the contribution from the blank was small and often below detection. This paper will consider results only from the duplicate high spikes. A bulk "standard soil" was obtained adjacent to the contaminated study area, carefully homogenized, and tested, and portions were supplied to each laboratory for use in all QC tests. A person other than the analyst spiked aliquots of this soil with known amounts of analytes, thereby permitting estimates of percent recovery.

The analyst could recognize which samples in a lot were intended for QC, but concentrations were unknown. The decision was made to use a standard soil for all laboratory QC to eliminate analytical differences caused by matrix effects; therefore, systematic differences in extraction efficiency from sample to sample were excluded from assessment of laboratory method control.

Correction for matrix effects is a separate issue that will not be considered here.

## Statistical Analysis

*Step 1.*—Individual percent recoveries from each laboratory and for each analyte were tested by the Dixon procedure for outliers (9) at a significance level of 5%. Before completion of 10 sets of duplicates, the test was based on the number of values available. After 10 acceptable sets of duplicates were collected, the last 20 values were used. The primary need for the Dixon test occurs before normal Shewhart $\bar{X}$ and $R$ control limits are established. If unreliable data are used to establish the original limits, this becomes a self-perpetuating system in which we continue to accept poor data. Less than 2% of the data were rejected by this test.

*Step 2.*—Omitting any outliers identified in *Step 1*, summary statistics (number of measurements ($n$), the laboratory mean ($\bar{X}$), the range ($R$), the variance ($S_x^2$), and the standard deviation ($S_x$)) were compiled for each analyte in each laboratory by standard computational procedures.

*Step 3.*—Before a grand mean recovery for all 4 laboratories and a pooled standard deviation were computed, the 4 laboratories were tested for significant differences in reproducibility by a range ratio test (10) at a significance level of 5%. In the 2

**Table 2. Summary of standard deviation estimates (%) for chromium**

| Standard deviations | Laboratory 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Repeatability ($S_O$) | 2.5 | 2.8 | 1.5 | 2.8 |
| Between lot ($S_L$) | 1.8 | 6.7 | 4.2 | 1.8 |
| Reproducibility ($S_R$)[a] | 3.1 | 7.2 | 4.4 | 3.3 |
| Ratio $S_R/S_O$ | 1.2 | 2.6 | 2.9 | 1.2 |

[a] Within-laboratory.

**Table 3. Average standard deviation estimates (%) for 8 analytes**

| Standard deviations | Laboratory 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Repeatability ($S_O$) | 3.36 | 2.81 | 2.58 | 2.76 |
| Between lot ($S_L$) | 4.06 | 4.48 | 4.83 | 3.58 |
| Reproducibility ($S_R$)[a] | 5.41 | 5.41 | 5.55 | 4.58 |
| Ratio $S_R/S_O$ | 1.57 | 2.13 | 2.33 | 1.68 |

[a] Within-laboratory.

**Table 4. Control limits on Shewhart charts: chromium analysis**

| | Laboratory | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| Laboratory mean ($\bar{\bar{X}}$) | 93.8 | 101.6 | 93.9 | 89.1 |
| Av. range for duplicates ($\bar{R}$) | 2.6 | 2.5 | 1.7 | 3.1 |
| Upper control limit on range ($UCL_R$) | 8.3 | 8.3 | 5.6 | 10.0 |
| Upper repeatability control limit on mean ($UCL_{\bar{X}}$)[a] | 98.6 | 106.3 | 97.2 | 94.8 |
| Lower repeatability control limit on mean ($LCL_{\bar{X}}$)[a] | 89.0 | 96.8 | 90.7 | 83.3 |
| Upper reproducibility control limit on mean ($URL_{\bar{X}}$) | 100.4 | 116.9 | 103.2 | 96.1 |
| Lower reproducibility control limit on mean ($LRL_{\bar{X}}$) | 87.2 | 86.3 | 84.6 | 82.1 |
| Upper modified control limit on mean ($UML_{\bar{X}}$) | 113.0 | 113.0 | 113.7 | 112.6 |
| Lower modified control limit on mean ($LML_{\bar{X}}$) | 84.0 | 84.0 | 83.3 | 84.4 |

cases in which variances were not homogeneous, the laboratory in question was excluded from the grand mean and pooled standard deviation calculations.

For each analyte, one variable of classification analysis of variance (ANOVA) was performed on the edited data. In a few cases the among-laboratory variability was significantly greater than the within-laboratory variability.

Although the procedures were conducted according to the same protocols in each laboratory, the inevitable differences associated with such complex analyses produce some systematic variations between laboratories. Technical judgment was involved in deciding how to use the information from these tests. For example, the chromium recoveries showed Laboratory 2 to have a much higher mean percent recovery than the other 3 laboratories. However, we were reluctant to exclude a laboratory with 101% mean recovery while retaining those with mean recoveries in the 90–94% range. Clearly, retention of Laboratory 2 in this example increases the grand mean recovery, but we believe that the final estimate is reasonable and should be generally within reach of other laboratories.

*Step 4.*—The grand mean for laboratories not excluded in *Step 3* was calculated for each analyte.

*Step 5.*—Because variability was surprisingly consistent among laboratories, a pooled standard deviation was calculated for each analyte.

*Step 6.*—Performance standards were estimated for each analyte from the grand mean ±3 times the pooled standard deviation for that analyte.

These performance standards represent the extremes within which we expect more than 99% of all in-control individual values to lie. Because these limits are based on the actual performance of the laboratories, they could also be designated "capability limits" and considered in setting specifications for acceptable recoveries. Because all analytes except one had grand mean percent recoveries of less than 100%, we believe that recoveries of some analytes may improve with further ex-

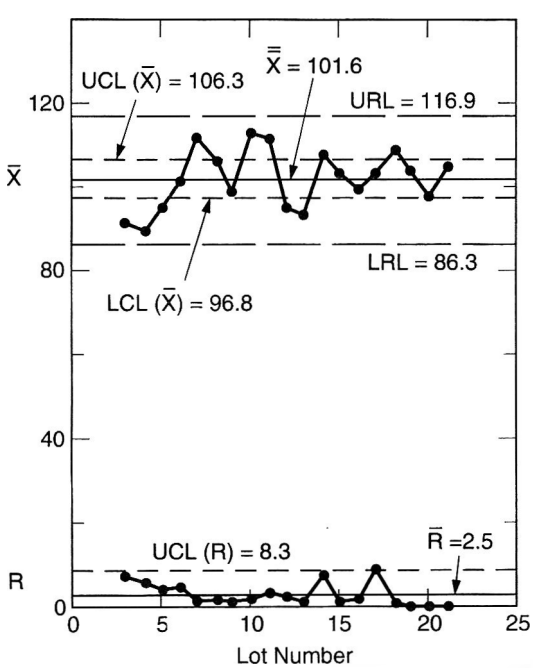


**Figure 1. Laboratory 2: chromium, duplicate spikes $\bar{X}$ and $R$ chart.**

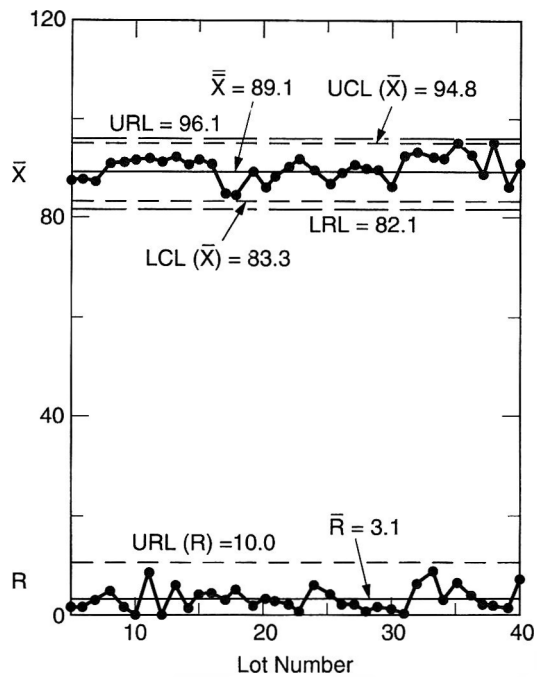


**Figure 2. Laboratory 4: chromium, duplicate spikes $\bar{X}$ and $R$ chart.**

**Table 5. Percent of means outside $S_O$ and $S_R$ control limits compared to ratio of reproducibility[a]/repeatability ($S_R/S_O$) for duplicate spike data**

| Analyte–laboratory | $S_R/S_O$ | Out-of-control, %, using $S_O$ limits | Out-of-control, %, using $S_R$ limits |
|---|---|---|---|
| Cd–1 | 1.41 | 17.2 | 17.2 |
| Cu–1 | 1.10 | 3.5 | 3.5 |
| Cr–1 | 1.24 | 6.9 | 0.0 |
| Pb–1 | 1.50 | 17.2 | 3.5 |
| Zn–1 | 1.30 | 6.9 | 0.0 |
| As–1 | 2.34 | 39.3 | 0.0 |
| DBCP–1 | 2.12 | 23.5 | 5.9 |
| Cd–2 | 4.20 | 68.4 | 5.3 |
| Cu–2 | 1.46 | 15.8 | 10.5 |
| Cr–2 | 2.57 | 52.6 | 0.0 |
| Pb–2 | 2.56 | 36.8 | 0.0 |
| Zn–2 | 1.74 | 10.5 | 5.3 |
| Hg–2 | 1.24 | 5.3 | 0.0 |
| As–2 | 1.67 | 36.8 | 10.5 |
| DBCP–2 | 1.60 | 25.5 | 2.1 |
| Cd–3 | 2.62 | 42.9 | 0.0 |
| Cu–3 | 2.75 | 50.0 | 0.0 |
| Cr–3 | 2.93 | 57.1 | 0.0 |
| Pb–3 | 2.21 | 28.6 | 0.0 |
| Zn–3 | 3.20 | 71.4 | 0.0 |
| Hg–3 | 1.58 | 15.8 | 0.0 |
| As–3 | 2.00 | 25.8 | 3.2 |
| DBCP–3 | 1.38 | 10.2 | 1.7 |
| Cd–4 | 2.09 | 58.3 | 2.8 |
| Cu–4 | 1.64 | 13.9 | 2.8 |
| Cr–4 | 1.18 | 5.6 | 0.0 |
| Pb–4 | 1.67 | 16.7 | 2.8 |
| Zn–4 | 1.50 | 5.6 | 1.4 |
| Hg–4 | 1.70 | 23.8 | 0.0 |
| As–4 | 2.04 | 28.0 | 0.0 |
| DBCP–4 | 1.58 | 20.9 | 3.0 |
| Means | 1.94 | 23.9 | 6.4 |

[a] Within-laboratory.

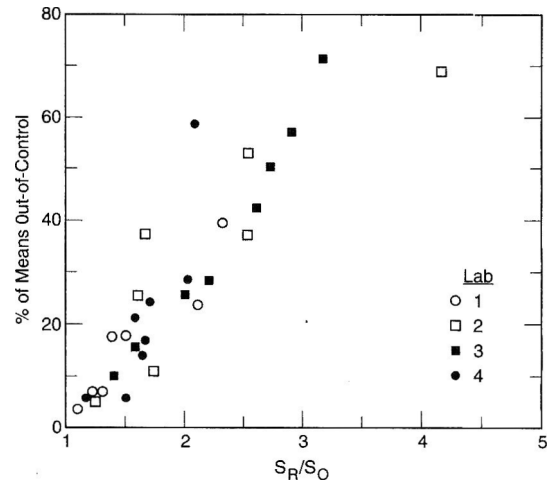


**Figure 3. Means outside repeatability control limits vs within-laboratory reproducibility/repeatability ratios.**

perience. Individual recovery values below the lower performance limits should be discarded as outliers to avoid any trend toward lower recoveries. Conversely, some high-side limits that were only slightly greater than 100% should be raised to accommodate efforts to approach grand mean recoveries of 100%. Nearly all of the values outside performance limits were on the high side and were associated with laboratories that obtained higher than average recoveries. For example, 7 chromium recoveries obtained by Laboratory 2 were between 109 and 113%, which is above the upper performance standard of 108%. These were the only values for chromium that were outside the limits, and we consider them to be acceptable values based on DQOs.

Ultimately, specification limits for a range of acceptable individual recoveries should be based on DQOs, but they should also be compatible with laboratory capability. On the basis of these considerations, specification limits were set at 82–115% recovery for these analytes in this study.

*Step 7.*—ANOVA was conducted on the individual data sets from each laboratory to separate the standard deviations for within-lot (repeatability), between-lot, and the combination of the 2, which we call within-laboratory reproducibility. The symbols for these 3 values are $S_O$ (repeatability), $S_L$ (lot-to-lot), and $S_R$ (within-laboratory reproducibility). Table 2 contains the estimates for chromium, and Table 3 gives similar values averaged across all 8 analytes.

*Step 8.*—Various ±3σ control limits were calculated. Shewhart limits based on repeatability were obtained by using the average range and conversion constants ($D_4$ and $A_2$) found in the text by Grant and Leavenworth (11).

The following equations were used:

$$\text{Upper control limit on range} = UCL_R = D_4\bar{R}$$

$$\text{Upper control limit on mean} = UCL_{\bar{\bar{X}}} = \bar{\bar{X}} + A_2\bar{R}$$

$$\text{Lower control limit on mean} = LCL_{\bar{\bar{X}}} = \bar{\bar{X}} - A_2\bar{R}$$

where $\bar{\bar{X}}$ is the laboratory mean with at least 20 subsample means.

**NOTE:** Our definitions of $S_L$ and $S_R$ differ from those used by AOAC (10), which include among-laboratory variability.

Control limits based on within-laboratory reproducibility (which includes repeatability plus lot-to-lot variations) were obtained by using standard deviation estimates ($S_R$ ) from ANOVA (Tables 2 and 3). Because subsamples were duplicates ($n = 2$), the following equations were used.

Upper within-laboratory reproducibility control limit on mean:

$$URL_{\bar{\bar{X}}} = \bar{\bar{X}} + 3S_R/\sqrt{2}$$

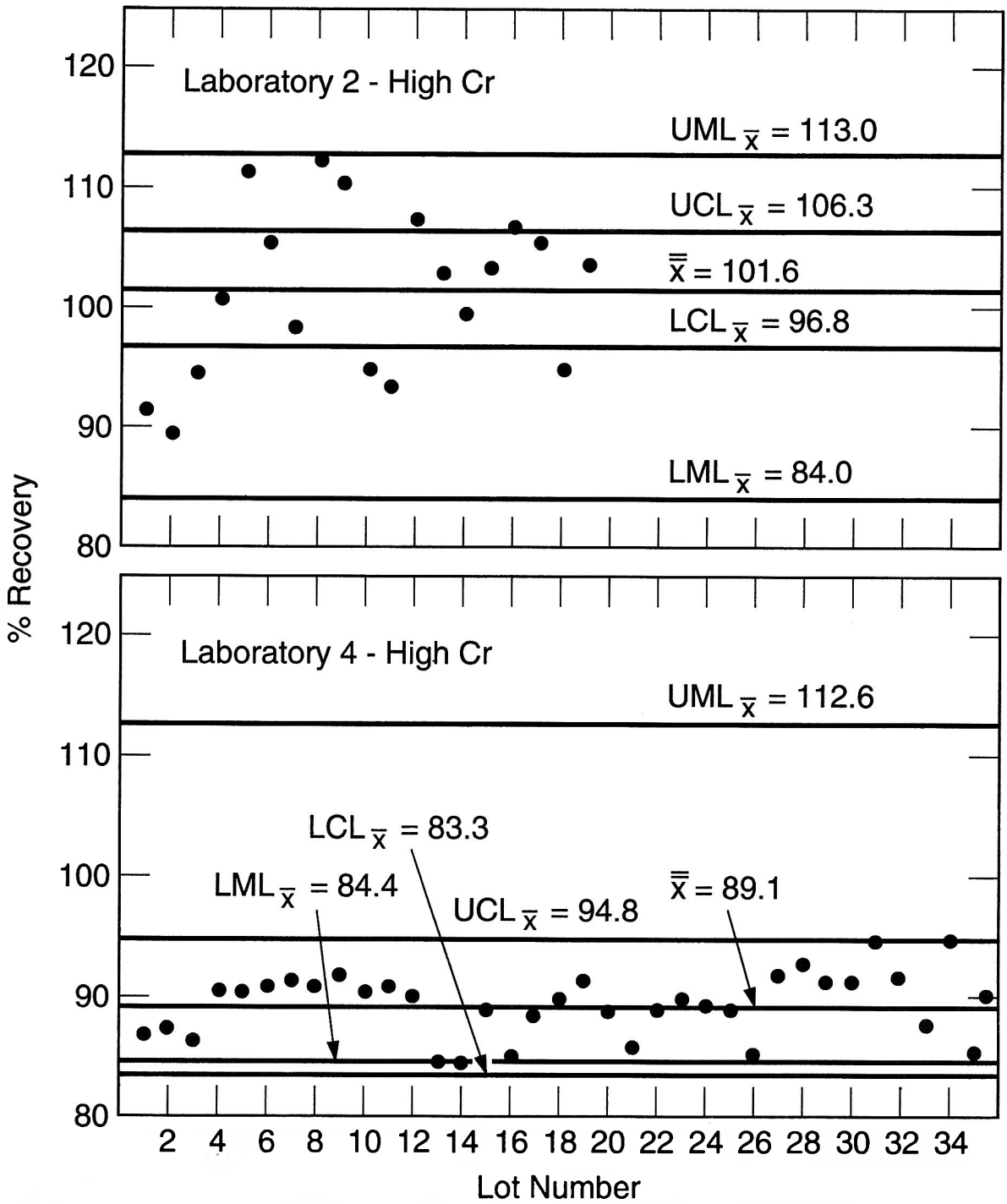


**Figure 4. $\bar{X}$ charts with both repeatability and modified control limits for duplicate chromium QC data, Laboratories 2 and 4.**

Lower within-laboratory reproducibility control limit on mean:

$$LRL_{\bar{\bar{X}}} = \bar{\bar{X}} - 3S_R/\sqrt{2}$$

Modified control limits were calculated based on specification limits for individual values of 82–115% and the conversion constant $M_3$ (11). These specifications were chosen to be within the performance capability of these methods for these analytes (see *Step 6*) and were adequate to meet data quality objectives. The following equations were used.

Upper modified limit on mean:

$$UML_{\bar{\bar{X}}} = 115.0 - M_3\bar{R}$$

Lower modified limit on mean:

$$LML_{\bar{\bar{X}}} = 82.0 + M_3\bar{R}$$

A summary of these limits together with $\bar{\bar{X}}$ and $R$ for chromium data from the 4 laboratories is given in Table 3.

## Discussion

Examination of the ratios for within-laboratory reproducibility ($S_R$) divided by repeatability ($S_O$) in Tables 2 and 3 reveals that lot-to-lot calibration variability makes a substantial contribution in all 4 laboratories. However, calibration variations exert a much greater effect with Laboratories 2 and 3 than with Laboratories 1 and 4. The control limits based on these 2 estimates of uncertainty are summarized for chromium analyses in Table 4. The contrasting situations for Laboratories 2 and 4 are presented in Figures 1 and 2, respectively. For Laboratory 2, half of the means are out-of-control on the basis of repeatability limits, even though all of the ranges are in control. However, none of the means is outside of the within-laboratory reproducibility limits. This finding is consistent with the $S_R/S_O$ ratio of 2.6 for chromium analyses by Laboratory 2. In contrast, Laboratory 4 (Figure 2) has only 2 means that are marginally out-of-control on the basis of repeatability limits, a finding that is consistent with the low $S_R/S_O$ ratio of 1.2.

We examined this phenomenon further by tabulating the ratios $S_R/S_O$ and the corresponding percent of out-of-control means, using limits based on both $S_O$ and $S_R$ for 31 data sets, i.e., 8 analytes from each of 3 laboratories and 7 from the fourth laboratory (Table 5). In contrast to the 23.9% out-of-control situations when limits are based on $S_O$, only 6.4% are out-of-control with limits based on $S_R$. It is also instructive to examine the correlation of percent of means out-of-control for $S_O$ limits with $S_R/S_O$ (Figure 3). The correlation coefficient, $r = 0.902$, is highly significant. A similar plot of percent of means out-of-control for $S_R$ limits vs $S_R/S_O$ produced only a scatter diagram showing no significant correlation.

Clearly, there is a strong argument here for using within-laboratory reproducibility limits. However, computations are less convenient than when the range is used, and a range chart is still required to generate within-lot precision information. An empirical adjustment is not practical because the contribution of lot-to-lot calibration error varies with method, laboratory, and time.

An alternative approach, especially useful when method capability equals or exceeds data quality objectives, is the use of modified control limits anchored on specification limits for individual recoveries.

Conceptually, the idea is to permit limited shifts in mean recoveries as long as individual recoveries remain within specifications. The more the difference between upper and lower specification limits exceeds 6 $S_R$ (from ±3 $S_R$), the easier it is to avoid rejecting data that meet DQOs. On the other hand, when 6 $S_R$ exceeds the specifications range or when the mean response is not near the center of the specification range, modified limits will reject lots that are in control according to either $S_O$ or $S_R$ limits. This reinforces the notion that a process can be in a state of statistical control without being able to generate data of acceptable quality.

These points are illustrated in Figure 4, where we again plot the chromium data with the modified control limits shown in Table 4. The rationale for 82–115% as specification limits was explained in *Step 6* of the statistical analysis in the *Experimental* section. Note that range charts are omitted from Figure 4 for convenience, but normally they must be included. If within-laboratory reproducibility is unreliable (range out-of-control), modified control limits should not be used because under these circumstances there can be no assurance of producing data within specifications. It is also important to recall that the modified limits are for means of $n = 2$ rather than for individual measurements. That is why modified limits are tighter than specification limits.

If both repeatability limits based on ranges and modified limits are placed on control charts, decisions can be unambiguously rendered about the state of control of the measurements and about the acceptability of the data. For Laboratory 2 (Figure 4), the repeatability control limits are much narrower than the modified limits, and they are nearly uniformly centered because the laboratory mean recovery is close to the center of the specifications. Clearly, within-lot repeatability is much better than specification limits require, but 11 of 19 means are outside repeatability limits because lot-to-lot calibration variation is fairly large. Still, none of the 19 means is outside of modified limits. In practice, as long as the range chart remains in control and mean recoveries stay between modified limits, the sample measurements should meet specifications in the absence of serious matrix effects. However, if a substantial trend developed or if 3 or 4 means in succession fell outside repeatability control limits but inside modified limits, this would require study of shifts in the measurement process even though the data were acceptable. Of course, a mean outside of modified limits would require immediate corrective action.

The results for Laboratory 4 (Figure 4) present a different scenario, with 24 of 26 means within repeatability control limits. Because $\bar{R}$ for Laboratory 4 is only moderately larger than for Laboratory 2 (3.1 vs 2.5), the difference in repeatability limits is not large enough to explain this difference. Instead, Laboratory 4 has a quite small lot-to-lot calibration variability. The problem is that the laboratory mean percent recovery is only 89.1%. Thus, the gap between $UML_{\bar{\bar{x}}}$ and $UCL_{\bar{\bar{x}}}$ is very large, but the $LML_{\bar{\bar{x}}}$ is actually higher (84.4%) than the $LCL_{\bar{\bar{x}}}$ (83.3%). Thus, means 13 and 14 are well within the $LCL_{\bar{\bar{x}}}$ but barely acceptable by the $LML_{\bar{\bar{x}}}$ criterion. Clearly, the data are in statistical control but the mean recovery is lower than desirable for the specifications. Because the need is to increase mean recovery, the use of modified control limits in conjunction with repeatability control limits would unambiguously accommodate this desired shift.

These examples illustrate some of the possible decision processes available if both repeatability and modified control limits are used. Clearly, other scenarios can be envisioned. Another advantage is retention of very simple computational procedures that allow timely use of QC data by analysts. The ease of deciding if a lot of data meets specifications makes this approach very advantageous.

## Acknowledgments

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MICROBIOLOGICAL METHODS

# Evaluation of Methods for Detection of *Listeria monocytogenes* in Foods: NMKL Collaborative Study

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**Eleven Nordic laboratories participated in a collaborative study on qualitative methods for the detection of *Listeria monocytogenes* in foods. Two enrichment methods (U.S. Department of Agriculture, Food Safety and Inspection Service method for meat, and modified U.S. Food and Drug Administration/International Dairy Federation method for dairy products) and 2 agar media (lithium chloride–phenylethanol–moxalactam (LPM) and Oxford agar) were compared. The effect of treatment of the enriched culture with KOH before plating was also studied. Food samples, blue and white mold cheese and canned corned beef, were inoculated with 3 levels of *L. monocytogenes*. Beef was also inoculated with a microbial flora derived from raw meat. The recoveries of *L. monocytogenes* using the 2 enrichment methods did not differ significantly. The Oxford agar was superior to the LPM agar. KOH treatment of the enriched culture did not significantly increase the detection rate of the organism. For cheese, the detection level of the methods was below 0.2 cfu *L. monocytogenes*/g, and for meat, the detection level was below 5 cfu *L. monocytogenes*/g.**

Interest in *Listeria monocytogenes* as a food-transmitted pathogen has increased rapidly during the past few years, and new methods for detection of this organism in foods are continuously being presented. Some of these methods are intended mainly for a specific type of food. The U.S. Department of Agriculture, Food Safety and Inspection Service (USDA–FSIS) has developed methods primarily intended for meat and poultry products (1–3). For analysis of dairy products, methods have been formulated by the U.S. Food and Drug Administration (FDA) (4) and the International Dairy Federation (IDF) (5).

Several studies that compare the USDA–FSIS method with the FDA method (or modifications of these methods) in recovering *L. monocytogenes* from foods have been published (e.g., 6–11). However, none of these studies is interlaborative.

In the collaborative study presented here, 11 Nordic laboratories evaluated different methods for detecting *L. monocytogenes* in meat and soft ripened cheese. Results obtained have been used as a basis for working out a Nordic Committee on Food Analysis (NMKL) method for the detection of *L. monocytogenes* in foods (12).

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## Collaborative Study

### *Design of the Study*

Two enrichment broth methods were compared. One method is comprised of enrichment in 2 steps with an increased concentration of acriflavine in the second step, according to the USDA–FSIS method (1, 2). The other method is comprised of enrichment in 1 step with 2 days of incubation of the broth, according to a provisional IDF method (5), but with the concentration of the inhibitory substances according to an FDA method (4). Isolation was performed on 2 media, the lithium chloride–phenylethanol–moxalactam (LPM) agar of the USDA–FSIS method (1, 2) and the more recently developed Oxford medium (13). The effect of KOH treatment of the enriched cultures before plating was also studied.

Soft ripened cheese and meat are foods that are frequently found to be contaminated with *Listeria* spp. Hence, blue and white mold cheese and canned corned beef were used as samples in the study. To approximate natural conditions, meat was inoculated with a competing flora derived from raw beef. Food was artificially contaminated with 3 levels of *L. monocytogenes*. Samples not inoculated with *Listeria* were included as controls. Cultures of *L. monocytogenes* and meat flora were provided as freeze-dried cultures that were added to food at the moment of sample preparation.

### *Pilot Study*

A preparatory, small-scale study was performed 6 weeks before the main study. Each collaborator received instructions, a vial containing freeze-dried *L. monocytogenes* for inoculation of cheese, and a vial containing *L. monocytogenes*, freeze-

dried together with a microbial flora derived from raw beef, for inoculation of meat. Methods and media used were as described for the main study.

### Preparation of Samples

(a) *Foods.*—Blue and white mold cheese ("Desirée blå ädelcamembert," Arla, Nyköping, Sweden, 140 g), and corned beef (Selecta, Três Rios, Brazil, 340 g can).

(b) *Bacteria.*—Cheese was inoculated with *L. monocytogenes* SLV L15:40; CCUG (Culture Collection, University of Gothenburg) 21829 (serotype 4, β+, lysovar 2389:2425:3274:2671:47:108:340, isolated from Vacherin Mont Ór red smear cheese, typed by J. Rocourt, Institut Pasteur). Meat was inoculated with *L. monocytogenes* NCTC 7973.

(c) *Preparation of freeze-dried cultures.*—Strains of *L. monocytogenes* were inoculated in brain heart infusion broth that was incubated 16–18 h at 35°C. The concentration of *L. monocytogenes* was then ca $10^9$ cfu/mL. After dilution, appropriate amounts of the cultures were transferred to the freeze-drying medium (2.5 g nutrient broth, 5 g inositol, and 100 mL distilled water) (14), and 0.5 mL aliquots were distributed in 4 mL glass vials. Material was frozen at −70°C and was then freeze-dried for ca 18 h using an Edwards freeze-dryer model Modulyo EF4. Vials were sealed with rubber stoppers under vacuum and labeled with code numbers. Collaborators could only identify which vials should be used for inoculation of cheese and meat, respectively, and which method to use, but not the inoculation level of *L. monocytogenes*. Vials were stored at room temperature in the dark until analysis.

Microbial flora for inoculation of meat samples was prepared from fresh beef cut into pieces and left at ambient temperature overnight. Peptone water (100 mL) was added, and the mixture was shaken. Peptone water was harvested and centrifuged 10 min at 1000 × *g*. The bacterial pellet was dispersed in 50 mL freeze-drying medium. For freeze-drying, 10 mL bacterial suspension was mixed with 10 mL *Listeria*-containing or *Listeria*-free freeze-drying medium, respectively.

The number of aerobic bacteria in the freeze-dried mixed cultures was determined using plate count agar (CM325, Oxoid Ltd, Hampshire, England) incubated 72 h at 30°C, *Enterobacteriaceae* using violet red bile glucose agar (CM485, Oxoid) incubated 24 h at 37°C, enterococci using Slanetz-Bartely agar (CM377, Oxoid) incubated 48 h at 44°C, and *Staphylococcus aureus* using Baird-Parker medium (CM275, SR54, Oxoid) incubated 48 h at 37°C. The presence of *Listeria* spp. was determined using both enrichment methods for the collaborative study.

The number of *L. monocytogenes* SLV L15:40 per vial was $2.0 \times 10^4$, $1.1 \times 10^3$, 50, and 0 cfu, which after preparation of samples corresponds to ca 80, 4, 0.2, and 0 cfu/g cheese, respectively. The number of *L. monocytogenes* NCTC 7973 per vial was $6.6 \times 10^4$, $1.0 \times 10^4$, $1.2 \times 10^3$, and 0 cfu, which after preparation of samples corresponds to ca 260, 40, 5, and 0 cfu/g meat. Final concentration of other bacteria in the meat samples was ca $4 \times 10^4$ cfu aerobic bacteria, $6 \times 10^2$ cfu *Enterobacteriaceae*, 0.5 cfu enterococci, and 0.5 cfu *S. aureus*/g meat. *Listeria* spp. were not detected in the meat flora.

(d) *Transport.*—Two weeks before the proposed start of the analysis, a complete set of instructions and data sheets, two 140 g packages of cheese, one 340 g can of corned beef, and 16 vials containing freeze-dried bacteria were sent to the collaborators. During transport, the samples were chilled with ice. Six collaborators received their test samples the day after the dispatching; 4 collaborators received their test samples 2 or 3 days later. The organizing laboratory (one of the collaborators) packed its samples in the same way as those sent away, and stored the parcel 54 h at 25°C 2 weeks before the analysis to imitate "worst transport conditions." After delivery, samples were stored at 4–6°C until initiation of the analysis. Seven collaborators started the analysis within an interval of 3 days; all collaborators started within an interval of 19 days.

### Statistical Calculations

Results of the different methods of analysis were studied using a 4-way, fully factorial ANOVA with enrichment broth, agar medium, KOH treatment, and inoculation level as factors, and with isolation of *L. monocytogenes* as the dependent variable. Results from cheese and meat were treated separately.

## METHODS

### Apparatus

Water baths, 45.0 ±0.2°C and 50.0 ±0.2°C; incubators, 30 ±1°C and 37 ±1°C; and homogenization equipment.

### Bacteria

*L. monocytogenes* (e.g., NCTC 7973) and *L. innocua*, used as control strains; *Staphylococcus aureus*, weakly β-hemolytic (e.g., ATCC 25923 or NCTC 1803) for the CAMP test.

### Media and Reagents

Chemical products and reagents should be of recognized analytical quality. If prepared media and reagents are not used immediately, they should be stored in the dark at 4 ±2°C, unless otherwise stated.

(a) *Enrichment broth UVM I.—Base medium.*—Dissolve 5 g proteose peptone, 5 g tryptone, 5 g meat extract (Lab Lemco powder, or equivalent), 5 g yeast extract, 20 g NaCl, 1.35 g $KH_2PO_4$, 12 g $Na_2HPO_4$, and 1 g esculin in 1000 mL distilled water. Autoclave 15 min at 121°C. Do not overheat. Cool medium immediately. *Supplement 1.*—Dissolve 40 mg nalidixic acid in 10 mL 0.05M NaOH. Sterilize by filtration (0.2 μm). *Supplement 2.*—Dissolve 25 mg acriflavine hydrochloride in 10 mL distilled water. Sterilize by filtration (0.2 μm). *Complete medium.*—Add 1.1 mL Supplement 1 and 1.1 mL Supplement 2 to 225 mL base medium.

(b) *Enrichment broth UVM II.—Base medium.*—Dissolve ingredients as for base medium UVM I in 1000 mL water. Add 5.0 mL Supplement 1. Dispense 10 mL portions into test tubes. Autoclave 15 min at 121°C. Do not overheat. Cool medium immediately. *Complete medium.*—Add 0.1 mL Supplement 2 to 10 mL base medium.

(c) *Enrichment broth.*—According to Lovett et al. (4).—*Base medium.*—Dissolve 30 g tryptone soya broth and 6 g yeast extract in 1000 mL distilled water. Adjust pH so that it is 7.3 ±0.1 (25°C) after autoclaving. Autoclave 15 min at 121°C. *Supplement 1.*—Dissolve 40 mg nalidixic acid in 10 mL 0.05M NaOH. Sterilize by filtration (0.2 μm). *Supplement 2.*—Dissolve 15 mg acriflavine hydrochloride in 10 mL distilled water. Sterilize by filtration (0.2 μm). *Supplement 3.*—Dissolve 50 mg cycloheximide in a mixture of 4 mL ethanol and 6 mL distilled water. *Complete medium.*—Add 2.5 mL each of Supplements 1, 2, and 3 to 225 mL base medium.

(d) *KOH solution.*—Dissolve 2.5 g KOH and 20 g NaCl in 1000 mL distilled water. Dispense 4.5 mL portions into screw-cap tubes, ca 16 mm diameter. Autoclave 10 min at 121°C. Keep at room temperature. Discard solution if pH falls below 12.3.

(e) *LPM agar.—Base medium.*—Dissolve 35.5 g phenylethanol agar (Bacto, or equivalent), 10 g glycine anhydride, and 5 g LiCl in 1000 mL distilled water. Autoclave 12 min at 121°C. Cool to 45°C in a water bath. *Supplement.*—Dissolve 1 g (sodium or ammonium) moxalactam in 100 mL 0.1M potassium phosphate buffer pH 6.0. Sterilize by filtration (0.2 μm). Dispense in 2 mL quantities. Keep at −20°C for no longer than 2 weeks, or at −70°C for no longer than 2 months. *Complete medium.*—Add 2 mL Supplement to the 45°C base medium. Base medium cannot be prepared in advance and reheated. Stir with a magnetic mixer and dispense portions of ca 10 mL into 90 mm Petri dishes. Store plates in a plastic bag in the dark at 4 ±2°C for no longer than 2–3 weeks.

(f) *Oxford agar.—Base medium.*—Dissolve 39 g Columbia blood agar (BA) base, 1 g esculin, 0.5 g iron (III) ammonium citrate, and 15 g LiCl in 1000 mL distilled water by heating gently to boiling. Adjust pH so that after autoclaving it is 7.0 ±0.2. Autoclave 15 min at 121°C. Cool to 50°C in a water bath. *Supplement*—Dissolve 400 mg cycloheximide, 20 mg colistin sulfate, 5 mg acriflavine, 2 mg cefotetan, and 10 mg phosphomycin in 10 mL ethanol–distilled water (1 + 1). Sterilize by filtration (0.2 μm). *Complete medium.*—Add supplement to base medium. Mix well. Dispense portions of ca 12 mL medium/Petri dish. Store plates in a plastic bag in the dark at 4 ±2°C for no longer than 2 weeks.

(g) *Blood agar (BA).*—Dissolve 33 g tryptose BA base, Bacto, or equivalent, in 1000 mL distilled water by heating gently to boiling. Autoclave 15 min at 121°C. Cool to 45–50°C. Add 50 mL defibrinated or citrate stabilized calf or beef blood brought to room temperature. Mix well. Dispense portions of ca 10 mL medium/Petri dish. A thin agar layer is important for the hemolysis to appear clearly. Store plates in a plastic bag in the dark at 4 ±2°C.

(h) *Brain heart infusion (BHI) agar.*—BHI or a similar nonselective medium.

(i) *Brain heart infusion (BHI) broth.*—BHI or a similar nonselective broth.

(j) *CAMP test agar.*—BA can be used. Sometimes sheep blood gives better results than calf or beef blood. Variations may occur between different batches of blood. A thin agar layer (8–10 mL/dish) is important for the CAMP effect to appear clearly. Store plates in a plastic bag in the dark at 4 ±2°C.

### Enrichment and Isolation

(a) *Sample preparation.*—Rinse the appropriate vial containing freeze-dried culture with 4 × 1 mL peptone water. Dilute to a total volume of 10 mL. Aseptically take out 25 g sample and transfer to a stomacher bag. Add 225 mL enrichment broth and 1.0 mL reconstituted freeze-dried culture. Homogenize for 30 s.

(b) *USDA–FSIS method.*—Use enrichment broth UVM I brought to room temperature. Incubate homogenate 24 h at 30°C. Transfer 0.1 mL UVM I culture to 10 mL UVM II broth. Incubate 24 h at 30°C. Using a loop, inoculate an LPM and an Oxford agar plate with material from the UVM II culture. Also, transfer 1 mL UVM II culture to 4.5 mL KOH solution. Mix with a vortex, and immediately inoculate half the area of an LPM and an Oxford agar plate with material from the KOH solution, using a sterile cotton swab. Streak out the culture on the remaining half of each plate using a loop.

Incubate the LPM agar at 30°C. Examine plates after 24 and 48 h with 45° transillumination at 7–35 × magnification (Henry illumination). *Listeria* colonies appear sparkling white or blue, like crushed glass. Compare with a control strain. Subculture, if possible, at least 3 presumptive *Listeria* colonies from the LPM agar plate on BA.

Incubate the Oxford agar at 37°C. Examine plates after 24 and 48 h. *Listeria* colonies are surrounded by a blackened zone. Compare with a control strain. Subculture, if possible, at least 3 presumptive *Listeria* colonies from the Oxford agar plate on BA.

(c) *FDA/IDF method.*—Use enrichment broth according to Lovett et al. (4), brought to room temperature for the meat samples, and 45°C for the cheese samples. Incubate homogenate 48 h at 30°C. Using a loop, inoculate an LPM and an Oxford agar plate with the enriched culture. Also, transfer 1 mL enriched culture to 4.5 mL KOH solution, mix, and inoculate LPM and Oxford agar plates with material from the KOH solution in the same way as for the USDA–FSIS method. Incubate and examine plates, and subculture presumptive *Listeria* colonies on BA as for the USDA–FSIS method.

### Confirmation

(a) *Hemolysis.*—Incubate the BA plates 24–48 h at 37°C. *L. monocytogenes* produces colonies surrounded by a narrow clear zone of β-hemolysis. For further confirmatory tests, isolate on a nonselective medium, such as BHI.

(b) *Catalase test.*—*Listeria* spp. are catalase positive.

**Table 1. Total number of positive isolations of *Listeria monocytogenes* in cheese: results of 11 collaborators**

| | UVM broth (USDA/FSIS method) | | | | Lovett broth (FDA/IDF method) | | | |
|---|---|---|---|---|---|---|---|---|
| | LPM agar | | Oxford agar | | LPM agar | | Oxford agar | |
| Inoculation level | No KOH | KOH | No KOH | KOH | No KOH | KOH | No KOH | KOH |
| High | 9 | 10 | 11 | 11 | 10 | 10 | 11 | 11 |
| Medium | 10 | 9 | 11 | 11 | 10 | 10 | 11 | 11 |
| Low | 8 | 9 | 10 | 11 | 9 | 10 | 11 | 11 |
| Total | 27 | 28 | 32 | 33 | 29 | 30 | 33 | 33 |

**Table 2. Percentage of *Listeria*-like growth of total growth on agar medium upon analysis of cheese: mean of results of 11 collaborators**

| | UVM broth (USDA/FSIS method) | | | | Lovett broth (FDA/IDF method) | | | |
|---|---|---|---|---|---|---|---|---|
| | LPM agar | | Oxford agar | | LPM agar | | Oxford agar | |
| Inoculation level | No KOH | KOH | No KOH | KOH | No KOH | KOH | No KOH | KOH |
| High | 99 | 100 | 94 | 93 | 100 | 99 | 99 | 100 |
| Medium | 100 | 99 | 96 | 96 | 88 | 90 | 98 | 99 |
| Low | 100 | 90 | 96 | 95 | 89 | 90 | 100 | 100 |

(c) *CAMP test.*—Make a thin, even streak from an overnight BHI broth culture of *S. aureus* straight across a CAMP plate. Streak a 37°C, overnight BHI culture of presumptive *L. monocytogenes* onto the CAMP plate. The streak should be perpendicular to and reach close to but not touch the *S. aureus* streak. Several isolates of *Listeria* spp. can be tested on the same plate. As controls, use *L. monocytogenes* and *L. innocua*. Incubate 20–36 h at 37°C. A positive reaction is a zone with enhanced hemolysis extending ca 2 mm from the test strain and within the weakly hemolytic zone of *S. aureus*. *L. monocytogenes* shows a positive CAMP reaction with *S. aureus*. The strength of the CAMP reaction may vary between different strains of *L. monocytogenes*. *L. innocua* shows a negative CAMP reaction (no enhanced hemolysis) with *S. aureus*.

In the collaborative study, a typical appearance on LPM or Oxford agar and BA, and a positive catalase and CAMP reaction were regarded as sufficient for confirmation of *L. monocytogenes*. When analyzing food samples, further tests are required for the complete confirmation of *L. monocytogenes* (3, 12).

## Results and Discussion

Results are summarized in Tables 1–7. The primary data of the individual collaborators are not included in this report.

One collaborator had problems with the determination of the catalase reaction of the *Listeria* strain (NCTC 7973) used for the meat samples; therefore, this collaborator also had difficulties with the confirmation of this strain. Results for meat samples of this collaborator were omitted from the statistical evaluations.

### *Enrichment Procedure*

Each collaborator isolated *L. monocytogenes* from all *Listeria* inoculated cheese samples with both enrichment procedures (Table 1). Altogether, 120 isolations were positive with the USDA–FSIS method (UVM broth) and 125 with the FDA/IDF method (Lovett broth) (Table 7). The difference is not statistically significant ($p > 0.05$).

Each collaborator isolated *L. monocytogenes* from all *Listeria* inoculated meat samples with both enrichment methods (Table 4). Altogether, 99 isolations were positive with the USDA–FSIS method and 94 with the FDA/IDF method (Table 7). The difference is not statistically significant.

From cheese samples that were not inoculated with *Listeria*, 2 collaborators isolated *L. monocytogenes* after enrichment in Lovett broth. This may reflect a true existence of low numbers of the organism in the cheese. In both cases, the cheese had been subjected to ambient temperatures; in one case due to problems during transport, in the other case deliberately at the organizing laboratory (see *Preparation of Samples, Transport* above).

From meat samples that were not inoculated with *Listeria*, no *L. monocytogenes* were isolated.

One reason for the slightly higher number of positive isolations obtained for cheese with the FDA/IDF method may be

**Table 3. Percentage of confirmed *Listeria monocytogenes* colonies upon analysis of cheese and number of investigated colonies (in parenthesis): results of 11 collaborators**

| | UVM broth (USDA/FSIS method) | | | | Lovett broth (FDA/IDF method) | | | |
|---|---|---|---|---|---|---|---|---|
| | LPM agar | | Oxford agar | | LPM agar | | Oxford agar | |
| Inoculation level | No KOH | KOH | No KOH | KOH | No KOH | KOH | No KOH | KOH |
| High | 100 (27) | 100 (30) | 97 (33) | 100 (33) | 100 (30) | 100 (30) | 100 (33) | 100 (33) |
| Medium | 100 (30) | 100 (27) | 100 (33) | 100 (30) | 100 (30) | 100 (30) | 100 (33) | 100 (33) |
| Low | 100 (24) | 100 (27) | 100 (30) | 100 (31) | 100 (27) | 100 (30) | 100 (33) | 100 (33) |

**Table 4. Total number of positive isolations of *Listeria monocytogenes* in meat: results of 10 collaborators**

| | UVM broth (USDA/FSIS method) | | | | Lovett broth (FDA/IDF method) | | | |
|---|---|---|---|---|---|---|---|---|
| | LPM agar | | Oxford agar | | LPM agar | | Oxford agar | |
| Inoculation level | No KOH | KOH | No KOH | KOH | No KOH | KOH | No KOH | KOH |
| High | 8 | 8 | 10 | 9 | 7 | 7 | 10 | 10 |
| Medium | 8 | 8 | 10 | 10 | 6 | 4 | 10 | 10 |
| Low | 5 | 4 | 10 | 9 | 7 | 4 | 10 | 9 |
| Total | 21 | 20 | 30 | 28 | 20 | 15 | 30 | 29 |

**Table 5. Percentage of *Listeria*-like growth of total growth on agar medium upon analysis of meat: mean of results of 10 collaborators**

| | UVM broth (USDA/FSIS method) | | | | Lovett broth (FDA/IDF method) | | | |
|---|---|---|---|---|---|---|---|---|
| | LPM agar | | Oxford agar | | LPM agar | | Oxford agar | |
| Inoculation level | No KOH | KOH | No KOH | KOH | No KOH | KOH | No KOH | KOH |
| High | 86 | 86 | 80 | 75 | 73 | 81 | 89 | 93 |
| Medium | 88 | 88 | 80 | 75 | 57 | 51 | 96 | 94 |
| Low | 78 | 73 | 70 | 68 | 64 | 56 | 93 | 91 |

that the broth was prewarmed at 45°C, while the broth was at room temperature in the USDA–FSIS method. Yousef et al. (15) recovered higher numbers of *L. monocytogenes* from *Listeria* contaminated Colby cheese homogenized with a stomacher in warm diluent (40°C), as compared to cool diluent (20°C). For meat, both enrichment broths were at room temperature.

Conclusions from comparisons of enrichment methods similar to those used in this study are not clear-cut: The results varied with the food analyzed and the conditions of the specific investigation (6–8). However, in several studies, a 2-stage enrichment method was found to reduce the competing microflora in cheese or meat (6, 9, 16).

### *Agar Medium*

For both cheese and meat, significantly ($p < 0.01$ and $p < 0.001$, respectively) higher numbers of positive *L. monocytogenes* isolations were obtained with the Oxford agar than with the LPM agar (Table 7). Several collaborators reported problems with poor and/or slow growth of *L. monocytogenes* on the LPM agar: 3 collaborators obtained no or very poor growth on the LPM agar after 48 h, and then extended the incubation time to 3 or 5 days. One collaborator obtained poor growth (less than 10 colonies) on several LPM plates, and another commented that "the Oxford agar is easier to examine; small amounts (of *Listeria* growth) cannot be detected on LPM plates." For the remainder of the collaborators, however, results with the LPM agar were as good as with the Oxford agar. The reason for this discrepancy between the collaborators is not clear. Buchanan et al. (17) found similar variations between results obtained with modified Vogel Johnson agar, and suggested that one reason might be production lot differences in the activity of the moxalactam. The Oxford agar does not contain moxalactam.

The percentage of *Listeria*-like growth of total growth was >80% on almost all plates, irrespective of what enrichment method was used (Tables 2 and 5). Collaborators were successful in recognizing *Listeria* colonies among other organisms; for both agar types the percentage of confirmed *L. monocytogenes* colonies was high, generally close to 100% (Tables 3 and 6).

Besides *Listeria* spp., some strains of enterococci hydrolyze esculin and blacken Oxford agar. These strains, however, usually grow poorly on this medium and exhibit a weak esculin reaction, usually after 40 h of incubation (Oxoid product information sheet). In the present study, no *Listeria* spp. false positives due to positive esculin reaction appeared.

Oxford agar was found to give higher recovery of *L. monocytogenes* from food than LPM agar, as in other studies (11, 18). In addition, an advantage of Oxford agar is that colonies belonging to *Listeria* spp. can be macroscopically recognized due to the esculin hydrolysis. LPM plates have to be examined by the more tedious Henry illumination microscopic technique.

### *Potassium Hydroxide Treatment*

The effect on the final results of the KOH treatment varied between collaborators. Considering the combined results of all collaborators, however, the differences for both cheese and

**Table 6. Percentage of confirmed *Listeria monocytogenes* colonies upon analysis of meat and number of investigated colonies (in parenthesis): results of 10 collaborators**

| | UVM broth (USDA/FSIS method) | | | | Lovett broth (FDA/IDF method) | | | |
|---|---|---|---|---|---|---|---|---|
| | LPM agar | | Oxford agar | | LPM agar | | Oxford agar | |
| Inoculation level | No KOH | KOH | No KOH | KOH | No KOH | KOH | No KOH | KOH |
| High | 100 (22) | 100 (24) | 97 (30) | 93 (28) | 95 (22) | 100 (21) | 100 (30) | 100 (30) |
| Medium | 100 (22) | 100 (22) | 93 (30) | 97 (30) | 100 (18) | 100 (12) | 100 (30) | 100 (30) |
| Low | 100 (15) | 100 (12) | 97 (30) | 93 (28) | 86 (22) | 79 (14) | 100 (28) | 100 (27) |

**Table 7. Number of positive isolations of *Listeria monocytogenes* in cheese and meat distributed on enrichment method, agar medium, and KOH treatment (summary of Tables 1 and 4)**

| | Enrichment method | | Agar medium | | KOH treatment | |
|---|---|---|---|---|---|---|
| | USDA/ FSIS | FDA/ IDF | LPM agar | Oxford agar | Without | With |
| Cheese | 120 | 125 | 114[a] | 131[a] | 121 | 124 |
| Meat | 99 | 94 | 76[b] | 117[b] | 101 | 92 |
| Total | 219 | 219 | 190 | 248 | 222 | 216 |

[a] Significant difference between agar medium (ANOVA; $p < 0.01$)
[b] Significant difference between agar medium (ANOVA; $p < 0.001$)

meat between the number of *L. monocytogenes* positive isolations obtained after KOH treatment as compared to no KOH treatment were not significant (Table 7). Also, there were no differences between KOH-treated and KOH-untreated cultures concerning *Listeria*-like growth on the plates (Table 2 and 5), or the rate of confirmed *L. monocytogenes* colonies (Tables 3 and 6).

The KOH treatment was introduced to reduce the competing flora. However, results of the present study show no significant positive effect of the treatment. Similar findings have been reported by Lammerding and Doyle (6) and Hitchins (10).

### *Limit of Detection*

Using Oxford agar, all collaborators succeeded in isolating *L. monocytogenes* from all *Listeria*-inoculated samples after enrichment according to the USDA–FSIS method and the FDA/IDF method (Tables 1 and 4). For cheese, the lowest inoculation level was 5 cfu/25 g; for meat, the lowest inoculation level was 120 cfu/25 g. Thus, 0.2 and 5 cfu/g cheese and meat, respectively, were detected by all collaborators with the methods tested.

Using methods similar to those compared in this study, detection limits ranging from 0.07 to 8.5 cfu/g have been reported (6, 8, 11, 18, 19). In fact, these 2 values were determined as detection limits, using the same method but analyzing 2 different kinds of seafood, crabmeat and surimi, respectively (19). Besides the influence of the food on the limit of detection of the method, the specific strain of *L. monocytogenes* seems to be of significance. Using LPM agar, Tiwari and Aldenrath (18) detected 0.12 cfu *L. monocytogenes* serotype 4b/g meat, but not 2.8 cfu *L. monocytogenes* serotype 3a/g meat.

### *Recommendation*

On the basis of the results of this collaborative study, an NMKL method for the detection of *L. monocytogenes* in foods was developed (12). The method incorporates enrichment either in UVM broth, according to the USDA–FSIS method, or in Lovett broth, according to the modified FDA/IDF method. In both cases, the enrichment broth should be prewarmed to 45°C if cheese or a similar fatty product is to be analyzed. For other foodstuffs, prewarming to 30°C is recommended. For foods suspected of having a high number of background microflora, the 2-stage selective enrichment of the USDA–FSIS method with increased concentration of acriflavine in the broth is recommended (6, 9, 16).

Material from the enrichment broth is subcultured on Oxford agar. The enriched culture is not KOH treated. The inoculation is performed using a swab and a loop (3). Four to 6 presumptive *Listeria* spp. colonies should be subcultured from the Oxford agar onto BA. Confirmation of *L. monocytogenes* is performed using physiological and biochemical tests. The CAMP reaction with both *S. aureus* and *Rhodococcus equi* is determined.

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PESTICIDE AND INDUSTRIAL CHEMICAL RESIDUES

# Column Extraction of Residues of Several Pesticides from Fruits and Vegetables: A Simple Multiresidue Analysis Method

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**Homogeneous sample pulp, prepared from fruits and vegetables of different water content with or without additional water, is adsorbed on the surface of activated Florisil to obtain a free-flowing powder, which is extracted in a glass column with ethyl acetate or methylene chloride–acetone (9 + 1, v/v). In most cases, no further cleanup is necessary for subsequent gas chromatographic measurement. The recovery of pesticide residues, including carbamate, organochlorine, organophosphate, synthetic pyrethroid, triazine, urea, and miscellaneous pesticides, is generally ≥ 80%, and is independent of the sample material. The column extraction is faster, less laborious, and less expensive than extractions based on traditional techniques, liquid partition, or other conventional chromatographic procedures.**

The regulatory control of pesticide residues in food involves large numbers of samples. The cost of the chemicals used and the time spent in the analysis of samples represent the major portion of the total costs of surveys. This study was initiated to improve the cost effectiveness of widely used multiresidue methods (1–5) without sacrificing the sensitivity of the method or the reliability of the results. To reduce the requirements for solvents and time, a column extraction method was chosen as an alternative.

Methods of this type have previously been applied to pesticide residue analysis. For the determination of organophosphorus compounds, plant materials were mixed with silica gel and extracted with petroleum ether; recoveries were 75% or above (6). Ground red pepper, cocoa beans, and coffee beans were mixed with Florisil™ and organochlorine residues were extracted with petroleum ether–methylene chloride (4 + 1); recoveries were above 80% (7). Wood (8) reported a method involving extraction and cleanup of organochlorine pesticide residues by column chromatography in which samples of fats, oils, and dairy products, among others, were mixed with celite and the residues were extracted with dimethyl sulfoxide. The eluate was adsorbed on Florisil and the pesticide residues were eluted with *n*-hexane; recovery values exceeded 70%. Steinwandter and Schluter (9) reported a multimatrix method with recoveries at or above 95% for 15 organochlorine pesticides extracted with petroleum ether, but this method has not yet been validated.

The first version of the method described here was published in 1985 (10). The number of pesticides covered by the method has been continuously extended, and it was validated and used regularly in the laboratories of the Plant Protection Organization in Hungary. The method was also tested and validated in Germany in 1989–1990. This paper represents the results obtained thus far, and demonstrates the applicability of the method to a great number of matrixes and pesticide residues.

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## METHOD

### *Apparatus*

(a) *Gas chromatograph.*—Equipped as follows: (*1*) $^{63}$Ni electron capture detector; interfaced to DB-5 fused-silica column, 60 m × 0.245 mm id, 1.0 μm film thickness (J & W Scientific, Folsom, CA 95630); splitless injection; hydrogen carrier gas. Operating conditions: injector 250°C, detector 350°C; temperature program, column 85°C, increase to 300°C at 2°C/min and hold 16 min. (*2*) $^{63}$Ni electron capture detector; interfaced to DB-1701 fused-silica column, 30 m × 0.25 mm id, 0.25 μm film thickness (J & W Scientific); splitless injection; hydrogen carrier gas. Operating conditions: injector 250°C, detector 350°C; temperature program, column 90°C, increase to 270°C at 4°C/min and hold 16 min. (*3*) Flame photometric detector (FPD-P); interfaced to HP-5 fused silica column, 30 m × 0.53 mm id, 2.65 μm film thickness (Hewlett-Packard Co., Palo Alto, CA 94303); splitless injection; nitrogen carrier gas. Operating conditions: injector 250°C, detector 350°C; temperature program, column 150°C, increase to 270°C at 4°C/min. (*4*) $^{63}$Ni electron capture detector; interfaced to CP-

**Table 1. Guide to amount of water and Florisil required for preparing various fruits and vegetables for extraction**

| Commodity | Water content, %[a] | Water added, mL[b] | Weight of pulp taken, g | Weight of Florisil, g |
|---|---|---|---|---|
| Cherries, sour | 84 | — | 5 | 8 |
| Cherries, sweet | 80 | — | 5 | 8 |
| Grapes | 81 | — | 5 | 8 |
| Melons | — | — | 5 | 8 |
| Pepper, green | 93 | — | 5 | 8 |
| Plums | 81–87 | — | 5 | 8 |
| Potatoes | 80 | — | 5 | 8 |
| Raspberries, red | 84 | — | 5 | 8 |
| Strawberries | 90 | — | 5 | 8 |
| Tomatoes | 90 | — | 5 | 10 |
| Apples | 84–87 | 50 | 6.25 | 10 |
| Apricots | 85 | 50 | 6.25 | 10 |
| Bananas | 76 | 50 | 6.25 | 10 |
| Broccoli | 89 | 50 | 6.25 | 10 |
| Cucumbers | 95 | 50 | 6.25 | 10 |
| Currants | 86 | 50 | 6.25 | 10 |
| Eggplant | 92 | 50 | 6.25 | 10 |
| Lemons | 87 | 50 | 6.25 | 10 |
| Oranges | 82 | 50 | 6.25 | 10 |
| Pears | 83 | 50 | 6.25 | 10 |
| Radish, black, red | 94–95 | 50 | 6.25 | 10 |
| Red beets | — | 50 | 6.25 | 10 |
| Sugarbeets | — | 50 | 6.25 | 10 |
| Brussels sprouts | — | 100 | 7.5 | 12 |
| Carrots | 88 | 100 | 7.5 | 12 |
| Celery | 94 | 100 | 7.5 | 12 |
| Green beans | 90 | 100 | 7.5 | 12 |
| Green peas | 83 | 100 | 7.5 | 12 |
| Kohlrabi | 90 | 100 | 7.5 | 12 |
| Lettuce | 94–95 | 100 | 7.5 | 12 |
| Turnip | 90 | 100 | 7.5 | 12 |

[a] Typical water content (5, 18).
[b] Approximate amount of water required for blending 200 g (precut) sample.

SIL 5 CB fused-silica column, 10 m × 0.32 mm id, 0.1 μm film thickness (Chrompack International B.V., Middelburg, The Netherlands); split injection (split ratio 1:10); nitrogen carrier gas. Operating conditions: injector 230°C, detector 250°C, column 200°C isotherm. (5) Nitrogen-phosphorus detector; interfaced to CP-SIL 5 CB fused-silica column, 10 m × 0.32 mm id, film thickness 0.1 μm (Chrompack); injector, nitrogen carrier gas. Operating conditions: injector 230°C, detector 250°C; temperature program, column 160°C, increase to 220°C at 8°C/min.

*Note*: The amount of extract injected was 1 μL. The portion of sample injected varied depending on the permissible load of the detectors and the columns.

(b) *High-speed blender.*—With glass, stainless steel, or plastic jar.

(c) *Evaporator.*—Rotary vacuum or Kuderna-Danish type.

(d) *Extraction column.*—400 × 20 mm id with PTFE stopcock.

### Reagents

(a) *Solvents.*—Distilled-in-glass grade water. Acetone, ethyl acetate, *n*-hexane, and methylene chloride, residue analysis quality, distilled-in-glass if necessary. Caution: Avoid contact of these solvents with skin and eyes. Do not use open flame or sparks.

(b) *Florisil.*—60–100 mesh, pretreated as follows: Reflux 2 kg Florisil in 5 L distilled water 1 h, decant water, and repeat procedure 3 times. Remove water with vacuum filtration. Dry Florisil at 150°C and reactivate at 600°C for 2 h. Cool adsorbent in desiccator kept tightly closed. Florisil is suitable for the method if 10 g can adsorb 6.0–6.5 g water without losing its free-flowing character.

### Sample Preparation

Chop or cut larger units to obtain an easily mixed material. Transfer 200 g representative portion of sample into the blender, and add distilled water if necessary for proper blending. Disintegrate sample material by high speed blending to obtain homogeneous pulp. Immediately weigh portion of the

**Table 2. Testing efficiency of solvents in column extraction method for apple, black currant, carrot, plum, and tomato samples fortified at 0.5 mg/kg**

| Solvent | Recovery of selected pesticides, % | | | | |
|---|---|---|---|---|---|
| | Lindane[a] | Fenitrothion | Propiconazole | Dichlobutrazol | Monocrotophos |
| *n*-Hexane | 85–90 | 58–62 | 5 | 5–15 | 0 |
| Benzene | 90–100 | 85–100 | 70–75 | 70–75 | 8–13 |
| Diethyl ether | 90–100 | 90–100 | 85–95 | 80–90 | 27–32 |
| $CH_2Cl_2$ | 95–100 | 95–100 | 95–100 | 80–90 | 55–65 |
| Chloroform | 95–100 | 95–100 | 95–100 | 80–90 | 55–65 |
| Ethyl acetate | 95–100 | 95–100 | 95–100 | 95–100 | 90–100 |
| $CH_2Cl_2$–*n*-hexane (1 + 1) | 95–100 | 95–100 | 65–70 | 60–70 | 27–32 |
| Diethyl ether–*n*-hexane (9 + 1) | 95–100 | 95–100 | 50–60 | 60–70 | 15–25 |
| $CH_2Cl_2$–acetone (9 + 1) | 95–100 | 95–100 | 95–100 | 95–100 | 65–75 |

[a] Subjected to further cleanup (1).

**Table 3. Recovery studies performed in Germany**

| Commodity | Active ingredient | Fortification, mg/kg | Rec. range, % | MDQ, ng[a] | LD, mg/kg[b] | Mode of detection[c] |
|---|---|---|---|---|---|---|
| Apples | Vinclozolin | 0.02 | 93–112 | 0.0125 | 0.005 | ECD |
| (pulp)[d] | Chlorpyriphos | 0.08 | 89–104 | 0.05 | 0.02 | ECD |
| Apples | Lindane | 0.005 | 91–103 | 0.0075 | 0.003 | ECD |
| | Quintozen | 0.005 | 93–95 | 0.0025 | 0.001 | ECD |
| | Vinclozolin | 0.005 | 99–100 | 0.0125 | 0.005 | ECD |
| Carrots | Dimethoate | 0.2 | 89–110 | 0.075 | 0.03 | ECD |
| | Permethrin | 0.2 | 85–101 | 0.125 | 0.05 | ECD |
| | Cypermethrin | 0.2 | 84–104 | 0.25 | 0.1 | ECD |
| Celery | Permethrin | 0.2 | 52–67 | 0.125 | 0.05 | ECD |
| | Linuron | 0.5 | 86–111 | 0.5 | 0.2 | ECD |
| | Chlorfenvinphos | 0.3 | 73–89 | 0.025 | 0.01 | ECD |
| Cucumber | Triadimefon | 0.1 | 86–106 | 0.025 | 0.01 | ECD |
| | Chlorfenvinphos | 0.3 | 76–88 | 0.0125 | 0.005 | ECD |
| | Pyrazophos | 0.2 | 68–96 | 0.125 | 0.05 | ECD |
| | Chlorfenvinphos | 0.3 | 83–90 | 0.125 | 0.05 | FPD-P |
| | Pyrazophos | 0.2 | 80–96 | 0.25 | 0.1 | FPD-P |
| Green beans | Procymidone | 0.3 | 99–109 | 0.05 | 0.02 | ECD |
| | Fenpropathrin | 0.4 | 67–78 | 0.125 | 0.05 | ECD |
| | Triazophos | 0.2 | 88–93 | 0.125 | 0.05 | FPD-P |
| Lettuce | Vinclozolin | 2.0 | 103–126 | 0.0125 | 0.05 | ECD |
| | Diazinon | 0.2 | 125–143 | 0.05 | 0.2 | ECD |
| | Quintozen | 0.1 | 101–109 | 0.0025 | 0.01 | ECD |
| Potatoes | Methidathion | 0.1 | 66–91 | 0.05 | 0.02 | ECD |
| | Phosphamidon | 0.5 | 91–117 | 0.125 | 0.05 | ECD |
| | Fenvalerate | 0.1 | 73–116 | 0.0125 | 0.005 | ECD |
| Tomatoes | Vinclozolin | 0.8 | 91–97 | 0.125 | 0.05 | ECD |
| (pulp)[d] | Triadimefon | 0.2 | 109–133 | 0.125 | 0.05 | ECD |
| | Endosulfan | 0.05 | 93–104 | 0.025 | 0.01 | ECD |

[a] Minimum detectable quantity.
[b] Limit for determination.
[c] For gas chromatographic conditions, see *Apparatus* (a), 1–3.
[d] In these cases the pulps were fortified with the active ingredients instead of the whole precut fruits or vegetables.

pulp ($W_p$) representing 5 g sample into mortar. Repeat homogenization, if necessary, to eliminate any segregation during weighing. Add enough Florisil ($W_f$) to obtain free-flowing powdered sample.

For guidance on $W_p$ and $W_f$, see Table 1. The weight of sample pulp ($W_p$) taken for extraction and the amount of Florisil ($W_f$) depend on the volume of water ($V$), in milliliters, added to the sample. They can be calculated in grams by the following formula:

$$W_p = (200 + V)/40$$

$$W_f = 1.6 \times W_p$$

### *Extraction*

Place 5 mm layer of anhydrous sodium sulfate on extraction column. Transfer powdery sample mixture onto column in uniform manner. Tap column as for column chromatography. Rinse mortar and pestle with ca 20 mL extracting solvent, methylene chloride–acetone (9 + 1) or ethyl acetate. Pour rinsing solvent carefully onto column, and adjust flow rate to 5 mL/min. Complete extraction with additional solvent, applying 50 mL altogether. Concentrate extract to 1 mL by means of the rotary vacuum evaporator, changing solvent, if necessary, to one suitable for detection or further cleanup. Alternatively, concentrate extract first on Kuderna-Danish evaporator and then under gentle stream of nitrogen just to dryness. Dissolve the dry extract in *n*-hexane.

### *Recovery Studies*

To determine extraction efficiency, sample pulps of fresh fruits and vegetables (apples, black currants, carrots, tomatoes, and plums) were fortified at 0.5 mg/kg with analytical stan-

**Table 4. Recovery studies performed in Hungary**

| Commodity | Active ingredient | Fortification, mg/kg | Rec. range, % | MDQ, ng[a] | LD, mg/kg[b] | Mode of detection[c] |
|---|---|---|---|---|---|---|
| Apples | Methidathion | 0.02 | 89–97 | 0.02 | 0.004 | NPD |
| | α-Endosulfan | 0.01 | 96–105 | 0.005 | 0.001 | ECD |
| | β-Endosulfan | 0.01 | 97–103 | 0.005 | 0.001 | ECD |
| | Fenarimol | 0.1 | 86–97 | 0.05 | 0.01 | ECD |
| | Parathion-methyl | 0.05 | 92–102 | 0.02 | 0.003 | NPD |
| | Dimethoate | 0.02 | 92–106 | 0.02 | 0.003 | NPD |
| | Brompropylate | 0.05 | 92–95 | 0.05 | 0.01 | ECD |
| | | 0.2 | 93–99 | 0.05 | 0.01 | ECD |
| | Quinalphos | 0.05 | 95–100 | 0.05 | 0.01 | NPD |
| | Heptenophos | 0.1 | 87–102 | 0.05 | 0.01 | NPD |
| Cucumber | Metalaxil | 0.1 | 86–97 | 0.05 | 0.01 | NPD |
| | Bupirimate | 0.2 | 83–94 | 0.1 | 0.02 | NPD |
| | Triadimefon | 0.1 | 90–103 | 0.02 | 0.005 | ECD |
| Potatoes | *p,p'*-DDT | 0.01 | 93–101 | 0.005 | 0.002 | ECD |
| | *p,p'*-DDE | 0.01 | 90–99 | 0.005 | 0.002 | ECD |
| | *p,p'*-DDD | 0.01 | 94–102 | 0.005 | 0.002 | ECD |
| | *o,p'*-DDT | 0.01 | 94–103 | 0.005 | 0.002 | ECD |
| | *o,p'*-DDD | 0.01 | 90–100 | 0.005 | 0.002 | ECD |
| | Phorate | 0.02 | 86–98 | 0.03 | 0.005 | NPD |
| | Diazinon | 0.05 | 91–103 | 0.02 | 0.003 | NPD |
| Oranges | Parathion-ethyl | 0.05 | 96–103 | 0.03 | 0.005 | NPD |
| | Triazophos | 0.1 | 90–102 | 0.02 | 0.004 | NPD |
| | Pyrazophos | 0.05 | 89–99 | 0.02 | 0.004 | NPD |
| | Permethrin | 0.2 | 91–103 | 0.05 | 0.01 | ECD |

[a] Minimum detectable quantity.
[b] Limit of determination.
[c] For gas chromatographic conditions, see *Apparatus* (a), 4 and 5.

dards dissolved in acetone. Recoveries of 5 pesticide active ingredients, including apolar (lindane) and polar (monocrotophos) pesticides, were tested in 7 replicates with solvents of different polarity (Table 2).

For recovery studies, analytical standard solutions of selected pesticides were added to different fruits and vegetables. In German investigations, whole or roughly precut commodities (apples, carrots, celery, cucumber, green beans, lettuce, and potatoes) were fortified at different mg/kg levels according to maximum residue limits stated in German regulation modalities. In 2 cases (tomatoes and apples), active ingredients were added to sample pulps after homogenization. In Hungarian investigations, thoroughly homogenized sample pulps (apples, cucumber, oranges, and potatoes) were fortified. The recovery ranges obtained with 5–7 replicates are shown in Tables 3 and 4.

## Results

Water content and texture of plant material may vary depending on the varieties and cultivars, as well as on the maturity of the crops. In addition to the type of blender, these 2 characteristics influence the amount of water necessary for proper blending. The amounts of sample pulp, water, and Florisil proposed in Table 1 were found to be generally appropriate for the samples analyzed in our laboratories. If the specified amount of water is in excess or does not lead to a well-blended mixture, the right volume must first be determined experimentally. The amount of water should be kept low to avoid the use of larger amount of Florisil, which would increase the cost of the analysis. Note that the amount of sample analyzed is always 5 g, which will be taken into account in the calculation of results.

Recoveries of selected pesticides obtained by the column extraction method using different solvents and solvent mixtures are summarized in Table 2. Yield percentages represent the efficiency of various solvents for the extraction method with Florisil. Gas chromatography/electron capture detection (GC/ECD) requires an additional cleanup step using a silica gel column, and this step often results in losses of lindane. These losses must be subtracted from the percentage given in Table 2.

Ethyl acetate was found to be the most efficient single component solvent; methylene chloride–acetone (9 + 1) gave the best results among the solvent mixtures tested. Ethyl acetate is the best solvent to use if the concentrated extract can be directly analyzed chromatographically. If further cleanup and changing of the extracting solvent is necessary, the methylene chloride–acetone (1 + 1) mixture is recommended for the following reasons: (*1*) It can be easily changed to an appropriate solvent

during evaporation; (*2*) its boiling point is much lower than that of ethyl acetate, enabling fast evaporation at a lower temperature, which is essential for the determination of compounds that are volatile and/or heat sensitive; and (*3*) both methylene chloride and acetone can be more easily purified than the ethyl acetate.

The required solvent volume for Florisil extraction was determined by analyzing 5 mL eluate fractions from different pesticide/sample matrix combinations using ethyl acetate or the methylene chloride–acetone mixture. Increasing the volume of extraction solvents from 50 to 100 mL improved the recovery by only 4–5%. As an example, the extraction profile of quinalphos from field-treated plum is shown in Figure 1. A similar trend was observed with other pesticide/sample combinations. Consequently, 50 mL solvent was considered sufficient for extraction. This still represents a relatively large solvent/sample ratio (10 mL/g) compared to other methods listed in Table 5 [2:1 (11, 12), 3:1 (1, 13, 14), 5:1 (15)]. This ratio is necessary, however, because the extraction is based on partition between the water adsorbed on the surface of Florisil and the solvent, as discussed later.

Recoveries of residues from some selected fresh plant materials were determined with a minimum of 5 replicates in both Hungary and Germany. Levels of fortifications were at or below the maximum residue limits registered. Tables 3 and 4 present the results of the recovery tests, including the following: the minimum detectable quantities (the amount of pesticide standard material, expressed in ng, which gives a peak height 2 times higher than the noise); the typical limits of determinations (values given are based on practical experiments concerning the permissible load of the columns and detectors, which may vary depending on the type of plant material; the values also account for lack of any significant difference in the recoveries, depending on the level of fortification); and the mode of detection.

In addition, the recoveries of a great number of pesticide residues were determined in Hungary by the analysis of different samples fortified at various levels. The commodities analyzed are listed in Table 1. The commodity/pesticide combinations depended on the actual agricultural use of pesticides. Generally, a further cleanup step was not necessary even if the determination was carried out with GC/ECD. The recoveries of pesticide residues (over 80% in most cases) were independent

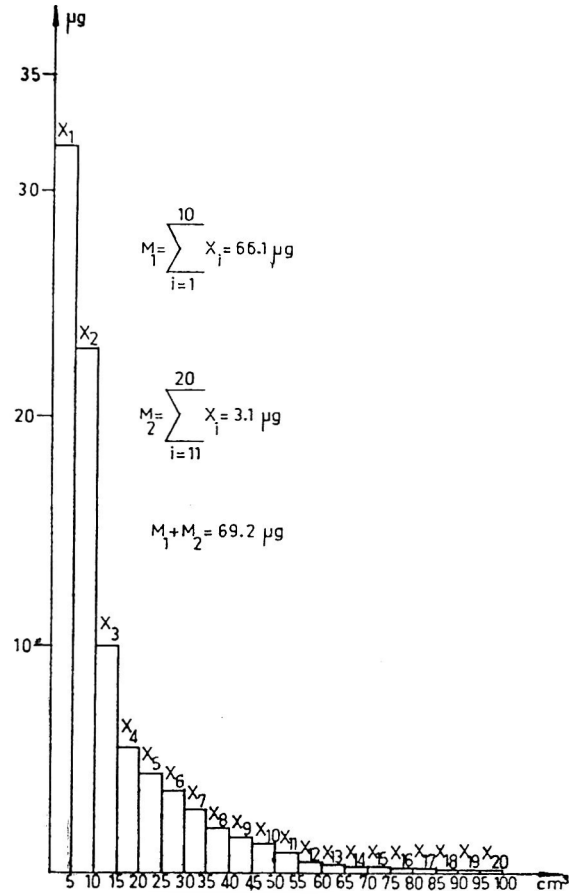


**Figure 1. Extraction profile of quinalphos from field-treated plum.**

**Table 5. Cost of chemicals used in extraction procedures[a]**

| Method | Weight of analytical sample, g | Extraction | Partition | Cost in U.S. dollars[b] |
|---|---|---|---|---|
| 12[a] | 100 | 200 mL acetone | 100 mL petroleum ether; 100 + 250 mL $CH_2Cl_2$ | 10.90 |
| 14 | 35 | 105 mL acetone | 210 mL *n*-hexane–$CH_2Cl_2$ (1 + 1); 2 × 70 mL $CH_2Cl_2$ | 8.05 |
| 16 | 35 | 105 mL acetone | 210 mL *n*-hexane–$CH_2Cl_2$ (1 + 1); 2 × 70 mL $CH_2Cl_2$ | 8.05 |
| 1 | 50 | 150 + 50 mL acetone | 200 mL $CH_2Cl_2$ | 6.40 |
| 15 | 100 | 200 mL $CH_3CN$ | 100 mL petroleum ether | 6.25 |
| 4 | 100 | 200 mL acetone | 100 mL $CH_2Cl_2$; 2 × 20 mL ethyl acetate | 5.10 |
| 13 | 25 | 75 mL $CH_2Cl_2$–MeOH (9 + 1); 50 mL $CH_2Cl_2$ | — | 2.65 |
| Proposed method | 5 | 50 mL ethyl acetate; 8–12 g Florisil | — | 2.15–2.80 |
| Proposed method | 5 | 50 mL $CH_2Cl_2$–acetone (9 + 1); 8–12 g Florisil | — | 2.35–3.00 |

[a] Based on MERCK price list 1989.
[b] Calculated in terms of 2.00 DM/U.S. dollar exchange rate.

**Table 6. Recovery of pesticide compounds tested in Hungary with column extraction method[a]**

| Active ingredients | Fortification, mg/kg | Rec., % | MDQ, ng | LD, mg/kg | Mode of detection | |
|---|---|---|---|---|---|---|
| Acetochlor | 0.05–0.5 | 85–96 | 0.05 | 0.01 | | ECD |
| Alachlor | 0.1–0.5 | 80–90 | 0.1 | 0.02 | | ECD |
| Aldrin | 0.01–0.1 | 92–102 | 0.002 | 0.0006 | | ECD |
| Alphamethrin | 0.2–0.5 | 82–93 | 0.02 | 0.005 | | ECD |
| Ametryne | 0.1–1.0 | 90–95 | 0.1 | 0.02 | | NPD |
| Atrazine | 0.05–0.2 | 87–100 | 0.1 | 0.02 | | NPD |
| Azinphos-methyl | 0.05–1.0 | 82–89 | 0.05 | 0.007 | | NPD |
| Aziprotryn | 0.1–2.0 | 90–98 | 0.1 | 0.02 | | NPD |
| Benalaxyl | 0.2–1.0 | 73–87 | 0.5 | 0.1 | | NPD |
| Benefin | 0.01–0.5 | 90–100 | 0.005 | 0.001 | | ECD |
| Benthiocarb | 0.2–2.0 | 80–95 | 0.5 | 0.1 | | NPD |
| Benzoylprop-ethyl | 0.1–0.5 | 87–100 | 0.05 | 0.01 | | ECD |
| Bitertanol | 0.1–0.5 | 90–95 | 0.5 | 0.1 | | NPD |
| Bromophos | 0.05–0.1 | 93–105 | 0.02 | 0.004 | PFD | NPD |
| Bromopropylate | 0.05–1.0 | 85–95 | 0.05 | 0.01 | | ECD |
| Bupirimate | 0.1–2.0 | 84–94 | 0.1 | 0.02 | | NPD |
| Butylate | 0.05–0.5 | 80–96 | 0.5 | 0.1 | | NPD |
| Captafol | 0.05–0.5 | 79–85 | 0.05 | 0.01 | | ECD |
| Captan | 0.05–0.5 | 83–93 | 0.02 | 0.005 | | ECD |
| Carbaryl | 0.2–2.0 | 92–98 | 0.5 | 0.08 | | NPD |
| Carbofuran | 0.2–2.0 | 90–101 | 0.5 | 0.08 | | NPD |
| Carbosulfan | 0.2–2.0 | 85–92 | 0.5 | 0.08 | | NPD |
| Chlorbromuron | 0.1–1.0 | 79–87 | 0.5 | 0.1 | | NPD |
| Chlorfenvinphos | 0.05–1.0 | 80–89 | 0.06 | 0.01 | PFD | NPD |
| Chloropropylate | 0.05–1.0 | 83–95 | 0.03 | 0.008 | | ECD |
| Chlorothalonil | 0.1–1.0 | 83–97 | 0.05 | 0.01 | | ECD |
| Chlorpyriphos | 0.05–2.0 | 90–98 | 0.01 | 0.002 | PFD | NPD |
| Ciobutid | 0.1–1.0 | 78–92 | 0.5 | 0.08 | | NPD |
| Cycloate | 0.05–0.5 | 82–99 | 0.5 | 0.05 | | NPD |
| λ-Cyhalothrine | 0.1–1.0 | 75–87 | 0.01 | 0.002 | | ECD |
| Cypermethrin | 0.05–1.0 | 76–89 | 0.01 | 0.002 | | ECD |
| Cyprofuram | 0.05–1.0 | 73–84 | 0.1 | 0.05 | | NPD |
| *o,p'*-DDD | 0.01–0.1 | 85–100 | 0.005 | 0.002 | | ECD |
| *o,p'*-DDT | 0.01–0.1 | 83–103 | 0.005 | 0.002 | | ECD |
| *p,p'*-DDD | 0.01–0.1 | 88–102 | 0.005 | 0.002 | | ECD |
| *p,p'*-DDE | 0.01–0.1 | 83–99 | 0.005 | 0.002 | | ECD |
| *p,p'*-DDT | 0.01–0.1 | 86–101 | 0.005 | 0.002 | | ECD |
| Deltamethrin | 0.05–0.5 | 77–89 | 0.005 | 0.001 | | ECD |
| Dialifos | 0.1–2.0 | 79–88 | 0.05 | 0.01 | | NPD |
| Diazinon | 0.05–0.5 | 91–103 | 0.02 | 0.004 | | NPD |
| Dichlobutrazol | 0.05–0.5 | 73–89 | 0.02 | 0.005 | | ECD |
| Dichlorvos | 0.01–0.5 | 84–92 | 0.02 | 0.005 | | NPD |
| Dichlozoline | 0.05–0.5 | 75–87 | 0.05 | 0.02 | | ECD |
| Dieldrin | 0.01–0.1 | 87–99 | 0.002 | 0.0006 | | ECD |
| Dienochlor | 0.01–0.2 | 87–101 | 0.002 | 0.0006 | | ECD |
| Dimethipin | 0.01–0.5 | 91–98 | 0.05 | 0.01 | | ECD |
| Dimethirimol | 0.01–0.5 | 82–93 | 0.1 | 0.02 | | NPD |
| Dimethoate | 0.02–1.0 | 89–106 | 0.02 | 0.003 | | NPD |
| Dinobuton | 0.1–1.0 | 74–86 | 0.1 | 0.02 | | NPD |
| Dinosebacetate | 0.05–1.0 | 81–96 | 0.02 | 0.005 | | ECD |
| Dioxacarb | 0.1–2.0 | 87–96 | 0.1 | 0.02 | | NPD |
| Diphenamid | 0.05–1.0 | 76–82 | 0.05 | 0.01 | | NPD |
| Ditalimphos | 0.05–1.0 | 82–92 | 0.02 | 0.003 | PFD | NPD |
| Dithianon | 0.05–1.0 | 87–96 | 0.1 | 0.01 | | NPD |
| DNOC | 0.01–0.5 | 69–79 | 0.005 | 0.001 | | ECD |

**Table 6. (Continued)**

| Active ingredients | Fortification, mg/kg | Rec., % | MDQ, ng | LD, mg/kg | Mode of detection | |
|---|---|---|---|---|---|---|
| Endosulfan | 0.01–0.1 | 87–105 | 0.005 | 0.001 | | ECD |
| Endrin | 0.01–0.2 | 91–101 | 0.003 | 0.0006 | | ECD |
| EPTC | 0.1–0.5 | 90–94 | 0.2 | 0.04 | | NPD |
| Etaconazole | 0.05–0.5 | 78–89 | 0.2 | 0.04 | | NPD |
| Ethirimol | 0.05–1.0 | 83–92 | 0.5 | 0.1 | | NPD |
| Etrimfos | 0.05–0.5 | 82–96 | 0.04 | 0.008 | | NPD |
| Fenarimol | 0.01–0.5 | 78–93 | 0.05 | 0.01 | ECD | NPD |
| Fenitrothion | 0.05–1.0 | 91–103 | 0.02 | 0.005 | NPD | PFD |
| Fenpropathrin | 0.1–1.0 | 77–87 | 0.2 | 0.05 | | ECD |
| Fenpropimorph | 0.1–1.0 | 79–85 | 0.5 | 0.1 | | NPD |
| Fenthion | 0.05–0.5 | 87–97 | 0.02 | 0.004 | NPD | PFD |
| Fenvalerate | 0.1–1.0 | 82–96 | 0.01 | 0.002 | | ECD |
| Fluazifop-butyl | 0.05–1.0 | 69–81 | 0.05 | 0.01 | | ECD |
| Flubenzimine | 0.05–1.0 | 65–78 | 0.1 | 0.05 | | ECD |
| Flurochloridone | 0.05–1.0 | 78–93 | 0.05 | 0.01 | | ECD |
| Fluvalinate | 0.05–1.0 | 82–96 | 0.02 | 0.006 | | ECD |
| Folpet | 0.01–1.0 | 87–95 | 0.02 | 0.005 | | ECD |
| Fonofos | 0.05–1.0 | 86–94 | 0.02 | 0.004 | | NPD |
| Formothion | 0.01–0.5 | 86–98 | 0.02 | 0.004 | NPD | PFD |
| Fosmethilan | 0.1–1.0 | 80–94 | 0.05 | 0.01 | | NPD |
| Haloxyfop | 0.05–0.5 | 78–89 | 0.1 | 0.04 | | ECD |
| HCB | 0.01–0.1 | 83–96 | 0.001 | 0.0002 | | ECD |
| α-HCH | 0.01–0.1 | 86–94 | 0.002 | 0.0004 | | ECD |
| β-HCH | 0.01–0.1 | 87–95 | 0.005 | 0.001 | | ECD |
| Heptachlor | 0.01–0.1 | 83–90 | 0.005 | 0.001 | | ECD |
| Heptachlor-epoxide | 0.01–0.1 | 80–94 | 0.005 | 0.001 | | ECD |
| Heptenophos | 0.1–1.0 | 78–102 | 0.05 | 0.01 | | NPD |
| Heptopargil | 0.1–1.0 | 69–78 | 1.0 | 0.15 | | PFD |
| Hexazinone | 0.1–1.0 | 82–93 | 0.1 | 0.02 | | NPD |
| Hexythiazox | 0.1–2.0 | 76–83 | 0.05 | 0.01 | | NPD |
| Iprodione | 0.1–2.0 | 82–96 | 0.05 | 0.01 | | ECD |
| Ioxynil | 0.1–1.0 | 79–83 | 0.01 | 0.003 | | ECD |
| Lindane | 0.01–0.2 | 86–99 | 0.002 | 0.0004 | | ECD |
| Linuron | 0.2–0.5 | 69–78 | 0.5 | 0.08 | | NPD |
| Malathion | 0.05–2.0 | 87–94 | 0.05 | 0.01 | | NPD |
| Mercaptodimethur | 0.05–2.0 | 81–97 | 0.5 | 0.08 | | NPD |
| Metalaxil | 0.1–1.0 | 76–89 | 0.05 | 0.01 | | NPD |
| Methamidophos | 0.05–1.0 | 82–93 | 0.05 | 0.01 | | NPD |
| Methidathion | 0.05–1.0 | 87–93 | 0.02 | 0.004 | | NPD |
| Metobromuron | 0.05–2.0 | 68–81 | 0.5 | 0.08 | | NPD |
| Metolachlor | 0.05–1.0 | 78–87 | 0.1 | 0.02 | | NPD |
| Metribuzin | 0.1–1.0 | 82–95 | 0.1 | 0.02 | | NPD |
| Mevinphos | 0.05–1.0 | 92–99 | 0.01 | 0.002 | NPD | PFD |
| Molinate | 0.2–2.0 | 78–93 | 0.3 | 0.05 | | NPD |
| Monocrotophos | 0.1–1.0 | 79–87 | 0.05 | 0.01 | | NPD |
| Nitrofen | 0.5–2.0 | 69–82 | 0.6 | 0.1 | | NPD |
| Nitrothale-isopropanol | 0.05–1.0 | 76–89 | 0.05 | 0.01 | | ECD |
| Nuarimol | 0.05–0.5 | 87–94 | 0.05 | 0.01 | | ECD |
| Oxazolidin | 0.1–1.0 | 72–87 | 0.1 | 0.02 | | NPD |
| Oxyfluorfen | 0.05–1.0 | 82–94 | 0.05 | 0.01 | | ECD |
| Parathion-ethyl | 0.05–1.0 | 96–103 | 0.03 | 0.005 | FPD | NPD |
| Parathion-methyl | 0.02–1.0 | 90–102 | 0.02 | 0.003 | FPD | NPD |
| Pendimethalin | 0.05–1.0 | 81–93 | 0.02 | 0.004 | ECD | NPD |
| Permethrin | 0.1–1.0 | 80–103 | 0.05 | 0.01 | | ECD |
| Phenkapton | 0.05–2.0 | 86–93 | 0.05 | 0.01 | NPD | PFD |

**Table 6. (Continued)**

| Active ingredients | Fortification, mg/kg | Rec., % | MDQ, ng | LD, mg/kg | Mode of detection | |
|---|---|---|---|---|---|---|
| Phenmedipham | 0.1–1.0 | 76–85 | 0.4 | 0.05 | | NPD |
| Phenthoate | 0.02–2.0 | 78–94 | 0.02 | 0.004 | NPD | PFD |
| Phorate | 0.01–0.5 | 87–98 | 0.03 | 0.005 | NPD | PFD |
| Phosalone | 0.05–1.0 | 73–86 | 0.05 | 0.01 | | NPD |
| Phosmet | 0.1–2.0 | 82–93 | 0.05 | 0.01 | | NPD |
| Phosphamidon | 0.05–1.0 | 80–96 | 0.05 | 0.01 | | NPD |
| Pirimicarb | 0.1–2.0 | 79–91 | 0.4 | 0.05 | | NPD |
| Pirimiphos-methyl | 0.05–0.5 | 87–94 | 0.02 | 0.004 | NPD | PFD |
| Procymidone | 0.1–1.0 | 87–96 | 0.01 | 0.002 | ECD | NPD |
| Prometryn | 0.05–2.0 | 86–98 | 0.1 | 0.02 | | NPD |
| Propachlor | 0.05–1.0 | 92–102 | 0.05 | 0.01 | ECD | NPD |
| Propiconazole | 0.1–2.0 | 78–89 | 0.05 | 0.01 | ECD | NPD |
| Prothoate | 0.05–1.0 | 81–94 | 0.05 | 0.01 | | NPD |
| Pyrazophos | 0.1–1.0 | 82–101 | 0.02 | 0.004 | NPD | PFD |
| Quinalphos | 0.05–1.0 | 90–108 | 0.05 | 0.01 | FPD | NPD |
| Secbumeton | 0.05–1.0 | 91–102 | 0.1 | 0.01 | | NPD |
| Terbacil | 0.1–1.0 | 78–90 | 0.2 | 0.02 | | NPD |
| Terbufos | 0.05–1.0 | 87–93 | 0.05 | 0.01 | NPD | PFD |
| Terbumeton | 0.1–1.0 | 91–99 | 0.1 | 0.01 | | NPD |
| Terbuthylazine | 0.1–2.0 | 83–96 | 0.1 | 0.02 | | NPD |
| Terbutryn | 0.1–2.0 | 91–101 | 0.1 | 0.02 | | NPD |
| Tetrachlorvinphos | 0.05–1.0 | 82–98 | 0.05 | 0.01 | | NPD |
| Tetradifon | 0.05–1.0 | 87–103 | 0.02 | 0.005 | | ECD |
| Thiometon | 0.05–1.0 | 91–98 | 0.05 | 0.01 | | NPD |
| Triadimefon | 0.1–1.0 | 87–98 | 0.1 | 0.03 | ECD | NPD |
| Triazophos | 0.05–1.0 | 90–102 | 0.02 | 0.005 | | NPD |
| Trifluralin | 0.05–1.0 | 91–100 | 0.01 | 0.005 | ECD | NPD |
| Vernolate | 0.1–1.0 | 84–97 | 0.1 | 0.05 | | NPD |
| Vinclozolin | 0.05–1.0 | 91–99 | 0.02 | 0.005 | ECD | NPD |

[a] Untreated samples were taken from fields of known spraying history. Recovery data are the average of a minimum of 5 experiments. Recovery ranges indicated were obtained from various crops.

of the sample matrix and the level of fortification. The results are summarized in Table 6.

Special care must be taken when compounds more polar than monocrotophos are determined because recoveries decrease proportionally with increasing polarity. Very polar compounds (e.g., phenoxyacetic acids and ethylene thiourea) cannot be recovered. Recovery of carbendazim exceeded 80% only with ethyl acetate. Thiophanate methyl partly decomposed in the pulp and formed carbendazim.

## Discussion

The analytical results may be greatly influenced by the manner in which samples are prepared for analysis. The first step of sample preparation is to separate the portion to be analyzed according to the objectives of the analysis, followed by appropriate size reduction and mixing of primary units to obtain a homogeneous mixture for weighing. The procedures recommended by the Food and Agriculture Organization/World Health Organization Codex Alimentarius Commission for sampling (16) and for the portion of commodities to be analyzed (17) were used in the present method.

A relatively large proportion of sample (200 g) was homogenized, compared to the 25, 35, 50, or 100 g quantities processed by other methods (1, 11–15, 18). This improved the reproducibility of parallel analytical results and reduced the deviation from the average residue content of the sample. Because only a 5 g portion of the homogenized sample was extracted, the thorough homogeneity of the blended sample was of utmost importance for obtaining reproducible results. It was advantageous to use glass jars for blending, so that the homogeneity of the pulp obtained could be checked visually.

Sample pulp is adsorbed onto the surface of Florisil during the preparation of the free-flowing powder. During extraction, the pesticide residues are partitioned between the water layer formed on the surface of the support and the extracting agent. The Florisil is thoroughly deactivated by the sample pulp and serves only as a support material in the partition/extraction process. This hypothesis is in agreement with the experimental results, namely: (*1*) The sample matrix does not influence the recovery of pesticide residues significantly (Tables 2–4); (*2*) the recovery of pesticides of various polarities depends on their solubility ratio in water and the extracting solvent; (*3*) the recoveries of residues decrease if Florisil is added to the pulp in

excess and, consequently, retains some of its adsorption activity.

The efficiency of the extraction was studied by analyzing field-treated samples obtained from supervised trials performed by the pesticide manufacturing companies. Samples were stored and transported deep-frozen to Hungary. They were analyzed in the company laboratories by specific methods developed for the respective compounds, as well as in our laboratories. The residue contents of the samples, as measured in the laboratories of the manufacturers, were compared with the average results obtained with 7 replicate analyses using the column extraction method. Results did not show significant difference at the 95% probability level for the 5 compounds tested (vinclozolin, triadimefon, methiocarb, metalaxyl, and pirimicarb). A detailed report on the comparative studies will be published elsewhere.

The time required for sample preparation, extraction, partition, and evaporation by the various methods (1, 11, 12) was compared by processing 8 samples using the same number of blenders and rotary evaporators. On average, the column extraction was completed within 1/4–1/5 of the time required by the other methods, mainly because filtration, partitioning, and drying were not required. Evaporation was also faster, in proportion to the solvent volumes used. In addition, the column extraction is less laborious and one analyst can handle more samples within the same time. The kinds and amounts of reagents used in the cited methods are shown in Table 5. The cost of reagents necessary for one extraction are calculated from the current prices of E. Merck (19) for pesticide residue analytical grade chemicals. The figures indicate that the cost of chemicals for the column extraction are approximately 40–50% of those used in other widely applicable multiresidue procedures (1, 4, 5, 11–14). Comparable costs were observed for the extraction procedure of Ohlin (15), who used an LC multiresidue method for the determination of pesticides in fruits and vegetables.

From the results obtained in the present study, we conclude that the column extraction method described here may replace more costly and time-consuming conventional extraction and partition steps, as well as highly sophisticated cleanup procedures of several multiresidue analytical methods commonly in use. At present, investigations are in progress to further simplify the column extraction method (20).

## Acknowledgments

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# Simultaneous Liquid Chromatographic Determination of Fenamiphos and Its Metabolites in Soil

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**A reversed-phase liquid chromatographic method has been developed for the determination of fenamiphos and the metabolites fenamiphos sulfoxide, fenamiphos sulfone, 3-methyl-4-(methylthio)-phenol, and 3-methyl-4-(methylsulfinyl)phenol. Trace quantities of the nematicide and its metabolites in soil can be determined simultaneously. The limit of detection of the method was 5 ppm. Recoveries of fenamiphos and its degradation products at fortification levels of 25, 50, and 100 ppm ranged from 99.2 to 100.8%. Standard deviations ranged from 0.29 to 0.70 ppm.**

Fenamiphos (ethyl 3-methyl-4-(methylthio)phenyl (1-methylethyl)phosphoramidate, Nemacur™ (I in Figure 1), is a systemic nematicide active against ecto and endo parasitic, free-living, cyst-forming, and root knot nematodes. It is applied in soil at 5–20 kg a.i./ha, and is useful in controlling nematodes on fruit, cereal, tobacco, and vegetable crops (1). Its acute oral $LD_{50}$ and percutaneous $LD_{50}$ for rats are 15.3–19.4 mg/kg and 50 mg/kg, respectively. In plants and soil (2), fenamiphos undergoes rapid degradation either by hydrolytic cleavage at the O–P bond or by oxidation of the methylthio moiety, producing 4 important metabolites, namely, fenamiphos sulfoxide (II), fenamiphos sulfone (III), 3-methyl-4-(methylthio)phenol (IV), and 3-methyl-4- (methylsulfinyl)-phenol (V). Earlier work on the determination of fenamiphos used gas chromatography (GC) for the analysis of the technical product and determination of its residues. The GC residue methods determine either the parent compound alone (3) or the total residue after oxidation of both the parent compound and sulfoxide with potassium permanganate (4). Another GC method individually determines the parent compound, sulfoxide, and sulfone in plants and soil (5). An improved method for the determination of fenamiphos and its 2 oxidative metabolites, using capillary GC and a thermionic detector, has also been reported (6).

Currently, liquid chromatography (LC) is assuming growing importance in pesticide analysis. An LC method has been described for the simultaneous determination of fenamiphos, its sulfoxide, and its sulfone in water (7). We report here a convenient method for the simultaneous determination of trace levels of fenamiphos and its 4 metabolic products in soil by reversed-phase LC.

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## Experimental

### Apparatus

(a) *Liquid chromatograph.*—Model 8000B, equipped with a UV-VIS variable wavelength detector (Model 4000 B), connected to a loop injection system (10 μL capacity), printer plot-type recorder and microprocessor-controlled data system; 10 μL of the sample was injected each time (Spectra Physics, Inc., Bedford, MA 01730).

(b) *$^1H$ NMR spectrometer.*—$^1H$ NMR spectra of fenamiphos and its degradation products were recorded on a 60 MHz 360-L spectrometer (EM Science, Gibbstown, NJ 08027) with TMS as the internal standard.

(c) *Mass spectrometer.*—Mass spectra were recorded at 70 ev on a JMS D-300 mass spectrometer using electron impact ionization (Jeol U.S.A., Inc., Peabody, MA 01960). Thin layer chromatography (TLC) of fenamiphos and its metabolites was carried out with $CHCl_3$–$CH_3OH$ (99 + 1) as the developing solvent system. Spots were visualized by exposing the TLC plate to iodine vapors in a glass chamber.

### Reagents

(a) *Water.*—Doubly distilled.

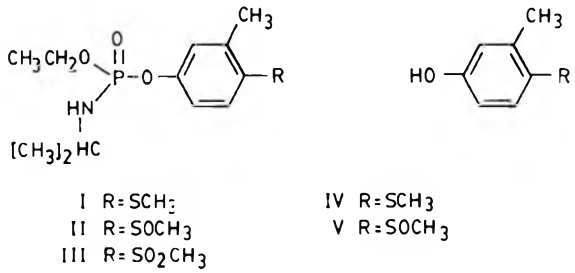
(b) *Methanol.*—Distilled, analytical grade. For LC, a mixture of methanol–water (80 + 20) was used in isocratic mode.

(c) *Fenamiphos.*—Analytical grade, 99.9% pure (Bayer, India).

All solvents were degassed and filtered through a filtration system before use (Millipore Corp., Bedford, MA 01730).

### Preparation of Metabolites

(a) *Ethyl 3-methyl-4-(methylsulfinyl)phenyl (1-methylethyl)phosphoramidate (II).*—Fenamiphos (200 mg), dissolved in dichloromethane, was added dropwise (5 mL) to a stirred solution of *m*-chloroperbenzoic acid (mCPBA) in dichloromethane (5 mL) at room temperature. The mixture was allowed to stand overnight in a freezer and then subjected to preparative TLC to obtain fenamiphos sulfoxide (II) as a viscous liquid. $R_f$ 0.19, $CHCl_3$–MeOH (90 + 1). $^1H$ NMR ($CDCl_3$): δ 1.15 (6H, d, $-CH(\underline{CH_3})_2$), 1.3 (3H, t, $-OCH_2-\underline{CH_3}$),




I R=$SCH_3$ IV R=$SCH_3$
II R=$SOCH_3$ V R=$SOCH_3$
III R=$SO_2CH_3$

**Figure 1. Fenamiphos and its metabolites.**

2.3 (3H, s, phenyl–$CH_3$), 2.55 (3H, s, –$SOCH_3$), 3.4 (1H, m, –$CH(CH_3)_2$), 4.2 (2H, m, –$OCH_2CH_3$), 4.9 (1H, m-NHR), 7.25 (2H, m, aromatic), 7.9 (1H, d, aromatic). MS (rel. int.): 320 ($M^+$, 68%).

(b) *Ethyl 3-methyl-4-(methylsulfonyl)phenyl (1-methylethyl)phosphoramidate (III).*—Fenamiphos (200 mg) was treated with 3 mL glacial acetic acid and 2 mL 30% hydrogen peroxide, and the mixture was heated on a water bath for 30 min. It was kept overnight at room temperature and then neutralized with 50 mL 10% sodium bicarbonate. The aqueous solution was extracted with chloroform and concentrated under vacuum to give a product that was further purified by preparative TLC to give sulfone as a viscous liquid. $R_f$ 0.38, $CHCl_3$–MeOH (99 + 1). $^1H$ NMR ($CDCl_3$): δ 1.15 (6H, d, –$CH(CH_3)_2$), 1.3 (3H, t, –$OCH_2CH_3$), 2.5 (3H, s, phenyl–$CH_3$), 2.93(3H, s, –$SO_2CH_3$), 3.45 (1H, m, –$CH(CH_3)_2$), 4.15 (2H, m, –$OCH_2CH_3$), 7.2 (2H, m, aromatic), 7.85 (1H, aromatic). MS (rel. int.): 336 ($M^+$, 5%).

(c) *3-Methyl-4-(methylthio)phenol (IV).*—Fenamiphos (300 mg) was treated with 10 mL 5% ethanolic sodium hydroxide solution and refluxed on a water bath for 1 h to give phenol, which crystallized from methanol as colorless crystals. $R_f$ 0.63, $CHCl_3$–$CH_3OH$ (99 + 1), mp 61–62°C. MS (rel. int.): 154 ($M^+$, 45%).

(d) *3-Methyl-4-(methylsulfinyl)phenol (V).*—3-Methyl-4-(methylthio)phenol (IV, 200 mg) solution in 20 mL methylenechloride was treated with 300 mg *m*-chloroperbenzoic acid at room temperature and allowed to stand overnight in a refrigerator. The reaction mixture was then filtered, the solvent was evaporated, and the viscous mass was subjected to column chromatography to obtain sulfoxide (V) (170 mg). $R_f$ 0.10, $CHCl_3$–MeOH (99 + 1). $^1H$ NMR ($CDCl_3$): δ 2.3 (3H, s, phenyl–$CH_3$), 3.75 (3H, s, –$SOCH_3$), 6.2 (1H, –OH, $D_2O$ exchangeable), 6.85 (2H, m, aromatic), 7.75 (1H, d, aromatic). MS (rel. int.): 170 ($M^+$, 10%).

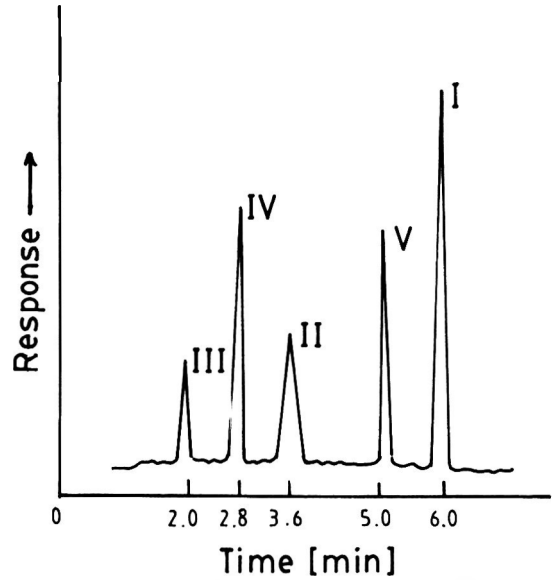



**Figure 2. Retention times of fenamiphos and its degradation products II, III, IV, and V by LC.**

### *Chromatographic Conditions*

The stationary phase was Lichrosorb RP-18 packed in a stainless steel column, 25 cm × 4.6 mm (Spectra Physics). The mobile phase was an isocratic mixture of methanol–water (4 + 1, v/v) maintained at a flow rate of 1 mL/min. UV detector was operated at a wavelength of 254 nm at a sensitivity of 0.04 AUFS. A 10 μL portion of sample was injected each time, and chromatograms were recorded at a chart speed of 1 cm/min. A microprocessor-controlled data system allowed the automatic calculation of detector response in terms of peak area.

### *Determination*

Under the reported chromatographic conditions, 500 ppm solutions of individual compounds were first injected and chromatographed to determine their retention times. Then, a mixture of all 5 compounds was injected.

Next, 50 mg portions of each of I, II, III, IV, and V were dissolved in 50 mL methanol and appropriately diluted to give 10, 20, 50, 100, and 500 ppm solutions. These solutions were used for calibration.

**Table 1. Relative retention times of fenamiphos metabolites**

| Structure[a] | Metabolite | Relative retention time, min |
|---|---|---|
| I | Ethyl 3-methyl-4-(methylthio)phenyl(1-methylethyl)phosphoramidate (fenamiphos) | 1 |
| II | Ethyl 3-methyl-4-(methylsulfinyl)phenyl(1-methylethyl)phosphoramidate | 0.33 |
| III | Ethyl 3-methyl-4-(methylsulfonyl)phenyl(1-methylethyl) phosphoramidate | 0.6 |
| IV | 3-Methyl-4-(methylthio)phenol | 0.46 |
| V | 3-Methyl-4-(methylsulfinyl)phenol | 0.83 |

[a] From Figure 1.

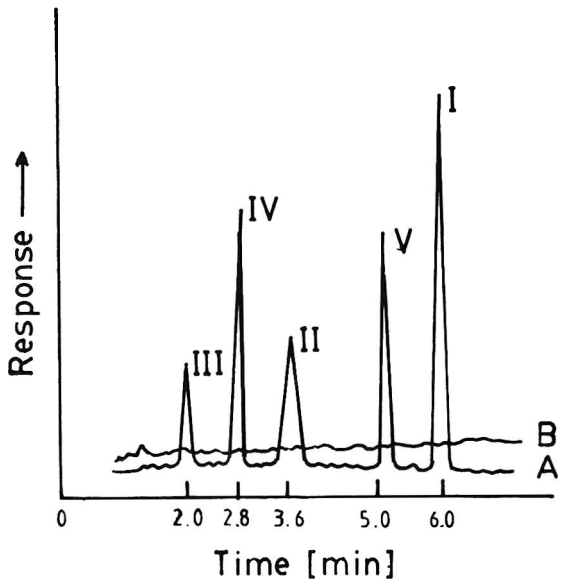



**Figure 3. Chromatogram of the soil extract: A, spiked with a mixture of I, II, III, IV, and V (25 ppm each); B, soil blank.**

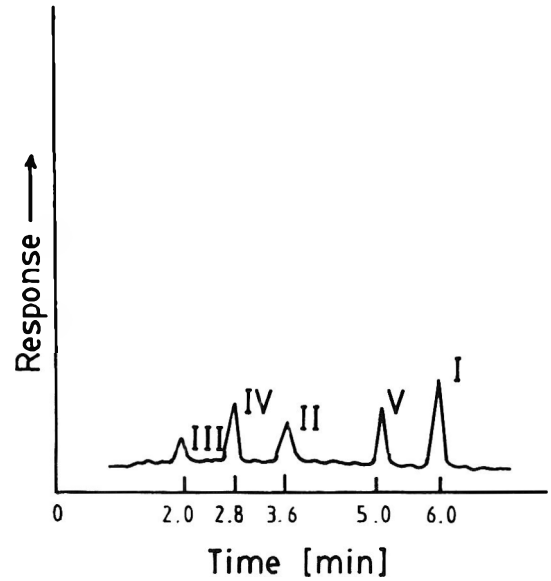



**Figure 4. Chromatogram of the soil extract spiked with a mixture of I, II, III, IV, and V (5 ppm each).**

The method was quantitatively validated by separately injecting 10, 20, 50, 100, and 500 ppm solutions of I, II, III, IV, and V in duplicate and measuring peak areas. Calibration graphs for all 3 compounds were prepared.

*Soil.*—The sample used for analysis was an alluvial soil (fine loam with a composition of 19% clay, 21% silt, 60% sand, and 0.35% organic carbon) having a pH of 7.2. A 10 g portion of this soil was treated with methanolic solutions containing 1, 2, or 4 mg of I, II, III, or IV and V, and then air dried for 1 h. Soil was extracted with 100 mL methanol by shaking on a wrist action shaker for 1 h, and then was filtered through Whatman No. 42 filter paper. A 25 mL aliquot of this extract, corresponding to 2.5 g soil, was evaporated under reduced pressure on a rotary evaporator at room temperature. The volume was adjusted to 10 mL. A 10 μL aliquot of this solution was injected and chromatographed following the injection of authentic samples of known concentrations of each component (standard). For simultaneous determination of all 5 compounds, 10 g soil fortified with a mixture (1, 2, or 4 mg) of each of the 5 compounds was extracted and chromatographed similarly. All treatments were done in triplicate.

The concentration of each compound in the extracted sample of soil was calculated by comparing the peak area of the sample with that of standard as follows.

$$C_i = A_i / K$$

where $C_i$ is the concentration of the component $i$; $A_i$ is the area of the peak corresponding to $i$; and $K$ = peak area of the standard/concentration of standard.

## Results and Discussion

Reversed-phase chromatography was chosen because of the ready solubility of the compounds in more polar solvents. The reported composition of the mobile phase, methanol–water (80 + 20), was chosen after various proportions of methanol, water, and acetonitrile were tried for optimum separation and sharp peaks. Under the reported conditions, fenamiphos sulfoxide (II) eluted first at 2 min, followed by its phenol (IV) at 2.8 min. Retention times were 3.6 min for fenamiphos sulfone (III) and 5 min for phenol sulfoxide (V). Fenamiphos (I) eluted last at 6 min. The identity of each component was established by injecting authentic samples of individual compounds. A chromatogram obtained by injecting a mixture of 10 μL standard solution containing 50 ppm each of I, II, III, IV, and V is shown in Figure 2. Table 1 shows the relative retention times of each component. Resolution of all 5 compounds was excellent. Calibration graphs obtained by plotting concentrations against peak areas were linear over the entire range of 10–500 ppm, with correlation coefficients of 0.96, 0.94, 0.97, 0.98, and 0.94 for I, II, III, IV, and V, respectively.

After LC conditions were optimized, the method was extended to the determination of fenamiphos and its 4 metabolites in fortified soil, first individually and then simultaneously. Soil was spiked with appropriate quantities of fenamiphos and its degradation products (mixture of I, II, III, IV, and V), extracted with methanol, and, after negligible cleanup, was analyzed by LC to simultaneously determine the concentration of all 5 compounds. The soil blank did not give any peak that interfered with these compounds. Figure 3 shows the chromatogram of soil extract spiked with a mixture of 5 compounds at 25 ppm; Figure 4 shows the chromatogram at the limit of detection (5 ppm of each compound). Results of the simultaneous determination of all 5 compounds in soil are given in Table 2. Recoveries ranged from 99.2 to 100.8%. Five-fold injections of 25, 50, and 100 ppm I, II, III, and IV and V were used to determine the standard deviation. Duplicate injections of each sample was the best way to obtain the desired precision at a 95%

**Table 2. Accuracy and precision of LC determination of fenamiphos and its metabolites**

| Structure[a] | Concn, ppm | Mean found, ppm | SD, ppm[b] | 95% Confidence interval, ppm | Relative mean error, % |
|---|---|---|---|---|---|
| I | 25 | 25.1 | 0.34 | 25.1 ± 0.2 | 0.96 |
| | 50 | 49.7 | 0.42 | 49.7 ± 0.4 | 0.78 |
| | 100 | 99.9 | 0.48 | 99.9 ± 0.4 | 0.40 |
| II | 25 | 24.9 | 0.30 | 24.9 ± 0.2 | 0.87 |
| | 50 | 49.6 | 0.42 | 49.6 ± 0.4 | 0.84 |
| | 100 | 99.6 | 0.64 | 99.6 ± 0.5 | 0.49 |
| III | 25 | 24.8 | 0.29 | 24.8 ± 0.2 | 0.97 |
| | 50 | 50.2 | 0.38 | 50.2 ± 0.3 | 0.64 |
| | 100 | 99.8 | 0.62 | 99.8 ± 0.3 | 0.31 |
| IV | 25 | 25.2 | 0.38 | 25.2 ± 0.3 | 1.2 |
| | 50 | 50.4 | 0.44 | 50.4 ± 0.4 | 0.84 |
| | 100 | 100.6 | 0.70 | 100.6 ± 0.6 | 0.58 |
| V | 25 | 25.1 | 0.40 | 25.1 ± 0.3 | 1.2 |
| | 50 | 49.8 | 0.49 | 49.8 ± 0.3 | 0.65 |
| | 100 | 100.1 | 0.50 | 100.1 ± 0.3 | 0.36 |

[a] From Figure 1.
[b] n = 5.

confidence interval. Hence, the accuracy and precision of this method for the determination of fenamiphos and its metabolites were adequate.

The lower limits of detection were also determined, and we found that a 10 μL injection of a 5 ppm solution gave a measurable peak for the least sensitive compound, fenamiphos sulfone (III), at 254 nm (signal-to-noise ratio ~ 3). At this level, fenamiphos (I), whose response is maximum at 254 nm, gives a signal-to-noise ratio of about 10.

The described LC method is simple, specific, and accurate for the determination of fenamiphos and its 4 important metabolites from alluvial soil. Because the method does not require any elaborate cleanup after extraction and the total run time by LC for each sample is less than 7 min, it is suitable for batch analysis.

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PESTICIDE FORMULATIONS

# Liquid Chromatographic Method for Determination of Cyanazine in Technical Products and Pesticide Formulations: CIPAC Collaborative Study

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Cyanazine, 2-[[4-chloro-6-(ethylamino)-1,3,5-triazin-2-yl]amino]-2-methylpropanenitrile, is a herbicide available as technical material, wettable powder, and suspension concentrate. A method for the determination of cyanazine was collaboratively studied by the Cyanazine Panel, and the report was prepared by the Pesticide Analytical Council-Great Britain for the Collaborative International Pesticides Analytical Council (CIPAC). In the method, cyanazine is determined by liquid chromatography (LC) using a normal-phase silica-based amino bonded phase column with a mobile phase of dichloromethane and 2-propanol, and UV detection at 254 nm.

## Collaborative Study

Of 11 participating laboratories, 10 successfully completed the analysis of 2 samples each of cyanazine technical material, wettable powder, and suspension concentrate. The study was based on replicate determinations performed on each of 2 different days. The method stipulates that duplicate injections for duplicate weighings should agree within ±1% relative to be acceptable. Both internal (2-nitrobenzenamine) and external calibrations are given to cover the potential interferences from cyanazine-related compounds (primarily simazine) and/or other interferences that might come from other sources such as the internal standard. To accommodate this approach, a system performance check is provided: Chromatograph the internal standard solution, the analytical standard solution, and a resolution check solution (cyanazine, 2-nitrobenzenamine and simazine) to determine if there are any interferences. If interferences are found, external calibration is used.

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Results of the study were presented at the 1983 CIPAC meeting in Brisbane, Australia.

The report has been evaluated by the General Referee and the Committee Statistician and reviewed by the Committee on Pesticide Formulations and Disinfectants. The method has been approved first action by the Official Methods Board at their May 1991 meeting. Association actions will be published in "Changes in Official Methods of Analysis" (1992) *J. Assoc. Off. Anal. Chem.* 75, January/February issue.

[1] A.R. Hanks is the AOAC General Referee on Pesticide Formulations: CIPAC Methods.

## Cyanazine in Technical Products and Pesticide Formulations—Liquid Chromatographic Method First Action 1991

### *CIPAC–AOAC Method*

Method Performance:
Technical product, 98%
$s_r = 0.2$; $s_R = 1.0$; $RSD_r = 0.2\%$; $RSD_R = 1.0\%$
Wettable powder, 50% (nominal)
$s_r = 0.3$; $s_R = 0.7$; $RSD_r = 0.5\%$; $RSD_R = 1.3\%$
Suspension concentrate, 45% (nominal)
$s_r = 0.4$; $s_R = 0.6$; $RSD_r = 0.8\%$; $RSD_R = 1.4\%$

### *A. Principle*

Cyanazine is dissolved in dichloromethane containing 2-nitrobenzenamine as internal standard, separated on 10 μm amino bonded phase column, and determined by peak area measurement.

### *B. Apparatus*

(a) *Liquid chromatograph.*—With constant flow (pulse free) pump and 10 μL sample loop, and able to generate more than 870 psi. Operating conditions: mobile phase flow rate ca 1.2 mL/min; detector sensitivity to obtain peak height 80–90% full scale for major peak; ambient temperature ±2°C (up to 30°C). Retention times: 2-nitrobenzenamine, 3.5 min; cyanazine, 5.6 min; and simazine, (efficiency test) 5.0 min. Number of theoretical plates (cyanazine) 4460.

(b) *Chromatographic column.*—Stainless steel, 250 × 4.6 mm, packed with amino bonded phase, 10 μm particle size (Lichrosorb-$NH_2$ is suitable). Capable of separating cyanazine from simazine (see *F. System Performance Check*). Column should be dedicated to normal phase use to assure performance.

(c) *Detector.*—UV at 254 nm.

(d) *Electronic integrator.*—Preferred for peak area measurement.

(e) *Recorder.*—Range to match output of LC detector.

### C. Reagents

(a) *Reference standards.*—Cyanazine (E.I. du Pont de Nemours & Co., Inc., Agricultural Products Dept, Walker's Mill, Barley Mill Plaza, Wilmington, DE 19898) and simazine (Ciba-Geigy Corp., Agricultural Div., PO Box 18300, Greensboro, NC 27419).

(b) *2-Nitrobenzenamine.*—Analytical reagent grade, free from impurities that co-elute with cyanazine (Fisher Scientific, Pittsburgh, PA 15219, or equivalent). (Caution: 2-Nitrobenzenamine is a highly toxic chemical. Avoid skin contact or breathing dust. Use suction bulb to pipet solutions.)

(c) *Dichloromethane (DCM).*—LC grade; <0.02% water.

(d) *2-Propanol.*—LC grade; <0.02% water.

(e) *Mobile phase.*—DCM–2-propanol (99 + 1). Mix 990 mL DCM and 10 mL 2-propanol. Degas before use.

### D. Preparation of Standard Solutions

(a) *Internal standard solution.*—0.5 mg 2-nitrobenzenamine/mL. Weigh ca 0.25 g 2-nitrobenzenamine into 500 mL volumetric flask, dilute to volume with DCM, and mix (Solution I). Pipet (use suction bulb) 25 mL Solution I into 50 mL volumetric flask, dilute to volume with DCM, and mix (Solution N).

(b) *Internal calibration standard solutions.*—Into each of four 100 mL volumetric flasks, accurately weigh 60, 80, 120, and 140 mg cyanazine reference standard. Accurately pipet 50 mL Solution I into each flask, dilute to volume with DCM, and mix (Solutions CA, CB, CC, and CD, respectively). Similarly, weigh ca 70 mg cyanazine standard into 50 mL volumetric flask, dilute to volume with DCM, and mix (Solution CO).

(c) *External calibration standard solutions.*—Proceed as in (b) but omit Solution I and preparation of Solution CO.

(d) *Column efficiency test solution.*—Into 50 mL volumetric flask, weigh ca 50 mg cyanazine, 13 mg 2-nitrobenzenamine, and 3 mg simazine reference standard, dilute to volume with DCM, and mix (Solution X).

### E. Preparation of Samples

(a) *Technical formulations.*—Accurately weigh amount of sample containing ca 0.1 g cyanazine into each of two 100 mL volumetric flasks. Accurately pipet 50 mL Solution I into each flask, dilute to volume with DCM, and mix (Solutions SA and SB). Filter through 5 μm filter. Prepare a single sample in same manner, substituting DCM for Solution I (Solution SO). (**Note**: If external calibration is used, prepare duplicate Solutions SO and use them in place of Solutions SA and SB.)

(b) *Wettable powders.*—Accurately weigh amount of sample containing ca 0.1 g cyanazine into each of two 150 mL glass-stoppered Erlenmeyer flasks. Accurately pipet 50 mL Solution I and 50 mL DCM into each flask. Stopper flasks and shake 10 min on mechanical shaker. Filter through 0.45 μm filter and continue as in (a), beginning, "Prepare a single sample...."

(c) *Suspension concentrates.*—Accurately weigh amount of sample containing ca 0.1 g cyanazine into each of two 150 mL glass-stoppered Erlenmeyer flasks. Accurately pipet 50 mL Solution I and 50 mL DCM into each flask, and add 5 g anhydrous $Na_2SO_4$ to each. Stopper flasks and shake by hand until formulation is partially dispersed. Shake additional 30 min on mechanical shaker, let settle, and filter through 0.45 μm filter. Continue as in (a), beginning, "Prepare a single sample...."

### F. System Performance Check

Using instrument conditions in *B*(a), inject 10 μL Solution CD and adjust instrument parameters to give height of major peak 80–90% full scale. Inject 10 μL of Solutions X, N, CO, and SO, and check chromatograms for interfering peaks; if any are present, external calibration, *G*(b), must be used. Solution X should exhibit baseline separation of the 3 compounds at about elution times in *B*(a).

### G. Determination

(a) *By internal calibration.*—Make duplicate injections of standard Solutions CA, CB, CC, and CD, and measure peak areas for cyanazine and 2-nitrobenzenamine. Determine response ratio (*R*) of each solution (area cyanazine/area 2-nitrobenzenamine). (**Note:** Method assumes constant volume injections.) Construct calibration curve to relate mean values of *R* to concentration of cyanazine (mg/mL) in each solution. Calibrate instrument at least once daily. Inject standard and sample solutions in following sequence CA, SA, SA, SB, SB, CB, etc. (**Note:** Bracket each group of 4 samples with injections of the 2 calibration solutions closest in concentration to the 4 samples.) Calculate *R* for each injection and read cyanazine concentration from each graph. For each subsequent injection of calibration solutions, calculate a calibration correction factor (*F*) as follows:

$$F = \text{initial value of } R \text{ /new value of } R$$

For each pair of calibration solution injections that bracket a group of 4 sample injections, multiply the value of *R* by [(*FA* + *FB*)/2] and read cyanazine concentration from calibration curve. If calibration correction factors *FA* and *FB* of each pair of calibration solutions differ by >2%, repeat both calibration and sample solution injections. If mean calibration correction factor [(*FA* + *FB*)/2] falls outside 0.95–1.05 range, stabilize operating conditions and recalibrate before proceeding with analysis.

(b) *By external calibration.*—If interfering peaks are noted in *System Performance Check*, **F**, construct curve that relates mean peak areas to cyanazine concentration and proceed as in (a), using peak areas instead of response ratios.

### H. Calculations

Calculate cyanazine as follows:

$$\text{Cyanazine, \%} = (C \times V \times P)/(W \times 1000)$$

where $C$ = cyanazine concentration in sample solution, mg/mL; $V$ = volume containing sample, mL; $P$ = % purity of standard; and $W$ = sample taken for analysis, g.

Ref.: JAOAC **75**, January/February issue (1992)
CAS-21725-46-2 (cyanazine)

**Table 1. Statistical analysis of collaborative results for LC determination of cyanazine in technical and formulated products[a]**

| Sample No. | Product | No. laboratories | Mean, % | $s_r$ | $s_R$ | $RSD_r$, % | $RSD_R$, % |
|---|---|---|---|---|---|---|---|
| 1 | Technical | 9 | 98.4 | 0.9 | 0.9 | 0.9 | 0.9 |
| 2 | Technical | 9 | 97.9 | 0.2 | 1.0 | 0.2 | 1.0 |
| 3 | Wettable powder | 8 | 51.0 | 0.3 | 0.7 | 0.5 | 1.3 |
| 4 | Wettable powder | 8 | 51.4 | 0.3 | 0.4 | 0.6 | 0.7 |
| 5 | Suspension conc. | 9 | 44.0 | 0.4 | 0.6 | 0.8 | 1.4 |
| 6 | Suspension conc. | 9 | 43.5 | 0.4 | 0.5 | 0.9 | 1.1 |

[a] Cyanazine technical, wettable powder, and suspension concentrate formulations.

## Results and Recommendation

Although both peak area and peak height measurements were used in the CIPAC collaborative study (separate measurements for each), only the use of peak area is recommended for inclusion in the method when adopted by AOAC because method performance is better using area measurements, and electronic area integrators are available in most laboratories. The statistical parameters generated by analysis of the data according to AOAC protocols indicate acceptable within- and between-laboratory precision (Table 1).

The General Referee recommends that the method be adopted first action as a CIPAC-AOAC method.

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PESTICIDE FORMULATIONS

# Titrimetric Determination of Maneb in Formulations Containing Fentin Acetate or Fentin Hydroxide: CIPAC Collaborative Study

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Maneb, [[1,2-ethanediylbis[carbamodithioato]]-(2-)] manganese, is an agricultural fungicide frequently used in The Netherlands as a maneb–fentin mixture. In this method, maneb is decomposed by a mixture of hydriodic and acetic acids, liberating carbon disulfide, which is passed by an air stream through lead acetate scrubbers to remove hydrogen sulfide. The carbon disulfide is then reacted in an absorber containing methanolic potassium hydroxide to form methyl xanthate. The xanthate solution is neutralized with dilute acetic acid and titrated with standard iodine solution to determine the original maneb concentration in the sample.

Although the analysis of dithiocarbamates via acid liberation, with subsequent trapping of carbon disulfide, has been used for many years, it was only recently observed that fentin acetate, as a coformulated active ingredient, could cause the incomplete recovery of maneb.

## Collaborative Study

An earlier CIPAC collaborative study reported low maneb recovery when in the same formulation with fentin, and this was subsequently confirmed in a limited study done by the Dithiocarbamate Panel. A study done by the Fentin Panel under the Dutch Pesticide Analytical Council (DUPAC) and reported at the 1985 CIPAC Symposium, again confirmed that fentin caused a decrease in analyzed maneb content. A collaborative study was then initiated with 17 laboratories representing 8 countries, and included the following samples: maneb wettable powder (80%), maneb–fentin acetate wettable powder (62%/4%), maneb–fentin acetate wettable powder (33%/11%), maneb–fentin hydroxide wettable powder (34%/10.5%), and maneb–fentin acetate suspension concentrate (34%/11%). The participants in the study were instructed to become familiar with the method by analyzing the maneb wettable powder until duplicate analyses were within 1% or better. Because maneb formulations can decrease in concentration upon storage, the participants were instructed to store the samples below 5°C and to keep sample bottles purged with nitrogen.

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The report has been evaluated by the General Referee and Committee Statistician and reviewed by the Committee on Pesticide Formulations and Disinfectants. The method has been approved first action by the Official Methods Board at their May 1991 meeting. Association actions will be published in "Changes in Official Methods of Analysis" (1992) *J. Assoc. Off. Anal. Chem.* **75**, January/February issue.

[1]A.R. Hanks is the AOAC General Referee on Pesticide Formulations: CIPAC Methods.

## Maneb in Pesticide Formulations Containing Fentin Acetate or Fentin Hydroxide—Carbon Disulfide Evolution Method First Action 1991

### *CIPAC–AOAC Method*

(Caution: See safety notes on acids and carbon disulfide.)

Method Performance:
Wettable powder, 62% (4% fentin acetate)
$s_r = 0.42$; $s_R = 1.11$; $RSD_r = 0.69\%$; $RSD_R = 1.79\%$
Wettable powder, 35% (11% fentin acetate)
$s_r = 0.20$; $s_R = 0.75$; $RSD_r = 0.58\%$; $RSD_R = 2.15\%$
Wettable powder, 35% (10.5% fentin hydroxide)
$s_r = 0.29$; $s_R = 0.67$; $RSD_r = 0.84\%$; $RSD_R = 1.93\%$
Suspension concentrate, 25% (11% fentin acetate)
$s_r = 0.20$; $s_R = 0.56$; $RSD_r = 0.80\%$; $RSD_R = 2.25\%$

### *A. Principle*

Maneb is decomposed by mixture of hydriodic and acetic acids, and liberated carbon disulfide is passed by air stream through lead acetate scrubbers to remove hydrogen sulfide. Carbon disulfide is then reacted in absorber containing methanolic potassium hydroxide to form methyl xanthate. Xanthate solution is neutralized with dilute acetic acid and titrated with standard iodine solution.

### *B. Apparatus and Reagents*

(a) *Carbon disulfide evolution system.*—(See Figure 1). With ground-glass joints, which must be absolutely gas tight. [Notes: (*1*) Do not use rubber connections. (*2*) Procedure should be performed in fume hood. (*3*) Use silicone grease only in small amounts.]

(b) *Hydriodic acid.*—57%.

(c) *Acetic acid.*—Glacial.

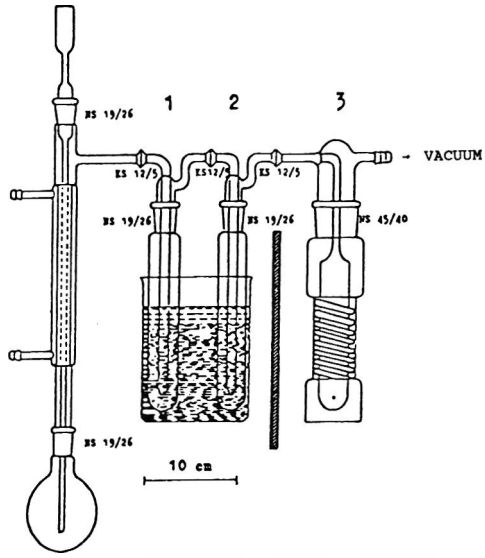


**Figure 1. Carbon disulfide evolution apparatus. Diameter of apertures: bottom end inner tube of condenser, 3 mm; inner tube of absorbers 1 and 2, 1.5 mm; side and bottom of absorber 3, 2 mm.**

(d) *Acetic acid solution.*—10%, v/v.

(e) *Digestion solution.*—Dilute 100 mL hydriodic acid to 1 L with glacial acetic acid. Freshly prepared solution can be used for 1 month. Solution may contain free iodine.

(f) *Lead (II) acetate aqueous solution.*—100 g/L.

(g) *Methanolic potassium hydroxide solution.*—0.5N. Dissolve 28 g KOH (or 33 g 85% KOH pellets) in anhydrous methanol.

(h) *Phenolphthalein indicator solution.*—0.1%.

(i) *Starch indicator solution.*—1%. See **922.03A**(f).

(j) *Standardized iodine solution.*—0.1N. Store in amber container to shield from light and restandardize as needed. See **939.13**.

(k) *Sodium diethyldithiocarbamate · trihydrate (NaDEC).*—Check purity as follows: Dissolve ca 0.5 g in 100 mL $H_2O$ and titrate directly with standard iodine solution (0.1N) using starch indicator; 1 mL 0.1N iodine solution = 0.02253 g NaDEC · $H_2O$.

### C. Preparation of Carbon Disulfide Evolution System

Place 50 mL lead acetate in each scrubber (absorbers 1 and 2) and 100 mL methanolic KOH solution in final absorber. Keep methanolic absorber cool by immersing it in beaker of melting ice. Turn on condenser water, heat water bath surrounding hydrogen sulfide scrubbers to ca 90°C, and maintain this temperature throughout determination. Apply slight suction from controlled vacuum supply to adjust air flow to ca 100 mL/min. To check both apparatus and reagents, perform entire procedure with NaDEC as test substance. Recoveries between 99.5 and 100.5% indicate satisfactory performance.

### D. Determination

In weighing boat, weigh (to nearest 0.1 mg) enough sample to contain ca 0.25 g maneb into dry round-bottom flask (see Figure 1). Avoid spilling sample on neck of flask; do not rinse or add water. Add a few glass beads and connect flask to condenser. Add 40 mL digestion solution through inner tube and swirl. Use heating jacket to bring contents of flask to boiling point. (Heat capacity of acetic acid is lower than that of water, so decomposition temperature is reached faster.) Control heating carefully until reaction mixture boils at steady rate. Do not let reaction mixture rise up inlet tube. Apply suction from vacuum to adjust level. Maintain moderate reflux for 30 min. Remove heating jacket, disconnect carbon disulfide absorber, and carefully wash contents into 600 mL beaker using ≤400 mL $H_2O$. Add 2 drops of phenolphthalein indicator solution and, under magnetic stirring, neutralize solution with dilute acetic acid. Then, add 3 drops in excess. Add 2.5 mL starch indicator solution, and immediately titrate with standard iodine solution until blue color persists for 1 min ($t$ mL). Perform blank determination, omitting sample ($b$ mL). To avoid end point fading in blank determination, transfer neutralized contents of carbon disulfide absorber to previously completed titration solution (in 1 L beaker), which is just faintly blue to starch indicator.

### D. Calculations

Calculate maneb as follows:

$$\% \text{ maneb} = [(t - b) \times N \times 13.266]/w$$

where $t$ = volume of iodine solution used for sample, mL; $b$ = volume of iodine solution used for blank, mL; $N$ = normality of iodine solution; and $w$ = sample weight, g.

Ref.: JAOAC **75**, January/February issue (1992)

CAS-12427-38-2 (maneb)

CAS-900-95-8 (fentin acetate)

CAS-76-87-9 (fentin hydroxide)

**Table 1. Statistical analysis of collaborative results for determination of maneb in formulations[a] containing fentin acetate or fentin hydroxide using carbon disulfide evolution with hydriodic–acetic acid mixture**

| Product | No. laboratories | Mean, % | $S_r$ | $S_R$ | $RSD_r$, % | $RSD_R$, % |
|---|---|---|---|---|---|---|
| M/FA-WP, 62%/4% | 15 | 62.42 | 0.42 | 1.11 | 0.69 | 1.79 |
| M/FA-WP, 35%/11% | 14 | 34.59 | 0.20 | 0.75 | 0.58 | 2.15 |
| M/FH-WP, 35%/10.5% | 15 | 34.75 | 0.29 | 0.67 | 0.84 | 1.93 |
| M/FA-SC, 25%/1[illegible]% | 15 | 24.97 | 0.20 | 0.56 | 0.80 | 2.25 |

[a] Maneb–fentin acetate wettable powder, maneb–fentin hydroxide wettable powder, and maneb–fentin acetate suspension concentrate formulations.

## Results and Recommendation

Results of the collaborative study were presented by The Netherlands representative to CIPAC, A. Martijn (Plantenziektenkundige Dienst, Postbus 9102, 6700 HC Wageningen, The Netherlands), at the 31st CIPAC meeting, 1987, in Cascais, Portugal (CIPAC Report 3368/R). Performance parameters, calculated according to ISO 5725, are shown in Table 1.

The General Referee recommends adoption first action of the CIPAC method for maneb in the presence of fentin acetate or hydroxide, as a CIPAC-AOAC method.

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A. Stevenson, Robinson Bros, West Bromwich, England

PESTICIDE FORMULATIONS

# Determination of Pirimiphos-Methyl in Technical Materials and Pesticide Formulations by Gas Chromatography: CIPAC Collaborative Study

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Pirimiphos-methyl, *O*-[2-(diethylamino)-6-methyl-4-pyrimidinyl] *O*,*O*-dimethyl phosphorothioate, is a broad spectrum contact and fumigant insecticide available as technical material, wettable and dustable powders, emulsifiable and aerosol concentrates, hot fog, and ultra-low volume (ULV) formulations. To analyze these formulations, the sample was dissolved in chloroform containing *n*-octadecane as the internal standard, and the concentration of pirimiphos-methyl was determined by gas chromatography (GC) using an SE-30 GC column and a flame ionization detector. Analyte to internal standard peak area ratios were used in the calculation of pirimiphos-methyl content.

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The report has been evaluated by the General Referee, Committee Statistician, and Committee Safety Advisor and reviewed by the Committee on Pesticide Formulations and Disinfectants. The method has been approved first action by the Official Methods Board at their May 1991 meeting. Association actions will be published in "Changes in Official Methods of Analysis" (1992) *J. Assoc. Off. Anal. Chem.* **75**, January/February issue.

[1]A.R. Hanks is the AOAC General Referee on Pesticide Formulations: CIPAC Methods.

## Collaborative Study

Two collaborative studies have been performed by the Substituted Pyrimidine Panel, Pesticide Analytical Council, Great Britain. In the first study, 15 laboratories analyzed 2 samples each of technical material (90%), 50% emulsifiable concentrate, and 1 and 2% dustable powders on 2 separate days. The results for the technical materials and one emulsifiable concentrate were not considered satisfactory, possibly due to small sample size and nonhomogeneity caused by temperature changes during transportation and storage. The second study,

specifying low temperature storage and attention to complete homogenization of the samples before use, was conducted by 12 laboratories using the same method as employed in the first study. Collaborators analyzed 2 samples each of technical material, 25 and 50% emulsifiable concentrate, 40% wettable powder, 50% ULV formulation, 65% aerosol concentrate, and 10% fogging formulation on 2 separate days. In either study, no significant differences were found between results for days 1 and 2.

## Pirimiphos-Methyl in Technical Products and Pesticide Formulations—Gas Chromatographic Method
## First Action 1991

### *CIPAC–AOAC Method*

(Pirimiphos-methyl is a cholinesterase inhibitor. Avoid contact with skin and eyes, and ingestion and inhalation of dust or vapor. Wear a laboratory coat, gloves, and safety glasses. Work in an efficient fume hood if possible. Dispose of unwanted materials to avoid accidental contamination.)

Method Performance:
Technical product, 89–91%
$s_r$ = 0.8; $s_R$ = 1.8; $RSD_r$ = 0.8%; $RSD_R$ = 2.0%
Wettable powder, 40% (nominal)
$s_r$ = 0.1; $s_R$ = 1.1; $RSD_r$ = 0.4%; $RSD_R$ = 2.7%
Emulsifiable concentrate, 49% (nominal)
$s_r$ = 0.5; $s_R$ = 0.8; $RSD_r$ = 1.0%; $RSD_R$ = 1.6%
Emulsifiable concentrate, 26% (nominal)
$s_r$ = 0.1; $s_R$ = 0.9; $RSD_r$ = 0.2%; $RSD_R$ = 3.6%
Ultra low volume formulation, 52% (nominal)
$s_r$ = 0.4; $s_R$ = 1.3; $RSD_r$ = 0.9%; $RSD_R$ = 2.6%
Aerosol concentrate, 65% (nominal)
$s_r$ = 0.6; $s_R$ = 2.3; $RSD_r$ = 0.9%; $RSD_R$ = 3.5%
Hot fogging concentrate, 12% (nominal)
$s_r$ = 0.2; $s_R$ = 0.5; $RSD_r$ = 1.4%; $RSD_R$ = 4.2%
Dustable powder, 1–2%
$s_r$ = 0.04; $s_R$ = 0.1; $RSD_r$ = 4.3%; $RSD_R$ = 6.5%

### *A. Principle*

Sample is dissolved in $CHCl_3$ containing *n*-octadecane as internal standard, and 1 μL is injected into GC system equipped with flame ionization detector. Peak areas are measured for pirimiphos-methyl and octadecane and compared with those from standard injection.

### *B. Apparatus*

(**a**) *Gas chromatograph.*—With flame ionization detector. Temperatures: column 215°C, injection port 240°C, detector 295°C; gas flows: nitrogen carrier gas 50 mL/min, adjust hydrogen and air flows as recommended by detector manufacturer; sample size 1.0 μL; retention times: internal standard 3.5 min, pirimiphos-methyl 5.1 min. Adjust parameters to ensure complete separation of peaks and peak heights ca 75% full scale on chart at retention times of interest.

(**b**) *Integrator.*—Automatic digital or chromatographic data system.

(**c**) *Column.*—Glass, 1.5 m × 4 mm id, packed with acid washed and silanized diatomaceous earth coated with 10% silicone elastomer (10% SE-30 on Chromosorb W-HP 100/200 mesh, Alltech Associates, Inc., Applied Science Labs, Deerfield, IL 60077, Cat. No. 8520 is suitable). With detector end of column disconnected, precondition column overnight at 300°C, continuously purging with nitrogen. After conditioning, pretreat column with three 10 μL injections of active site derivatizing agent (e.g., Silyl-8) at 1 min intervals before reconnecting column.

### *C. Reagents*

(*Caution*: Chloroform is a suspected carcinogen. All operations in which chloroform is used to prepare analytical solutions, e.g., extracting, pouring, dispensing, and diluting, should be conducted in an efficient fume hood. Clean glassware and dispose of unwanted solutions in a manner that prevents release of chloroform into general working areas.)

(**a**) *Octadecane internal standard solution.*—Weigh ca 2 g *n*-octadecane (Eastman Organic Chemicals, 1187 Ridge Rd W, Rochester, NY 14650, Cat. No. 4825), dissolve in $CHCl_3$, and dilute to 1 L. Inject 1 μL portion into LC system to check for interfering compounds. Store solution tightly capped to avoid evaporation (Solution I).

(**b**) *Pirimiphos-methyl standard solution.*—Equilibrate to room temperature pirimiphos-methyl standard of known purity (ICI Americas, Inc., PO Box 208, Goldsboro, NC 27530). Be sure that standard is homogeneous and free from crystals. Accurately weigh ca 200 mg equilibrated standard into 100 mL flask; add 25 mL internal standard solution by buret (or pipet), stopper, and shake to dissolve. Store solution tightly capped to avoid evaporation (Solution C). Prepare similar solution using 25 mL $CHCl_3$ in place of internal standard solution (Solution $C_0$).

### *D. Determination*

(**a**) *Technical and liquid products.*—Equilibrate sample to room temperature and mix thoroughly to ensure homogeneity. Accurately weigh, in duplicate, amount of sample containing ca 200 mg pirimiphos-methyl into separate 100 mL flasks; add 25 mL internal standard solution by buret (or pipet) to each, stopper, and shake to dissolve (Solutions SA and SB). Prepare similar amount of sample using 25 mL $CHCl_3$ in place of internal standard solution (Solution $SA_0$).

(**b**) *Wettable and dustable powders.*—Accurately weigh, in duplicate, amount of sample containing ca 200 mg pirimiphos-methyl into separate 100 mL flasks; add 25 mL internal standard solution by buret (or pipet) to each, stopper, and shake to dissolve. Let solid matter settle; chromatograph supernatant liquid (Solutions SA and SB). Prepare similar amount of sample using 25 mL $CHCl_3$ in place of internal standard solution (Solution $SA_0$).

Inject 3 or more portions of pirimiphos-methyl standard solution to obtain integration parameters and stabilize instrument. Monitor response factor until results agree within ±0.5%. Inject

**Table 1. Statistical analysis of collaborative results for GC determination of pirimiphos-methyl in technical and formulated products**

| Product | No. laboratories | Mean, % | $s_r$ | $s_R$ | $RSD_r$, % | $RSD_R$, % |
|---|---|---|---|---|---|---|
| Technical, 90% | 15 | 91.03 | 0.75 | 1.80 | 0.83 | 1.97 |
| Technical, 90% | 15 | 90.71 | 0.82 | 1.64 | 0.91 | 1.81 |
| Technical, 90% | 12 | 90.14 | 0.28 | 1.69 | 0.31 | 1.87 |
| Wettable powder, 40% | 12 | 40.22 | 0.14 | 1.08 | 0.35 | 2.69 |
| Dustable powder, 1% | 15 | 0.91 | 0.03 | 0.05 | 3.75 | 6.01 |
| Dustable powder, 1% | 15 | 0.96 | 0.06 | 0.04 | 4.25 | 6.51 |
| Dustable powder, 2% | 15 | 1.78 | 0.10 | 0.02 | 1.28 | 5.61 |
| Dustable powder, 2% | 15 | 1.90 | 0.10 | 0.04 | 1.93 | 5.49 |
| Emulsifiable conc., 25% | 12 | 25.75 | 0.06 | 0.94 | 0.22 | 3.63 |
| Emulsifiable conc., 50% | 12 | 48.78 | 0.33 | 0.68 | 0.68 | 1.39 |
| Emulsifiable conc., 50% | 15 | 48.31 | 0.44 | 0.55 | 0.92 | 1.14 |
| Emulsifiable conc., 50% | 15 | 48.84 | 0.48 | 0.79 | 0.99 | 1.61 |
| Aerosol conc., 65% | 12 | 64.31 | 0.58 | 2.25 | 0.90 | 3.50 |
| Hot fog, 10% | 12 | 12.60 | 0.17 | 0.53 | 1.38 | 4.20 |
| Ultra-low volume, 50% | 12 | 51.39 | 0.44 | 1.34 | 0.85 | 2.61 |

1 μL portions of Solutions I, $C_o$, and $SA_o$ and examine chromatograms for interfering peaks caused by impurities. Inject 1 μL portions of standard solution (C) and sample solutions (SA and SB) in sequence: $C_1$, $SA_1$, $SA_2$, $C_2$, $SB_1$, $SB_2$, and $C_3$.

Calculate response factor, $R$, for each injection as follows:

$$R = \text{area pirimiphos-methyl peak/area internal standard peak}$$

Calculate pirimiphos-methyl in sample as follows:

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

where $R$ and $R'$ = average response factor for sample and standard solutions, respectively; $W$ and $W'$ = mg sample and standard, respectively; and $P$ = purity (%) of standard.

Ref.: JAOAC **75**, January/February issue (1992)

CAS-29232-93-7 (pirimiphos-methyl)

## Results and Recommendation

The results of both studies were presented at the 28th CIPAC (Collaborative International Pesticides Analytical Council) meeting in Baltimore, Maryland, by M.J. Edwards (ICI Plant Protection Division, Yalding, Kent ME18 6HN, England, CIPAC documents 3174M and 3173R). The statistical parameters for these results were analyzed by Peter D. Bland (ICI Agricultural Products, Western Research Center, 1200 S. 47th St, Box 4023, Richmond, CA 94804-0023) according to AOAC guidelines, and the performance parameters are provided in Table 1. Similar parameters were obtained for like samples in the 2 studies. However, the parameters for the ULV sample were noted to be high relative to the emulsifiable concentrate samples of similar concentration and even higher than those of the technical material; this may be attributable to sample nonhomogeneity.

The General Referee recommends the method be adopted first action as a CIPAC-AOAC method.

## Acknowledgments

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## TECHNICAL COMMUNICATIONS

# Development of a Deer Rapid Identification Field Test (DRIFT) by Modified Agar-Gel Immunodiffusion

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**A deer rapid identification field test (DRIFT) has been developed for detection of deer meat (venison) in a wide variety of meat products. The test is an adaptation of previously developed field screening immunodiffusion tests for detection of other individual species. DRIFT was demonstrated to be specific, sensitive, and accurate in the analysis of 100 samples. This test is especially well suited for use by state officials in charge of game regulations.**

A deer rapid identification field test (DRIFT) has been developed as a screen test for detection of deer tissue substitution or adulteration in any raw whole, ground, or emulsified meat product. The test employs an agar-gel immunodiffusion plate with a printed template for accurate placement of stabile freeze-dried reagent paper discs and sample discs saturated in meat tissue fluid. This basic method was established and described in previous publications on the overnight rapid bovine identification test (ORBIT) (1), poultry rapid overnight field identification test (PROFIT) (2), porcine rapid identification method (PRIME) (3), serological ovine field test (SOFT) (4), and rapid equine serological test (REST) (5). Subsequent to a collaborative study (6), the method was adopted AOAC official final action (7). This paper reports a further adaptation of the method, requiring suitable reagent modifications for detection or verification of the presence of deer tissue in any raw meat product.

## Experimental

### *Reagent Modifications*

Prepare reference anti-deer antibody discs by impregnating blank filter paper discs (BBL No. 31039, Becton, Dickinson and Co., Cockeysville, MD 21030) with 40 μL goat anti-white-tailed deer serum (No. 5116-1381, Organon Teknika-Cappel, Durham, NC 27704). Prepare deer reference antigen discs by impregnating additional blank paper discs with 40 μL white-tailed deer serum albumin Fraction V (Environmental Diagnostics, Inc., Burlington, NC 27215) at 0.1% concentration in phosphate-buffered saline, pH 7.2. Let both sets of discs absorb their reagents and freeze-dry overnight as previously described (1). For weak antiserum, recharge antibody discs with 40 μL goat anti-deer serum, and relyophilize. Prepare immunodiffusion plates as previously described (1) with the following modifications for agar preparation: substitute Oxoid purified agar (Code L-28) for Difco purified agar, which is no longer in production. For 1 L agar, add 10 g agar to 975.71 mL buffered saline and dissolve until clear on a hot plate. Substitute Thiazin red R stain (No. 88370, Fluka Chemical Corp., Ronkonkoma, NY 11779) for Lanaperl fast pink dye. Prepare a 2.0% (aqueous) stock solution of Thiazin red R stain and sterilize through a 0.45 μm filter (Millipore Corp., Bedford, MA 01730). Add 14.29 mL stock stain solution to 985.71 mL clear agar on the hot plate, mix, and filter stained agar under vacuum through a single layer of Miracloth (Calbiochem-Behring, La Jolla, CA 92037) before sterilization in autoclave. Final Thiazin red R concentration in agar is 1:3500, which distinguishes DRIFT from ORBIT, PROFIT, PRIME, SOFT, and REST plates of other colors. Cool sterile stained agar to 60°C. From a sterile stock 5.0% (aqueous) merthiolate solution, add sufficient quantity to effect a final concentration of 1:10 000.

### *Reaction Characteristics*

Specificity (Table 1) for DRIFT was assessed by reacting blank paper sample discs saturated in strictly homologous (white-tailed deer) and heterologous species meat tissue fluids against reference antibody discs. Heterologous tissues included some species originating within the *Cervidae* (deer-related) family for which positive detection was desirable.

Sensitivity, as applied to ground meat mixtures, was assessed by testing prepared sample composites of known amounts of white-tailed deer or New Zealand red deer added to ground red meat base tissues. Three replicates were tested at each adulterant level (1–22% by weight). Presence of a visible sample immunoprecipitin band that completely fused with the reference band was taken as evidence of detection at a given percentage level of adulterant.

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**Table 1. Specificity of DRIFT performed on ground and whole muscle tissues of various species using goat anti-white-tailed deer reagent discs**

| Species | Drift plate reaction[a] |
|---|---|
| Equine | - |
| Bovine | - |
| Pig | - |
| Ovine | - |
| Chicken | - |
| Turkey | - |
| Red kangaroo | - |
| White-tailed deer | + |
| Mule deer | + |
| Red deer (New Zealand) | + |
| American elk | + |
| Moose | + |
| Reindeer | + |

[a] +, Sample produced immunoprecipitin line of identity (complete fusion) with deer reference line; –, sample produced no reaction.

### *Shelf Stability*

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

### *Sample Analysis*

To determine the accuracy and reliability of DRIFT, 65 meat samples of a wide variety were first analyzed using the AOAC method (7) with reagent modifications for deer detection described above. Thirty-five additional unknown samples were then analyzed in a blind, in-house laboratory trial (Table 2). Species origins of all 100 samples were confirmed by the Ouchterlony agar-gel immunodiffusion technique (8).

## Results and Discussion

Specificity determinations using reference antibody discs prepared with goat anti-white-tailed deer are shown in Table 1. Even though the antiserum used to develop DRIFT was produced using whole white-tailed deer serum as the immunogen, the test detected and gave the desired positive reactions for all other deer-related muscle tissues examined within the *Cervidae* family. These included mule deer, New Zealand red deer, American elk, moose, and reindeer. Unrelated red meat and poultry tissues all produced negative reactions, as expected.

Sensitivity determinations indicated that white-tailed deer was detectable at the 3% level in either beef or pork base tissue, while New Zealand red deer was found to be detectable at 10% in beef base tissue and 7.5% in pork base tissue (data not shown). These sensitivity limits (3–10%) are comparable to levels measured for similar previously developed tests for detection of other species (1–6). These limits are considered adequate for screening tests of this nature.

**Table 2. Results of DRIFT pretrial and laboratory trial on a wide variety of meat product samples**

| Product | Species composition[a] | No. of samples | No. of samples with deer | Positive samples |
|---|---|---|---|---|
| *Prelaboratory trial* | | | | |
| Frank emulsion | Bovine | 3 | 0 | 0 |
| Bologna emulsion | Bovine | 1 | 0 | 0 |
| Gyro | Ovine, bovine | 1 | 0 | 0 |
| Sausage | Bovine, ovine, pig | 2 | 0 | 0 |
| Sausage | Bovine, deer, (pig) | 2 | 2 | 2 |
| Sausage | Pig | 1 | 0 | 0 |
| Sausage | Pig, (bovine) | 1 | 0 | 0 |
| Sausage | Pig, (deer) | 8 | 8 | 8 |
| Ground beef | Bovine | 4 | 0 | 0 |
| Ground beef | Bovine, (chicken) | 2 | 0 | 0 |
| Ground beef | Bovine, (equine) | 2 | 0 | 0 |
| Ground beef | Bovine, (deer) | 1 | 1 | 1 |
| Ground lamb | Ovine, (bovine) | 1 | 0 | 0 |
| Ground deer | Deer | 3 | 3 | 3 |
| Whole deer | Deer | 28 | 28 | 28 |
| Whole beef | Bovine | 1 | 0 | 0 |
| Whole lamb | Ovine | 1 | 0 | 0 |
| Whole pork | Pig | 1 | 0 | 0 |
| Whole chicken | Chicken | 1 | 0 | 0 |
| Whole horse | Equine | 1 | 0 | 0 |
| Total | | 65 | 42 | 42 |
| *Laboratory trial* | | | | |
| Sausage | Bovine, deer, (pig) | 2 | 2 | 2 |
| Sausage | Pig | 1 | 0 | 0 |
| Sausage | Pig, (deer) | 8 | 8 | 8 |
| Ground beef | Bovine | 2 | 0 | 0 |
| Ground beef | Bovine, (equine) | 1 | 0 | 0 |
| Ground beef | Bovine, (deer) | 1 | 1 | 1 |
| Ground deer | Deer | 2 | 2 | 2 |
| Whole deer | Deer | 14 | 14 | 14 |
| Whole lamb | Ovine | 1 | 0 | 0 |
| Whole pork | Pig | 1 | 0 | 0 |
| Whole chicken | Chicken | 1 | 0 | 0 |
| Whole horse | Equine | 1 | 0 | 0 |
| Total | | 35 | 27 | 27 |

[a] Identity of species in all samples was confirmed by Ouchterlony agar-gel immunodiffusion technique (8) using antispecies sera and extracts of authentic reference tissue. Species given in parenthesis represent known adulterant tissue present in the test samples.

Tests of shelf stability revealed that antigen and antibody discs stored for 1 year at 4°C produced immunoprecipitin bands of intensity equal to that of freshly prepared reagent discs. Reference antigen discs stored at room temperature lost considerable reactivity after only 2 months, while reference an-

tibody discs did not lose reactivity in 1 year. Therefore, we recommend that both reference antigen and antibody discs be stored in the refrigerator for maximum shelf life.

Results of the pretrial and laboratory trial analyses of samples are shown in Table 2. Of 100 total samples analyzed, 69 contained deer proteins and gave positive DRIFT reactions. Thirty-one samples, devoid of deer proteins, gave negative reactions. The absence of any false positive or false negative reactions demonstrates the accuracy and reliability of DRIFT. However, we recommend that positive results always be confirmed using the traditional Ouchterlony immunodiffusion technique (8) or by isoelectric focusing (9), especially when legal action may result from violative results.

With the exception of REST, all predecessors of DRIFT (ORBIT, PROFIT, PRIME, and SOFT) are presently commercially available in kit form. Perhaps commercial production of DRIFT kits should be considered. Authorities in charge of enforcing state game regulations could make excellent application of DRIFT to provide evidence of poaching or illegal sale of deer meat where applicable. Popularity of deer farming in New Zealand and Europe has motivated recent interest and successful attempts in this regard in the United States (10). Growth of deer farming may necessitate more frequent testing to verify the presence of deer tissue in edible meat products in the near future.

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TECHNICAL COMMUNICATIONS

# Precolumn *o*-Phthalaldehyde Derivatization and Reversed-Phase Liquid Chromatography of *S*-Methylmethioninesulfonium in Satsuma Mandarin Juice

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**Experiments were conducted to determine the amount of *S*-methylmethioninesulfonium (MMS) in Satsuma mandarin juice using a precolumn *o*-phthalaldehyde (OPA) derivatization and reversed-phase liquid chromatography. Favorable analytical conditions that allowed MMS analysis were achieved by using OPA/3-mercaptopropionic acid (MPA) as a derivatization agent with fluorescence detection (excitation at 330 nm, emission at 450 nm). A C18 reversed-phase column with 5 μm particle size and a length of 250 mm was used. Resolution of MMS/OPA/MPA derivative was accomplished with a linear gradient eluent (30mM sodium acetate buffer solution, pH 7.3, and 70% (v/v) methanol). Quantitative analysis of MMS by the internal standard method using β-alanine gave highly reproducible result with a coefficient of variation less than 3%. Recovery of MMS added to juice samples was 105%. MMS content in Satsuma mandarin juice was 28.2μM.**

*S*-Methylmethioninesulfonium (MMS) is widely distributed in nature and has been reported as a constituent of Satsuma mandarin, tomato, sweet corn, green tea, milk, soybean, asparagus, and cabbage. MMS has considerable biological and medicinal interest. It is a precursor of dimethylsulfide (DMS), which is the characteristic component of the off-flavor produced by heating Satsuma mandarin juice (1, 2). Shimoda et al. reported on a method that determined MMS in Satsuma mandarin juice. Satsuma mandarin juice was injected directly into a GC injection port heated at 200°C. DMS produced by heat degradation of MMS in the injection port was quantitatively analyzed by using a flame photometric detector. MMS content was calculated from the DMS amount (3). Kovatcheva described a method to determine MMS in plant products using an amino acid analyzer (4). Ohtsuki et al. reported MMS in the extracts of various kinds of teas, such as green teas, black teas, and oolong teas, by the use of an amino acid analyzer (5, 6).

Recently, the determination of amino acids by reversed-phase liquid chromatography (RPLC) in combination with precolumn *o*-phthalaldehyde (OPA) derivatization and fluorescence detection has gained wide popularity for its sensitivity, speed, comparative simplicity, and lower cost (7, 8).

Classically, amino acids have been separated by automatic amino acid analyzers that involve postcolumn derivatization. Both ion exchange and RP packing have been used in the analysis of amino acids. However, the RP mode now appears to be the method of choice. C18 columns are widely used on separation of amino acids.

Precolumn, off-line derivatization is often used in LC because it does not impose any restrictions on the chromatographic system in terms of the mobile phase composition, reaction temperature, duration, etc. However, postcolumn, online derivatization does impose restrictions on the mobile phase composition, reaction temperature, etc. Also, postcolumn, online derivatization requires a special reactor of small volume to avoid band broadening (9).

In this paper, we use the precolumn off-line derivatization method and C18 column RPLC method for the determination of MMS in Satsuma mandarin juice.

## Experimental

### *Reagents*

(a) *Sodium acetate trihydrate, sodium borate.*—Special grade (Wako Pure Chemical Industries Ltd, Osaka, Japan).

(b) *Methanol.*—LC grade (Wako).

(c) *OPA.*—Biochemical grade (Wako).

(d) *S-Methylmethioninesulfonium chloride (MMS-Cl).*—Greater than 99% pure (Tokyo Kasei Kogyo Co. Ltd, Tokyo, Japan).

(e) *β-Alanine (β-Ala), L-glutamine (Gln), and 3-mercaptopropionic acid (MPA).*—(Tokyo Kasei Kogyo.)

(f) *L-Aspartic acid (Asp), L-glutamic acid (Glu), L-asparagine monohydrate (Asn), L-serine (Ser), glycine (Gly), L-threo-*

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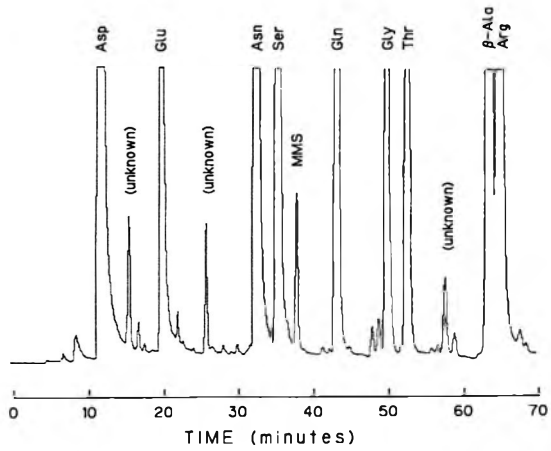


**Figure 1. Typical chromatogram of MMS–OPA–MPA derivative and amino acid–OPA–MPA derivatives from the Satsuma mandarin juice. Abbreviations: Asp = L-aspartic acid; Glu-L-glutamic acid; Asn = L-asparagine; Ser = L-serine; MMS = *S*-methylmethioninesulfonium; Gly = glycine; Thr = L-threonine; β-Ala = β-alanine (internal standard); Arg = L-arginine.**

*nine (Thr), and L-arginine monohydrochloride (Arg).*—Analytical grade (Takara Kohsan Co. Ltd, Tokyo, Japan).

All Satsuma mandarins were purchased from local markets.

### Apparatus

(a) *Elgastat UHQ Purification System.*—Used in the preparation of buffer solutions and standard samples (Elga Ltd, Lane End, U.K.).

(b) *CN-type membrane filters.*—Used for sample preparation, 0.45 μm pore size (Advantec Toyo Corp., Tokyo, Japan).

(c) *LC system.*—Consisted of 2 Model LC-6AD LC pumps, a Model SCL-6B system controller for gradient programming, a Model SIL-6B automatic sample injector to add reagents or dilute samples, CTO-6A column oven with a preheater, and a Model DGU-1A degasser for degassing of mobile phases with helium (Shimadzu, Kyoto, Japan).

(d) *Spectrofluorometer.*—Model RF-535, used to routinely monitor fluorescence (Shimadzu). Measurements were made at an excitation wavelength of 330 nm and an emission wavelength of 450 nm, using a 12 μL flow cell and a xenon lamp. Chromatographic data were recorded and processed by a Model Chromatopac C-R4A system (Shimadzu).

(e) *Reversed-phase LC column.*—Inertsil ODS-2 250 × 4.6 mm, 5 μm (Gasukuro Kogyo Inc., Tokyo, Japan).

(f) *Mini-guard column.*—OS-type 10 × 4.6 mm, 5 μm (Gasukuro Kogyo Inc.).

### Standard Preparation

Satsuma mandarins were reamed. Juice was passed through a 20 mesh sieve and adjusted to pH 8.7. Juice was heated in an autoclave at 120°C for 10 min to eliminate MMS. Aliquots of 0, 5, 10, 20, and 30 μL 2mM MMS-Cl were added to separate 1 mL portions of MMS-free juice containing 10 μL 100mM β-Ala (internal standard), respectively. Standard samples were centrifuged at 1000 × *g* for 20 min. Supernatants were passed through a membrane filter and analyzed.

### Sample Preparation

Sample preparation was the same as standard preparation, excluding the MMS-free treatment and MMS-Cl addition. Prepared juice containing MMS was analyzed with the OPA derivatization method.

### Precolumn Derivatization of MMS with OPA

*Preparation of OPA–MPA derivatization reagent.*—A 50 mg portion of OPA was dissolved in 4.5 mL methanol, then 50 μL MPA and 0.5 mL 0.1M sodium borate buffer (pH 9.5) were added. The reagent mixture was kept in the dark at 4°C. Fresh mixtures were prepared each week (8).

*Derivatization procedure by automatic sample injector.*—Methanol (0.2 mL), 0.2 mL 0.1M sodium borate buffer (pH 9.5), and 50 μL OPA–MPA derivatization reagent were added to 50 μL MMS standard or 50 μL juice sample in 2 mL vials with PTFE (polytetrafluoroethylene) screwcaps. After thorough mixing and a reaction time of 1.7 min, 1 μL of the resulting solution was injected. All of these processes were automated using an automatic sample injector.

### Chromatography and Quantitation

*Chromatographic conditions.*—Gradients were prepared by mixing 2 mobile phases, A and B. Mobile phase A was 30mM sodium acetate, pH 7.3, with water. Mobile phase B was 70% methanol (v/v). Both mobile phases were degassed with helium sparking before use. Linear gradient was from 0% mobile phase B. Mobile phase B increased to 5% for 10 min, then increased from 5 to 15% for 50 min, and then from 15 to 25% for 10 min. An additional step to 100% B for 20 min was used to flush retained components from column. Program was then returned to 100% A for 15 min to regenerate column. Flow-rate was 1.0 mL/min. Temperature of column was maintained at 40°C.

*Quantitation.*—Fluorescence detection was made at an excitation wavelength of 330 nm and an emission wavelength of 450 nm. Fluorescence response was measured at sensitivity dial setting of High (H), response dial setting of Medium (M), and attenuation dial of 32 on the detector. Peak areas of MMS derivative and β-Ala derivative (internal standard) were obtained using the data processor, Chromatopac C-R4A. Linearity was plotted for the ratio of peak area (MMS derivative–β-Ala derivative) as a function of MMS concentration. Analytic concentration was quantitated by comparing peak area of MMS derivative with that of β-Ala derivative (internal standard).

*MMS recovery experiments.*—Aliquots of 5 or 10 μL 2mM MMS-Cl were added to 1 mL orange juice containing 10 μL

100mM β-Ala as internal standard, respectively. Duplicate samples were analyzed.

## Results and Discussion

The primary purpose of this paper is the RPLC determination of MMS in Satsuma mandarin juice by precolumn OPA derivatization and fluorescence detection. Derivatization of MMS and separation of the derivative can be accomplished efficiently using the described system. Satsuma mandarin juice contains a large amount of free amino acid. Figure 1 shows a typical chromatogram of MMS–OPA–MPA derivative and amino acid–OPA–MPA derivatives from the Satsuma mandarin juice. Peak intensities of amino acid–OPA–MPA derivatives were very strong compared with peak intensity of the MMS–OPA–MPA derivative. The pH of mobile phase A (30mM sodium acetate buffer) must be carefully maintained, because the retention time of the MMS–OPA–MPA derivative is extremely pH sensitive when compared to its amino acid–OPA–MPA derivatives. Identification of a peak of the MMS–OPA–MPA derivative was confirmed by the disappearance of the peak on the alkaline heat-treated juice, and the appearance at the same position of a peak by addition of MMS-Cl to the MMS free juice. The regression equation obtained by a least square method was $Y = 7.212 \times 10^{-4} X + 3.511 \times 10^{-4}$, where $Y$ is the peak area ratio, MMS derivative–β-Ala derivative (internal standard), and $X$ is the MMS concentration in μM.

Linearity was good ($\gamma = 0.9996$). MMS content of Satsuma mandarin juice was 28.2μM. Coefficient of variation (CV) was 2.78% for 5 measurements. Recovery of MMS (added as MMS-Cl, 10 or 20μM) from spiked Satsuma juice averaged 104.9% for 4 measurements. CV of recovery was 0.77%. Limit of detection for MMS-Cl aqueous solution was approximately 0.1μM. MMS contents of additional 2 samples of Satsuma mandarin juice measured by the present method were 24.8 and 30.3μM, respectively.

The present results demonstrate that OPA-based MMS analysis can be used for Satsuma mandarin juice.

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## GENERAL REFEREE REPORTS

# Committee on Pesticide Formulations and Disinfectants

## Pesticide Formulations: Organohalogen Insecticides; Other Insecticides, Synergists, and Repellents

DAVID F. TOMKINS
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### *Organohalogen Insecticides*

*Benzene Hexachloride and Lindane.*—New associate referee needed for this area.

*DDT.*—Continued study.

*Dicofol.*—Continue study.

### *Other Insecticides, Synergists, and Repellents*

*d-trans Allethrin.*—Continue study.

*Cyfluthrin.*—Continue study.

*Dipropyl Isocinchomeronate.*—Planning a collaborative on gas chromatography method in 1992 (MGK Repellant No. 326)

*Cyromazine (Larvadex).*—Continue study.

*Cypermethrin.*—Continue study.

*Rotenone and Other Rotenoids.*—Continue study.

*Pyrethrins.*—Continue study.

### *Recommendations*

(1) Drop *Resmethrin* from active study status.

This report of the General Referee was presented at the 105th AOAC Annual International Meeting, August 12–15, 1991, at Phoenix, AZ. 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*, 2nd supplement, 1991.

## Pesticide Formulations: Organothiophosphorus Insecticides

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During the past year, the protocol for 1 collaborative study was received and a collaborative study on this topic should start during the coming year. One method is being recommended for final action. One method that is final action is being superseded by a superior method and is being recommended for repeal back to official first action. Two topics that have been inactive are being discontinued. Five topics are being continued.

*Azinphos-Methyl (Guthion).*—The HPLC method has been finalled at the last Annual Meeting. Associate Referee Stephen C. Slahck intends to start a mini-collaborative study on new formulations of this product. Continue study.

*Ethoprop.*—A protocol for a collaborative study has been received and the collaborative study should begin in the coming year. Continue study.

*Fenitrothion.*—The official final action GC method (**985.07**) has been superseded by a superior GC method for water-dispersible powder and emulsifiable concentrate formulations (**989.02**). The Associate Referee Dwight L. Mount has not received any negative comments about the new method, which has been official first action for 2 years.

The Associate Referee and the General Referee recommend adoption of **989.02** as official final action.

The General Referee recommends that the old official final action GC method (**985.07**) be repealed from final to first action prior to full repeal next year.

*Methidathion (Supracide).*—The Associate Referee for this topic has resigned and has not been replaced. The General Referee recommends that this topic be discontinued.

*Oxydemeton-Methyl (Metasystox-R).*—Associate Referee Stephen C. Slahck reports that no negative comments have been received on the official first action LC method. Another year is needed before it can be adopted official final action. Continue study.

*Parathion and Methyl Parathion.*—There are official first action GC (**978.06**) and LC (**978.07**) methods for parathion, and GC (**977.04**) and LC (**977.05**) methods for methyl parathion that have been official first action for many years. The LC methods use a silica column with water-saturated chloroform as mobile solvent. There is a question as to the stability of the silica column when used with water-saturated chloroform. Chloroform is also a suspected carcinogen. The General Ref-

eree recommends repeal of the 2 LC methods next year after it has been announced and comment received.

The GC methods are suitable for final action with a few editorial revisions. The GC column packing used is a mixture of 1.5% SE-30 plus 1.5% OV-210. An equivalent column packing having similar McReynolds constants should be included as an option. OV-17 column packing has these characteristics. The official final action GC method for parathion in microencapsulated pesticide formulations (**980.11**) uses OV-17 for the column packing. I would recommend adding the words "...or a column packing having similar McReynolds constants, such as OV-17." The General Referee recommends official final action for these 2 methods next year after the announcement has been made and comments received. Continue study.

*S,S,S,-Tributylphosphorotrithioate (DEF).*—There has been no activity on this topic. Discontinue study.

### *Recommendations*

(1) Adopt as official final action the official first action GC method for fenitrothion in water-dispersible powder and emulsifiable concentrate formulation (**989.02**).
(2) Repeal the official final action GC method for fenitrothion (**985.07**) to official first action.
(3) Discontinue the following topics: methidathion (supracide), and *S,S,S*-tributylphosphorotrithioate (DEF).
(4) Continue study on the following topics: azinphos-methyl, ethoprop, fenitrothion, oxydemeton-methyl, and parathion and methyl parathion.

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## Pesticide Formulations: Other Organophosphorus Insecticides

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During the past year, all 3 topics were inactive. One topic will be discontinued, the other 2 topics will be continued.

The following is a status report of the topics assigned to this General Refereeship.

*Crotoxyphos.*—There has been no activity on this topic. Discontinue study.

*Dichlorvos (DDVP).*—Two infrared methods (**964.04** and **966.07**) have been official first action for many years. These methods are long and involved and require a column cleanup of some formulations before running the IR determination. The formulations used in the collaborative study are no longer commercial products. The only U.S. manufacturer of dichlorvos does not use IR; instead, they use a gas chromatographic method that they believe to be a superior method.

The General Referee recommends that the 2 infrared methods be repealed next year after notice has been given and comments received. Continue study.

*Fenamiphos (NEMACUR).*—Associate Referee Carl Gregg conducted a collaborative study a year ago. He did not receive the required 8 laboratories with valid data. The Associate Referee intends to restart a collaborative study. Continue study.

### *Recommendations*

(1) Continue study on *Dichlorvos* and *Fenamiphos.*
(2) Discontinue study on *Crotoxyphos.*

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## CIPAC Studies

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The 35th CIPAC meeting and annual symposium were held at the Federal Biological Research Center for Agriculture and Forestry (BBA), Braunschweig and the Forum of the Federal Research Center for Agriculture, Braunschweig-Völkenrode, Germany, respectively. The annual informal Food and Agriculture Organization (FAO) of the United Nations meeting on specifications for pesticides was held in conjunction with the 35th CIPAC meeting and annual symposium. These meetings were sponsored by the German Federal Ministry of Food, Agriculture, and Forestry along with CIPAC.

As for 1990 in Tunisia, F. Sanchez Räsero, Estation Experimental de Zaidin, Granada, Spain, chaired the FAO meetings and again great progress was made in the review and approval of specifications for pesticides. Continued and rapid progress was greatly supported by past CIPAC Chairman, J. Henriet (Gembloux, Belgium), through his work for FAO. Also, the establishment of priorities for review, and, especially, the early transmittal of proposed specifications for review to the participants helped actuate the specifications. CIPAC Chairman H. Bosshardt (Swiss Federal Research Station, Wodenswil, Switzerland) presided over the CIPAC meeting, promoting coordination and cooperation among the participating countries and industries while maintaining an excellent working atmosphere that was both serious and fun. W. Dobrat (BBA and meeting host) and J.R. Plimmer (USDA retired, currently ABC Laboratories, Columbia, Missouri) shared the duties of symposium chairman. A welcome and opening to the technically and scientifically excellent CIPAC symposium was given by Professor H. Kohsiek, Director of the Department of Plant Protection Products and Application Techniques (BBA). The symposium closing summary was provided by J.R. Plimmer.

It should be noted that during the past year the founder and long time chairman of CIPAC, R. de B. Ashworth, passed away. His service, contributions and long devotion to the purposes of CIPAC were recognized during the meetings and highlighted through a tribute given by J. Henriet, also a founding member of CIPAC.

The meeting location, changes in the political order in parts of Europe, and a general increased interest in the work of CIPAC resulted in good attendance at all meetings: approximately 90 for the FAO sessions, 200 for the CIPAC symposium, and 60 for the CIPAC technical sessions. The next CIPAC meeting is tentatively scheduled for May 25–June 1, 1992, in Bangkok, Thailand.

During this past year the CIPAC-AOAC interim first action LC method for deltamethrin was approved first action. Also during this time, the following methods were approved CIPAC-AOAC first action: maneb in formulations containing fentin acetate or fentin hydroxide, cyanazine in formulations by LC and pirimiphos-methyl in formulations by GC. One method, methamidophos in formulations by LC, should be nearing final review by the AOAC Methods Committee on Pesticides and Disinfectants, while the General Referee is preparing additional methods for Committee review.

Method actions taken this year by CIPAC are reported in Table 1, a report that would not be possible without the very timely efforts of the CIPAC Secretary A. Martijn (Plantenziektenkundige Dienst, Wogeningen, The Netherlands). Methods reported adopted by CIPAC as full methods were previously reported upon when they were first adopted as provisional, provided such methods were found to meet minimum criteria for review by AOAC. Thus, in the following, only those methods newly adopted as provisional by CIPAC and, which after very brief review, appear to be candidates for submission to the Methods Committee are given in additional detail.

*Dichlobenil.*—Samples of granular and technical material were dissolved or extracted with acetone. Analysis was accomplished by GC on a 30 m DB-17 capillary column with detection using flame ionization and quantitation employing an internal standard. Thirteen laboratories from 7 countries returned data for statistical analysis. Neither outlier nor straggler data were detected.

*Metalaxyl.*—Packed GC on an OV-101 column and flame ionization detection were applied in the analysis of technical material, seed treatments and wettable powders using internal standardization. The 13 participating laboratories included representation from 8 countries.

*Thiabendazole.*—Fifteen laboratories from 7 countries analyzed technical, wettable powder, flowable, liquid, and dust samples by reversed-phase LC using external standardization and detection at 302 nm. Only 1 outlier and 4 stragglers (2 Grubles and 2 Cochran) were found out of 80 data points.

*Bromadialone.*—Five samples (2 technical, 2 liquid concentrates, and 1 solid concentrate) were analyzed using a reversed-phase LC method with internal standardization and detection at 254 nm. Of the 60 data points reported by 15 laboratories located in 7 countries, only 4 were rejected as outliers.

*Fentin Acetate or Hydroxide.*—A total of 14 laboratories representing 7 countries applied an LC method using reversed-phase chromatography with internal standardization and 220 nm detection in the analysis of 5 samples: 4 fentin acetate products containing maneb (2 wettable powders, 1 water dispersible granule, and 1 suspension concentrate) and 1 fentin hydroxide plus maneb wettable powder. Generally good results were obtained for all but the water dispersible granule formulation, where sample preparation may be a problem.

*Omethoate.*—Five samples (1 technical and 4 soluble concentrates) were analyzed in 8 countries by 17 laboratories. Significant outlier data were identified for the 50% soluble concentrate, which is likely attributable to a sampling problem. A reversed-phase LC method with detection at 220 nm and external standardization was used.

*Ethiofencarb.*—Two technical, 2 soluble concentrate, and 1 emulsion product were analyzed by reversed-phase LC with

**Table 1. Summary of the decisions taken at the 35th CIPAC meeting**

| Code no. | Name | Status of method |
|---|---|---|
| 73. | dichlobenil | The capillary GC method for dichlobenil technical and formulations, CIPAC/3633, adopted as *provisional* CIPAC method. |
| 95. | carbetamide | The provisional CIPAC method for carbetamide technical and formulations, CIPAC/3602, adopted as *full* CIPAC method. |
| 103. | fentin | The HPLC method for fentinacetate/hydroxide technical and formulations (including mixtures with maneb), CIPAC/3639, adopted as *provisional* CIPAC method. The method for water dispersible granules accepted as *tentative* method. |
| 115. | thiometon | The provisional CIPAC method for thiometon technical and emulsifiable concentrates, CIPAC/3589, accepted as *full* CIPAC method. |
| 171.a. | oxydemeton-methyl | The HPLC method for oxydemeton-methyl technical and formulations, *J. Assoc. Off. Anal. Chem.* (1990) 73, 431, adopted as *provisional* AOAC-CIPAC method. |
| 202. | omethcate | The HPLC method for omethoate technical and formulations, CIPAC/3641, adopted as *provisional* CIPAC method. |
| 275. | tolylfluanid | The provisional method to remain provisional. |
| 323. | thiabendazole | The HPLC method for thiabendazole technical and formulations, CIPAC/3645, adopted as *provisional* CIPAC method. |
| 335. | imazalil | The GLC method for imazalil technical, CIPAC/3643, adopted as *full* CIPAC method. |
| 355. | methamidphos | The provisional method for methamidophos technical and formulations, CIPAC/3578, accepted as *full* CIPAC method. |
| 363. | ethiofencarb | The HPLC method for ethiofencarb technical and formulations, CIPAC/3635, adopted as *provisional* CIPAC method. |
| 365. | metalaxyl | The GC method for metalaxyl technical and formulations, CIPAC/3649, adopted as *provisional* CIPAC method. |
| 371. | bromadiolone | The HPLC method for bromadiolone technical and formulations, CIPAC/3629, adopted as *provisional* CIPAC method. |
| 394. | propetamphos | The provisional method for propetamphos technical and formulations, CIPAC/3592, accepted as *full* CIPAC method. |
| 399. | propamocarb | The provisional method for propamocarb technical and aqueous solutions, CIPAC/3580, accepted as *full* CIPAC method. |
| 409. | edifenphos | The provisional method for edifenphos technical and formulations, CIPAC/3584, accepted as *full* CIPAC method. |
| 417. | carbosulfan | The HPLC method for carbosulfan technical and formulations, CIPAC/3631, adopted as *provisional* CIPAC method. |
| 456. | isoprothiolane | The provisional method for isoprothiolane technical and formulations, CIPAC/3582, accepted as *full* CIPAC method. |
| 464. | bensultap | The provisional method for bensultap technical and formulations, CIPAC/3586, accepted as *full* CIPAC method. |
| MT 168 | | Determination of suspension stability of water dispersible granules, CIPAC/3543. The chemical determination adopted as referee method. |
| MT 171 | | Dustiness of water dispersible granules, CIPAC/3463. Method also accepted for granules and to be re-named "Dustiness of granular formulations." |
| MT 172 | | Flowability of water dispersible granules, CIPAC/3608. Provisional method accepted as *full* CIPAC method. |
| MT 173 | | Emulsion stability of dilute emulsions, CIPAC/3595. Provisional method adopted as *full* method. |
| MT 174 | | Dispersibility of water dispersible granules, CIPAC/3610. Provisional method adopted as *full* CIPAC method. |

254 nm detection, external standardization, and gradient elution for removal of late eluting components. Results of statistical analysis on data from 10 laboratories representing 5 countries were generally good, with the reproducibility for the oil in water emulsion being an exception. In the case of the emulsion, insufficient formulation stability apparently resulted in the formation of a sediment requiring shaking to restore homogeneity.

*Carbosulfan.*—Reversed-phase LC with 280 nm detection and internal standardization was used to analyze 6 samples (1 technical, 1 granule, 2 emulsifiable concentrates, 1 seed treatment, and 1 wettable powder). One of the 8 participating laboratories produced the only outliers detected.

Four preliminary studies were presented that provided ample evidence to encourage full collaborative studies in the future: iprodione by LC with 6 participating laboratories analyzing 5 samples; bifenox by LC with 6 participating analyzing 5 samples; fenvalerate by LC with 5 laboratories studying 3 samples; and flucyloxuron by LC with 6 laboratories analyzing 2 samples.

### *Recommendations*

(1) Adopt as first action the CIPAC method for methamidophos in formulations by LC.
(2) Continue study on all other topics.

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## Disinfectants

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*Hard Surface Carrier Test (formerly Use Dilution Test).*—The Hard Surface Carrier Test method was submitted and accepted for publication in the AOAC *Journal* after minor revisions. The test will be presented for first action approval by the Official Methods Board at the 105th Annual Meeting. Current work by the Associate Referee and his associates are focusing on the use of hard water and a soil load. Continued study is recommended.

*Tuberculocidal Test.*—Studies to improve the AOAC Tuberculocidal Activity test method are underway. Current studies within this research include developing growth curves of 4 Mycobacterium species in several TB culture medias, determining the bacterial load on carriers of different materials, and determining the resistance of the Mycobacteria to quaternary ammonium compounds. Continued study is recommended.

*Virucidal Test.*—A cooperative agreement to develop a standardized virucidal test was recently led by the U.S. Environmental Protection Agency Office of Pesticide Programs (OPP) with a view to integrate the test method into the AOAC process. Continued study is recommended.

*Sporicidal Test.*—Proposals to conduct research to upgrade the AOAC Sporicidal Test or develop a new method are being reviewed by OPP. Continued study is recommended.

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# Committee on Drugs and Related Topics

## Drugs I

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*Acetaminophen in Drug Mixtures.*—No additional work has been done on this topic. The method is currently in official first action status.

*Diethylpropion Hydrochloride.*—No additional work has been done on this topic. The method is currently in official first action status.

*Phenothiazine and Related Drugs.*—No additional work has been done on this topic. The method is currently in official first action status.

*Salicylic Acid in Acetylsalicylic Acid Preparations.*—No additional work has been done on this topic. The method is currently in official first action status.

*p-Aminobenzoic Acid and Salicylic Acid.*—No additional work has been done on this topic. The method is currently in official first action status.

### *Recommendations*

(1) Adopt as official final action the methods for *Acetaminophen in Drug Mixtures*, *Diethylpropion Hydro- chloride*, *Phenothiazine and Related Drugs*, *Salicylic Acid in Acetylsalicylic Acid Preparations*, and *p-Aminobenzoic Acid and Salicylic Acid.* Discontinue each of these topics.
(2) Discontinue study on all other topics.

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## Drugs II

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*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 recoveries and a low standard deviation were obtained for all 3 analytes in liquid cough-cold products. Based on the collaborative study results, he is recommending that the proposed method be adopted first action.

*Belladonna Alkaloids.*—A replacement for this vacant Associate Refereeship is being sought. The General Referee Edward Smith is reporting an LC procedure that enables one to directly determine both scopolamine and atropine and the individual enantiomers of each alkaloid. This will be 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, initially presented (2), was expanded to include other chiral stationary phases and scopolamine. The scope of this topic would also include the determination of the individual alkaloids as well as insuring the suitability of the procedure to detect any decomposition product present.

*Colchicine in Tablets.*—Associate Referee Richard D. Thompson reported that he is actively pursuing methodology for the determination of the decomposition product colchiceine. He is still planning a collaborative study of an LC method for the determination of colchicine in tablets containing colchicine in combination with probenecid.

*Curare Alkaloids.*—Associate Referee John R. Hohmann reported that the topic be discontinued because the new USP monograph (3) LC procedure for commercial tubocurarine chloride injections yielded reproducible results.

*Dicyclomine Capsules.*—A new Associate Referee is needed to follow up on the proposed capillary GLC method for dicyclomine in capsules (4) to injections and tablets. The proposed GLC method resolves any decomposition products from the dicyclomine and the internal standard.

*Epinephrine and Related Compounds by LC-Electrochemical Detectors.*—Associate Referee John Newton reported no further work has been accomplished. In view of his inability to arrange research time due to a change in his work assignments, he is resigning his associate refereeship.

*Ergot Alkaloids.*—Associate Referee Thomas C. Knott reported a summary of the status of the proposed LC methods. Because he is now assigned to a different subject, no further work was accomplished this year on this topic. A new Associate Referee is being sought for this topic.

*Morphine Sulfate.*—Associate Referee Ada C. Bello again reported no adverse comments were received on the first action method **989.08**, Morphine Sulfate in Bulk Drug and Injections, Liquid Chromatographic Method (5). The Associate Referee recommends that the first action method for the determination of morphine sulfate and certain contaminants in the bulk drug and injection be adopted as official final action. It is also now official in the USP (6). (The required notice of recommendation has been published in *The Referee* to facilitate the recommendations of the Associate Referee and the General Referee.) The Associate Referee recommends that the topic be discontinued following the successful adoption of the procedure as final action.

*Physostigmine and Its Salts.*—Associate Referee Norlin W. Tymes reported he is going to evaluate proposed LC methods by Wang and Wilken (7), which are suitable for the analysis of decomposed samples of physostigmine containing eseroline and rubreserine.

*Rauwolfia Alkaloids (Reserpine and Rescinnamine).*—A new Associate Referee is needed for this topic.

*Rauwolfia Serpentina.*—Associate Referee Ugo R. Cieri reported no further work on this topic. More validation work is needed before any collaborative study is undertaken.

### *Recommendations*

(1) Adopt as official final action the official first action method **989.08** for the determination of morphine sulfate and certain contaminants in injections and bulk drug material.
(2) Adopt as official first action the proposed LC procedure for the simultaneous determination of chlorpheniramine maleate, pseudoephedrine HCl, and dextromethorphan HBr in liquid cough-cold products.
(3) Declare open any topic that has been inactive for an extended period (more than 2 years). New Associate Referees are needed for the following topics: Belladonna Alkaloids, Ergot Alkaloids, Rauwolfia Alkaloids, and Rauwolfia Serpentina. If no new Associate Referees are identified and appointed these topics will have to be dropped.
(4) Appoint a new Associate Referee for Dicyclomine Capsules.
(5) Discontinue the topics Curare Alkaloids, Epinephine, and Related Compounds by LC-Electrochemical Detectors, and Morphine Sulfate.
(6) Continue study on all other topics.

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## Drugs III

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*Halogenated Hydroxyquinoline Drugs.*—No additional work is anticipated on this topic. The method is currently in first action status.

*Haloperidol.*—No additional work is anticipated on this topic.

*Hydralazine.*—No additional work has been done on this topic. The method is currently in first action status.

*Medicinal Gases.*—The proposed collaborative study of this method has not been initiated nor is the prospect of conducting this study in the near future very encouraging.

*Metals in Bulk Drug Powders.*—No work has been accomplished on this topic in several years.

*Penicillins.*—No additional work has been done on this topic. The method is currently in first action status.

*Salts of Organic Nitrogenous Bases.*—No work has been accomplished on this topic in several years.

*Recommendations*

(1) Adopt as final action the methods for halogenated hydroxyquinoline drugs, hydralazine, and penicillins. Discontinue each of these topics.
(2) Discontinue study on all other topics.

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## Drugs IV

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*D- and L-Amphetamines (LC Separations).*—Two methods are currently being studied for this topic. Continue study on the method that is in first action status. Associate Referee Thomas Doyle is planning a collaborative study on a published method for D- and L-amphetamines.

*Benzodiazepines.*—The method for flurazepam in bulk drug and capsules by Associate Referee Eileen Bargo has been accepted for first action.

*Diazepam.*—Discontinue topic.

*Dicloxacillin.*—Associate Referee Mei-Chick Hsu is planning a collaborative study on a published method.

*Fumagillin.*—Discontinue topic because of difficulty in obtaining samples. Associate Referee John Brackett will be transferred to another topic.

*Heroin.*—Associate Referee Charles Clark has initiated a collaborative study on a GC method for heroin hydrochloride.

*Miconazole Nitrate.*—Associate Referee Judith Genzale is planning a collaborative study on miconazole nitrate in creams and suppositories.

*Recommendation*

Continue study on all other topics.

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## Drugs V

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*Chlorpropamide.*—The HPLC method, **986.37**, has now been first action for 6 years. No adverse comments have been received. In fact, this method is the United States Pharmacopeia XXII method for chlorpropamide. Both the 1989 and 1990 General Referees published and posted reports included the recommendation that this method be adopted final action. The July 1990 issue of *The Referee* included a statement of intent to recommend **986.37** for final action. Again, *The Referee* makes this same recommendation.

The Associate Referee has retired and his laboratory sees no point in keeping the topic open. Discontinuation is recommended.

*HPLC Screening for Anabolic Steroids.*—The developmental work was published (*J. Assoc. Off. Anal. Chem.* (1990) **73**, 904–926) and now the matter of a suitable protocol for a collaborative study must be resolved. Only a present-absent decision is involved. Hopefully a limited collaborative study involving a few typical steroids will serve the purpose of making this method suitable for official status.

*Progestins in Tablets-Automated Methods.*—While the Associate Referee believes the method studied several years ago is quite viable for large-scale testing, he sees no likelihood of his laboratory's getting to work on this project. Discontinuation is recommended.

*Steroid Acetates.*—The LC methods (**988.25** and **988.26**) have been first action since 1988. No adverse comments have been received. Notification of intent to recommend final action was published in the May 1991 issue of *The Referee*. Adoption as final action is now recommended.

The Associate Referee is no longer able to conduct laboratory studies and further study of the dosage forms studied is not warranted. Discontinuation of the topic is recommended.

*Steroid Phosphates.*—The methods in **988.27** include LC quantitative and TLC identification procedures for dexamethasone bulks and elixirs plus a GLC method for alcohol in elixirs. These have been first action since 1988. No adverse comments have been received. Notification of intent to recommend final action was published in the May 1991 issue of *The Referee*. Adoption as official final action is now recommended.

The Associate Referee has found the USP method for dexamethasone tablets to be unsatisfactory. She intends to study this matter. Continued study is recommended.

A change in the topic title is in order. While earlier work was on phosphates, the Associate Referee has been and intends to continue working on the steroid alcohols. The title *Steroids* is recommended.

*Recommendations*

(1) *Chlorpropamide.*—Adoption of **986.37** as final action. Discontinuation of topic.
(2) *Conjugated Estrogens by Liquid Chromatography.*—Continue study.
(3) *HPLC Screening for Anabolic Steroids.*—Continue study.
(4) *Pentaerythritol Tetranitrate.*—Continue study.
(5) *Progestins in Tablets-Automated Methods.*—Discontinuation of topic.
(6) *Steroid Acetates.*—Adoption of **988.25** and **988.26** as final action. Discontinuation of topic.
(7) *Steroid Phosphates.*—Adoption of **988.27** as final action. Continue study. Change title to *Steroids.*

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## Drug Residues in Animal Tissues

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*Benzimidazole in Cattle Tissue.*—LeVan's procedure for determining 800–1600 ppb fenbendazole in cattle liver has been approved first action.

*Clopidol in Chicken Tissues.*—Associate Referee has left the Food and Drug Administration (FDA). She no longer works in a laboratory.

*Enzyme Immunoassay for Antimicrobial Compounds.*—No report.

*β-Lactams in Milk by Liquid Chromatography.—No report.*

*β-Lactams Residues in Milk, Qualitative Methods.—No report.*

*β-Lactams Residues in Milk, Quantitative Methods.—No report.*

*β-Lactams Residues in Milk by Devotest.—No report.*

*Screening Procedures for β-Lactams in Milk, Elisa.*—An Associate Referee was appointed this year. No report.

*Sulfamethazine in Milk, Chromatography Methods.*—The Committee Chair returned the report of the collaborative study for revisions. The revision will likely be completed in August of 1991. The Associate Referee will recommend that the procedure be adopted first action for determining sulfamethazine at the 10 ppb level and above in raw milk.

*Sulfonamides in Milk Chromatography.*—Michael Smedley of FDA was appointed Associate Referee. He initiated a study of a multiresidue procedure for 8 sulfonamides under joint FDA/AOAC sponsorship. Because of the difficulty in meeting the study criteria of both organizations in the short time available, he decided to withdraw the study from AOAC sponsorship. Efforts are still being made to satisfy the requirements of both organizations.

*Tetracyclines in Tissues, Chromatographic Methods.*—The General Referee has reviewed MacNeil's protocol for a collaborative study of an LC procedure for determining oxytetracycline, chlortetracycline, and tetracycline at the 0.1 to 2.0 ppm levels in muscle and kidney. A complete study protocol package, to be forwarded to the committee, has been requested from the Associate Referee. Collaborators have been assembled. Some preliminary studies are now underway.

*Tetracyclines in Tissue by Microbiology.*—No report. We are seeking a replacement for the Associate Referee who resigned.

*Recommendations*

Discontinue clopidol in chicken tissue; β-lactam residues in milk, qualitative methods; and β-lactam residues in milk, quantitative methods. Continued study on all other topics.

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## Cosmetics

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The last 4 current topics were discontinued last year because of lack of time and opportunity to pursue them on the part of the Associate Referees. With all the current topics gone, the possibility of discontinuing the Cosmetic General Refereeship had been discussed. However, over the course of this year there have been a number of requests for information on the AOAC involvement in cosmetic analysis, especially from Europe and Latin America. As an indication, I have received over 15 requests for reprints of the 1990 General Referee Report alone.

This apparent interest in cosmetic analytical chemistry will be pursued. An appropriate candidate will be sought to lead this area forward and seek the development of several studies of immediate concern to the field. The goal will be to have this person in place before the end of 1991.

The 3 methods (see below) in Chapter 15 of *Official Methods of Analysis* that are still first action will be reviewed with the Chapter Editor and the respective authors. By October 1,

1991, it will be decided whether these methods are to be declared surplus or recommended for final action.

### *Recommendations*

(1) *Water and Ethyl Alcohol in Cosmetics.*—Continue first action status (**966.22**).
(2) *Zirconium (Soluble) in Antiperspirant Aerosols.*—Continue first action status (**976.24**).
(3) *Eye Irritants in Cosmetic Constituents.*—Continue first action status (**973.59**).

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## Forensic Sciences

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Robert D. Koons of the Forensic Science Research and Training Center at the Federal Bureau of Investigation Academy in Quantico, VA, has submitted a completed collaborative study, "Flameless Atomic Absorption Spectrophotometric Determination of Antimony and Barium in Gunshot Residue Collection Swabs." The report is undergoing minor revisions before adoption and publication.

Koons has been appointed the Associate Referee for the reestablished topic of *Gunshot Residues.*

Recently there has been an increasing dependence of the courts on scientific evidence. At the same time the courts have increased the burden of proof necessary for successful prosecution. This has resulted in a greater interest in laboratory certification. Along with laboratory certification, the need arises for strong laboratory quality assurance programs, analytical protocols, check samples, and validated methods. The long time fear of the forensic community for "Official Methods" can be overcome with the development of a body of validated methods.

A survey is being planned that will search for suitable methods and laboratory resources.

### *Recommendations*

Reestablish the associate referee topic and appoint an Associate Referee.

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# Committee on Foods I

## Dairy Chemistry

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*Fat in Raw Milk, Babcock.*—D.M. Barbano reports that this method continues to be used by a large number of laboratories. Research effort is continuing to resolve issues dealing with the differences in amount of fat measured when comparisons are made to modified Mojonnier data. Because Babcock results usually read high by 1% of the fat, an adjustment downward in tempering bath temperature is being explored. The difference is particularly apparent with cream. A collaborative study is under development to study the feasibility of alterations in test procedures to negate these differences.

*Fat in Milk, Modified Mojonnier Ether Extraction Method.*—D.M. Barbano reports that this method has been in use by Federal Milk Market Administration laboratories, and other laboratories, since first action approval. This method has been evaluated every 2 months over a 3 year period with no comments given that would require alteration of the method procedure or the need for further study.

*Nitrogen (Total) in Milk–Kjeldahl Methods.*—D.M. Barbano reports that this method is used by many laboratories, principally MA laboratories. In the Federal Market Administrator laboratories, performance is monitored every other month.

*Nonprotein Nitrogen in Whole Milk–Kjeldahl Method, Protein Nitrogen Content in Milk–Kjeldahl Method-Direct Method,* and *Protein Nitrogen Content of Milk–Kjeldahl Method-Indirect Method.*—According to D.M. Barbano, these methods are used in a few laboratories with no comments about method adequacy and performance.

*Raw Milk Sampling at the Farm.*—D.T. Metzger reports that the collaborative study has been successfully completed and is in written form. The submitted manuscript has passed statistician's and GR's review, and has been accepted for publication in the *Journal of AOAC International.*

*Infrared Spectroscopic Method for Milk Components.*—D.M. Barbano reports that a collaborative study has been planned.

*Fat in Foods, Robotic Mojonnier.*—R.L. Bradley reports that a collaborative study is in its initial stage.

*Moisture in Cheese.*—R.L. Bradley reports that a collaborative study is planned.

*Somatic Cells Assay by Fluorescence (Foss).*—During 1990, when no GR was in place for Dairy Chemistry, an AR directed collaborative study and report was prepared to show equivalence in method performance using Fossomatic 360. Foss has been asked to prepare the data for formal submission.

One new AR has been appointed; David Sertl—*Iodine in Milk.*

No other reports were received from Associate Referees.

### *Recommendations*

(1) Retain *Fat in Raw Milk–Babcock Method*, **989.04**, as first action approval.
(2) Recommend as official final action *Fat in Milk–Modified Mojonnier Ether Extraction Method*, **989.05**.
(3) Recommend as official final action *Fat, Lactose, Protein, and Solids in Milk* (mid-infrared spectroscopic method) as approved final action. This method was given first action status in 1972, and use of A, B, and A and B filters was given first action status in 1988.
(4) Recommend IDF standard **105:1981** as the official technology for calibration of all Gerber and Babcock glassware, to replace **989.04(B)(3).**
(5) Recommend as official first action *Automated Raw Milk Sampling at the Farm.*
(6) In **972.16,** *Fat, Lactose, Protein, and Solids in Milk* (mid-infrared spectroscopic method), change the cross-references to read **972.16B** ... and lactose (**896.01, 930.28**, or **984.15**); **972.16H** ... ≥ 8 preanalyzed (**896.01, 930.28**, or **984.16**); **972.16N** ... (e.g., **905.02, 920.105, 896.01, 930.28**, or **984.15**).
(7) In **989.05** *Fat in Milk (Modified Mojonnier)*, delete the manufacturer's name at the end of the second sentence, **989.05B(a)**. (Kimble does not manufacture Mojonnier flasks.)

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## Flavors

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*Additives in Vanilla Flavoring.*—Active work in this topic has been completed with the acceptance of the liquid chromatography (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 has been 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.

A request has been presented for the development of an AOAC method for moisture in vanilla beans. There is at present no AOAC method for this determination. The method required in the *Code of Federal Regulation* standard of identity for vanilla extract uses an azeotropic distillation with a mixture of toluene and benzene. The vanilla industry has found that a method that uses benzene is inconsistent with current industrial hygiene requirements, and the industry wishes to develop an alternate method that eliminates the use of benzene. The general referee recommends that this topic be established as a new associate refereeship.

### *Recommendations*

(1) Designate method **960.37(C)(b)** as surplus. This is a complicated method for preparation of pure vanilla extract from vanilla beans for use as a reference material in certain qualitative tests. The general referee has found that this method is rarely used, and that the other, simpler method **960.37(C)(a)** is satisfactory for most purposes.

(2) Designate method **973.23** as official final action. This is a gas chromatography method for determination of alcohol in flavors. It was designated official first action in 1973, and the general referee is unaware of any problems with the method which would preclude official final action status.

(3) Designate methods **975.24**, **950.44(C)**, **950.45(C)**, **920.147(C)**, **920.148(A)**, and **975.25** as official final action. These methods are for the determination of alcohol in various specific flavoring materials. All 6 methods are simple references to method **973.23**, the general method for alcohol in flavoring materials.

(4) Designate method **960.36** as official final action. This is a qualitative paper chromatographic method for detecting the presence of nonvanilla resins in vanilla extract. It was designated official first action in 1960, and the general referee is unaware of any problems with the method that would preclude official final action status.

(5) Designate method **920.133** as official final action. This is a method for evaluating the presence of caramel color in vanilla extract. It was designated official first action in 1920, and the general referee is unaware of any problems with the method that would preclude official final action status.

(6) Designate method **982.19** as official final action. This is an LC method for determination of glycyrrhizic acid and its salts in licorice products. It was designated official first action in 1982, and the general referee is unaware of any problems with the method that would preclude official final action status.

(7) Designate method **984.17** as official final action. This is an LC method for determination of sugars in licorice extracts. It was designated official first action in 1984, and the general referee is unaware of any problems with the method that would preclude official final action status.

(8) Establish an associate referee topic for moisture in vanilla beans.

(9) Continue study in all topic areas.

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## Food Additives

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*Antioxidants.*—A collaborative study of the method for the determination of 9 antioxidants in butter oil (Page, B.D. (1983) *Food Additives Analytical Manual I*, 62–71) was completed. The antioxidants studied were propyl gallate (PG); 2,4,5-trihydroxybutyrophenone (THBP); *tert*-butylhydroquinone (TBHQ); nordihydroguairetic acid (NDGA); 2- and 3-*tert*-butyl-4-hydroxyanisole (BHA); 2,6-di-*tert*-butyl-4-hydroxymethylphenol (Ionox-100); 3,5-di-*tert*-butyl-4-hydroxytoluene (BHT); octyl gallate (OG); and dodecyl gallate (DG). Recoveries of the 9 antioxidants at levels ranging from 1 to 10 ppm ranged from 81 to 112%.

A study of the effects of singlet oxygen on the oxidation of lipids in the presence of TBHQ, BHA, and BHT was conducted.

Because of the abundance of sensitizers in foods, singlet oxygen is believed to play an important role in the oxidation of lipids in foods. The study results showed that TBHQ gave the most protection, BHA gave intermediate protection, and BHT gave no protection under the conditions studied.

*Brominated Vegetable Oils (BVOs).*—A replacement Associate Referee has not been found, nor has anyone volunteered to collaboratively study the capillary gas chromatography (GC)/acid-catalyzed esterification method for identifying and quantitating BVOs developed by Lawrence.

*Indirect Additives from Packaging.*—The following summarizes the activities over the past year in the Associate Referee's laboratory.

(*1*) 2-Butoxyethoxy ethanol, a common surfactant component in adhesive formulations, has been demonstrated to readily penetrate PET microwave susceptor food contact films. Other alcohols present in susceptor adhesive formulations would be expected to penetrate the PET film in a like manner.

(*2*) A solvent wash procedure for the determination of total nonvolatile migrants following microwave heating was found to significantly underestimate the amounts of UV absorbing chemicals observed to migrate to food. Additional observations suggest that the procedure probably underestimates the total chemical migration as well.

(*3*) The migration of styrene, 2,6-dimethylbenzoquinone, and benzaldehyde readily occur from blended polyphenylene oxide/polystyrene food trays to fatty food simulants at relatively mild temperatures of 110 to 125°C. Also, when in contact with oil at these temperatures, the material physically degrades. These results indicate the package as tested (estimated 75/25 blend of PS/PPO) is not suitable for fatty foods such as bacon, which could reach a temperature in excess of 110°C during microwave cooking. Also, they may not be suitable for any fatty foods if the fat separates from the food during cooking.

(*4*) Experimental measurements on the migration of Irganox 1010 (I-1010) from polypropylene (PP) to foods and food simulating liquids (FSL) were successfully validated. Evaporated skimmed milk and chicken broth, both having less than 1% fat, may be simulated by 2 radically different FSLs, water and corn oil, respectively, and have been verified for PP/I-1010 at 100°C. This illustrates that the way fat is incorporated in food is very important with regard to its effect on polymer additive migration.

(*5*) Potential alternative analytical techniques such as radiolabeled chemical doping, inverse gas chromatography, isotopic dilution, and fluorescence quenching for studying high temperature additive migration phenomena have been evaluated. Diffusion coefficients of some polymer adjuvants can be determined from inverse gas chromatography. To measure bulk diffusion, the experimental temperature must be maintained between the glass transition temperature, Tg, and the melt temperature or decomposition temperature of the polymer, whichever is lower. The fluorescence quenching method may be useful for studying migration of aromatic compounds if they can function as quenchers for a chromophore previously spiked to food. One possible scenario is anthracene-derived monomer migrating out of polymer to quench naphthalene fluorescence. This method would permit continuous, nonintrusive monitoring of additive migration, but would also involve a complicated optical arrangement to satisfy food cell heating and agitation requirements. A report on this review is being prepared.

(*6*) A microwave susceptor volatiles draft protocol has been written for distribution by the Food and Drug Administration (FDA) to interested parties. The procedure is similar to the ASTM susceptor volatiles protocol, except that it recommends use of standard additions for quantitation and uses standard microwave heating for all susceptors tested. The FDA procedure is based on qualitative and quantitative data gathered from the microwave susceptor survey and contains recovery data for certain susceptor volatiles.

*Nitrosamines in Foods.*—Associate Referee Sen submitted a report on his work in determining volatile and nonvolatile nitrosamines in foods and beverages. A summary of his research over the past year is described below.

(*1*) Methodology for Nonvolatile Nitrosamines.—A method was developed for the determination and mass spectrometric (MS) confirmation of 1-methyl-2-nitroso- 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (MNTHCC) and 2-nitroso-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (NTHCC) in foods. The method is based on (a) extraction of the acidified food sample with methanol, (b) removal of fats and lipids by partitioning of the extract with *n*-hexane, (c) cleanup on an acidic alumina extraction cartridge, and (d) determination by a liquid chromatography (LC) postcolumn chemical denitrosation/thermal energy analyzer (TEA) technique [Havery, D.C. (1990) *J. Anal. Toxicol.* **14**, 181–185] or by conventional LC-TEA analysis after esterification of the compounds with diazomethane. Confirmation was performed by LC/MS of the free acids and also by GC/MS of the methyl ester. Recoveries of MNTHCC and NTHCC added to various foods (e.g., cured meats, fried bacon, Chinese and Japanese pickled or fermented vegetables, soy sauce) at 50–100 ppb spiking levels ranged between 84–100%. The minimum detection limit of the method was estimated to be about 10 ppb for each compound. The formation of both MNTHCC and NTHCC in nitrosated samples of several Japanese and Chinese pickled vegetables, soy sauce, and cheeses was demonstrated. The unnitrosated samples in most cases were negative.

Work has continued on the problem of nitrosamine formation in cured pork products packaged in elastic rubber nettings. The concern is that nitrite used for curing meat products can react with the amines in the rubber and produce both volatile and nonvolatile nitrosamines. Fifteen samples of unused nettings were analyzed. They contained only traces (nondetected to 8 ppb) of *N*-nitrosodibutylamine (NDBA), but fairly high levels (45–660 ppb) of *N*-nitrosodibenzylamine (NDBzA). After deliberate nitrosation of the nettings, much higher levels of both NDBA (100–760 ppb) and NDBzA (0.8–12.8 ppm) were produced. These results suggested that the nettings had the potential to form both NDBA and NDBzA if used for packaging cured meat products.

Sen also made the recommendation for a change in the status of the method for determining NDMA in nonfat dry milk powder (**984.16**). Since 1984, the method has been used in the

Associate Referee's laboratory with no problems. In addition, the method has been used successfully by other laboratories in Canada and the United States. To date we have received no adverse remarks or comments. Therefore, it is recommended that the method be given official final action status.

*Polydimethylsiloxane in Foods.*—The successful collaborative study on determination of polydimethylsiloxane residues in pineapple juice by atomic absorption spectroscopy was presented at the AOAC 104th Annual International Meeting. The General Referee recommends the method be given official first action status.

*Polycyclic Aromatic Hydrocarbons.*—During the past year, work has focused on evaluating/developing methodology for determining PAHs in seafood. The following 3 projects were involved: (*1*) An evaluation of 3 analytical methods for the determination of PAHs in seafood, (*2*) preliminary studies of the relationship between organoleptic properties and PAH contamination of seafood harvested from oil contaminated waters, and (*3*) verification of the modified LC/fluorescence method for determination of PAHs in seafood.

The 3 methods evaluated included a GC/MS procedure, an LC/fluorescence procedure, and a modification of the latter method. On the basis of these findings, it was concluded that the cleanup procedure used in the modified LC/fluorescence method is cheaper, faster, and more reliable. Furthermore, the instrumentation needed is less expensive and more widely available than that required for the GC/MS method. Initial studies of the relationship between organoleptic properties and muscle tissue levels of PAH contamination were completed. Thirty-four organoleptically screened samples of various types of seafood (mussels, chiton, snails, sole, greenling, herring, cod, and octopus) were received from the Alaska Department of Fish and Game. The samples were cleaned, homogenized, and stored in a freezer. Seven samples were analyzed by the modified method. Six were organoleptically positive and 1 was negative. PAH levels in the negative sample were generally less than 1 ppb. In the positive samples, 9 apparent PAHs were found, ranging in levels from 1 to 325 ppb. These data suggest that there may be a relationship between organoleptic properties and the actual residue levels of petroleum hydrocarbons found in the edible tissues of seafood. However, further analyses and controlled studies must be conducted before a final conclusion can be drawn.

*Sulfiting Agents in Foods.*—A report describing the procedure for determining free and reversibly bound sulfite by formaldehyde capture/LC has been published (*Food Addit. Contam.* (1990) **7**, 575–581). Sulfite levels have been determined by Monier-Williams and LC methods in a variety of foods including dried fruit, dried mashed potatoes, shrimp, canned mushrooms, fruit juices, and vegetables. The levels found range from 0 ppm in orange juice to 3330 ppm as sulfur dioxide in dried fruit. Cooking did not cause a significant reduction in the sulfite levels found in uncooked shrimp, hominy, or mashed potatoes. The samples were also analyzed by LC to determine the proportion of free and reversibly combined sulfite as well as to compare the LC procedure with the optimized Monier-Williams procedure. To verify that sulfur dioxide rather than an artifact was being distilled, the foods were analyzed by the optimized Monier-Williams method (with and without a 2% formaldehyde trap) and subsequent LC analysis.

*Urethane in Foods.*—Ethyl carbamate (EC), also known as urethane, is an animal carcinogen and a by-product of fermentation. Methods were developed and published for determining EC in alcoholic beverages and a variety of fermented foods by Canas et al. (*J. Assoc. Off. Anal. Chem.* (1988) **71**, 509–511; and *J. Assoc. Off. Anal. Chem.* (1989) **72**, 873–876).

EC has been found in distilled spirits, wines, and, at much lower levels, in a variety of fermented foods and beverages. To date, 279 fermented foods and beverages have been analyzed. These include malt beverages, bread, soy sauce, cheese, yogurt, cider, wine vinegar, olive, pickle, relish, condiment sauce, cultured buttermilk, sour cream, and orange juice. EC levels were detected only in yeast fermented foods and beverages. EC levels in bread and malt beverage samples ranged from nondetected to 13 ppb. EC levels in soy sauce ranged from nondetected to 93 ppb with a mean of 16.5 ppb. EC levels in nonyeast fermented foods were generally not detected.

Experimentation has demonstrated that heat increases the level of EC in wines. Toasting increases the level of EC in bread an average of 2.6 times its fresh level. When soy sauce is heated at 70°C for 48 h, the level of EC increases an average of 3.4 times.

### *Recommendations*

(1) Adopt as official first action the interim first action method for determination of polydimethylsiloxane residues in pineapple juice by atomic absorption spectroscopy.
(2) 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**).
(3) Continue study on all other topics.

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## Meat, Poultry, and Meat and Poultry Products

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During the past year, 1 method was approved by the Official Methods Board and granted official first action. One new collaborative study was initiated, 4 new topic areas were established, and 4 new Associate Referees were appointed.

*Bouillons and Consommes.*—It is recommended that this topic be discontinued for lack of activity, as suggested by Margreet Lauwaars, AOAC European Representative.

*Glutamic Acid and Monosodium Glutamate.*—No Associate Referee report received.

*Gluten in Meat.*—This topic was transferred to the Committee on Foods II during 1990 as a result of General Referee's 1990 report recommendation. Reporting on this topic by this General Referee will be discontinued.

*Heme, Nonheme, and Total Iron in Meat, Fish, and Poultry Products.*—This is a newly established topic as of July 2, 1991. Arthur W. Mahoney, Utah State University, Logan, UT has volunteered to be the Associate Referee.

*Hydroxyproline in Meat.*—Associate Referee, Kurt Kolar, Swedish Meat Research Institute reports no new activity since his study comparing microwave and Kjeldahl digestion, reported in the 1990 General Referee's report. He recommends that this topic be discontinued. Kolar reported on a calibration study of the Infratec Food and Feed Analyzer using near IR transmission spectroscopy. The calibration included moisture, fat, protein, and hydroxyproline determinations in beef and pork. This work will be reported in *Die Fleischwirtschaft.*

*Immunological Methods for Meat and Poultry Products.*—The Associate Referee is no longer employed or actively working in this area. Recommend topic be discontinued.

*Ion Chromatography Analysis of Meat and Meat Products.*—Associate Referee Mark Paine reports some initial progress on the development of an ion chromatography method for detecting and quantitating nitrite, nitrate, sulfite, ascorbate, and benzoate. Problems are encountered with interference from high chloride content of meat products. He intends to continue working on this problem.

*Liquid Chromatographic Methods for Meat and Poultry Products.*—Associate Referee Sher Ali has organized a collaborative study on a liquid chromatography (LC) method for the analysis of carbamates in liver. He reports a delay in this study because of higher priority analytical needs in his laboratory. It is recommended that this work continue. He reports renewed interest in methodology for preservatives in meats and suggests a collaborative study of a method he published in the AOAC *Journal* in 1985, "Rapid Quantitative Method for Simultaneous Determination of Benzoic Acid, Sorbic Acid, and 4 Parabens in Meat and Nonmeat Products by Liquid Chromatography."

*Liquid Chromatography of Creatinine in Soups and Bouillons.*—This is a newly established topic as of July 2, 1991. R. M Maeyer, Nestle Nederland b.v., The Netherlands, has volunteered to be the Associate Referee.

*Microwave Techniques for Meat Analysis.*—No Associate Referee report received.

*Protein Determination in Meat by Combustion Method.*—Associate Referee, Joseph G. Sebranek, Iowa State University, reports that 11 laboratories are participating in a collaborative study designed to evaluate the combustion (Dumas) method for determination of protein in meat and meat products using new instrument designs currently available. This method has advantages over the Kjeldahl in terms of safety and environmental concerns. The 11 laboratories completed analyzing practice samples followed by some instrument adjustments. The results of the 30 collaborative study samples have been reported by most laboratories. Data from the study will be analyzed once received from all 11 laboratories.

*Crude Protein.*—Associate Referee David Christians reports that there has been no activity on this topic and anticipates none in the near future. On the basis of his comments, it is recommended that this topic be discontinued.

*Near Infrared Proximate Analysis of Meat Products.*—Larry Hands, University of Oklahoma, was appointed Associate Referee for this topic. A protocol for a collaborative study to be conducted in the near future is being prepared.

*Proteins in Meat.*—A collaborative study was conducted in 1986 comparing copper and mercury catalysts in the Kjeldahl method. A resubmission of the paper after additional statistical analysis was again rejected. The paper is undergoing additional data analysis to address current problems. Because of the critical need for alternate catalysts to the mercury catalyst for environmental reasons, it is recommended that this study be completed or a new study conducted.

*Rapid Moisture Analysis.*—Recommend topic be discontinued.

*Robotic Methods.*—Associate Referee Randy Simpson reports that methods development efforts in the Food Safety and Inspection Service laboratory, Athens, GA, has concentrated on investigating tissue extraction techniques suitable for automation with robotic equipment. Considerable success is reported on adapting the technique of suspending the sample matrix on a solid carrier (matrix solid phase dispersion) for use with robotics. Promising results are reported on the automation of ivermectin and nicarbazin methods.

*Specific Ion Electrode Applications.*—Frank McGovern, Associate Referee, reports no activity, but he plans to initiate a collaborative study involving the sodium ion electrode next year.

*Minimum Processing Temperature.*—No Associate Referee report received.

*Total Fat.*—Max Foster, Associate Referee, conducted a collaborative study and prepared a manuscript, "Total Fat in Meat Using the SOXTEC Fat Analyzer: A Collaborative Study." The study was voted on and approved by the Official Methods Board and granted official first action.

*Volatiles in Meat and Poultry, and Meat and Poultry Products.*—Jeffrey P. Donohue was appointed Associate Referee. He reports that future studies for his laboratory have not been finalized. His current work involves the use of a dynamic headspace and purge and trap method with capillary gas chromatographic separation and mass spectroscopic detection.

The analysis of volatiles in meat and poultry has been a very active area of research in 1990. There have been approximately 24 papers published, over 15 in the application of flavor and fragrance, 5 in food chemistry, and several in application area of food toxicology. Two Ph.D. dissertations on work in volatile components of meat were also accepted in 1990. Although not all of these papers involved headspace or purge and trap techniques, the analysis of volatiles in meat or poultry is an active area of research in several laboratories.

*Recommendations*

(1) Discontinue the topic *Bouillons and Consommes.*
(2) Discontinue the topic *Hydroxyproline in Meats.*
(3) Discontinue the topic *Immunological Methods for Meat and Poultry Products.*
(4) Discontinue the topic *Crude Protein.*
(5) Discontinue the topic *Rapid Moisture Analysis.*
(6) Continue study in all other topics.

This report of the General Referee was presented at the 105th AOAC Annual International Meeting, August 12–15, 1991, at Phoenix, AZ. 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*, 2nd supplement, 1991.

## Mycotoxins

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Keeping up-to-date with conferences and books relating to mycotoxins has now been made much easier with publication of the *Mycotoxicology Newsletter*. This is now in its second year, and it is edited by U.L. Diener, Department of Plant Pathology, Auburn University, AL 36849, who welcomes contributions.

Of the mycotoxins that are not covered by the Associate Referee reports below, patulin has now been evaluated by the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (1). A provisional tolerable weekly intake of 7 μg/kg body weight was set. Although a maximum intake of patulin by children from apple juice was estimated to be only 0.26 μg/kg body weight/day, there was concern over occasional samples of heavily contaminated apple juice. While on the subject of international evaluations of health risks from mycotoxins, attention is also drawn to a book on environmental health criteria for ochratoxins, trichothecenes, and ergot alkaloids sponsored by the International Programme on Chemical Safety (2).

*Aflatoxin M.*—Associate Referee Robert D. Stubblefield (U.S. Department of Agriculture (USDA), Peoria, IL) reports that the pilot study on an immunoaffinity column method for determination of aflatoxin $M_1$ in milk has been successfully completed by 4 laboratories. The methodology for this International Dairy Federation (IDF) collaborative study is satisfactory, but the protocol does not include artificially contaminated samples. The organizer of the study, L.G.H. Tuinstra, has been informed that AOAC would require recovery data from use of spiked samples, and the Associate Referee has proposed that standard solutions of aflatoxin $M_1$ from sealed ampules be added to the collaborators' commercial milk samples.

The joint United Nations Environment Programme (UNEP)/Food and Agriculture Organization/World Health Organization Food Contamination Monitoring Program includes a study on aflatoxin $M_1$ in lyophilized milk. Of the participants, 30% used liquid chromatography (LC). Two AOAC official methods are included: **974.17** (3), (Pons method) (4); and **980.21** (3), (Stubblefield method) (5). The use of the first method, **974.17**, is decreasing each year, while use of the second method remains steady, at 20%. Laboratories using method **974.17** had significantly lower results than laboratories using other methods. The study found an increase (10%) in the use of immunoassay methods.

IDF Group E33 reported that the final version of the revised IDF Standard 111:1982 "Determination of Aflatoxin $M_1$" (essentially AOAC **980.21**) was approved. The new number will be published as IDF Standard 111A:1990. The revision of the Stubblefield method includes changing 1-dimensional thin-layer chromatography (TLC) to 2-dimensional TLC, and adding the procedure for LC.

In 1990, the Southeast United States and Texas experienced a severe drought that intensified the presence of aflatoxin in corn crops. The state of Georgia monitored its milk supply and found most samples contained trace concentrations of aflatoxin $M_1$ (6) and 1 farmer dumped 1400 gallons of contaminated milk exceeding the 0.5 ng/mL limit. Texas experienced aflatoxin contamination for the third straight year (7). George Latimer, State Chemist, reported that Texas farmers had accumulated large amounts of contaminated corn; therefore, they requested permission from the Food and Drug Administration to ammoniate corn containing less than 1000 ng/g, and to blend corn containing less than 500 ng/g to reduce the levels to ≤200 ng/g. The FDA refused permission, but Texas decided to go ahead with their plan anyway (8). A Texas A & M aflatoxin project reported a rapid detection method for $M_1$ in milk, a modified selective adsorption of mycotoxins (SAM) technique (9). The 20 min test is sensitive to less than 0.5 ng aflatoxin $M_1$/mL.

Other recent developments in methodology for aflatoxin $M_1$ include 2 automated assay systems (10, 11), and an evaluation of an enzyme immunoassay that uses antibody-coated polystyrene beads, or a "bead method" (12). The latter was comparable in performance to an LC method.

A survey of raw milk in Czechoslovakia for aflatoxin $M_1$ using immunochemical analysis found a 7% incidence in the 0.1–0.5 ng/mL range (13). Aflatoxin $M_1$ was not detected in 95% of commercial milk from a large dairy plant (positive samples had only low concentrations, ranging from 0.025 to 0.1 ng/mL).

There was a report of the presence of *Aspergillus flavus* and aflatoxins $B_1$, $B_2$, and $M_1$ in an Arkansas corn sample associated with the deaths of 3 horses (equine aflatoxicosis) (14). The toxin concentrations were 114, 10, and 6 ng/g respectively. It is unusual to find aflatoxin $M_1$ in corn samples.

The book *Mycotoxins in Dairy Products* (15) contains a good review of aflatoxin $M_1$, including its occurrence, toxicity, and methods of analysis. The published proceedings of the 1990 International Dairy Congress include a presentation on the determination of aflatoxins in milk and other dairy products by the Associate Referee (16).

The Associate Referee will resign effective September 3, 1991. He recommends that the new Associate Referee consider surplussing method **974.17** in 1992, and that methods **980.21**, **986.16**, **982.24**, and **982.25** continue in official final action status.

*Aflatoxin Methods.*—Associate Referee Mary W. Trucksess (FDA, Washington, DC) notes that recently more evidence on the mode of action of aflatoxin has been found, and a specific mutation has been identified that may be responsible for hepatocellular carcinoma (17, 18). In 1990, 2 guidelines for aflatoxins in food were proposed. The Food Codex Committee on Food Additives and Contaminants had agreed to set a maximum level of 10 μg/kg of total aflatoxins in all foods, excluding milk and dairy products (19). However, the Committee decided in meetings March 4–9, 1991, in The Netherlands not to establish a maximum level for total aflatoxins for all foods at the present time because of the objections by several delegations (20). These delegations maintained that a single level for all commodities was not logical for 2 reasons: (*1*) Levels for commodities that will undergo further processing can be higher than the level for ready-to-consume products because processing or sorting can reduce aflatoxin levels, and (*2*) sampling variability must be taken into account. In the United States, the Peanut Administrative Committee has proposed and implemented the level of 15 ng/g for aflatoxins in raw peanuts for human consumption (21). This is in response to the peanut industry's desire to provide consumers with the best product possible and to ensure only wholesome peanuts enter edible market channels.

This year many of the published methods have been centered on immunochemical techniques, including enzyme linked immunosorbent assays (ELISAs), which can give either qualitative or quantitative results, and assays involving immunoaffinity column cleanup, which is coupled with LC or TLC for separation and quantitation. Both immunochemical approaches have been applied to a wide variety of matrixes: peanuts (22), peanut butter (23, 24) cottonseed (25), rodent feed (26), and human serum (27, 28) (See also *Immunochemical Methods*). The Federal Grain Inspection Service of USDA has decided to use Vicam's Aflatest™ (immunoaffinity cleanup column) for aflatoxin in corn, sorghum, wheat soybeans, milled rice, and processed commodities (29). The method using this immunoaffinity column coupled with quantitation by solution fluorometry or LC with postcolumn derivatization was subjected to an AOAC/International Union of Pure and Applied Chemistry (IUPAC) collaborative study (30), and it was approved by AOAC as official first action for corn, peanuts, and peanut butter at ≥10 ng total aflatoxin/g. Many immunoassay kits are now commercially available, and these are products of biotechnological development that is a rapid growing field of science. The format, the design of the assays, and in some cases the reagents used have been and are constantly modified. For example, the size of the device used in a previously studied and AOAC approved immuno-dot cup method (31) has been reduced. Hence, the amount of test sample and reagents required are modified to accommodate the capacity of the smaller device. Details of the changes will be published in the AOAC Changes of Methods supplement.

The collaboratively studied ELISA method (Biokits) for determination of total aflatoxin levels in peanut butter was adopted as official first action by the Official Methods Board at the 1991 AOAC Annual Meeting in Phoenix, AZ.

The collaborative study of an LC method using precolumn derivatization with trifluoroacetic acid, which was adopted by AOAC as official first action for the determination of aflatoxins in peanut butter and corn at concentrations ≥13 ng/g, has been published (32). A European collaborative study has been performed on a method for determination of aflatoxin $B_1$ in feedstuffs (33). On the basis of the LC (postcolumn iodination) results, the method has been recommended to the European Community as an official method. Degradation of aflatoxins in common reversed-phase LC solvents was studied by Beaver (34).

An extensive review concerning detoxification of aflatoxins in foods and feeds by physical and chemical methods has been published (35). In a feeding study of growing lamb, the addition of 2% hydrated sodium calcium aluminosilicate to the diets was found to greatly reduce the toxic effects resulting from exposure to aflatoxin at the level of 2.6 mg/kg feed (36). A paper entitled "Laboratory Safety Considerations in the Handling of Natural Toxins" presented at the American Chemical Society 1990 annual meeting (37) will be published soon. Included are guidelines for handling mold cultures, moldy commodities, and pure toxins including aflatoxins; preparing samples for analysis as well as for animal feeding studies; and decontaminating the laboratory.

The Associate Referee has been developing an LC immunoaffinity column method and making preparation for 2 collaborative studies. One is a repeat study of an ELISA method for corn (31); the other is the Mycosep No. 224 column cleanup method. It is recommended that study of automating solvent efficient methods be continued and the feasibility of using supercritical fluid chromatography for aflatoxin analysis be explored. The Associate Referee also recommends an editorial clarification in **989.06** Aflatoxin $B_1$ in Cottonseed Products and Mixed Feed. Enzyme-Linked Immunosorbent Screening Method: insert Absorbance Measurement (optical density) after Method Performance.

*Alternaria Toxins.*—Edgar E. Stinson has retired. The new Associate Referee is Angelo Visconti (National Research Council, Institute of Toxins and Mycotoxins, Bari, Italy).

Recent developments in this area include studies on natural occurrence of *Alternaria* toxins in Italian mandarins infected with *Alternaria alternata* (38), and in sorghum and ragi in India (39). Normal and reversed-phase LC systems were evaluated for determination of tenuazonic acid, alternariol, alternariol methyl ether, and altenuene in inoculated tomatoes and apples (40), with a normal phase (silica) system found to be the most suitable. A reversed-phase LC method for altertoxins I and II using electrochemical detection has been applied to their determination at sub-μg/g levels in various foodstuffs infected by *A. alternata* (41).

*Citrinin.*—Associate Referee David Wilson (University of Georgia, Tifton, GA) reports that little progress has been made in solving the difficulties of citrinin estimation. The best LC separation of citrinin seems to be accomplished using an acid-buffered silica gel column (42). However, this method does not lend itself to collaborative study because of the time and effort required to prepare the buffered silica gel column. Therefore, efforts are currently underway to test nonsilica based LC columns. Reversed-phase LC of citrinin using a mobile phase of pH 3.8 containing the ion-pairing reagent tetrabutylammonium phosphate was reported by Vail and Homann (43). The Associate Referee recommends continued study on citrinin.

*Cyclopiazonic Acids.*—Associate Referee Joe W. Dorner (USDA, Dawson, GA) reports that progress is being made in the development of an ELISA for α-cyclopiazonic acid. He also notes 2 reports on α-cyclopiazonic acid presented at the 1990 AOAC meeting. In a new LC method for determination of cyclopiazonic acid in corn (44), the toxin is extracted with methanol–2% sodium bicarbonate (70 + 30), partitioned into chloroform following acidification, and separated on a $C_{18}$ column with gradient elution of 0–4mM $ZnSO_4$ in 85% methanol. The second report showed that cyclopiazonic acid accumulated in the eggs (particularly egg whites) of chickens that were administered cyclopiazonic acid as well as in the milk of sheep that consumed the toxin (45). In view of the continued interest in α-cyclopiazonic acid, the Associate Referee recommends continued study on this mycotoxin.

*Ergot Alkaloids.*—Associate Referee George M. Ware (FDA, New Orleans, LA) reports that a collaborative study of an LC method for ergot alkaloid determination in wheat and rye has not been completed. The collaborative study was initiated in 1989, yet the Associate Referee has received only 7 returns. AOAC requires a minimum of 8 laboratories for a statistically valid collaborative study. The Associate Referee will confer with the General Referee with respect to the final disposition of this study. He recommends that the study be completed and will contact 1 or 2 of the laboratories to encourage them to finish their analyses. If necessary, new standards and test materials will be supplied.

A short review of the occurrence of ergot and ergot alkaloids in Canada was part of a paper on the potential for occurrence of mycotoxins and toxigenic fungi in cereal grains in western Canada (46). Recent reports on methodology for ergot alkaloids include an LC method for quantitating ergovaline in endophyte-infested tall fescue (47), hydrophobic uptake and voltammetry of ergonovine maleate, ergotamine tartrate and ergocristine at lipid-coated electrodes (48), and a TLC method for ergot alkaloids in plants (49). New ergot alkaloids continue to be reported from fungal endophytes of grasses; ergobalansine from *Balansia obtecta* differs from other ergot peptide alkaloids in that the characteristic proline residue has been replaced by an alanine residue (50).

The Associate Referee recommends that method development work be conducted on a suitable solid phase extraction cleanup for ergot alkaloids in grains. This should save time, solvent cost, and solvent waste. He plans to adapt the current liquid-liquid partition procedure to a solid phase extraction cleanup procedure for ergot alkaloids.

*Fumonisins.*—Associate Referee Ronald D. Plattner (USDA, Peoria, IL) reports that research on fumonisins has been an active area in the past year. There was a 2-day conference dedicated to fumonisins April 24, 25, 1991 at Raleigh, NC. The Gordon Research Conference on mycotoxins and phycotoxins in Plymouth, NH, June 24–28, 1991 had several talks devoted to fumonisins. Fumonisins were associated with on-farm animal toxicoses (primarily equine leukoencephalomalacia (ELEM) and porcine pulmonary edema) in the United States. Most of these cases were associated with consumption of corn screenings from the 1989 corn crop (51). There appears to be a much lower incidence of problems with the 1990 corn crop.

A method for determination of fumonisin $B_1$ and $B_2$ was published by the South African group (52). This method involves extraction, cleanup on a strong-anion exchange column, derivatization of the free amino group at C-2 with *o-phthaldialdehyde (OPA) and mercaptoethanol to form a fluorescent derivative, and determination by LC with a fluorescence detector. The report claims detection limits of 50 and 100 ng/g for fumonisins $B_1$ and $B_2$, respectively, in corn and corn feeds. A collaborative study of this method under the auspices of IUPAC is underway. This method was used to determine fumonisins in a limited study in southern Africa, where significantly higher levels of both fumonisins $B_1$ and $B_2$ were present in healthy corn samples from an area with a high rate of human esophageal cancer than in corresponding samples from a low rate area (53). Two other methods using a fluorescent derivative of the free amine have been reported (51, 54). An additional method that involves hydrolysis followed by gas chromatography/mass spectrometry (GC/MS) of a derivative of the aminopentaol backbone of fumonisin $B_1$ has been reported (55).*

Samples have been exchanged informally among several groups interested in fumonisin assays and in developing methods for analysis. The Associate Referee reports that agreement in fumonisin $B_1$ concentrations he has seen from analyses in laboratories using different methods are encouraging. A small study of interlaboratory variability for fumonisin $B_1$ analyses using a set of 5 samples containing from 2 to 1800 μg/g of fumonisin $B_1$ was reported (56). Four laboratories analyzed the samples by the fluorescamine procedure (57). Additionally, 2

of the laboratories did a second assay by another method. John Reagor of the Texas Veterinary Medical Diagnostic Laboratory has agreed to send samples and compile results on fumonisins for the American Association of Veterinary Laboratory Diagnosticians.

In the Associate Referee's laboratory, good agreement in analyses for fumonisin $B_1$ in corn and corn products by LC (OPA derivative) and by hydrolysis GC/MS is routinely achieved. The GC/MS method has good sensitivity and high selectivity but, because the method does not use an internal standard, the precision of the GC/MS procedure is poor. To improve precision, deuterium labeled fumonisin $B_1$ has been produced (58) for use as an internal standard. When this labelled fumonisin is added to extracts to compensate for variability (in the hydrolysis, cleanup, derivatization and GC/MS steps) precision is greatly improved.

The Associate Referee recommends continued evaluation of the published methods for fumonisins with particular emphasis on the sample extraction and cleanup steps before beginning a formal AOAC collaborative study.

*Immunochemical Methods.*—Associate Referee James J. Pestka (Michigan State University, East Lansing, MI) reports on new general references that are of relevance to mycotoxin immunoassays. First, the new journal *Food and Agriculture Immunology* is an excellent source for new information on immunoassay of mycotoxins and other chemicals. Manuscripts dealing with these topics are being requested. Second, the recent book "Immunoassays for Trace Chemical Analysis" (59) discusses several mycotoxin immunoassays. Thirdly, the proceedings of the Third Conference on Improvement of Comparability and Compatibility of Laboratory Assay Results in Life Sciences Immunoassay Standardization was published (60). In an opening comment that mirrors concerns of the AOAC, Bergmeyer (61) noted that immunological methods now make up 50% of all clinical chemical methods used and that clinical chemists "find it hard to understand the far higher relative standard deviations that may be returned by immunoassays compared to traditional methods (perhaps as much as an order of magnitude higher: 50% vs 5%) and why the status of standardization of immunoassays is so poor." Eighteen papers follow that describe calibration, matrix effects, reference materials, analytical variation, and performance characteristics that may be of particular interest to analytical chemists employing recently introduced mycotoxin immunoassay kits. Finally, in a review, Kaufman and Clower (62) discussed current approaches by the Environmental Protection Agency (EPA), FDA, USDA, AOAC, and IUPAC for evaluation and acceptance of immunoassay kits.

An immunoaffinity column procedure coupled with solution fluorometry or LC has been adopted as official first action by AOAC as an AOAC-IUPAC method for analysis of total aflatoxins in corn, peanuts, and peanut butter (30). Additionally, several studies described the comparison of aflatoxin immunoassays to conventional chemical methods (22, 63–65). Lee et al. (25) reported the integration of ELISA with conventional chromatographic procedures for quantitation of aflatoxin $B_1$ in individual cotton bolls, seeds, and seed sections.

New developments and improvements continue in the aflatoxin immunoassay area. An ELISA for versicolorin A and related aflatoxin biosynthetic precursors has been described (66). A new immunoblot approach called ELISAGRAM was devised that combines the sensitivity and selectivity of competitive ELISA with the capacity of high performance thin layer chromatography (HPTLC) to separate structurally related haptens (67). The procedure involves (*1*) separation of haptens by HPTLC, (*2*) blotting of the HPTLC plate with nitrocellulose that is coated with hapten-specific monoclonal antibody, (*3*) incubation of nitrocellulose with hapten-enzyme conjugate to identify unreacted antibody binding sites, (*4*) detection of bound enzyme conjugate with a precipitating substrate, and (*5*) visual or densitometric assessment of inhibition bands indicative of a cross-reacting hapten. Multiple standard curves for the aflatoxins, zearalenone, and α-zearalenol could be constructed using scanning densitometry.

Advancements in epidemiological assessment of human aflatoxin exposure were noted. Autrup et al. (68) found evidence for human antibodies that recognize an aflatoxin epitope in groups with high and low exposure to aflatoxins. Wild et al. (69) evaluated 3 methods for quantitation of aflatoxin–albumin adducts and applied them to human exposure assessment.

To assess the potential for mycotoxin contamination of the human food supply following the 1988 U.S. drought, 92 grain food samples purchased from retail outlets in 1989 were surveyed for aflatoxin $B_1$, zearalenone, and deoxynivalenol (DON or vomitoxin) by ELISA (70). Only 1 sample (buckwheat flour) was found to contain aflatoxin $B_1$ (12 ng/g), whereas zearalenone was found in 26% of the samples at a mean level of 19 ng/g. In contrast, the ELISA for DON indicated positive results in 50% of the samples at a detection level of 1.0 μg/g. Positive samples were corn cereals, wheat cereals, wheat flour/muffin mixes, rice cereals, corn meal, oat cereals, wheat- and oat- based cookies/crackers, corn chips, popcorn, and mixed grain cereals. The mean DON content of the positive samples was 4.7 μg/g, and the minimum and maximum mean levels were 1.3 and 19 μg/g, respectively. When positive ELISA samples were also analyzed by LC, a strong correlation between the 2 methods was found.

Research continues on the immunoassay of *Fusarium* toxins. Teshima et al. (71) reported on the development of a monoclonal antibody to zearalenone with no cross-reactivity for the zearalenols or zearalanols. A radioimmunoassay has been described for nivalenol in barley (72), and a chromatographic enzyme immunoassay was reported for T-2 toxin (73). When the reactivity of a previously described monoclonal antibody for DON was evaluated further, it was found to cross-react with isotrichodermin, 3-acetylDON, 15-deacetylcalonectrin, and 3,15-dideacetylcalonectrin (74). A generic antibody against group A trichothecene mycotoxins was used in a competitive ELISA for detection of T-2 toxin and its metabolites in the urine of rats and monkeys after a single dose of T-2 toxin (75).

*Ochratoxins.*—Associate Referee Stanley Nesheim (FDA, Washington, DC) reports that the collaborative study of an LC method for ochratoxin A presented at the AOAC Annual Meet-

ing last year (76) has been approved by the Committee on Foods I. The method was adopted for corn and barley as Official First Action by the Official Methods Board at the 1991 AOAC Annual Meeting in Phoenix, AZ. The Associate Referee is planning to evaluate immunochemical methods for ochratoxin A. Presently, 2 different immunochemical test kits are commercially available.

This year several new reports of the natural occurrence of ochratoxin A were noted, including its finding in 2 of 10 samples of Turkish corn (77), and in pig serum and mixed feeds in Japan (78). Both ELISA and LC were used to analyze livestock sera in the latter report. Some good news is that the FDA, in a survey of 351 samples collected across the United States, found no ochratoxin A (79). The samples included green coffee beans, cereals, soybeans, beans, peas, corn, and processed food products from these commodities. Meat and meat products were not included. To get a better picture of the incidence of ochratoxin A, IUPAC has initiated a worldwide survey (questionnaire sent to selected countries) (80). The information requested included: product, geographic origin, year, number of samples analyzed, detection limit of method used, confirmatory procedure used, % positive samples, and references.

The health risks of ochratoxin A based on exposure estimates and a hazard assessment has been performed on ochratoxin A, and the results compared with similar risk assessments on aflatoxin $B_1$ and zearalenone (81). Ochratoxin A is reported to be an important western Canadian storage mycotoxin (82). Current results are summarized and related to animal disease. In a paper submitted to *Pure and Applied Chemistry* in 1991, the significance of ochratoxin A as a factor in human and animal health was reviewed (83).

*Trichothecenes.*—Associate Referee Robert M. Eppley (FDA, Washington, DC) notes that surveys of cereals from diverse locations around the world for the presence of the trichothecenes have continued to demonstrate widespread natural occurrences of the 8-ketotrichothecenes (84–90). Limited surveys of wheat from China (85), Poland (86), U.S.S.R. (87), Argentina (88), and The Netherlands (89) have reported the presence of DON, with the highest level of 38 μg/g in a moldy wheat sample from Poland. Other trichothecenes, including nivalenol (NIV), 3-acetylDON, and 15-acetylDONs were found when the samples were analyzed for these toxins. Zearalenone was frequently present along with the trichothecenes. In the survey of grains harvested in The Netherlands (89), DON and NIV were also detected in barley, oats, corn, and rye samples.

A recent publication (91) investigating the production of DON and NIV by single ascospore isolates of *Gibberella zeae* strains showed the presence of 2 groups, one producing DON and its acetyl derivatives and the other producing NIV and 4-acetylNIV. Within the DON-producing group, 3-acetylDON and/or 15-acetylDON could be produced in a single isolate, while in the NIV group, some isolates could also produce lower levels of DON, but no acetylDON derivatives. Another publication investigated the production of mycotoxins by *Fusarium* species growing on soybeans (92). Most of the cultures did not produce as well on the soybeans as they did on rice, but 1 isolate of *Fusarium poae* produced more HT-2 toxin on soybeans than on rice, indicating soybeans could be contaminated with trichothecenes under favorable conditions. One other publication (93) noted that 40–100% of the DON and zearalenone could be removed from cereals by dehulling; however, 13–19% of the grain material and 22–32% of the protein were lost in the process.

As reported last year, several methods have been published for the determination of multiple trichothecenes. Two additional methods (94, 95) were noted this year. One method (94) has GC/electron capture detection (ECD) as the determinative step, similar to the method of Scott et al. (96), but has a different extraction and cleanup procedure. The other method (95) involves hydrolysis of the trichothecenes to their parent alcohols, followed by analysis. In this method, NIV and DON are detected and quantitated by LC, and NIV, DON, scirpentriol, and T-2 tetraol are determined by GC/ECD of the trifluoroacetyl derivatives. All of these multitrichothecene screening methods need to be evaluated to select one or more methods for possible collaborative study.

The Associate Referee recommends the following: (*1*) Studies be continued on the development of screening methods for the determination of trichothecenes in grains and foods, (*2*) a method capable of determining 5 or 6 of the most frequently occurring trichothecenes be selected for a preliminary collaborative study, and (*3*) studies be continued on other determination methodologies, including immunoassays, as they become available.

*Zearalenone.*—Associate Referee Glenn A. Bennett (USDA, Peoria, IL) reports that the ELISA screening method for zearalenone in corn, wheat, and feed has been reviewed by AOAC Committee on Foods I. As a measure of continuing interest in zearalenone, he has noted that over 40 reports were published during the last year dealing with natural occurrence, animal effects, assays of fluids from exposed animals, and new conjugates of the mycotoxin zearalenone. A comparative study was done on the natural occurrence of *Fusarium* toxins (DON, 15-acetylDON, NIV, and zearalenone) and the incidence of esophageal cancer in high- and low-risk areas of the People's Republic of China. Although DON was significantly higher in corn and wheat from the high-risk area, levels of zearalenone could not be implicated in this comparative study (85). Other reports show that zearalenone is a common metabolite of *Fusarium crookwellense* from different geographic areas (97), that endogenous *Fusarium* isolates produce zearalenone in silo maize (98), that zearalenone was present in 35% of cattle fodders examined in Serbia with levels ranging from 0.2 to 12.0 μg/g (99), and that 42% of cereals and mixed feed examined in Germany were contaminated with zearalenone (100). Interestingly, 9 of 37 isolates from cereals in Jordan were identified as *Gibberella fujikori* and *G. fujikori var intermedium*, and were reported to produce zearalenone at a mean level of 0.78 μg/g (101). Additional studies on the zearalenone-sulfate conjugate reported last year (102) demonstrate that *Rhizopus arrhizus* catalyzes sulfation of zearalenone at the C-4 hydroxyl group (103). Another conjugate, zearalenone 4-β-D-glucopyranoside, was synthesized and used to confirm the structure of the same

conjugate isolated from corn tissue cultures. An LC method using a gradient solvent system was developed to separate zearalenone, β-zearalenol, and the glucopyranoside (104). Zearalenone glucoside is decomposed during digestion in swine with release of zearalenone (105). Zearalenone has also been reported to be bound to nonextractable starch, hemicellulose, and lignin fractions in corn cell suspension cultures (106).

A sensitive tandem affinity chromatography/ELISA procedure was used to examine milk for zearalenone. No zearalenone was detected in the 12 commercial samples tested (107). Also, 92 grain food samples from retail outlets were surveyed by ELISA to assess the levels of mycotoxins in food products following the 1988 drought (see also *Immunochemical Methods*). Zearalenone was found in 26% of the samples at a mean level of 19 ng/g (70). A related method for zearalenone detection uses a novel type of immunogen, 5-aminozearalenone-bovine serum albumin, which exhibits very little cross-reactivity to zearalenone metabolites (71). Attention is also drawn to the ELISAGRAM (see *Immunochemical Methods*), whose applications include zearalenone and α-zearalenol (67).

Research on the effects of low levels of zearalenone on animals show that zearalenone did not have serious effects on the reproductive efficiency of young gilts at levels below 0.5 μg/g feed (108). Also, when 2520 breeding sows were monitored for reproductive characteristics, few effects were observed, although zearalenone was present in the fodder and feed at levels up to 1.1 μg/g. Vulvovaginitis was observed in 6 gilts (0.24%) and there were no abortions, premature parturitions, or changes in the milk glands (109). Studies conducted to determine if immunization against zearalenone would prevent zearalenone toxicosis in gilts demonstrated that immunization actually potentiated the estrogenic effects and urinary excretion of zearalenone equivalents (110). A sensitive GC/MS method has been developed for the quantitative analysis of zearalenone and α-zearalenol in urine of ruminants. The procedure has a detection limit of 1 ng/ml for both compounds, and it was used to confirm natural mycotoxicoses of cows (111).

Two reports were published on the destruction or inhibition of zearalenone. Ammoniation of highly contaminated barley did not destroy the toxicity of the zearalenone (112). Conversely, malathion was shown to inhibit endogenous zearalenone production in higher plants (113).

The Associate Referee recommends that studies continue to determine the extent of binding of zearalenone in cereal grains and development of procedures to measure "unextractable" and conjugated forms.

### *Recommendations*

(1) Adopt as official first action the method for determination of total aflatoxin levels in peanut butter by ELISA (Biokits).
(2) Make an editorial clarification to **989.06**: insert Absorbance Measurement (optical density) after Method Performance.
(3) Appoint new Associate Referee on *Aflatoxin M.*
(4) Continue study on all topics.

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## Plant Toxins

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There is growing interest in plant toxins, particularly in the area of herbal products in the "health food" market (1, 2). Substantial progress has been made toward obtaining the analytical reference materials required for several collaborative studies that are being planned. The planned associate refereeship for cyanogenic glycosides will be initiated upon development of a protocol for a method for cyanogenic glycosides in cassava products.

*Glucosinolates.*—(D. Ian McGregor, Agriculture Canada Research Station, Saskatoon, Saskatchewan.) Methods evaluation studies by the International Organization for Standards (ISO) and the American Oil Chemists Society (AOCS) are being considered for the possible recommendation for adoption or additional study.

*Hydrazines.*—(Joseph M. Betz, Natural Products and Instrumentation Branch, FDA, Washington, DC.) The joint International Union of Pure and Applied Chemistry (IUPAC)/AOAC collaborative study of the liquid chromatographic (LC) method of Stijve et al. (3) for agaritine in cultivated mushrooms is pending the procurement of additional quantities of agaritine. A review of hydrazines in the cultivated mushroom (*Agaricus bisporus*) has been published (4). The Nordic Committee of Senior Officials for Food Questions concluded that while there is "serious concern as to possible human health risk from consumption of the cultivated mushroom," additional toxicological and epidemiological information is needed to determine the human health risk.

*Hypoglycin in Ackee Fruit.*—(G. William Chase, Center for Nutrient Analysis, FDA, Atlanta, GA.) The initiation of the interlaboratory review and subsequent collaborative study of the ion exchange procedure for hypoglycin in fresh and canned ackee fruit (5) awaits the availability of sufficient reference standards. The Centers for Disease Control, Atlanta, GA, is planning a study of the health problems that have been associ-

ated with the consumption of ackee fruit in Jamaica (personal communication, R. H. Hill, Jr, CDC). For these epidemiological studies, reliable methods of analysis are required.

*Phytoestrogens.*—(Shia S. Kuan, Natural Toxins Research Center, FDA, New Orleans, LA.) Considerable efforts have been devoted to the optimization of conditions for the hydrolysis and extraction of phytoestrogens for soy-based products. This ruggedness testing has indicated that the reported procedure (6) is not yet suitable for collaborative study.

*Pyrrolizidine Alkaloids.*—(Robert M. Eppley, Natural Products and Instrumentation Branch, FDA, Washington, DC.) The pyrrolizidine alkaloids (PAs) continue to be the plant toxins of greatest interest, as evidenced by the number of publications on this subject.

Publications in the past year on analytical methods included a study of the supercritical fluid extraction of PAs from *Seniceo inaequidens* (7) and a review of gas chromatographic/mass spectrometric methods for PAs (8).

Two reports of particular interest were the chemo- and karyotaxonomic studies of some rhizomatous species of the genus *Symphytum* (9), and collaborative studies on the determination of PAs in *Symphytum* extracts (10). In addition, a book (11) and a comprehensive review article (12) on the PAs have been published.

Studies in the laboratories of the Associate Referee have focused on the development of a rapid method for the determination of PAs in comfrey products.

The Associate Referee recommends that the evaluation of methods for the determination of PAs be continued and that a survey of PAs in comfrey be conducted.

*Steroidal Alkaloids.*—(Allen S. Carman, Natural Toxins Research Center, FDA, New Orleans, LA.) All preparations for the collaborative study of an LC method for the potato glycoalkaloids (13) have been completed. This joint AOAC/Nordic Committee on Food Analysis (NMKL)/IUPAC study will be initiated upon final approval of the protocol. The Nordic Working Group on Food Toxicology and Risk Assessment (14) has published a report on the health risks of the potato glycoalkaloids. They concluded that a total glycoalkaloid concentration of 200 mg/kg of potato was the maximum level that should be present in potatoes for human consumption. However, they recommended that in new potato varieties, the average total glycoalkaloid concentration should not exceed 100 mg/kg.

### *Recommendations*

(1) Complete collaborative study of LC method for solanine and chaconine in potatoes in coordination with NMKL and IUPAC. Continue study in other areas.
(2) Continue study in all other associate refereeship areas.

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## Seafood Products

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*Decomposition by Gas and Liquid Chromatography.*—Associate Referee Walter Staruszkiewicz, Jr, Center for Food Safety and Nutrition, U.S. Food and Drug Administration (FDA), is organizing a collaborative study of the gas chromatography (GC) method for cadaverine and putrescine.

*Drained Weight of Block Frozen, Raw, Peeled Shrimp.*—Associate Referee Gerald Maus of Central Analytical Labs, Kenner, LA, has no report.

*Flow Injection Analysis (FIA).*—Associate Referee James Hungerford of the Seafood Products Research Center, Bothell, reports that the flow-injection method for histamine (1) was used by Seattle and Los Angeles Districts of FDA to screen hundreds of subsamples of Mahi-Mahi (genus *Coryphaena*, also called dolphin or dolphin fish) for histamine. The method, which was first developed using high-pressure liquid chromatography (LC) and postcolumn pumps, was implemented on a commercially available flow injection system based on low pressure (peristaltic) pumps. Many of the same Mahi-Mahi subsamples were analyzed by high pressure LC (2). Linear regression analysis of histamine (FIA) vs histamine (high pressure LC) over the 0–2600 ppm range free base showed excellent agreement between the 2 methods (slope = 0.92, R = 0.98, n = 156). In the range of regulatory interest (0–600 ppm), the FIA method gave somewhat higher predicted histamine levels than high pressure LC (slope = 1.1, R = 0.96, n = 130). A combination of the FIA and high pressure LC methods could be applied in concert as the basis of an efficient, automated, and comprehensive approach to the determination of histamine in fish. FIA would function as the rapid, initial screening method fish (by FIA) for histamine, with confirmation of positive results by high pressure LC. A precollaborative study of the FIA method for histamine is being organized; participants are solicited.

Enzyme inhibitors present in spoiled fish have been proposed as potentiators of histamine toxicity in Scombroid poisoning (3). Therefore, the selectivity-optimized conditions used in the FIA method for histamine are now being applied as part of an FIA system for the detection of inhibitors of diamine oxidase (DAO), one of the enzymes responsible for the regulation of histamine and other biogenic amines in mammals (4). The proposed FIA system, which is based on immobilization of the enzyme to sepharose, is capable of rapid, generic detection of DAO-inhibitors and simultaneous determination of histamine. No prior knowledge of the inhibitors' structural class is required. Mahi-Mahi samples implicated in a Scombroid poisoning outbreak showed inhibition of DAO (and histamine levels) much higher than fresh samples.

*Rancidity in Seafood.*—Associate Referee John French of the Fishery Industrial Technology Center, Kodiak, AK, is comparing sensory scores of rancidity with chromatographic data, including chromatograms of aldehydes and organoperoxide intermediates.

*Volatile Amines (TMA, DMA) by Gas Chromatography.*—Associate Referee Ronald C. Lundstrom of the National Marine Fisheries Service, Gloucester, MA, has evaluated lots of Chromosorb 103 column packing and found that only 2 of 6 were acceptable. Laboratories to participate in a precollaborative study should contact Lundstrom.

*Ciguatoxins-Biochemical Methods.*—Associate Referee Douglas Park, Department of Nutrition and Food Science, University of Arizona, will conduct a joint AOAC/International Union of Pure and Applied Chemists collaborative study of the solid phase immunobead assay (SPIA) (5) for ciguatoxin (CTX) and related polyethers. The study will be conducted in 2 stages: An initial, precollaborative study (with 4 to 5 laboratories participating) will be followed by a full collaborative study with at least 15 laboratories participating. Naturally contaminated ciguatoxic fish tissue, artificially contaminated fish tissue, standard solutions, and practice solutions will be provided. Laboratories interested in participating should contact Park.

*Ciguatera by Liquid Chromatography (LC).*—Associate Referee Robert Dickey, of the Fishery Research Branch, U.S. Food and Drug Administration (FDA), Dauphin Island, AL, reports that fluorescent derivatization and LC methods for the determination of chromophore-poor CTX continue to be investigated. Progress, and therefore, reporting, are hindered by the scarcity of CTX standard. LC methods for the determination of maitotoxin also are progressing slowly with no known reports released this year. Structure elucidation of the complex maitotoxin molecule remains an obstacle to analytical methods development. The LC-fluorometric method for the determination of okadaic acid using bromoacetylpyrene as labeling reagent has been suggested for collaborative study.

*Cyanobacterial Peptide Toxins.*—Associate Referee Judith Pace, U.S. Army Medical Research Institute of Infectious Diseases, Frederick, MD, has no report.

*Diarrhetic Shellfish Poisons (DSP).*—Takeshi Yasumoto, Tokoku University, Faculty of Agriculture, Department of Food Chemistry, Sendai, Japan, is comparing LC methods for the toxins associated with DSP. The methods are based on the use of precolumn derivatization with fluorescent labels followed by separation on bonded reversed-phase columns. Because of their parallel efforts to develop methods for toxins of the okadaic acid group, Yasumoto will coordinate some of his efforts with Robert Dickey of FDA.

*Domoic Acid.*—Associate Referee Michael Gilgan of the Department of Fisheries and Oceans, Halifax, Nova Scotia, Canada, reports that since 1988 only low levels of domoic acid have been reported, and that documented outbreaks of amnesiac shellfish poisoning have not occurred.

*Neurotoxic Shellfish Poisoning.*—Associate Referee Daniel G. Baden of the Rosentiel School of Marine and Atmospheric Sciences, University of Miami, submitted the following report:

Neurotoxic shellfish poisons, or the brevetoxins, are responsible for human shellfish poisoning, fish kills, and evolution of a respiratory irritant—most probably toxin absorbed to

particles of lysed marine dinoflagellate. The problem of human health concern following consumption of tainted shellfish has been largely reduced using intensive field programs where the toxigenic organism *Ptychodiscus brevis* is counted in specific volumes of seawater. Mouse bioassay is instituted when and if cell counts reach a specific prescribed number.

A collaborative study has been initiated under Department of Commerce funding, to evaluate a developed enzyme-linked immunoassay for detection of the brevetoxins in food sources and in the water column. The assay is based on specific goat antitoxin antibodies, which have been purified through protein g and toxin affinity columns. The assay involves initial adsorption of the hydrophobic toxins to plastic 96-well microtiter plates, followed by washing and blocking with nonfat dry milk. Antibrevetoxin antibody is next applied, followed by rabbit antigoat peroxidase antibody conjugates, and ABTS substrate.

The process of laboratory selection for collaborative study is presently underway. Kit prototypes are being assembled, and spiked and authentic samples are in preparation. Results are to be evaluated by an independent statistical consulting group. The fully evaluated results should be available by March 1992.

Further development of the assays include modification to both a dot-blot format and an immunochromatographic format, both designed as field assays. Application of the 96-well and modified formats to potential ciguatoxic samples is currently under survey, and the potential for a "universal" polyether toxin test kit is being evaluated.

*Paralytic Shellfish Poisons, Immunoassays.*—Associate Referee Patrick Guire, Biometric Systems, Inc., wishes to retire as referee due to a change in research direction.

*Paralytic Shellfish Poisons, LC Determination.*—Associate Referee James Hungerford of the Seafood Products Research Center, Bothell, WA, reports that various poly(styrene-divinylbenzene) columns are being tested for their ability to resolve neosaxitoxin and saxitoxin. These 2 toxins showed the greatest interlaboratory variations in a precollaborative study (6) of the LC method developed by Sullivan et al. (7). Particle size appears to be more of an influence than the separation efficiency. Columns of smaller (5 μm) particle size have poor injection-to-injection reproducibility, even though they provide improved separation of the less basic toxins (especially $B_2$ and GTX IV). The poor reproducibility of the 5 μm columns may be due to swelling that occurs when the level of acetonitrile (a component of the second mobile phase) is increased during gradient elution. The swelling (detected as a substantial increase in back-pressure) is more severe with the tightly-packed 5 μm particles. Wide-pore (300A) columns of 8 μm particle size resolve the toxins as well as the more conventional 110A pore columns of comparable (10 μm) particle size. Because of a shortage of toxin standards, Hungerford wishes to resign as Associate Referee of this topic.

*Tetrodotoxins (TTX).*—Associate Referee Sherwood Hall wishes to resign as referee of this topic.

*Toxins.*—Efforts to develop and validate analytical methods for seafood toxins must be seen in perspective. Natural toxins research, particularly the study of marine toxins, is now a rapidly-moving and very competitive field. In an ideal world, the development of analytical methodology for natural toxins follows a straightforward and rapid path from the initial development of the method through the validation process. However, as methods (particularly separation methods) for the toxins are developed and improved they often reveal the presence of previously unknown toxin forms. These "new" toxins may belong to a new class of toxins; more often they are simply new variations (epimers, biosynthetic intermediates, or metabolites) of a familiar toxin class. This process has occurred many times over, illustrated by the initial discovery of saxitoxin, followed by the discovery of multiple forms, and most recently the discovery of a new saxitoxin species, possibly a biosynthetic intermediate (8). Another example is the discovery of the many variations on the tetrodotoxin nucleus (9). Because they are isolated and used primarily in the course of research, these new toxin forms are in even shorter supply than the already scarce familiar forms. The end result is that the development of analytical methods that are not doomed to rapid obsolescence is often very difficult. Equally discouraging is that method development and refinement, which should address the detection of the new toxin forms, cannot be performed by more than a few research groups. At the method validation stage, even these lucky few will have to delay collaborative studies for years until they have isolated the toxins in sufficient quantity and purity.

There are many possible approaches to detecting natural toxins, and chromatographic methods represent only one. If protecting public health is the ultimate goal of the analysis, chromatography may actually be too specific in some applications. Bioassays, which at the organism level are still used to monitor the shellfish supply (10), may be more practical than specific chemical analyses in some applications. When a new type of seafood intoxication appears, it is rarely practical to try a large number of specific detection methods, particularly when complete sets of toxin standards are unavailable for even the best known toxins. This limitation is especially true when regulatory action must be taken to avoid additional outbreaks even before the toxin identity is known. Two examples of this type of circumstance can be given here. In one such instance (11), 4 individuals eating striped mullet (*Mugil cephalus*) after preparation in-the-round became ill with ciguatera-like symptoms, among them the characteristic reversal of temperature sensation and paralysis. Mouse bioassay allowed regulatory action, and a guinea pig atrium assay for sodium channel activity provided some preliminary information on the nature of the toxin (including that it did not have the neurological mode of action expected of CTX). In another case, 3 people died following exposure to a toxin with palytoxin-like symptomatology. In this case also, mouse bioassay provided the initial information in an emergency situation.

Bioassays using cultured nerve cells may provide a means to realize the same advantages of animal bioassays (essentially generic detection of neurotoxins and reduced requirements for multiple toxin standards), without the controversy and at reduced cost. Some work along these lines has been published (12). To encourage physiologists and cell biologists to bring their talents to AOAC and into the realm of applied method-

ology, the General Referee encourages the development of cell bioassays and the establishment of an Associate Referee position.

### *Recommendations*

(1) Following completion of a precollaborative study, conduct a collaborative study of the FIA method for histamine.
(2) Conduct a collaborative study of the GC method for cadaverine and putrescine.
(3) Appoint an Associate Referee for the topic *Biogenic Amines by Liquid Chromatography.*
(4) Appoint an Associate Referee for the topic *Conjugated Dienes as Indicators of Decomposition.*
(5) Delete second sentence in **976.16** and replace with "Cooking procedure and cooking time are based on heating coldest part of product to final internal temp of 160°F (70°C); when this temperature is reached, cooking is ceased and cooking time recorded." The remainder of the paragraph to read as is, beginning "Cooking times vary...."
(6) Appoint a new Associate Referee for *Tetrodotoxin*, and a new Associate Referee for *Paralytic Shellfish Poisons–LC Determination.*
(7) Encourage development of assays based on a biochemical or physiological mechanism of toxicity by appointing an Associate Referee for *Enzymatic Detection of Toxins* and an Associate Referee for *Cell Bioassay.*

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GENERAL REFEREE REPORTS

# Committee on Foods II

## Alcoholic Beverages

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For this General Referee report year, 3 new Associate Referees and a new AOAC-American Society of Brewing Chemists (ASBC) Liaison Officer have been appointed. Bruno Trombella will be the new AR for *Ascorbic Acid in Wine by LC*. Durward Walker will be the new AR for *Polydimethylsiloxane in Wine*. Tony Riberio will be the new AR for *Glycerol Monooleates in Wine*. Mark Schwiesow will be the new AOAC-ASBC Liaison Officer. Congratulations to a fine new group of AOAC volunteers, and I feel very confident each of you will make a positive contribution to the AOAC in these challenging endeavors.

In the interest of clarifying some possible confusion regarding differences between the densitometric methods in the 14th and 15th editions of the *Official Methods of Analysis* (OMA), the following information should be helpful.

The original references in the OMA, 14th Ed. for the densitometric method for alcohol by volume cited the Mettler/Paar DMA 55D (**9.026–9.031** and **9.119–9.121**). The 15th Ed. cites a replacement instrument (**942.06** and **983.12**). The correct citation should be as follows: Density meter—Mettler/Paar DMA 55D, or equivalent. In addition, the calibration part of the method was simplified to eliminate the barometric pressure correction.

The original reference for the digital density meter method for specific gravity in beer and wort cited the Mettler/Paar DMA 46. The 15th edition cites a replacement instrument. The correct citation should be as follows: Density meter—Mettler/Paar DMA 46, or equivalent (**988.06**).

The collaborative study results on the gas chromatographic (GC) method for determination of ethyl carbamate (urethane) in distilled spirits and wines using capillary GC and a thermal energy analyzer detector have been submitted and evaluated by the AR, and recommended for adoption as interim official first action. Although the submitted statistical evaluation indicates higher than desired variability, I concur with the recommendation for adoption as interim official first action.

Recent studies for lead in alcoholic beverages have been in the news lately, and perhaps a collaborative study should be initiated and performed. An associate refereeship should be established, and if anyone is interested, please contact me.

*Recommendations*

(1) Adopt as interim official first action the GC method for ethyl carbamate in alcoholic beverages using capillary GC and TEA detection.
(2) Continue study on all topics.

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## Cereals and Cereal Products

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*Phytate in Foods.*—No activity was reported this past year. Associate Referee Barbara Harland was away on sabbatical working in an unrelated research area.

*Iron in Flour.*—Associate Referee Jim Martin has successfully completed the project concerning the reduction of points from 11 to 4 for the concentration curve of the colorimetric analysis of iron in flour (**944.02B**). The completed report is currently being reviewed by the Statistician and General Referee of the Committee on Foods II.

*Minerals in Cereal Products.*—Associate Referee Yasmin Neggers is completing a study in which a general ashing method is being compared with official ashing methods (**922.03** and **936.07**) for cereal products. The method under consideration is straightforward, simple, and requires no ashing aids other than demineralized water. Samples are charred initially on a hot plate from ambient temperature then over a Bunsen burner until all traces of moisture are evaporated. Samples are then placed in a cold muffle furnace and allowed to equilibrate to 450°C. Ashing is continued to constant weight. The proposed method avoids temperatures of 550–700°C prescribed in the present official methods. These higher temperatures may cause volatilization of the chlorides of 3 nutritionally important minerals, Zn, Cu, and Fe. Mineral analysis will be performed by atomic absorption spectrometry on samples ashed by the proposed method and the official methods. If the proposed ashing method yields higher recoveries, a collaborative study of the method will follow. Also, applicability of the

**Table 1. Recommendations on recent actions taken by the AACC approved methods committee**

| AACC method | AOAC reference | Title of AACC method | Recommendation by cereals AOAC GR |
|---|---|---|---|
| 32–05 | **985.29** | Total dietary fiber | List as AACC-AOAC method |
| 39–11 | None | NIR for protein in wheat flour | Evaluate for adoption by NIR Associate Referee |
| 46–11a | **984.13** | Crude protein Cu catalyst mod. | List as AACC-AOAC method cross-list in cereals chapter |
| 02–32a | **950.03** | Neutralizing value of acid-reacting... | List as AACC-AOAC method cross-list in cereals chapter |
| 39–10 | None | NIR method for protein detn | Evaluate for adoption by NIR Associate Referee |
| 45–21 | **976.22** | Zearalenone (ol) in corn | List as AACC-AOAC method cross list in cereal chapter |
| 46–13 | **960.52** | Crude protein Micro-Kjeldahl | List as AACC-AOAC method cross-list in cereals chapter |
| 46–15 | **960.04** | Crude protein 5 min Biuret | List as AACC-AOAC method cross-list in cereals chapter |
| 12–10 | **948.05** | Residual $CO_2$ in baking powder | List as AACC-AOAC method cross-list in cereals chapter |
| 40–70 | **965.09** | Elements by AAS | List as AACC-AOAC method cross-list in cereals chapter |
| 40–71 | None | Na & K by AAS | Consider for adoption |
| 45–41 | **986.17** | Deoxynivalenol in wheat TLC method | List as AACC-AOAC method cross-list in cereals chapter |
| 46–16 | **988.05** | Crude protein Kjeldahl/Cu $TiO_2$ | List as AACC-AOAC method cross-list in cereals chapter |
| 32–07 | **985.29** (modified) | Total dietary fiber modified-Lee | Consider for adoption |
| 61–01 | **935.34** | Benzoyl peroxide in flour | List as AACC-AOAC method cross-list in cereals chapter |

official atomic absorption method (**968.08**) for minerals in feeds will be investigated for potential use with ready-to-eat and ready-to-cook cereals.

*Fat Acidity in Flour.*—A study is presently underway in the General Referee's laboratory to determine the influence of fat soluble pigments (carotenes) extracted from cereal products on the visual endpoint of phenolphthalein during fat acidity analysis by the official method (**939.05**). A previous work (*J. Assoc. Off. Anal. Chem.* (1990) **73**, 626–627) concerning the removal of toluene from the titration medium indicated that higher fat acidity values may result in a medium of alcohol alone. Using several cereal samples containing bran as test materials, the present study involves a comparison of fat acidity values obtained on evaporated pet ether extracts of whole grain flours. Potentiometric titration is used in alcohol–toluene (official method), and in alcohol alone, noting the transition pH of the phenolphthalein and the initial appearance of the first faint pink color of the dye that persists. On the basis of the results of this preliminary investigation, a collaborative study to remove toluene from the official method may be in order.

*Gliadin in Gluten-Free Products.*—Associate Referee W. Hekkens has completed the collaborative study on gliadin in gluten-free products and has submitted the data for evaluation by the General Referee and the Statistician of the Committee on Foods II. The review is in progress.

*β-D-Glucan in Cereal Products.*—Lou Zygmunt, Associate Referee, has completed the collaborative study of the enzymatic analysis of (1 → 3) (1 → 4)-β-D-glucan in cereal products in which 11 laboratories participated. The collaborative report is currently being prepared for review by the General Referee and Statistician of the Committee on Foods II.

*Generic Combustion Method for Crude Protein in Cereals and Oilseeds.*—Associate Referee Ron Bicsak has completed the collaborative study in which crude protein content determined by the

Kjeldahl method (**954.01**) was compared with a combustion method [*J. Assoc. Off. Anal. Chem.* (1989) **72**, 770–774)]. Results indicated that the combustion method gave higher recoveries than the Kjeldahl method. The completed collaborative study is currently being reviewed by the General Referee and Statistician of the Committee on Foods II.

*Near Infrared Reflectance Analysis for Protein in Wheat Flour.*—Donald E. Koeltzow (Chief, Quality Assurance and Research Division, USDA-FGIS and AACC NIR Chairperson) recently accepted the Associate Refereeship for NIR analysis of cereal products. He is currently working toward the adoption of existing NIR methods (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 joint AACC-AOAC methods.

### *Recommendations*

(1) As AOAC Liaison to the AACC, the General Referee makes the following recommendations on recent actions taken by the AACC Approved Methods Committee (Table 1).

(2) Continue all work in progress.

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## Dietary Fiber

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### *Recommendations*

(1) Accept as official first action the total dietary fiber (TDF) method, and the methods for soluble dietary fiber (SDF), and insoluble dietary fiber (IDF) developed by S.C. Lee. This TDF method, a modification of the AOAC-TDF method, will be an alternative procedure to the TDF method previously accepted as final.

(2) Accept as official first action the method for IDF submitted by the General Referee to the AOAC. This method is based on a modification of a method for TDF approved as final action by the AOAC.

(3) Test celite for uniformity in the determination of SDF, a method for which there was a high RSD (probably due to celite differences). Preliminary results will be presented at the August meeting.

(4) Proceed with the initiation of collaborative study of a chemical method for dietary fiber by O. Theander. Preliminary results of this study will be presented at the August meeting.

(5) Recommend that J. Jeraci continue with his development of urea-enzyme method for dietary fiber.

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## Fats and Oils

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*Emulsifiers.*—Associate Referee Theresa Lee evaluated the application of capillary supercritical fluid chromatography (SFC) to the quantitative determination of mono- and diglycerides in commercial monoglyceride emulsifiers (1). A capillary SFC methyl silicone column (SB-methyl 100, 10 m × 100 μm, 0.5 μm film thickness, Lee Scientific) was used with a flame ionization detector. Both underivatized mono- and diglycerides and their propionyl esters were analyzed for monoglyceride content (2). Results were also compared to those obtained by gas chromatography (GC). Similar results were obtained for SFC analyses of underivatized and derivatized monoglyceride emulsifiers, with relative standard deviations less than 4% (total monoglyceride content, 92–95%). Student's *t*-tests did not indicate any significant mean difference (95% confidence level) between the GC and SFC methods. The Associate Referee noted that the advantages of SFC compared to GC include analysis without prior derivatization and a marked reduction in the time required for elution of diglycerides (from less than 1 h to about 20 min). However, because SFC instrumentation may not be generally available, the Associate Referee is considering a collaborative study of the GC method (2) using both packed and capillary columns.

The International Union of Pure and Applied Chemistry (IUPAC) capillary GC method for determination of mono- and diglycerides in both concentrates and fats and oils has been published in *Pure and Applied Chemistry* (3), and the General Referee recommends adoption of the IUPAC method. See et al. (4) described a packed column GC method for analysis of monoglyceride and propylene glycol emulsifiers as the TMS derivatives using octadecyl glyceryl ether (batyl alcohol) as an internal standard.

*Hydrogenated Fats.*—The Associate Referee resigned during the past year. The IUPAC Commission on Oils, Fats, and Derivatives reported results of a collaborative study of a capillary column GC method for determining *n*-3 and *n*-6 unsatu-

rated fatty acids in hydrogenated and unhydrogenated vegetable fats and oils (5). Moderate polarity stationary phases were specified. $RSD_R$ values for 20–40% *n-3 or n-6 fatty acids were in the range of 3–7%* (average 5%). Statistical results of IUPAC collaborative studies performed in 1987–88 were presented in the previous General Referee report (6). The General Referee recommends adoption of the IUPAC capillary GC method for *n*-3 and *n*-6 unsaturated fatty acids as official first action.

The Associate Referee performed an AOAC-AOCS collaborative study in 1988 of a general method for capillary column GC determination of C-18 monoene and *cis,cis*-methylene interrupted unsaturated diene and triene fatty acids as well as overall fatty acid composition of vegetable oils, shortenings, and margarines (hydrogenated and unhydrogenated fats and oils). The method specifies use of a 60 m × 0.25 mm fused silica capillary column coated with SP-2340 polar stationary phase. The statistical results were presented in a previous General Referee report (7). The method is capable of good precision as demonstrated by the collaborative results. The General Referee recommends adoption of the AOAC–AOCS capillary GC method as official first action.

*Lower Fatty Acids.*—The Associate Referee resigned during the past year.

*Marine Oils.*—The Associate Referee R.G. Ackman and Jeanne Joseph prepared a report of results of the 1988 collaborative study of the capillary column GC method for determination of fatty acid composition of marine oils. The report was accepted for publication in the *Journal of AOAC International* and the method was approved as official first action.

The proximity of 24:1 components in fish oils to the docosahexaenoic acid (22:6, *n*-3) peak in GC analyses on Carbowax 20M based capillary columns prompted the Associate Referee to investigate the isomers in detail (8). Capillary GC analysis of fish oils usually shows a single 24:1 peak. Isolation by urea adduct formation and reversed-phase liquid chromatography (LC) followed by oxidative fission (ozonolysis) and analysis by capillary GC and GC/MS demonstrated the presence of at least 5 isomers with 24:1, *n*-9 the dominant (60–90%) isomer.

*Olive Oil Adulteration.*—Associate Referee E. Fedeli continued as advisor and a principal participant in an International Olive Oil Council (IOOC) program to validate modern methods for analysis of olive oil products.

The Associate Referee has been involved in development of a number of Italian methods of analysis of fats and oils, including analyses of isomeric fatty acids (NGD-C74-1989), β-carotene concentration, fatty acid composition, triglyceride composition, revised peroxide value, sterol and erythrodiol content of olive oils, and wax content of olive oil (NGD-C80-1989). A new capillary GC method was proposed for rapid determination of free and esterified minor components (sterols, alcohols, and triterpene alcohols) in olive oil and other vegetable oils (9). Grob et al. (10, 11) applied a coupled LC/GC method to the determination of fatty alcohols, wax esters, and free and esterified sterols to distinguish pressed and solvent extracted olive oils. Aitzetmuller (12) reviewed the use of LC for the determination of triglycerides, tocopherols, and other components in olive oil and other oil and fats.

Marini et al. (13) investigated the application of spectrophotofluorimetric analysis of olive oil to determine virgin in refined olive oils. As little as 1% virgin olive oil could be detected in refined oil. Guth and Grosch (14) investigated the distinctive odorants present in virgin olive oils. Mariani et al. (15) proposed use of a new analytical method based on capillary GC analysis of the triglycerides to determine the presence of high oleic seed oils in olive oil. Sacchi et al. (16) investigated the use of C13-NMR for control of olive oil quality.

The IUPAC Commission on Oils, Fats, and Derivatives completed study of an LC method for determination of triglycerides in vegetable oils in terms of their partition numbers (17). The method is especially applicable to the detection of small quantities of linoleic-rich oils (soybean oil, sunflower oil, etc.) in oils such as olive oil, which contain high levels of oleic acid. The IUPAC Commission has also performed studies of the LC method for determination of the nature and percentage of individual triglycerides in olive oil and other fats and oils. (18). In addition, the International Olive Oil Council (IOOC) evaluated the LC method to observe detection of low levels of sunflower oil in olive oil, based on determination of trilinolein content. A new regulation in Germany for olive oil restricting the amount of trilinolein to 0.5% was introduced in the EEC. The General Referee recommends adoption as official first action of the IUPAC method for LC determination of triglycerides in vegetable oils.

*Oxidized Fats.*—Associate Referee M.M. Blumenthal is continuing investigation of methods of analysis of frying fats including rapid tests for free fatty acids and total polar compounds. Burkow and Henderson (19) described a rapid gel permeation LC method using a light-scattering detector to isolate and quantify polymers in autoxidized fish oils. Chu (20) performed a comparative study of analytical methods (dielectric constant, Food Oil Sensor, total polar compounds, refractive index, Fritest, acid value, iodine value, anisidine value, carbonyl value, viscosity, and color) employed for evaluation of soybean oil quality. Food Oil Sensor, total polar compounds, refractive index, and iodine value were found to be more reliable than the other methods for assessment of oil quality.

The IUPAC Commission on Oils, Fats, and Derivatives completed study of a gel permeation LC method for determination of polymerized triglycerides in oils and fats (21). Between laboratory relative standard deviations ($RSD_R$) of 5–12% were obtained for oils with 5–10% polymerized triglycerides, with increasing $RSD_R$ values for oils with less than 5% polymerized triglycerides. The General Referee recommends adoption as first action of the IUPAC method LC determination of polymerized triglycerides in oils and fats.

*Pork Fats in Other Fats.*—The topic has no Associate Referee.

*Sterols and Tocopherols.*—Associate Referee R.J. Reina developed and is evaluating a new method for capillary GC determination of sterol and triterpene diol content of vegetable oils. The method involves thin-layer chromatography (TLC) fractionation of the unsaponifiable matter after passage

through an alumina column, followed by acetylation of the isolated sterol and triterpene bands and then capillary GC of the acetate derivatives on a 25 m CP-Sil 8 CB capillary column. Homberg and Bielefeld (22) pointed out that TLC separation of the 4-methyl sterols and triterpenes from the sterols is necessary to obtain accurate determinations of sterol composition by GC.

Gordon and Griffith (23) observed that campesterol and stigmasterol, and to a lesser extent β-sitosterol, are lost during physical refining of vegetable oils. Steryl esters are lost to a much lesser extent. These authors described capillary GC and LC methods for analysis of steryl esters isolated by TLC as a means of identifying oils and mixtures of oils. Teng (24) described the use of normal-phase LC for determination of oxysterols present in tissues and food. Warner and Mounts (25) reported methods for separation, detection, and quantification of sterols and tocopherols by LC with an evaporative light-scattering detector. Ulberth (26) evaluated the capillary GC determination of tocopherols and cholesterol as the underivatized compounds, silyl ethers, or heptafluorobutyryl esters. Luscombe and Bond (27) demonstrated the use of reversed-phase LC and electrochemical detection for determination of tocopherols. Coors (28) discussed the problems in determining fat and oil adulteration by means of LC analysis of the tocopherols. Andrikopoulos et al. (29) described LC analysis of phenolic antioxidants, tocopherols, and triglycerides. The compounds were separated in the same run on a reversed-phase C-18 column by gradient elution with water (pH 3)–acetonitrile–methanol–isopropanol and UV detection. Absorbance was measured at wavelengths of 215 and 280 nm.

The IUPAC Commission of Oils, Fats, and Derivatives performed collaborative studies of an LC method for determination of tocopherols and tocotrienols in vegetable oils and fats (30). The method was adopted as method **2.432**. The results of statistical analysis of the collaborative study data were included in an earlier General Referee report (31), and the method was also adopted by the American Oil Chemists' Society (32). The General Referee recommends adoption of IUPAC Method **2.432** as official first action.

*Other Topics.*—Because of the potential carcinogenic risk with the use of carbon tetrachloride in the determination of iodine value, the IUPAC Commission of Oils, Fats, and Derivatives performed collaborative studies of the Wijs method (**920.159**) for determination of iodine value using cyclohexane as well as carbon tetrachloride as the solvent (33–35). The results showed excellent agreement between cyclohexane and carbon tetrachloride. The IUPAC method requires a reaction time in the dark (after adding the Wijs reagent) of 1 h for fats and oils having an iodine value below 150, and 2 h for fats and oils with an iodine value above 150 to assure sufficient reaction time. The General Referee recommends that **920.159** be revised to specify the 1 and 2 h reaction times, and also to allow use of cyclohexane as an alternate solvent, stating that the test portion can be added to the reaction flask containing 20 mL of cyclohexane–glacial acetic acid (1 + 1).

The standard method for determination of peroxide value (**965.33**) requires use of acetic acid–chloroform (3 + 2). The American Oil Chemists' Society (36) performed an interlaboratory study to evaluate the use of isooctane as a replacement solvent for chloroform. Results of the study showed good one-to-one correlation between the results obtained for the 2 solvents, with oils having peroxide values in the range of 1 to 300. The General Referee recommends revision of **965.33** to allow use of acetic acid–isooctane (3 + 2) as an alternate solvent.

*Commission on Oils, Fats and Derivatives, Applied Chemistry Division, IUPAC.*—The 44th meeting of the Commission was held in Hamburg, Germany, August 8–10, 1991. Delegates representing 13 countries attended the meeting chaired by Commission Chairman W.D. Pocklington. The Commission reviewed reports submitted by working group coordinators covering methods for determination of fatty acids by capillary GC, detection of cross contamination of edible oils transported in bulk (including a report on contamination of edible oils by nonedible previous cargoes with results of a Leatherhead Food RA collaborative study of a GC/MS method), determination of residual chlorinated hydrocarbons in edible oils and fats, determination of peroxide value using isooctane instead of chloroform as solvent, detection of thermally treated oils (including detection of refined oils in cold pressed oils), determination of lipid oxidation, determination of arsenic, cadmium, selenium, and mercury in oils, determination of chlorophyll pigments, determination of unsaponifiable matter, and evaluation of environmental aspects of analytical methods (including efforts to reduce the amounts of solvents and reagents used in IUPAC methods).

New topics discussed included determination of sterols by capillary GC, determination of iodine value of fish oils, determination of organophosphorus pesticides, and replacement of toxic and carcinogenic reagents and solvents in existing IUPAC methods. Wessels informed the Commission of new EEC regulations for olive oil comprising some 40 pages and replacing the 1058/77 regulations concerning olive oil characteristics. The next meeting of the Commission will be held in Budapest, Hungary, September 9–11, 1992.

### *Recommendations*

(1) Adopt as first action the IUPAC method for determination of mono- and diglycerides.
(2) Adopt as first action the IUPAC method for capillary GC determination of *n*-3 and *n*-6 unsaturated fatty acids.
(3) Adopt as first action the AOAC–AOCS capillary GC method for determination of fatty acid composition of hydrogenated and unhydrogenated fats and oils.
(4) Adopt as first action the IUPAC method for LC determination of triglycerides in vegetable oils.
(5) Adopt as first action the IUPAC gel permeation LC method for determination of polymerized triglycerides.
(6) Adopt as first action IUPAC Method **2.432**, determination of tocopherols and tocotrienols in vegetable oils and fats by LC.
(7) Revise **920.159** (Wijs Iodine Value) to specify 1 h reaction times for oils with iodine values below 150, or 2 h reaction times for oils with iodine values above 150.

Also, allow cyclohexane–acetic acid (1 + 1) as an optional solvent, in place of carbon tetrachloride.

(8) Revise **965.33** (Peroxide Value) to allow isooctane as an optional solvent, in place of chloroform.

(9) Continue study on all other topics.

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## Fruits and Fruit Products

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*Adulteration of Apple Juice.*—John Heuser and Ed Elkins recently conducted a collaborative study on the detection of adulteration in apple juice using the L-Malic Acid/Total Malic Acid Ratio. The report, which is currently under review, may be summarized as follows.

The L-Malic Acid/Total Malic Acid Ratio is currently being used to determine economic adulteration of apple juice. L-Malic acid is the predominate acid in apple juice and no D-malic acid should be present. Synthetic malic acid is cheap but contains 50% D-malic acid and may be used to increase the acidity of apple juice. Fourteen laboratories participated in a collaborative study to determine the interlaboratory variability of this method. Ten samples of apple juice were sent to each laboratory. Authenticity varied from 100 to 0%. L-Malic/Total Malic Ratios of 0.9 or less are indicative of an adulterative sample. The coefficients of variation in all cases were acceptable, i.e., around 5%. The General Referee recommends 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). E.D. Coppola, A. Smith, J. Provost, and J. Speroni presented an invited paper on "Recent Developments in Assessing Cranberry Adulteration" at the AOAC Northeast Regional Section

Meeting at Newport, Rhode Island, on May 15, 1991. 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.

His firm's method and their data base on organic acids (mainly quinic) to calculate percent cranberry juice continues to be used by state and private laboratories to assess cranberry juice content and authenticity of cranberry products. His organization 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. The Associate Referee recommends continued study on this topic and the General Referee concurs in his recommendation.

*Adulteration of Orange Juice by Pulpwash and Dilution.*—No activity has been reported. The Associate Referee recommends continued study and the General Referee concurs.

*Fruit Juices, Identification and Characterization.*—Associate Referee R.E. Wrolstad reports that the interest and need of improved methodology for detecting adulteration in fruit juices is substantiated by the growth and activities of the Technical Committee for Juice and Juice Products (TCJJP), which was formed in 1989. Betsy Woodward of the Florida Department of Agriculture and Consumer Services is Chairwoman of the Board of Directors, and Kristen Chadwell of the Florida Department of Citrus is Secretary-Treasurer. Three Board of Directors meetings were held this past year, and an open meeting is scheduled for Thursday morning, August 15th, 1991, in conjunction with the AOAC Annual International Meeting in Phoenix, AZ. Included on the agenda will be proposals for formalizing association with the AOAC, either through cooperative agreements or by becoming a technical division of AOAC.

A hands-on Technical Committee Methods Workshop is being planned for August 29th and 30th, 1992, in conjunction with the 1992 AOAC Meeting in Cincinnati.

The Associate Referee recommends continued study and the General Referee concurs in his recommendation.

*Determination of Naringin and Neohesperidin in Orange Juice.*—The Associate Referee reports that he has received results from 11 of 14 collaborators. Eliminating outliers, statistics for naringin and neohesperidin were as follows: intralaboratory relative standard deviation ($RSD_R$), less than 15%; total relative standard deviations ($RSD_R$), 20–40% for less than 10 ppm of a component, less than 20% for component concentrations greater than 10 ppm. Poor performance was determined to be caused by the following:

(*1*) Absorption of components by filter materials when the filtered volume is small. Early testing of several syringe filters did not show any significant changes in component amounts when using several different filter material and pore diameters. In this case, the high speed centrifuging at 10 000 rpm (12 100 × *g*) allowed at least 2 mL of sample to pass through a filter. Some collaborators were not able to duplicate this and, therefore, were not able to obtain as much filtered sample. Subsequent testing of different filters revealed that 50–80% of naringin and neohesperidin were absorbed by some filter materials (one of which was recommended for use) when small volumes (150 μL) are filtered. Two filters by different manufacturers have been found that do not adsorb flavanone glycosides.

(*2*) HPLC columns used by some collaborators caused severe peak tailing of the components of interest. Early investigations found that 2 C18 columns from different manufacturers (both 4.6 × 150 mm × 3 packing) performed well with mobile phases containing 0.1% aqueous acetic acid–acetonitrile (82 + 18 and 81 + 19). It is now evident that 0.5–1.0% aqueous acetic acid is necessary to minimize peak tailing of the flavanone glycosides on some C18 columns.

(*3*) Several collaborators experienced interferences in some samples due to late eluting peaks from pervious samples and poor injection timing.

Provided the 2 new filters are used for sample preparation, the method should be adequate to detect the presence of grapefruit juice in orange juice using a lower limit of 10 ppm naringin and 3 ppm or less neohesperidin for confirmation. Orange juices containing 10 ppm naringin from blends with sour orange or k-early juices (legal at 5 and 10%, respectively) will contain at least 8 ppm neohesperidin. The method will be retested using the new filters and C18 columns with minimal tailing for flavanone glycosides. The Associate Referee recommends continued study on this topic and the General Referee concurs in his recommendation.

*Moisture in Dried Fruits.*—No activity has been reported. The Associate Referee recommends continued study and the General Referee concurs.

*Sodium Benzoate in Orange Juice.*—In the national survey of commercial single-strength and reconstituted frozen concentrated orange juice, benzoic acid was found in the 0.6–1.7 ppm range in 13 samples, and 0.1–150.1 ppm of sorbic acid was found in 10 samples. Due to reports of the natural presence of benzoate in orange fruit, we are no longer reporting values below 0.5 ppm. Three samples showed both benzoate and sorbate. A total of 1232 samples were tested from July 1, 1990 through June 6, 1991.

Results have been received from all participants in the collaborative study of benzoate in orange juice. We are currently preparing data for the report. One area of concern involves the proper handling of the blank (unspiked sample). Due to the possible natural presence of benzoate in orange juice and the increased sensitivity in some HPLC systems, a few of our participants reported 0.1–2.0 ppm benzoate in the blank. Whether this value should be considered in our data to determine variability between laboratories or disregarded as natural presence is still unanswered. The Associate Referee recommends continued study and the General Referee concurs in his recommendation.

*Stable Carbon Isotope Ratio Analysis of Fruit Products.*—No activity has been reported. The Associate Referee recommends continued study and the General Referee concurs.

*Application of Computer Pattern Recognition Analysis of Trace Elements for Determination of Geographical Origin of Orange Juice.*—Collaborators (9 laboratories) were contacted to begin the AOAC collaborative study on the metals and geographic origin. Frozen concentrated orange juice (FCOJ) sam-

ples from 3 Brazilian processing plants and a Citrus Research and Education Center pilot plant were collected and stored in the freezer for this study. Using FCOJ samples from Florida and Brazil, samples of blended Florida-Brazil juices were prepared for an AOAC collaborative study. A protocol procedure, which was approved by the General Referee and the statistician, for "Trace Elements Analyses by inductively coupled plasma/atomic emission spectrometry (ICP/AES) for Determination of Geographical Origin of Orange Juice" was sent to all collaborators. Also, 8 blind duplicate (total of 16) samples of blended FCOJ for Florida and Brazil were shipped for ICP/AES trace metal analyses to these laboratories. So far, the Associate Referee has not received the results from any of them.

Multiple replicates of the same samples were digested via microwave method and analyzed by ICP/AES for 15 elements in our laboratory. This was done to obtain the training data base for blending ratio determination classification by artificial neural network analysis of trace metals. Results are as follow:

| | | | |
|---|---|---|---|
| Blended Florida: Brazil | (01:0) | 100% | Florida |
| Blended Florida: Brazil | (19:1) | 95% | Florida |
| Blended Florida: Brazil | (09:1) | 90% | Florida |
| Blended Florida: Brazil | (04:1) | 80% | Florida |
| Blended Florida: Brazil | (07:3) | 70% | Florida |
| Blended Florida: Brazil | (03:2) | 60% | Florida |
| Blended Florida: Brazil | (01:1) | 50% | Florida |
| Blended Florida: Brazil | (00:1) | 00% | Florida |

The blending ratio determination is the most difficult problem in the citrus industry; however, the artificial neural networks were able to separate all categories successfully.

The Associate Referee recommends continued study and the General Referee concurs.

### *Recommendations*

Continue study on all topics.

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## Nonalcoholic Beverages

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The recently appointed associate referee for the analysis of safrole in sassafras root, Marvin Carlson, has a liquid chromatographic procedure that will separate and quantitate safrole in various spices. He plans to study various commodities, such as nutmeg oil and sassafras root.

Irv Rabinowitz, associate referee for quinine, plans to develop a method that will differentiate quassia from quinine in various beverages.

### *Recommendations*

(1) Appoint an Associate Referee for the determination of pyrrolizidine alkaloids in herbal tea.
(2) Continue study on all topics.

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## Processed Vegetable Products

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Two new Associate Referees were appointed during the past year. No studies were completed this year. A brief summary of the status of each topic follows.

*pH Determination.*—This method has official final action status for pH of acidified foods. The Associate Referee reports no progress on plans for the study of the suitability of this method for extension to low-acid and acid foods, or on plans for the study of the suitability of new electrode and instrument developments. Interested persons are invited to submit comments and participate in continued study of this topic. The Associate Referee recommends that work continue on this topic.

*Sodium Chloride.*—This topic was discontinued after considerable effort over several years failed to obtain a new Associate Referee. After the topic was discontinued, J. Anderson Williams expressed a strong interest in the topic and volunteered to become Associate Referee. Williams was recommended for appointment as Associate Referee, and was appointed Associate Referee by the AOAC on June 17, 1991. Williams has indicated interest in reviewing the analytical

methods in this field to identify current needs, and in pursuing needed method development and improvement. Interested persons are invited to submit comments. It is recommended that this topic be reestablished.

*Total Solids by Microwave Moisture Methods.*—This method has official first action status. No response has been received to the request that consideration be given to amending this method (for the determination of total solids in processed tomato products by microwave drying oven) by the possible addition of an instrument calibration and/or warm-up procedure. This procedure would be in accordance with what was specified in the original collaborative study. Results of a related study were published by Wang (*J. Assoc. Off. Anal. Chem.* (1987) **70**, 758–759). This issue was reported in 1988, 1989, and 1990. No further action has occurred. Discontinuation of this topic is recommended.

*Water Activity in Foods.*—The method for water activity determination has official final action status. The Associate Referee reports no progress on the proposed studies to clarify the needed frequency for instrument calibration and to develop the means to significantly reduce the time required for calibration, or on studies to investigate product-instrument sensor interactions and new instrument developments.

Interested persons are invited to submit comments and participate in continued study of this topic. The Associate Referee recommends that work continue on this topic.

*Sugars in Processed Vegetables by Liquid Chromatography (LC).*—Peter H. Yu has been appointed Associate Referee to this new topic. Yu has completed a literature search on "The Determination of Sugars in Processed Vegetables by LC," as well as a preliminary evaluation of the LC method used in his laboratory. When this method was used to analyze some vegetable samples (carrots, corn, beans, broccoli, and peas), interference was encountered in the region of the sugar peaks (fructose, glucose, and sucrose). Plans are to conduct studies to eliminate this interference problem, before developing a collaborative study proposal. Interested persons are invited to submit comments and participate in the study of this topic. The Associate Referee recommends that work continue on this topic.

### *Recommendations*

(1) *pH Determination.*—Continue study.
(2) *Sodium Chloride.*—Re-establish topic.
(3) *Total Solids by Microwave Moisture Methods.*—Discontinue topic.
(4) *Water Activity in Foods.*—Continue study.
(5) *Sugars in Processed Vegetables by LC.*—Continue study.

This report of the General Referee was presented at the 105th AOAC Annual International Meeting, August 12–15, 1991, at Phoenix, AZ. The recommendations were reviewed by the Committee on Foods II. See the report of the committee, this issue.

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## Spices and Other Condiments

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*Capsicum Spices and Oleoresins: Pungency.*—Associate Referee Mark Parrish, McCormick & Co., reports that he has results from 3 of 5 laboratories in a preliminary study.

*Curcumin in Turmeric.*—Associate Referee Ted Lupina, Kalsec, reports that he has 10 laboratories to perform a collaborative study. Samples are targeted to be sent in September 1991.

*Moisture in Dried Spices; Vacuum Oven Method.*—Associate Referee Lou Sanna, Santa Maria Chili Co., reports that samples have been sent for the collaborative study.

*Steam Volatile Oil in Cassia: Sample Preparation.*—Associate Referee Phil Guarino, McCormick & Co., reports that a preliminary study comparing various mills/grinders has been completed and that a protocol is being prepared for the next phase.

*Vinegar: Corn-Derived Acetic Acid in Apple Cider Vinegar.*—Associate Referee Dana Krueger, Krueger Food Labs, recommends that the carbon isotope method be adopted official first action.

*Water Activity of Spices.*—Associate Referee Sue Schena, Cal-Compack Foods, reports that samples have been sent to 8 laboratories for a preliminary study.

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## Sugars and Sugar Products

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*Color, Turbidity, and Reflectance–Visual Appearance.*—No activity is reported.

*Corn Syrups and Sugars.*—Associate Referee R. Bemetti will submit report at a later date.

*Enzymatic Methods.*—Newly appointed Associate Referee Guenther Henniger has submitted the following "state of the art" summary of enzymatic analysis of sugars and sugar products.

(*1*) D-Glucose in food, drugs, and agricultural samples is determined with hexokinase and glucose-6-phosphate dehydrogenase, a specific procedure with a high reliability: no

cross-reactions and interferences with other ingredients of samples are known. The glucose oxidase procedure cannot be recommended because of possible interferences by reducing substances, purity problems with the enzymes (different grades are commercially available and designed for special fields of application), and because of measurements against standards.

(*2*) D-Fructose is measured (after D-glucose in the same assay system: reaction vessel is the cuvette) with the enzymes hexokinase, glucose-6-phosphate dehydrogenase, and phosphoglucose isomerase. This procedure is the only enzymatic one used routinely.

(*3*) Inverted sugar (a mixture of D-glucose and D-fructose) is analyzed as mentioned for D-glucose and D-fructose. Chemical procedures based on the reductometric properties of these sugars are not satisfactory; this is demonstrated by the existence of many modifications to the Fehling, Lane-Eynon, or Luff-Schoorl methods.

(*4*) Sucrose is inverted enzymatically by means of β-fructosidase. The liberated D-glucose and D-fructose are measured with the a.m. procedures.

(*5*) Lactose is a "sugar" (carbohydrate) that is determined in another group of samples: milk, dairy products, samples containing milk, and milk products. The enzymatic determination of lactose in milk (by means of the enzymes β-galactosidase and galactose dehydrogenase) has already been published in *Official Methods of Analysis*, **984.15**.

During sugar production, the following organic acids can also be measured by enzymatic means: (**a**) L-Lactic acid, which is formed from sugars during production, especially under alkaline conditions; and (**b**) Citric acid: concentration should be known to assess procedures in sugar production.

*Gas Chromatographic Methods.*—Associate Referee Mary An Godshall has submitted the following report.

A survey of articles in the previous years' issues of the AOAC *Journal* showed there were no articles written on the use of gas chromatography (GC) to analyze sugars in foodstuffs. A similar survey of the previous 7 months of *J. Chromatogr.* produced only 3 articles relating to analysis of sugars by GC. Two of these were on foodstuffs (apples and industrial sorbitol solutions). The third dealt with a specialized detector.

GC analysis of sugars on a routine basis has lost ground over the years to liquid chromatography (LC) and ion chromatography because of ease of sample preparation and increasing sensitivity of these newer techniques.

Only a few sugar companies still use GC for analysis—Amalgamated in the United States, and several in Europe. The South African sugar industry, which pioneered the GC method for cane payment, is switching over to LC.

GC continues to be a viable technique for many compounds such as pesticides, drug residues, and volatiles. Methods are being developed and collaborated in these areas. GC also continues to be a useful research tool for sugar analysis, especially in structural studies of polysaccharides and amino sugars.

It is unlikely that any GC methods for sugars will undergo collaborative testing in the future. The demand is for simple methods that do not require the use of dangerous or unpleasant derivatizing reagents such as pyridine and the chlorosilanes.

*Glucoamylase Activity.*—Newly appointed Associate Referee Michael T. Elder reports that the collaborative study on glucoamylase activity in industrial enzyme preparations has been completed by collaborating labs. All raw data have been collected and submitted to John Phillips for statistical evaluation. The study report is in progress.

*Honey.*— Associate Referee Jonathan White reports that the new Internal Standard Stable Carbon Isotope Ratio method for honey has been applied to a considerable number of honey samples that had isotope ratio values in the area that requires additional testing before decision. Results showed 88% of these samples to be admixed with corn or cane sugars. Because these honeys originated from an area in which corn sugar is not generally available, cane sugar is indicated. Had the thin-layer chromatography method now required by the AOAC *Official Methods of Analysis* been used, adulteration of few, if any, of these samples would have been detected.

The possibility of contamination of the protein isolated in the procedure by yeast cells from fermented honeys will be examined and, if necessary, dealt with.

The Associate Referee recommends, and the General Referee agrees with, the following: (*1*) The performance parameters for the determination of a stable carbon isotope ratio of honey (method **978.17**) obtained from the study on sugar (corn or cane) in honey be added to the current description of the method. (*2*) The title of the method for sugars (corn or cane) in honey (see 1990 General Referee Report) be changed to "Corn and Cane Sugar Products in Honey." (*3*) The Sofer procedure be included in "Corn and Cane Sugar Products in Honey" as an alternate combustion procedure. (*4*) The final paragraph under **978.17D** should be deleted, and the following submitted: "Samples with $\delta^{13}C$ less negative than −23.1‰ are considered adulterated with corn and cane sugars only after confirmation with the internal standard isotope ratio method."

*Lactose Purity Testing.*—Associate Referee Janice R. Saucerman reports no activity at this time.

*Liquid Chromatographic Methods.*—No report at this time.

*Maple Sap, Maple Syrups, and Maple Products.*— No report at this time. A new Associate Referee is needed.

*Polarimetric Methods.*—Associate Referee R.W. Plews reports that the search for a suitable nontoxic clarifying reagent to replace basis lead acetate in the determination of polarization is continuing. Basis lead acetate is being replaced because of environmental concerns.

Experiments are expected to proceed with aluminum and zinc chlorides, as well as with the determination of polarizations without defecation of solutions. Some collaborative work is being considered for the determination of polarizations of other sugars and intermediate syrups.

Conversion to the new International Sugar Scale, designated °Z, from the previous scale is as follows:

$$°S \times 0.99971 = °Z$$

*Stable Carbon Isotope Ratio Analysis.*—Associate Landis W. Doner reports that the method, "Detecting Adulteration of Frozen Concentrated Orange Juice with Sugar Beet-Derived Syrups on the Basis of $\delta^{18}O/^{16}O$ Measurements in Water: a Col-

laborative Study" has been approved by Foods Committee II, as recommended in the 1990 General Referee Report. The method is at present being rewritten, with additional methods description, for AOAC *Official Methods of Analysis*, and will be submitted for official first action.

*Standardization of Methods of Sugar Analysis.*—No report at this time.

*Sugars in Cereals.*—Associate Referee L. Zygmunt reports no activity, pending Food and Drug Administration (FDA) clarification on classification of food carbohydrates. Investigations into chromatographic analysis of low levels (<1%) of sugars in foods, using a pulsed amperometric detector, have begun.

*Sugars in Syrups.*—The method recommended for official first action in the 1990 General Referee Report is once again recommended for adoption.

*Sulfites in Sugar Products.*— Associate Referee R. Riffer reports as follows: H.-J. Kim, K.R. Conca, and M.J. Richardson [*J. Assoc. Off. Anal. Chem.* (1990) **73**, 983–989] determined levels of sulfur dioxide in grapes via 3 methods, and then compared the results. The 3 techniques used were: modified Monier-Williams; acid distillation/ion exclusion chromatography; and alkali extraction/ion exclusion chromatography. In each of the 2 chromatographic methods, electrochemical detection was used.

The latter stage of the Monier-Williams distillation yields false-positive material that is somewhat offset by incomplete recovery of true sulfite. The authors recommend limiting distillation to 60 min. However, the FDA-approved method [*Fed. Regist.* **51**, No. 13, pp. 25012–25018, July 9, 1986], which was the AOAC *Official Methods of Analysis* (14th Edition) modified in FDA laboratories, specifies a distillation of 105 min. Even with the recommended reduction in distilling time, the authors note that a correction must be made to compensate for incomplete recovery.

This work adds to the well-documented shortcomings of the various Monier-Williams procedures, particularly at levels of 10 ppm and below. The authors recommend the alkali extraction method as being rapid, sensitive, straightforward, and free from interference.

*Weighing, Taring, and Sampling.*—A change has been made by U.S. Customs in frequency of sampling of raw sugar cargoes. A report will follow.

### *Recommendations*

(1) It is recommended that the performance parameters for determination of stable carbon isotope ratio of honey (**978.17**) obtained from this study be added to the current description of that method, that the title be changed to "Corn and Cane Sugar Products in Honey," that a suitable description of the Sofer procedure be included as an alternate combustion method, and that the final paragraph under **978.17D** be deleted and the following added: "Samples with $\delta^{13}C$ less negative than –23.1‰ are considered adulterated with corn or cane sugars only after confirmation with the internal standard isotope ratio method."

(2) A new Associate Referee be appointed for the subtopic *Maple Sap, Maple Syrups, and Maple Products.*

(3) The method for "Detecting Adulteration of Frozen Concentrated Orange Juice with Sugar Beet-Derived Syrups on the Basis of $\delta^{18}O/^{16}O$ Measurements in Water" be adopted official first action (see 1990 Report).

(4) The LC method for "Sugars in Syrups" be adopted official first action (see 1990 Report).

This report of the General Referee was presented at the 105th AOAC Annual International Meeting, August 12–15, 1991, at Phoenix, AZ. 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*, 2nd supplement, 1991.

## Vitamins and Other Nutrients

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Eleven papers in this topic area were presented as a poster session at the 105th meeting of the association. Thirteen additional nutrient papers were published in the *Journal of the AOAC* during the last year.

Jacob Scheiner resigned as Associate Referee for Biotin and Willy Schüep has replaced him.

### *Recommendation*

Continued study on all topics.

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# Committee on Residues

## Metals and Other Elements

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*Atomic Absorption Spectrophotometry (AAS).*—Associate Referee Milan Ihnat reports that an extensive interlaboratory certification campaign has been completed for chemical characterization of 10 agricultural/food reference materials comprising bovine muscle powder, whole egg powder, whole milk powder, corn bran, corn starch, wheat gluten, microcrystalline cellulose, soft winter wheat flour, hard red spring wheat flour, and Durum wheat flour. Estimates of material homogeneity indicated excellent uniformity for elements of interest. Analyses were performed using diverse methods, including on atomic absorption and emission spectrometry, mass spectrometry, neutron activation analysis, molecular light absorption spectrometry and fluorometry, electrochemistry, Kjeldahl method for nitrogen, combustion elemental analysis, ion chromatography, volumetry, and gravimetry. Recommended concentration values are available for Al, As, B, Ba, Ca, Cd, Cl, Co, Cr, Cu, F, Fe, Hg, I, K, Mg, Mn, Mo, N, Na, Ni, P, Pb, S, Se, Sr, V, and Zn. These materials representing a wide range of matrixes, add substantially to the existing world repertoire of reference materials, and are expected to be useful for analytical method quality control for reliable determination of major, minor, and trace elements in foods and feeds. They will shortly be available to analysts for quality control of AAS and other analytical methods, as well as method development.

*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.

*Hydride Generating Technique.*—Associate Referee Stephen Capar reports no progress on this topic and that he no longer works directly on this topic. His organization's work on a continuous flow hydride generation apparatus for AAS determination of arsenic and selenium in foods is scheduled to be published (*Anal. Lett.* (1991) **24**). A collaborative study of the method is not currently planned due to other higher priority projects.

*Lead in Calcium Supplements.*—Associate Referee Patricia Maroney-Benassi did not report on this topic.

*Methyl Mercury in Fish and Shellfish*—Associate Referee Susan Hight reports there are no current activities or future plans for this topic. She has received no adverse comments on the official first action rapid gas chromatographic (GC) method for methyl mercury in fish and shellfish (**988.11**), and she recommends the method for official final action.

*Neutron Activation Analysis (NAA).*—Associate Referee William C. Cunningham is the AOAC Liaison Officer to the American Society for Testing and Materials' Task Group on Nuclear Methods of Chemical Analysis, and he participates on the group's activities on issues related to the development of standard NAA methods. With assistance from the group, he has developed several analytical guidelines for general NAA that improve the quality of NAA investigations of biological materials. These guidelines were developed specifically for the method to be submitted to AOAC for collaborative study on determining the level of sodium in biological materials by NAA. Inclusion of these guidelines will result in a generic method applicable to many laboratories and other elements. Test samples for the collaborative study were prepared and characterized with sodium concentrations ranging from microgram/gram to percent levels. Cunningham's preliminary investigations on the feasibility of using powdered infant formula as a reference material for element analysis has led to a joint developmental effort between the U.S. Food and Drug Administration, the U.S. Department of Agriculture, and the National Institute for Standards and Technology (NIST). A large supply of this material is expected to be received at NIST for initial characterization before the end of 1991. Both element concentration and organic nutrient information will be generated for this material that will subsequently be promoted and distributed to the public by NIST.

*Organotin in Foods.*—Associate Referee Allen Uhler reports that modifications have been made to the analytical method for analysis of tributyltin (TBT) and its degradation products dibutyltin (DBT) and monobutyltin (MBT), and that the methods have been published (*Environ. Toxicol. Chem.* (1989) **8**, 971–979; in *Oceans '89, The Global Ocean* (1989) Institute of Electrical and Electronics Engineers, New York, NY, pp. 508–511; *Bull. Environ. Contam. Toxicol.* (1991) in press). The methods are based on solvent-chelation extraction, *n*-pentyl derivatization, and analysis by GC with flame photometric detection at an emission wavelength of 610 nm. Methods for the analysis of TBT and its degradation products in shellfish and finfish tissue were submitted in 1989 to the U. S. Environmental Protection Agency (EPA) Office of Pesticides Programs in support of a TBT Data Call-In. These methods have been accepted by EPA, and are in use for EPA-mandated toxicology and environmental monitoring programs. Currently, detection limits for TBT in tissue samples are approximately 3 μg/kg (wet weight basis). The analytical method is now at a stage where it is relatively mature and reliable. Inter-

laboratory studies have been conducted with the U.S. Navy and split samples analyses have been conducted with Texas A & M University. There is reasonable agreement in the results. An AOAC collaborative study is being considered. Uhler also reports progress on a species-specific method for the analysis of triphenyltin (TPT) and its metabolites diphenyltin and monophenyltin in potatoes, sugar beets, molasses, and refined sugar. TPT is a much more unstable compound than TBT, and as such, the method for this compound is challenging. The method is based on solvent-chelation extraction, *n*-butyl derivatization, and analysis by GC with flame photometric detection at an emission wavelength of 610 nm. Detection limits are about 5 μg/kg. He plans to publish the method in 1991 and is considering an interlaboratory study in 1992 or 1993.

*Organometallics in Fish.*—Associate Referee Walter Holak did not report on this topic.

### *Recommendations*

(1) Adopt as official final action the official first action rapid GC method for determining methyl mercury in fish and shellfish (**988.11**).
(2) Continue official first action status of the liquid chromatographic/AAS method for methyl mercury in seafood (**990.04**).
(3) Continue official first action status of the GF/AAS method for copper, iron, and nickel in edible oils, and copper and iron in edible fats (**990.05**).
(4) Discontinue study on *Hydride Generating Techniques* due to lack of activity in the area.
(5) Continue study on all other topics.

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This report of the General Referee was presented at the 105th AOAC Annual International Meeting, August 12–15, 1991, at Phoenix, AZ. The recommendations were reviewed by the Committee on Residues. See the report of the committee, this issue.

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## Multiresidue Methods

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*Comprehensive Multiresidue Methodology.*—(Associate Referee Dr. S. Mark Lee, California Department of Food and Agriculture (CDFA), Sacramento, CA.) The multiresidue method, along with recovery information, reported on last year has been published in *Fresenius Z. Anal. Chem.* (1991) **339**, 376–383. Continued investigations of the published method have resulted in proposed changes for the determination of organohalogen pesticides. Specifically targeted for method performance investigations were chlorpyrifos, dicloran, hexachlorobenzene, iprodione, lindane, methoxychlor, PCNB, and *cis/trans*-permethrin. Changes made in the methodology, over that reported last year, include the addition of a Florisil cartridge cleanup step followed by electron capture detection (ECD). Extensive work was reported to show an improved linear range and reliable performance with this detection system compared to one equipped with an electrolytic conductivity detector. Recovery comparisons were developed using the CDFA method reported on last year, the new or revised CDFA method, and that described in **985.22**. All recoveries were calculated against standard solutions prepared in "blank" crop matrix.

Results of the comparison study, performed at individual predefined limits of quantitation, demonstrated performance improvement with the modifications compared to the previous CDFA method direct comparison with the performance of **985.22** is more difficult because the Associate Referee applied a factor of 15% to correct for sample weight concentration that occurs in "salting" out the aqueous phase in the modified approach. It was postulated this occurs in **985.22** as well, but was not investigated. Results of the **985.22** recovery study did show a "high" recovery bias when electrolytic conductivity detection was used, but when a Florisil cleanup step was added and ECD used, the results appear comparable to the weight "corrected" CDFA method results. The validity of using the correction factor is still under investigation.

It was reported that the investigations in attempting to improve the CDFA method were specifically targeted toward the 9 pesticides listed above that are to be included in an extensive survey program. No method modifications or changes for determining OP or *N*-methylcarbamates from the method reported last year were reported to have been made.

*Fumigants.*—(Associate Referee James L. Daft, Food and Drug Association, Kansas City, MO.) Two years ago, the Associate Referee submitted a collaborative study protocol for multifumigant residues in grains, grain products, and citrus fruit. This protocol was considered acceptable by the General Referee and the Committee Statistician. No further work has been accomplished toward initiating a collaborative study, though the Associate Referee plans to do so as time permits.

The Associate Referee's efforts have been diverted to methyl bromide analyses and he has worked closely with the Associate Referee on this topic. See the General Referee report on *Organohalogen Pesticides* for further details.

*Gel Permeation Chromatography (GPC) Cleanup.*—(Associate Referee W. Frank McCullough, ABC Laboratories, Columbia, MO.) No work was accomplished this year. The Associate Referee stated that he lacks time to work on the project and requested that the committee accept his resignation.

*Low Moisture–High Fat Samples.*—(Associate Referee Gregory Beard, Hershey Chocolate USA Laboratory Services, Hershey, PA.) The Associate Referee indicated he plans to conduct a limited collaborative or interlaboratory study in late 1991 or early 1992. No details were received other than he plans to use a short GPC cleanup of fatty materials to determine organohalogen and organophosphorus pesticide residues and widebore capillary gas chromatography (GC) in the determinative step. He currently is working on a faster, more efficient procedure to extract oilseeds.

*Miniaturized Multiresidue Methods for Fat Containing Foods.*—(Associate Referee D. Ronald Erney, FDA, Detroit, MI.) The Associate Referee continued with research efforts to develop a small scale method for organohalogen (OH) and organophosphorus (OP) pesticide residues in milk. A poster entitled "Rapid Procedure for Pesticide Residue Analysis in Milk" is being presented at this meeting detailing his results to date. Last year, the Associate Referee reported on a method that appeared to give quantitative recoveries (90–110%) for heptachlor epoxide, endosulfan $SO_4$, chlorpyrifos, diazinon, and acephate. This year it is reported that these recoveries were probably erroneous because of detector "enhanced" response caused by sample matrix effects. This year, using a slightly modified version of the same method and using standards prepared in "blank" sample matrix, recoveries are reported to range from 66–80% for the same 6 pesticides at the same levels of fortification; 0.01–0.03 ppm on the whole product basis. RSDs for 7 replicate analyses were reported to range from 3 to 8% for the 6 pesticides.

The method studied involves extracting 10 g of milk in a 50 mL centrifuge tube with 4 portions (10, 5, 5, and 5 mL) of acetone–$CH_3CN$ (2 + 8) followed by periods of standing, shaking, and centrifuging. After the first extraction, 2 mL water are added with the combined solvents. After the second extraction, 1 mL is added. After each centrifuging, supernatants are decanted into a separatory funnel followed by partitioning 4 times with 50 mL portions of $CH_2Cl_2$. After drying through $Na_2SO_4$, 25 mL $CH_3OH$ are added to the $CH_2Cl_2$ and the extract is taken to dryness ($CH_3OH$ is added once more) by rotary evaporation. The residue is taken up in $CH_3CN$ and cleaned up by elution through a C18 cartridge. After removal of $CH_3CN$ by rotary evaporation in the presence of $CH_3OH$, OH and OP residues are determined by ECD/GC and FPD/GC respectively.

The method is reported to be more of a "screening" approach than a quantitative one, but the Associate Referee has demonstrated that polar OP pesticides such as acephate can be recovered at approximately 70%. He has also recovered other polar pesticides with similar or greater success, and feels that this recoverability is a major benefit over other approaches that concentrate only on the nonpolar fat soluble residues. He plans to investigate ways to avoid the use of matrix/standards and to concentrate on further development of the method application to polar pesticides.

*Miniaturized Multiresidue Methods for Nonfatty Foods.*—(Associate Referee Charles H. Parfitt Jr, FDA, Washington, DC.) An extensive recovery comparison study between the official method **985.22** and a miniaturized approach has been completed. The study used 6 crops, 7 representative organohalogen and organophosphorus pesticides, 2 fortification levels, duplicate analysis at each fortification level, and 2 variations of the miniaturized approach. In addition, 6 incurred residues in 3 of the crops were encountered and included in the study. The Associate Referee is presenting the results at this meeting with a poster entitled "Miniaturized Multiresidue Approach to Determine Pesticide Residues in Fresh Fruits and Vegetables."

Pesticides studied for recovery comparisons were acephate, omethoate, monocrotophos, dimethoate, chlorpyrifos, α-BHC, and dieldrin. Fortifications levels were 0.1 and 0.5 ppm in lettuce, bell pepper, potato, strawberry, tomato, and pear extracts. Incurred residues were E-mevinphos/captan in strawberries, chlorpyrifos/methamidophos in tomatoes, and parathion/captan in pears. The 2 variations of the miniaturized approach differed only in the final solvent concentration step: one used the common Kuderna Danish (KD)/steambath approach; the other used a commercial (TurboVap™) concentrator.

Recovery comparisons for all 3 approaches were made from the same acetone extract of 100 g of produce as described in **985.22**: 20 mL for the miniaturized approaches and 80 mL for the official method were used. The miniaturized method follows the solvent partitioning ratios described in **985.22**, but it is uniquely different in that aqueous/solvent phases are separated by "freezing out" the water and decanting the solvent. The "freeze out" step is accomplished by immersing the sample/solvent mixture (contained in 125 mL Erlenmeyer flasks) in a dry ice/acetone bath for a few minutes. This step is very rapid, eliminates the need for separatory funnels, requires minimum laboratory space, and eliminates the potential for emulsion formation. It is reported to take 25–33.3% as much solvent per sample as **985.22**, and samples can be processed in half the time. Wide bore capillary GC using either phosphorus selective flame photometric or halogen selective electrolytic conductivity detection was used for the determinative step for all recoveries. Chlorpyrifos recoveries were determined by both systems.

Reported recoveries and precision, based on percent RSDs ($n$ = 12), are comparable for the 3 approaches except for α-BHC and acephate with TurboVap concentration. As much as 50–60% loss occurred on a few occasions. It was reported these losses occurred when the evaporation went to complete dryness and the concentrator tubes were not removed from the apparatus immediately thereafter. Results of the recovery study and incurred residue findings are summarized in Tables 1 and 2.

**Table 1. Combined average (%) recoveries and % RSDs (n = 12) from 6 crops**

| 0.1 ppm | Miniature TurboVap | | Miniature K-D steam | | AOAC 985.22 | |
|---|---|---|---|---|---|---|
| Acephate | 88 | (9.6) | 89 | (10.3) | 90 | (9.1) |
| Omethoate | 110 | (7.0) | 108 | (9.3) | 108 | (8.1) |
| Monocrotophos | 113 | (7.9) | 109 | (9.3) | 105 | (7.9) |
| Dimethoate | 99 | (9.8) | 102 | (10.3) | 102 | (6.7) |
| Chlorpyrifos (FPD) | 98 | (8.4) | 101 | (11.4) | 101 | (8.8) |
| Chlorpyrifos (HECD) | 104 | (8.7) | 106 | (10.5) | 104 | (6.2) |
| α-BHC | 76 | (27.6) | 103 | (13.1) | 107 | (12.4) |
| Dieldrin | 102 | (7.8) | 101 | (8.4) | 102 | (6.2) |
| **0.5 ppm** | **Miniature TurboVap** | | **Miniature K-D steam** | | **AOAC 985.22** | |
| Acephate | 80 | (12.1) | 83 | (13.2) | 93 | (11.0) |
| Omethoate | 98 | (11.6) | 104 | (11.7) | 110 | (9.7) |
| Monocrotophos | 105 | (6.8) | 107 | (6.4) | 110 | (7.2) |
| Dimethoate | 97 | (3.3) | 100 | (5.0) | 104 | (4.8) |
| Chlorpyrifos (FPD) | 94 | (5.2) | 98 | (5.2) | 102 | (3.9) |
| Chlorpyrifos (HECD) | 95 | (8.8) | 103 | (8.6) | 101 | (6.7) |
| α-BHC | 71 | (22.4) | 105 | (11.1) | 100 | (9.1) |
| Dieldrin | 96 | (6.5) | 100 | (6.8) | 100 | (7.5) |

**Table 2. Comparative duplicate findings (ppm) of incurred residues in 3 crops**

| | Miniature TurboVap | | Miniature K-D Steam | | AOAC 985.22 | |
|---|---|---|---|---|---|---|
| Strawberry | | | | | | |
| E-mevinphos | 0.071 | 0.047 | 0.085 | 0.078 | 0.102 | 0.064 |
| Captan | 4.66 | 3.93 | 4.83 | 4.92 | 4.29 | 4.18 |
| Tomato | | | | | | |
| Chlorpyrifos | 0.011 | 0.012 | 0.009 | 0.010 | 0.010 | 0.010 |
| Methamidophos | 0.022 | 0.020 | 0.022 | 0.020 | 0.022 | 0.022 |
| Pear | | | | | | |
| Parathion | 0.011 | 0.010 | 0.011 | 0.011 | 0.010 | 0.010 |
| Captan | 1.31 | 1.48 | 1.61 | 1.81 | 1.70 | 1.68 |

Future plans include development of a scaled-down extraction step based on 25 g product and 50 mL acetone. Various homogenization techniques with crops containing incurred residues will be studied and compared to **985.22** performance. Applicability of the total miniaturized approach (including extraction) to additional crops and the pesticides *N*-methylcarbamates, pyrethroids, and triazoles are also planned. Pending successful completion of the planned investigations, an interlaboratory trial of the procedure may be conducted within the year.

*Sweep Codistillation.*—(Associate Referee Barrie Magor, Australian Government Analytical Laboratories, Melbourne, Australia.) The Associate Referee was officially appointed to this position only recently. He indicated in his letter of acceptance that he would pursue obtaining results from the Australian National Pesticide Residue Proficiency Testing Program (NPRPTP) for potential acceptance as an official AOAC collaborative study. Prior results obtained under this program would have fulfilled almost all collaborative study criteria as far as number of laboratories, recoveries obtained, and precision for the cleanup of organohalogen pesticide residues by sweep codistillation (Unitrex) procedures. The Associate Referee is an active researcher/user of sweep codistillation and is a participant in the NPRPTP. In his position as a Senior Chemist with the Australian Government Analytical Laboratories, the Associate Referee indicated he would be seeking involvement in the development of future studies under this program, which potentially could assure all AOAC criteria would be followed.

*Synthetic Pyrethroids.*—(Associate Referee Darryl E. Johnson, FDA, Minneapolis, MN.) Although a method based on modifications to **985.22** was completed last year with quantitative recovery information for 13 synthetic pyrethroids from 3 different crops, no further work on this project was completed. The Associate Referee stated that he will not be able to conduct the planned interlaboratory study of the method and recommended the project be discontinued or reassigned.

### *Recommendations*

(1) *Comprehensive Multiresidue Methodology.*—Continue investigation/validation of reported sample weight concentration relationship with "salting out" procedures and publish findings. 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.*—Proceed with previous (1990) plans to conduct both an interlaboratory trial and a collaborative study following the approved protocol.

(3) *Gel Permeation Chromatography Cleanup.*—Discontinue as a topic area. Investigations of GPC cleanup are ongoing by the Associate Referee for *Low Moisture–High Fat Samples.*

(4) *Low Moisture–High Fat Samples.*—Submit a detailed protocol of the planned interlaboratory study of the GPC cleanup method for review. Also, submit a progress report on extraction studies.

(5) *Miniaturized Multiresidue Methods for Fat Containing Foods.*—Continue development of the miniaturized milk method with emphasis on its application to polar pesticides and metabolites. Investigate alternatives to the use of standards prepared in sample matrix diluent. If recoveries can be demonstrated to be consistently achievable at ≥80% at levels approximating 0.0005 ppm, then proceed with plans to initiate the collaborative study process.

(6) *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.

(7) *Sweep Codistillation.*—Submit results and design of the latest NPRPTP samples for AOAC review and comment. Continue with research efforts and plans to assist and design future sample studies that potentially could be coordinated to serve both the AOAC and Proficiency Testing Program.

(8) *Synthetic Pyrethroids.*—Appoint a new Associate Referee to this topic area. After appointment, the new Referee should prepare plans for an interlaboratory trial of the method that has been developed.

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## Organohalogen Pesticides

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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's review paper, which documents differences and similarities among current methods for polychlorinated dibenzo-*p*-dioxins and dibenzofurans, was published (*J. Assoc. Off. Anal. Chem.* (1991) **74**, 375–384). The Referee also reports the following advances in methodology for dioxins and furans:

(*1*) Methodology similar to that used for determination of dioxins and furans in tissue was used to determine brominated dioxins and furans in human adipose tissue (U.S. Environmental Protection Agency (EPA) document 560/5-90-005, April 1990).

(*2*) FDA Chicago District Laboratory, Dioxin Group published a description of their current method for determination of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in finfish and shellfish as FDA Laboratory Information Bulletin 3561.

(*3*) The National Council of the Paper Industry for Air and Stream Improvement (NCASI) and FDA Chicago District Laboratory, Dioxin Group have each developed an extraction and cleanup procedure for determination of dioxins and furans in bleached paper products intended for use in food packaging. The methods are intended to detect and confirm the presence of dioxins and/or furans in paper products at levels of 1–2 ppt or less. Both methods use high resolution gas chromatography (GC) coupled with high resolution mass spectrometry (MS) as the determinative step. NCASI has expressed interest in validating its method via AOAC collaborative study.

The Associate Referee will chair a session on "Levels in Food" at Dioxin '91, the 11th International Symposium on Chlorinated Dioxins and Related Compounds, to be held September 23–27, 1991, in Research Triangle Park, NC.

*Chlorophenoxy Alkyl Acids and Pentachlorophenol.*—(Marvin Hopper, FDA, Total Diet Research Center, Kansas City, MO.) The Associate Referee has not been able to devote time to this project during the past year. He does not foresee being able to work on this project in the future, because of other priorities. Collaborative study of this method may be dependent upon finding another researcher to plan and perform the study.

*Methyl Bromide.*—(Joseph Ford, USDA, Gulfport, MS.) The Associate Referee reports that 2 manuscripts, each describing essentially the same method for methyl bromide in processed nuts, have been submitted to *J. Assoc. Off. Anal. Chem.* and are currently undergoing review. Manual application of the method is described in a manuscript by James Daft, FDA, Kansas City, while a manuscript by Mr. Ford describes an automated version. It is expected that the manuscripts will be published together in a forthcoming issue of the journal.

The Associate Referee plans to examine the manual version of the method further, because it is a better candidate for collaborative study than the automated version. The latter requires special equipment that could preclude obtaining the necessary number of collaborators. After the evaluation, he will develop a protocol for collaborative study.

The Referee's laboratory analyzed 1100 samples of processed nuts for methyl bromide during FY 90; 3 samples were found to contain methyl bromide residues at levels of 0.014, 0.017, and 0.03 ppm, respectively. In the current fiscal year, the laboratory is examining processed nuts, dried fruits, dried beans, and rice; thus far, after examining 32 samples of processed nuts and 130 samples of dried fruits and beans, 1 residue (0.035 ppm) was found in blackeyed peas.

As residue data on these commodities are collected, they are reported to EPA. Manufacturers of methyl bromide and segments of the food industry have surveyed other commodities for methyl bromide residues, and these data have also been submitted to EPA.

The Referee recommends that he organize a symposium, for presentation at the 1992 Annual International Meeting on development of policy for use of methyl bromide. The symposium would provide an opportunity for cooperation among the agencies responsible for various aspects of regulation of methyl bromide.

*PCB Determination by Measurement of Specific Congeners.*—(Kimmo Himberg, Crime Laboratory, National Bureau of Investigation, Helsinki, Finland.) The Associate Referee provided preliminary information about changes in the method he intends to collaborate. Further details are promised in the

near future. Briefly, the method now employs saponification of the homogenized sample, replacing the previous Soxhlet extraction. It also uses a GC column of normal phenylmethyl silicone phase rather than a polar one, which caused unwanted coelutions of congeners. He reports that results of intralaboratory precision and accuracy tests have been mainly good and that ruggedness testing is underway.

*PCBs in Blood.*—(Virlyn Burse, Centers for Disease Control, Atlanta, GA.) At the Annual International Meeting, the Associate Referee will present a poster on quality control related to use of the official first action method for PCBs in blood serum, **990.07**. The presentation will display several years' quality control data from two laboratories using the method with packed column GC. It will also include data from a single laboratory using the method with capillary column GC over a shorter time period.

The Referee continues to plan extension of the official status of this method to cover selected pesticides, in the presence of PCBs, in blood serum. Collaborative study of this application of the method is important, because epidemiologists and environmentalists still routinely use residues of organochlorine insecticides in human tissues as a primary indicator of human environmental exposure to pesticides.

The Referee has proposed the following projects to his supervisors to request the necessary funding for FY 92.

(*1*) The purchase or preparation of radiolabeled ($^{13}C$) versions of at least some of the pesticides most often found in human tissues: hexachlorobenzene, β-BHC, DDE, DDT, dieldrin, heptachlor epoxide, mirex, octachlor epoxide, and *trans*-nonachlor.

(*2*) The use of isotope-dilution analysis of serum fortified with labeled and nonlabeled pesticides, in the presence of PCBs, to measure the adequacy of the method in extracting residues from the serum. GC/MS, probably using capillary GC columns, would be used in the isotope-dilution analysis.

(*3*) The collaboration of the official method, or a modification of it, through the protocol required by the AOAC. The collaborative study would not involve radiolabeled materials, but the results of the collaborative study would be compared to the results obtained with the isotope-dilution method. The collaborative study would include serum containing both in vivo and in vitro PCBs, as was done in the previous collaborative study.

This project has not yet been funded by the Referee's agency. The expense associated with purchase or preparation of radiolabeled pesticides may delay initiation of the project.

### *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. Review the NCASI method for dioxins and furans in paper products and guide the organization in their decisions about AOAC collaborative study of the method.

(2) Complete the manuscript that describes the method for chlorophenoxy alkyl acid and phenol residues. Study the extraction efficiency of the method using field-incurred residues. Prepare a protocol for an interlaboratory study and perform the study. Prepare for and perform a collaborative study on the method if the interlaboratory study is successful.

(3) Revise for publication, as needed, the manuscripts describing 2 versions of a method for methyl bromide in processed nuts. Perform further validation of the manual version of the method. Develop a protocol for collaborative study. Pursue with the AOAC plans for a symposium at the 1992 annual meeting on methyl bromide regulation, toxicology, and methods of analysis.

(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 first 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**.

(7) Make official final action the official first action status of the method for PCBs in blood serum, **990.07.**

### *References*

(1) Burse, V.W., Korver, M.P., Needham, L.L., Lapeza, C.R., Jr, Boozer, E.L., Head, S.L., Liddle, J.A., & Bayse, D.D. (1989) *J. Assoc. Off. Anal. Chem.* **72**, 649–659

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## Organonitrogen Pesticides

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Five Associate Referee reports were received. Richard Krause resigned as an Associate Referee, and the Associate Referee for *LC Methods for Meat and Poultry Products* has been transferred to AR for *Carbamate Insecticides*. Associate Referees are required for an additional 12 topics.

*Anilazine.*—An Associate Referee is required to select and conduct an interlaboratory trial of a method for anilazine residues in fruit and vegetables.

*Benzimidazole-Type Fungicides.*—An Associate Referee is required to study a method for benomyl, thiophanate methyl, and carbendazim, which converts all compounds to carbendazim and subsequently determine it by HPLC or GLC.

*Captan and Related Fungicides.*—Associate Referee Dalia Gilvydis reports that the interlaboratory study of her method for captan, captafol, and folpet has been accepted for publication in the *Journal of AOAC International*, and that additional collaborators will be sought in the next year.

*Carbamate Herbicides.*—An Associate Referee is required to select and test a method for the carbamate herbicides asulam, desmedipham, and phenmedipham.

*Carbamate Insecticides.*—Richard Krause reports that he is resigning his position as Associate Referee. The Associate Referee on *LC Methods for Meat and Poultry Products* has been transferred from the Committee on Meat, Poultry, and Meat and Poultry Products, and will assume the position of Associate Referee for *Carbamate Insecticides*. Sher Ali has submitted a method based on LC determination of 10 *N*-methyl carbamates using postcolumn derivatization with OPA and fluorescence detection (*J. Agric. Food Chem.* (1989) **72**, 586–592) for review and comment by the General Referee.

*Carbofuran.*—An Associate Referee is required to conduct a collaborative study of a method for carbofuran phenolic metabolites and 3-hydroxy carbofuran glucoside residues in crops, and a study 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).*—Interlaboratory data developed by the National Food Processors Association on the Conditt et al. method (*J. Assoc. Off. Anal. Chem.* (1988) **71**, 735–739) has not yet been submitted for review as an official method.

*Organonitro Pesticides.*—Associate Referee Richard Krause reports that he is resigning as Associate Referee.

*Diquat and Paraquat.*—Associate Referee Brian Worobey reports that comments have been received from the General Referee and Statistical Consultant on the report of his collaborative study of the HPLC method for diquat and paraquat. The study will be submitted to the committee on revision of the manuscript.

*Dithiocarbamate Fungicides.*—An Associate Referee is required to select and test a method for the differentiation and quantitative determination of dimethyl and ethylene bis(dithiocarbamates) 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 HPLC method for maleic hydrazide residues.

*Sodium o-Phenylphenate.*—An Associate Referee is required to select and collaboratively study a method for *o*-phenylphenol residues.

*Substituted Ureas.*—Associate Referee Ronald Luchtefeld reports that an additional 5 collaborators are required before a collaborative study of his postcolumn photolytic HPLC method for urea herbicides can be initiated.

*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 he has been unable to perform further development work on his GLC method for triazines.

### *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 before initiation of a collaborative study of the HPLC method for carbamate insecticides in liver. Conduct an interlaboratory trial of the method.
(3) Review data on the Conditt method for daminozide as it becomes available for the possible adoption as an official method.
(4) Revise the manuscript describing the collaborative study conducted by the Associate Referee incorporating suggestions of the General Referee and Statistical Consultant, and resubmit to the Methods Coordinator for further review. Continue study to broaden the scope of the method.
(5) Obtain additional collaborators and initiate study of the Associate Referee's multiresidue HPLC method (*J. Assoc. Off. Anal. Chem.* (1987) **70**, 740) for urea herbicides in onions and potatoes.
(6) Appoint Associate Referees for the topics of anilazine, benzimidazole-type fungicides, carbamate herbicides, carbofuran, chlorothalonil, dithiocarbamate fungicides, glyphosate, maleic hydrazide, organonitro compounds, sodium *o*-phenyl phenate, and thiolcarbamate herbicides.

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## Organophosphorus Pesticides

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There are 3 Asscciate Referee topics assigned to this General Referee.

*Extraction Procedures.*—Vacant.

*Organophosphorus Pesticide Residues.*—(Ronald R. Laski, U.S. Food and Drug Administration, Buffalo, NY.) The Associate Referee did not submit a report this year.

*Phosphine.*—Vacant.

### *Recommendations*

(1) *Extraction Procedures.*—Appoint an Associate Referee to the following: Study efficiency of procedures for extracting field-incurred residues of organophosphorus pesticides and their metabolites from crops; extend the study of Watts (*J. Assoc. Off. Anal. Chem.* (1971) **54**, 953–958); and develop improved extraction procedures for incorporation into existing multiresidue methods.

(2) *Organophosphorus Pesticide Residues.*—Continue study on recovery of organophosphorus (parent and metabolite) chemicals through LUKE multiresidue procedure (**985.22**) followed by oxidation to "total sulfones" (*Analyst* (1984) **109**, 483–487).

(3) *Phosphine.*—Appoint an Associate Referee to study the topic of *Phosphine* residues, including the solvent soaking method for extraction of fumigants in grains (**977.18B**).

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## Radioactivity

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*Cesium-137.*—Methods for determining cesium-137 in milk, foods, and biological materials at lower levels are being considered. (Also, the search for an Associate Referee is continuing.)

*Iodine-131.*—A collaborative study for the determination of iodine-131 in milk at very low-levels has been completed. The data have been submitted to the Committee Statistician for calculation. The data will then be evaluated.

*Plutonium-239.*—The search for a new Associate Referee has been completed. A letter of availability is expected soon. Upon receipt, the appointment will be made.

A method has been selected for collaborative study. It will be tested before submittal for the study.

*Radium-228.*—The write-up for the collaborative study is expected in August 1991. The report will be submitted for evaluation and recommendations. If acceptable to the General Referee, it will be submitted to the Committee on Residues for possible interim first action status.

*Strontium-89 and -90.*—Effram Revas, the newly appointed Associated Referee, resigned due to change in employment. Search for and appoint a new Associate Referee for this topic. New Referee will prepare a protocol for the collaborative study of the method of Baratta and Reavey (*J. Agric. Food Chem.* (1969) **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.*—A selection has been made for an Associate Referee for this topic. Appointment will be made upon receipt of a letter of approval from applicant's supervisor. First order of business will be to select suitable method for tritium in foods.

### *Recommendations*

(1) Submit the study for iodine-131 in milk at low-levels.

(2) Submit the study for radium-228 when completed.

(3) Search of new Associate Referees for *Cesium-137* and *Strontium-89 and -90.*

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# Committee on Microbiology and Extraneous Materials

## Analytical Mycology and Microscopy

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*Vegetable Substitutes in Horseradish.*—A method has been developed to detect adulterants in horseradish. The method relies on diagnostic histological features of horseradish root and its known adulterants such as turnip root, parsnip root, potato tuber, and corn starch, as viewed through a polarizing microscope with crossed polars and a first-order red plate. A collaborative study was begun 2 years ago. Results have been received from some of the collaborators. Completion of the study has been delayed due to difficulty obtaining collaborators with sufficient expertise and time to complete the study. Preliminary results indicate that some analysts are having difficulty identifying the various starches. The Associate Referee plans to revise and simplify the method and conduct another collaborative study. Final evaluation will occur when all collaborators have submitted their results.

*Microscopic Mold Count Methods, Use of Compound Microscope.*—The optional substitution of the compound microscope in mold count methods specifying a wide-field microscope is being evaluated for comparability of counts. Use of the compound microscope should lead to less eye strain during fragment counting as compared with a wide-field microscope. The Associate Referee has developed a slide adaptor that will accommodate the large rot fragment counting slide on a standard compound microscope stage. Movement of the slide with the mechanical stage, as opposed to movement by hand on a wide-field stage, should result in smoother movement of the rot fragment slide during counting. In-house research is complete and the mold count data from the compound and wide-field microscopes are being evaluated for comparability.

*Mold by Chemical Detection.*—Refinements are being investigated for a method that measures the mold content of a test sample by measuring the level of chitin as glucosamine found in the test sample. The chitin in the test sample is deaminated by heating the sample at 121°C in 14M KOH, and then digestion with nitrous acid generated in situ. The resulting glucoaldehydes are measured colorimetrically. A series of commercial catsups were analyzed for glucosamine content and the results compared to that obtained with the Howard mold count. Correlation between the 2 methods was low. The samples were sent for analysis by total mold count. The total mold count procedure should not be affected by variation in mold particle size as the Howard mold count is. Directions for future work will be decided after the data are analyzed.

*Mold Counting–Standardizing Plant Tissue Concentrations.*—Physical separation techniques are being explored for use in developing a method to standardize tissue concentrations among different forms of a product, e.g., tomato paste, puree, juice, and catsup. Tissue concentrations should be standardized so that products with equivalent amounts of tissue would be evaluated on an equivalent basis, because mold contamination is associated with the tissue. Difficulties arise with products having a wide range of particle sizes or with added sugars, which would eliminate the use of a refractometer. This topic was recently returned to the original Associate Referee who is just beginning to resume his research.

*Mold in Reconditioned Nutmeg.*—A method is being developed to quantitate mold in reconditioned nutmeg. Cracked nutmeg can be examined macroscopically for mold; however this method is tedious and it is subject to piece-by-piece variation. An approach involving grinding the nutmeg and then using a variation of the Howard mold count or rot fragment count is being explored. Work in this area has been temporarily suspended due to other assignments.

*Mold (Geotrichum) in Canned and Frozen Fruits, Vegetables, and Juices.*—This topic was recently returned to the original Associate Referee who is just beginning to resume his research.

*Yeasts and Molds, Mycological Media for Isolation.*—A new method for the isolation of fungi from foods using media containing NaCl to inhibit spreader molds has been published. A collaborative study was planned, but work in this area was suspended due to illness of the Associate Referee. The Associate Referee will continue evaluating the efficacy of other mycological media for the isolation of fungi from foods and cosmetics.

### *Recommendation*

Continue study on all topics.

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## Environmental Sanitation Microbiology

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There was some activity in this committee this year. There were 2 projects for consideration for precollaborative and collaborative studies.

The first involved the use of Defined Substrate Technology as a test for total coliforms and *E. coli* on surfaces.

The second was an inquiry from England requesting the use of an ATP-based system as a means of assessing the overall cleanliness of surfaces.

Because a standard does not exist for the microbiological sampling of surfaces, it was felt that an overall protocol had to be developed. In order to do so, it would be necessary to bring together a group of AOAC people, including the statistician, to agree on a means of testing. When one of the interested parties has finished a precollaborative test phase, this protocol development will be instituted by the General Referee.

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## Cosmetic Microbiology

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This report provides an update on the status of the 3 topics reported on last year (*J. Assoc. Off. Anal. Chem.* (1991) **74**, 157).

*Evaluation of the Cosmetic Toiletry and Fragrance Association's (CTFA) Guideline Method for Determination of Preservative Efficacy.*—Associate Referee R. J. Spielmaker (Amway Corp.) and his collaborators are planning to conduct a collaborative study in the Fall of 1991. They will use a CTFA-based method to evaluate the efficacy of preservation of non-eye-area water miscible cosmetic and toiletry formulations. Five different products 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 $1 \times 10^8$–$9 \times 10^8$ colony forming units/g. Investigators interested in becoming involved in planning a similar collaborative study of eye-area products should contact the General Referee. CTFA has published results of a survey (by R. J. Spielmaker) on the preservative test methods most used by cosmetic companies (*Cosmetic and Toiletries* (1990) **105**, 79–82).

*Isolation of Microbes from Cosmetics.*—No Associate Refereeship has been established in this topic although there is a need for rapid quantitative methods for assessing recovery of stressed surviving bacteria from preserved cosmetics. In relation to this, there is a crucial need to evaluate the performance of neutralizers in the microbial analysis of cosmetics (D. Brannan, personal communication). Investigators interested in collaborative studies of this and other aspects of the incidence of microbes in cosmetics should contact the General Referee. U.S. Food and Drug Administration (FDA) microbiologists (A. D. Hitchins, J. McCarron, and T. T. Tran) have revised the cosmetic microbiology methodology chapter for AOAC's *Bacteriological Analytical Manual* (to be submitted). A rapid (24 h) bacteriological bioluminescent screening for cosmetics and toiletries has been developed (P. K. Nielsen and E. Van Dellen: 105th AOAC Annual International Meeting).

The incidence of fungi in shared-use cosmetic test kits at retail outlets, determined in an FDA nationwide survey, is being published by P. B. Mislevic, R. Bandler, and G. Allen (*J. Assoc. Off. Anal. Chem.*, submitted). The related bacterial prevalence results have been summarized (T. T. Tran & A. D. Hitchins (1990) *Abstracts, Annual Meeting of the American Society for Microbiology* **90**, 322).

*Preservative Efficacy in Solid Cosmetics.*—This difficult topic has often discouraged investigators; therefore, it is exciting to see that significant progress is occurring. A direct-contact bacterial challenge method for evaluating solid cosmetic preservative efficacy has just been published by T. T. Tran et al. (*Int. J. Cosmet. Sci.* (1990) **12**, 175–183). The method is also applicable to yeasts and molds (T. T. Tran and S. W. Collier (1991) *Abstracts Annual Meeting American Society for Microbiology* **91**, Q312). This method is flexible in that the challenge microorganisms can be used in adapted or unadapted physiological states as required. A ring-trial of the method is planned in preparation for a regular collaborative study. Investigators interested in collaborative study of the solid cosmetic preservative efficacy methodology should contact T. T. Tran, FDA, HFF-234, Washington, D.C. 20204.

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## Drug- and Device-Related Microbiology

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*Packaging Integrity Testing.*—(Anna Placencia, Associate Referee.) A method of testing the penetration of viruses through surgical gowns and other materials was developed. Plans are to collaborate this method after some further work.

A collaborative study is being planned on a method for detecting leaks in medical device packages.

American Society for Testing and Materials (ASTM) standard on the rating of microbial penetration through packaging materials is under balloting by ASTM.

A computer controlled chamber is being developed for testing both medical packages and materials for microbial penetration.

All other methods development is inactive at this time.

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## Food Microbiology—Dairy

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The Dairy Microbiology section of AOAC is on the verge of initiating 3 collaborative studies:

(*1*) Vitek Systems, Hazelwood, MO: impedance detection of coliform in milk. Expected start of study: Fall 1991.

(*2*) Corporate Microbiological Services, Belgium: *Salmonella* detection from dairy products by motility enrichment on modified semisolid rappaport-vassiliadis medium. Expected start of study: January 1992. Expected date of report: October 1992.

(*3*) Radiometer America, Westlake, OH: impedance detection of coliform in milk. Expected start of study: January 1992.

An International Dairy Federation initiated collaborative study will tentatively be submitted for approval by September 16, 1991 entitled: "Isolation of *Listeria monocytogenes* from Dairy Foods: IDF Collaborative Study," G.A. Prentice, R.M. Twedt, and A.D. Hitchins.

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# Food Microbiology—Nondairy

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## *Collaborative Studies*

*Clostridium perfringens, Iron Milk Test for Recovering Clostridium perfringens from the Marine Environment.*—In the AOAC method for the enumeration of *Clostridium perfringens* in foods, **976.30**, a 50 g sample is blended with 450 mL peptone dilution water, and dilutions are plated (either pour plated or spread plated) to tryptose-sulfate-cycloserine agar. Typical colonies are picked, and cultures are Gram stained and tested for sporulation. Gram positive, nonmotile, sporeforming bacilli that reduce nitrates to nitrites, produce acid and gas from lactose, and liquefy gelatin are provisionally identified as *C. perfringens*. Suspect cultures not meeting the above criteria must be confirmed by determination of acid and gas from salicin and raffinose. Salicin is usually not fermented (but a few strains produce acid), and acid is usually produced from raffinose.

As an alternative to the AOAC procedure, Associate Referee Carlos Abeyta has proposed using a rapid screening procedure based on the stormy fermentation of iron milk. This reaction is characterized by the rapid coagulation of milk followed by the fracturing of the curd into a spongy mass that usually rises above the medium surface. It has been reported that stormy fermentation in iron milk is highly indicative of the presence of *C. perfringens* (1, 2). The alternate procedure is based on a most probable number (MPN) technique using iron milk as the enrichment medium. Tubes exhibiting stormy fermentation are streaked to modified *Clostridium perfringens* (mCP) medium consisting of the following ingredients (g/100 mL): Tryptose, 3.0; yeast extract, 2.0; sucrose, 0.5; L-cysteine, 0.1; $MgSO_4 \cdot 7H_2O$, 0.01; bromcresol purple, 0.004; and agar, 1.5. After autoclaving and cooling to 50°C, the following ingredients are added: D-cycloserine, 40 mg; polymyxin B sulfate, 2.5 mg; 0.5% phenolphthalein diphosphate, 2 mL; and 4.5% $FeCl_3 \cdot 6H_2O$, 0.2 mL. Cultures exhibiting a positive stormy fermentation reaction and a positive phosphatase test on mCP agar are considered *C. perfringens*.

Eleven laboratories participated in a collaborative study to compare the iron milk stormy fermentation procedure with the existing method, **976.30**, for the recovery of *C. perfringens* from samples of oysters and marine sediment. A preliminary review of the data has indicated comparability of the 2 methods for the analysis of oysters. For the analysis of the sediment samples, however, there was an apparently significant difference between the 2 methods in favor of the AOAC method, this difference being attributed to iron-binding between the iron milk medium and one or more factors in the sediment samples. Thus, the Associate Referee is not planning to recommend the method for the analysis of sediments. After statistical analysis of the data, a collaborative study manuscript will be prepared.

*Listeria, MICRO-ID System.*—This system (Organon Teknika Corporation, Durham, NC) is a self-contained unit consisting of reagent-impregnated discs that will reportedly identify the genus to species level in 24 h. These reagents can detect the presence of specific enzymes and/or metabolic products produced by *Listeria* species. These reagents include substrates that react with bacterial enzymes and include detection systems that react with metabolic end products formed during incubation to produce a readily identifiable color change.

A precollaborative study comparing the accuracy of MICRO-ID *Listeria* kit to conventional biochemical procedures in the *Bacteriological Analytical Manual* (BAM) (3) was conducted. A total of 73 isolates (37 isolates of *L. monocytogenes*; 5 isolates each of *L. ivanovii*, *L. innocua*, *L. seeligeri*, and *L. welshimeri*; 3 isolates each of *L. murrayi* and *L. grayi*; and 10 non-*Listeria* isolates) was tested. The 2 procedures agreed on 72 (98.6%) of the isolates tested.

In addition to the above precollaborative study, co-Associate Referees Donald Higgins and Barbara Robison conducted a collaborative study. Each of 14 laboratories received 51 *Listeria* and 9 non-*Listeria* cultures for identification with the MICRO-ID system. All isolates were identified by conventional biochemical analyses in the originating laboratory only. In addition to using the MICRO-ID *Listeria* kits, the collaborators determined the hemolytic reaction of each isolate by performing the CAMP test and by stabbing sheep blood agar. Identification of *Listeria* was based on the octal code obtained from the strip and the hemolytic reactions of the isolates. The MICRO-ID *Listeria* system and the conventional biochemical procedure agreed for 98.0% of the *L. monocytogenes* isolates, 77.1% of the *L. seeligeri* isolates, 90.0% of the *L. ivanovii* isolates, 96.4% of the *L. grayi/L. murrayi* isolates, 72.5% of the *L. welshimeri* isolates, and 100% of the *L. innocua* isolates. A large percentage of the errors in the identification of the *L. seeligeri* and *L. ivanovii* cultures was due to inaccurate reading of the CAMP and hemolysis tests rather than to errors in the MICRO-ID *Listeria* reactions.

On the basis of the above precollaborative and collaborative studies, the Co-Associate Referees recommend that the MICRO-ID *Listeria* system be adopted official first action for the species identification of foodborne and environmental *Listeria* isolates and for screening and elimination of non-*L. monocytogenes* isolates. The General Referee has recommended revisions in the collaborative study manuscript.

*Listeria, Vitek AutoMicrobic System.*—The Vitek AutoMicrobic System (McDonnell Douglas, Hazelwood, MO) for the identification of *Listeria* spp. was subjected to a collaborative study, the details of which were provided in the 1990 General Referee Report (4). Based on the results of that collaborative study, Associate Referee Loralyn Weiss recommended that this system be adopted official first action. In his 1990 report (4), the General Referee recommended minor revisions in the collaborative study manuscript. Subsequently, the Associate Referee submitted a revised manuscript. The manuscript is still not acceptable, and the General Referee has recommended revisions.

*Salmonella, Assurance Enzyme Immunoassay.*—The Assurance system (BioControl Systems, Inc., Bothell, WA) is a colorimetric *Salmonella* enzyme immunoassay, configured in a microwell format, and employs a proprietary formulation of polyclonal antibodies to *Salmonella*. The conjugates normally used in enzyme immunoassays are very large polymers of several hundred thousand dalton molecular weight. The size and shape of such molecules limit the binding efficiency to specific epitopes on the cell surface. This physical restriction is referred to as steric interference. In addition to the polyclonal antibody mentioned above, the Assurance system uses another antibody, referred to as a linking antibody, which, because of its small size and configuration, facilitates efficient immunochemical coupling.

In a precollaborative study, Associate Referee Philip Feldsine compared the enzyme immunoassay and the reference culture method (**967.25–967.28**) for the detection of *Salmonella* in 1000 samples (680 artificially contaminated, 150 naturally contaminated, and 170 control samples) representing 20 food types. *Salmonella* recoveries and false negative rates were comparable for the 2 methods. Moreover, no false positive reactions were observed with the immunoassay.

Subsequent to the completion of the precollaborative study, the Associate Referee conducted a collaborative study. Thirty-two laboratories participated in a study comparing the 2 methods for the recovery of *Salmonella* from nonfat dry milk, dry egg, black pepper, soy flour, chocolate, and ground poultry. Of the 1020 samples analyzed, there was agreement between the 2 methods for 97.2% of the samples analyzed. The false negative rates for the 2 methods were comparable for all foods, except for ground poultry, where the immunoassay procedure detected a significantly higher number of confirmed positive samples when compared to the reference culture method (**967.25–967.28**).

On the basis of these findings, the Associate Referee has recommended that the Assurance enzyme immunoassay for detection of *Salmonella* in foods be adopted official first action, and the General Referee concurs.

*Salmonella, GENE-TRAK Colorimetric DNA Hybridization Method.*—Because this method (**990.13**) uses DNA probes that are nonreactive with subgenus V *Salmonella* organisms, GENE-TRAK Systems (Framingham, MA) has proposed adding a DNA probe for subgenus V organisms to the probe solution. These probes are reported to be specific for subgenus V organisms and they do not appear to hybridize to other members of the *Enterobacteriaceae*. Moreover, the probes are reported not to interfere with the activity of the other probes already used with the method.

A second proposed change involves the combination of the neutralization and probe solutions into a single solution called the hybridization solution. This change simplifies the test by eliminating one assay step and shortens assay time by at least 15 min.

A third proposed change involves color coding some of the solutions to serve as visual clues that the assay steps have been performed properly. The addition of thymophthalein and cresol red to the lysis and hybridization solutions, respectively, has been proposed.

Finally, the assay has been further simplified by reducing the number of required washing steps. These minor modifications are reported to simplify and shorten the test format and broaden the inclusivity of the assay.

Associate Referee E. Patrick Groody conducted a study evaluating the modified assay. The new probe set detected all of 453 *Salmonella* strains representing at least 239 serovars and 50 somatic antigen groups, including 8 strains in subgenus V. Moreover, none of 225 non-*Salmonella* enteric organisms was reactive with the modified probe set. In a comparative study, the modified DNA hybridization assay and the reference culture method (**967.25–967.28**) were equally effective in recovering *Salmonella* from 240 artificially inoculated food samples and 80 naturally contaminated poultry samples.

On the basis of these findings, the Associate Referee recommends that the modified colorimetric DNA hybridization procedure be adopted official first action to replace the current official first action method (**990.13**), and the General Referee concurs.

*Staphylococcal Enterotoxin, Tecra Enzyme Immunoassay.*—The Tecra staphylococcal enterotoxin visual immunoassay (Bioenterprises Pty. Ltd., Roseville, New South Wales, Australia) is designed to provide a rapid, sensitive, and specific test for the detection of staphylococcal enterotoxins in foods. The enzyme immunoassay 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-labelled 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 each of 15 collaborators received duplicate samples of mushrooms, nonfat dry milk, lobster bisque, beef pasta, and chicken. Duplicate samples of each of the 5 food types were inoculated with one of 5 staphylococcal enterotoxin types (A, B, C, D, or E) at concentrations ranging from 4 to 10 ng/g.

The Associate Referee reports that all collaborators obtained correct results, with no false positive or false negative reactions being observed. The Associate Referee is preparing a manuscript detailing the results of this collaborative study.

*Vibrio vulnificus, Identification by Gas Chromatography of Cellular Fatty Acids.*—Bacteria grown under controlled laboratory conditions exhibit constant, but unique, fatty acid profiles, which can be used to differentiate genera, species, and even subspecies. Gas chromatography (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 acid 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 so as to identify the unknown culture.

Associate Referee Warren Landry conducted a collaborative study on the identification of *Vibrio vulnificus* by cellular fatty acid composition using the Hewlett Packard 5898 A Microbial Identification System (MIS) (Microbial ID, Newark, DE). Each of 16 laboratories was sent 10 cultures of *V. vulnificus* and one culture each of *V. parahaemolyticus*, *V. mimicus*, *V. cholerae*, *V. damsela*, *V. fluvialis*, and *V. alginolyticus*. The efficiency of a computer library recently generated by the Associate Referee was compared to that of the Hewlett-Packard library that is supplied with the instrument. The new library correctly identified 97.4% of the *V. vulnificus* isolates, whereas the Hewlett-Packard library identified only 67% of the *V. vulnificus* isolates. The Associate Referee attributed the higher efficiency of the new library, as compared to that of the Hewlett-Packard library, to the use of more isolates and the use of environmental isolates in creating the library entry.

The Associate Referee recommended that the identification of *V. vulnificus* isolates by GC of cellular fatty acids be adopted as an official first action method. Because the collaborative study protocol was not in accordance with AOAC-recommended guidelines for conducting collaborative studies, the General Referee did not concur with the Associate Referee's recommendation. The Associate Referee is planning to repeat the collaborative study.

### *Associate Referee Reports*

*Bacillus cereus, Enzyme Immunoassay for Enterotoxins.*—Associate Referee Reginald Bennett has initiated a preliminary study evaluating the efficiency of the Tecra *Bacillus cereus* diarrheal enterotoxin visual immunoassay (Bioenterprises Pty. Ltd, Roseville, New South Wales, Australia) for identifying 24 enterotoxigenic cultures. As a further expansion of this study, the Associate Referee will include an additional 100 *B. cereus* cultures as well as artificially contaminated and naturally contaminated samples in the evaluation. The results of this study will determine the feasibility of conducting a collaborative study.

*Hydrophobic Grid Membrane Filter Methods.*—Two hydrophobic grid membrane filter (HGMF) procedures (QA Laboratories Ltd, Toronto, Canada) have recently received official first action status. The first of these methods, **990.11**, uses lactose monensin glucuronate agar and buffered MUG agar for the enumeration of total coliforms and *Escherichia coli*, respectively. The second of these methods, **991.12**, uses EF-18 agar for the detection of *Salmonella*. Associate Referee Phyllis Entis reports being in contact with several laboratories using either one or both of these 2 methods. No adverse reports have been received regarding the performance of either method. Moreover, at least one of these laboratories is using EF-18 agar as a streak-plate isolation medium in addition to bismuth sulfite, Hektoen enteric, and xylose lysine desoxycholate agars as recommended in **967.26**.

The official first action method, **999.12**, replaces official final action method, **985.42**, the latter method having been repealed to official first action at the 1990 meeting. It is now recommended that the official first action method, *Salmonella* in Foods, Hydrophobic Grid Membrane Filter Screening Method, **985.42**, be repealed.

The Associate Referee reports that HGMF procedures for *Listeria monocytogenes* and for yeasts and molds are in the developmental stage. Thus, there are no plans for any collaborative studies in the immediate future.

*Listeria, Assurance Enzyme Immunoassay.*—This system (BioControl Systems, Inc., Bothell, WA) is a colorimetric, polyclonal enzyme immunoassay with a mechanism of action similar to that of other enzyme immunoassays described in this report. With this assay, the final result is read spectrophotometrically.

Associate Referee Philip Feldsine has submitted a protocol for a precollaborative study, and the General Referee has recommended revisions.

*Listeria, GENE-TRAK Colorimetric DNA Hybridization Method.*—GENE TRAK Systems (Framingham, MA) has developed a colorimetric DNA hybridization 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 sequences. 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 bound to the solid support. Detection of hybridization is achieved by using a colorimetric detection system comprised 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.

Associate Referee Michael Curiale reports that in a precollaborative study the DNA hybridization procedure was comparable to reference culture methods recommended by the U.S. Food and Drug Administration and the U.S. Department of Agriculture. Moreover, a protocol for a collaborative study has been approved at the committee level, and the Associate Referee reports that this study is in progress.

*Listeria, Identification by GC of Cellular Fatty Acids.*—The MIDI Microbial Identification System (MIS) (Microbial ID, Newark, DE) is a computer assisted microbial identification system that identifies microorganisms based on cellular fatty acids. Microorganisms are grown under standardized condi-

tions, their cellular fatty acids are extracted, and these extracts are chromatographed using capillary GC. The resulting fatty acid patterns are then compared to a database 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 has developed a *Listeria* database for the MIS using soybean casein digest broth and incubation at 30°C for 24–48 h. Hemolysis is used in conjunction with the results of fatty acid analysis to obtain final species identification. To differentiate *L. grayi* and *L. murrayi*, nitrate reduction is also required.

The Associate Referee is currently conducting a precollaborative study comparing the MIS and the conventional biochemical procedure in the BAM (3) for the identification of 97 *Listeria* strains and 21 non-*Listeria* strains. The study includes a comparison of the results obtained with the MIS when the strains are cultured using soybean casein digest broth from 3 different manufacturers.

*Listeria, Listeria-Tek Enzyme Immunoassay.*—The *Listeria*-Tek system (Organon Teknika Corporation, Durham, NC) is a colorimetric, monoclonal enzyme immunoassay for *Listeria*. Its mechanism of action is similar to that of other enzyme immunoassays described in this report. With this assay, the final result is read spectrophotometrically.

A precollaborative study comparing the *Listeria*-Tek assay with conventional culture procedures recommended by the U. S. Food and Drug Administration and the U. S. Department of Agriculture has been completed. A total of 1135 naturally contaminated, artificially inoculated, and uninoculated samples was tested. The conventional culture methods detected 708 (62.4%) confirmed positive samples, whereas the *Listeria*-Tek assay detected 781 (68.8%) positive samples, of which 762 (67.1%) were confirmed.

A protocol for a collaborative study has been submitted, and the General Referee has recommended revisions.

*Listeria, Tecra Enzyme Immunoassay.*—This assay (Bioenterprises Pty. Ltd., Roseville, New South Wales, Australia) is a colorimetric enzyme immunoassay and uses a proprietary formulation of polyclonal antibodies to identify *Listeria*. The mechanism of action is similar to that of other enzyme immunoassays described in this report. With this assay, results may be read either visually or spectrophotometrically.

Associate Referee Michael Knight has submitted a protocol for a precollaborative study, and the General Referee has recommended revisions.

*Pectin Gel Methods.*—With methods in place for the aerobic plate counts in foods, **988.18**, and for the total coliform count in dairy products, **989.11**, Associate Referee Jonathan Roth has reported the development of a new pectin gel medium (RCR Scientific, Inc., Goshen, IN) for the enumeration of total coliforms and *Escherichia coli* based on their production of galacturonidase and glucuronidase, respectively. After completion of a precollaborative study, the Associate Referee is planning to conduct a collaborative study.

*Reflective Colorimetry Method for Automated Microbiology.*—A reflectance colorimeter has been combined with an X-Y positioning mechanism and incubator to measure microbial activity in foods. Microwells are filled with samples, dyes, and media. The instrument obtains color measurements from the bottom of the microwells at frequent intervals. These measurements are then related to biochemical changes caused by the organisms. When these changes occur, they indicate the concentration of the biological units of interest. A computer calculates and prints out the estimated number of microorganisms.

Associate Referee Gary Richardson reports that potential collaborators who purchased the Omnispec reflective colorimeter (Wescor, Inc., Logan, UT) obtained low correlations with plating techniques when analyzing refrigerated foods containing low numbers of bacteria. Thus, the use of preincubation procedures is being investigated. In one example, incubation of raw milk at 7°C for 48 h and then introduction into the instrument at 32°C gave a significantly improved correlation with the standard plate count and the psychotrophic bacteria plate count. After completion of these preincubation treatment studies, a collaborative study will be conducted.

*Salmonella, Immunodiffusion Method.*—After the immunodiffusion or *Salmonella* 1-2 TEST (BioControl Systems, Inc., Bothell, WA) (**989.13**) received official first action status at the 1988 meeting, unfavorable evaluations of the method were published (5, 6). The manufacturer has proposed alternate enrichment steps of the procedure.

The currently approved AOAC official first action immunodiffusion method (**989.13**) for processed and low contamination products recommends preenrichment of the sample in a nonselective medium at 35°C for 24 ±2 h followed by inoculation of the test unit. The modified enrichment steps include a 24 h preenrichment step followed by selective enrichment in tetrathionate broth at 35°C for 24 ±2 h before inoculation of the test unit. In the currently approved AOAC official first action immunodiffusion method (**989.13**) for raw flesh foods and highly contaminated products, samples are directly enriched in tetrathionate broth and incubated at 35°C for 24 ±2 h before inoculation of the test unit. The modified enrichment steps proposed by the manufacturer for these types of foods include a preenrichment step at 35°C for 24 ±2 h followed by selective enrichment in tetrathionate broth at 43°C for 24 ±2 h before inoculation of the test unit.

Associate Referee Russell Flowers has conducted a collaborative study evaluating these alternate enrichment steps and reports that the results obtained with the original method (**989.13**), the revised procedure, and the conventional reference method (**967.25–967.28**) were equivalent.

On the basis of this foregoing data, the Associate Referee has prepared a protocol to reverify in a more expansive study, the equivalence of the original method (**989.13**), the alternate procedure, and the conventional reference method (**967.25–967.28**). A final report presenting all of the above results will be prepared at the conclusion of the study.

*Salmonella, Modified Semi-Solid Rappaport-Vassiliadis Method for Cocoa and Chocolate.*—A procedure for the rapid detection of *Salmonella* has been 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 precollaborative study comparing 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). Statistical analysis of the data demonstrated that the 3 methods were equivalent. A protocol for a collaborative study has been approved by the committee.

*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**.

Associate Referee Michael Curiale conducted a precollaborative study comparing the reference culture method, **967.25–967.28**, and the Oxoid culture method for the recovery of *Salmonella* from 356 random food samples, 20 types of artificially inoculated foods, and 3 types of naturally contaminated foods. The methods were comparable except for the analysis of 3 types of artificially contaminated foods (egg powder, coconut, and ground pork). These discrepant results were attributed to unnoticed temperature fluctuations during incubation and/or to some inherent peculiarities in those particular food lots. In an expanded evaluation of the Oxoid procedure, additional samples of these 3 food types were analyzed. No significant differences were observed between the reference culture method, **967.25–967.28**, and the Oxoid procedure.

Subsequently, a collaborative study was performed, and the Associate Referee reports that the results are being collated and a manuscript is being prepared.

*Salmonella, Salmonella-Tek Enzyme Immunoassay.*—Several modifications of this colorimetric monoclonal enzyme immunoassay (Organon Teknika Corporation, Durham, NC), **986.35** and **987.11**, are being proposed. These modifications are (*1*) incubation of tetrathionate broth and M broth at 42 ±0.5°C, (*2*) addition of 10 μg/mL novobiocin to the M broth, (*3*) elimination of the need to agitate the microtiter plates during antibody capture, and (*4*) elimination of the requirement for centrifugal concentration of the M broth culture for all food enrichments. The purpose of the first 2 modifications is to reduce the number of false positive reactions by minimizing the growth of competitor organisms and to allow for improved recovery of *Salmonella* organisms, whereas the second 2 modifications 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*, Associate Referee Russell Flowers conducted a preliminary study 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 conventional culture method, **967.25–967.28**, than with the proposed *Salmonella*-Tek procedure. In addition to these samples, 10 replicates of each of 10 naturally contaminated samples were analyzed by the culture method, **967.25–967.28**, and the proposed *Salmonella*-Tek method. No significant differences were observed between the 2 methods.

Protocols for precollaborative and collaborative studies have been submitted, and the General Referee has recommended that the protocols be revised.

*Salmonella, Tecra Enzyme Immunoassay.*—This official final action method (**989.14**) is a colorimetric polyclonal enzyme immunoassay for the rapid detection of *Salmonella* in foods. Bioenterprises Pty. Ltd. (Roseville, New South Wales, Australia) is the manufacturer of this kit. This kit is distributed in the United States under various trade names: Tecra (Vitek Systems, Inc., Hazelwood, MO), Report (3M Microbiology Products, St. Paul, MN), and BioPro (International BioProducts, Inc., Redmond, WA). The components and instructions are the same for all of the kits marketed under these various trade names.

It is recommended that in addition to the use of the name, Tecra, in **989.14**, the alternate names, Report and BioPro, be included.

*Salmonella, Escherichia coli, and Other Enterobacteriaceae, Vitek AutoMicrobic System.—Associate Referee Michael Knight conducted a survey of 50 users of this official first action method (990.13) (Vitek Systems, Inc., Hazelwood, MO) and none of those laboratories contacted had experienced any problems. All of those laboratories contacted had reported using this system as their primary identification system for enteric organisms.*

*Staphylococcus aureus, GENE-TRAK Colorimetric DNA Hybridization Method.*—The Colorimetric GENE-TRAK *Staphylococcus aureus* Assay (GENE-TRAK Systems, Framingham, MA) has been developed for the rapid detection of this organism in processed foods. A 2-stage enrichment procedure (4 h in peptone followed by 22 ±2 h in modified Giolitti-Cantoni broth) is required before performing the 3 h hybridization assay. Presumptive results are available within 24 h after initiation of analysis as compared to 2–4 days required with the current method, **987.09**.

The assay involves solution hybridization between target ribosomal RNA (rRNA) molecules and synthetic deoxyribonucleotide probes directed against *S. aureus*-specific rRNA

sequences. The mechanism of action is similar to that for the GENE-TRAK colorimetric DNA hybridization assay for *Listeria* described earlier in this report.

Associate Referee Gwen Reynolds reports that this assay successfully detected all of 176 *S. aureus* isolates tested and was nonreactive with 89 strains of non-*aureus Staphylococcus* cultures as well as with 35 other organisms not belonging to the genus *Staphylococcus*. A protocol for a precollaborative study has been submitted, and the General Referee has recommended that the protocol be revised.

*Total Coliforms and Escherichia coli, ColiComplete Discs.*—It has been reported that the ColiComplete system (BioControl Systems, Inc., Bothell, WA) simultaneously determines the total coliform count and *Escherichia coli* reactions 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 presence-absence format.

In the MPN fermentation method, lactose is metabolized by the enzyme, β-galactosidase, resulting in the production of carbon dioxide gas. This gas is trapped in the inverted fermentation vial indicating the potential presence of coliforms.

In the ColiComplete procedure, 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside is the indicator nutrient. A water insoluble indigo blue derivative is formed by, first, cleavage of 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside into the 5-bromo-4-chloro-indoxyl 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.

Associate Referee Philip Feldsine conducted a precollaborative study demonstrating comparability between the ColiComplete 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. A protocol for a collaborative study has been approved at the committee level.

*Vibrio cholerae, Elevated Temperature Method.*—The official final action method, Elevated Temperature Enrichment Method for *Vibrio cholerae* in Oysters, **988.20**, is based on the ability of almost all strains of *V. cholerae* to grow at 42°C, while related competitive microflora associated with oysters do not. This differentiation results in a higher confirmation rate of suspect colonies as *V. cholerae*.

Peru is experiencing its largest cholera epidemic ever, and this epidemic appears to be spreading to other South American countries. The U. S. Food and Drug Administration is currently analyzing imported samples of Peruvian seafood (primarily shrimp and finfish) as well as Ecuadorian and Colombian shrimp for *V. cholerae*. Because the AOAC method is restricted to the analysis of oysters, the U. S. Food and Drug Administration is using the BAM (3) procedure, which does not use an elevated temperature. Thus, Associate Referee Angelo DePaolo is considering another collaborative study to expand the applicability of **988.20** to additional types of seafood (shrimp, finfish, and crabs).

### *Recommendations*

(1) Adopt as official first action the *Salmonella* Assurance Enzyme Immunoassay for the detection of *Salmonella* in foods.

(2) Adopt as official first action the modified colorimetric DNA hybridization procedure to replace the current official first action method, *Salmonella* in Foods, Colorimetric Deoxyribonucleic Acid Hybridization (GENE-TRAK *Salmonella* Assay) Screening Method, **990.13**.

(3) Repeal the official first action method, *Salmonella* in Foods, Hydrophobic Grid Membrane Filter Screening Method, **985.42**.

(4) Include the trade names Report and BioPro, in addition to the trade name Tecra, in the official final action method, *Salmonella* in Foods, Colorimetric Polyclonal Enzyme Immunoassay Screening Method, **989.14**.

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This report of the General Referee was presented at the 105th AOAC Annual International Meeting, August 12–15, 1991, at Phoenix, AZ. 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*, 2nd supplement, 1991.

## Filth and Extraneous Materials in Foods and Drugs

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Flotation methods for the extraction of light filth from fish paste and sauce nct containing spice by Larry E. Glaze, and light filth from dried soybean curd by Marvin J. Nakashima were approved official first action by the Official Methods Board.

The following new topics and Associate Referee appointments were approved: Alkaline Phosphatase in Foods, Electrophoresis Detection Method—George C. Ziobro; Bean Paste, Light Filth by Flotation Method—John R. Bryce; Condimental Sauces Containing Soy Sauce, Thickeners, and Spices, Light Filth by Flotation Method—Marvin J. Nakashima; Grains (Whole), Internal Infestation by ELISA Method—G. Barrie Kitto; and Grains and Seeds (Whole), External Light Filth by Flotation Method—James Gallman.

Larry E. Glaze resigned as Associate Referee for Light Filth in Fish Paste and Sauces, Light Filth in Grain Products, and Light Filth in Bean Paste following his transfer to a new position. John R. Bryce has been appointed Associate Referee for these topics.

The following topics and Associate Refereeships are being discontinued: Fish (Canned) and Fish Products, Light Filth by Flotation Method—WilFred Sumner; Mammalian Feces, Coprostanol by Thin Layer Chromatography Method—George C. Hoskin; Onion and Garlic Powders and Granules, Filth by Sedimentation Method—James Buhlert; and Spirulina, Light Filth by Flotation Method—Marvin J. Nakashima. Methods development research will continue on all other topics.

Proper wet sieving technique is essential for the removal of as much fat and fine product material as possible before the extraction step in filth methods. Holding the sieve at approximately a 30° angle under the aerator spray, as described in **970.66B(a)**, provides effective wet sieving while avoiding the loss of filth from splashing. The use of a sieve handle or an equivalent device helps maintain the proper angle of the sieve and also prevents burns when using hot water (1).

In the method for Light Filth in Dried Bean Curd and some other new methods, the use of a sieve handle or its equivalent is recommended to help ensure that proper wet sieving technique is followed. To facilitate reference to the sieve handle in these and future methods, and to make information about this useful device more readily available to analysts, its inclusion in the apparatus section of the chapter on extraneous materials should be considered.

Specifically, the following addition to **945.75B** is being recommended: *Sieve handle.*—For holding 8 in. diameter sieves during wet sieving. See Figure **945.75D**. Constructed from 1 in. od hard aluminum tube, 14 in. long; 1/4 in.–28, 2 in. brass bolt; 1/4 in.–28 brass wing nut; and 2 1/4 in. brass washers. Cross drill 1/4 in. hole. Saw tube lengthwise perpendicular to 1/4 in. hole. Cut off 2 in. of upper half of slit tube. Spread split tube about 2 in. and flatten about 2 in. of top and bottom halves. Bend 1/4 in. right angle lip on each flattened end.

Reference to the sieve handle under wet sieving technique, **970.66B(a)**, is also being recommended as follows: After the sentence "Hold sieve under aerator...at approx. 30° angle." Add "Use of a sieve handle, **945.75B(t)**, or similar device helps in maintaining the proper angle of the sieve."

### *Recommendations*

(1) Discontinue study on the following topics: Fish (Canned) and Fish Products, Light Filth by Flotation Method; Mammalian Feces, Coprostanol by Thin Layer Chromatography Method; and Spirulina, Light Filth by Flotation Method.

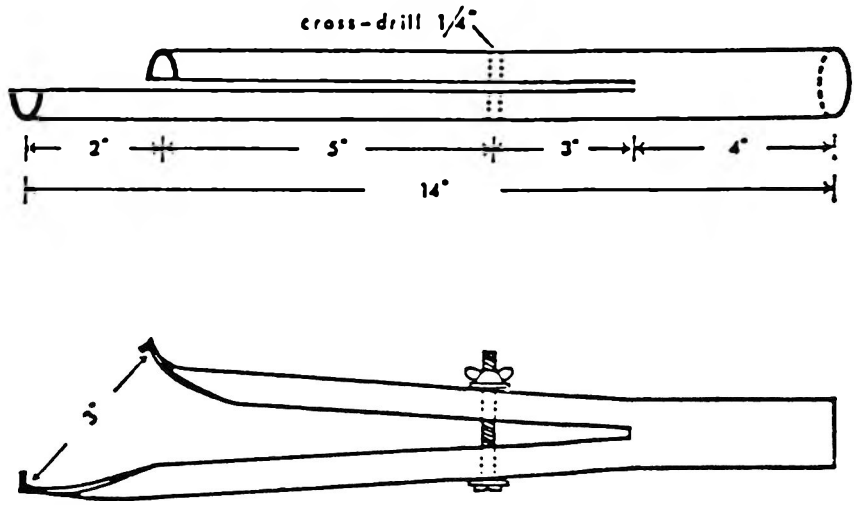


**Figure 945.75D.—Sieve handle.**

(2) Continue study on all other topics.
(3) Add "*Sieve handle*" as described under apparatus, **945.75 B(t)**, and under wet sieving technique, **970.66 B(a)**.

### *References*

(1) Brickey, P.M., Jr, Gecan, J.S., Thrasher, J.J., & Eisenberg, W.V. (1968) *J. Assoc. Off. Anal. Chem.* **51**, 872–876

This report of the General Referee was presented at the 105th AOAC Annual International Meeting, August 12–15, 1991, at Phoenix, AZ. The recommendations were reviewed by the Committee on Microbiology and Extraneous Materials. See the report of the committee, this issue.

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## Water Microbiology

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There has been no interlaboratory testing activity during this past year. Three inquiries have been received from individuals who wish to test methods by the AOAC procedures. The appropriate information for beginning the process has been forwarded to these individuals.

The inquiries came from:

Kristen Brenner, U.S. Environmental Protection Agency, Cincinnati, OH. The method to be considered is a membrane filter procedure that enumerates coliforms and *E. coli* in drinking water. Jim Datin, Access Corp., Branford, CT. The method to be considered is a broth tube procedure that detects coliforms and *E. coli* in marine surface waters.

Bill Watkins, U.S. Food and Drug Administration, Davisville, RI. The method to be considered is a membrane filter procedure for enumerating *E. coli* in marine surface waters.

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# Committee on Feeds, Fertilizers, and Related Materials

## Antibiotics in Feeds

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*Bacitracin in Feeds.*—In a previous report (Ragheb, H.S. (1991) *J. Assoc. Off. Anal. Chem.* **74**, 163), Associate Referee Anil Desai of A.L. Laboratories, Inc. modified extraction of bacitracin MD by performing double extraction (rather than the single extraction step). Results showed a positive bias. The Associate Referee gives the following explanation for the bias: If a feed sample has theoretical potency of 25 g bacitracin activity/ton and 33 g were weighed and extracted with 90 mL (first extraction volume), the bacitracin activity theoretically would be 0.4239 units/mL. If the volume of the second extraction aliquot is 40 mL, then bacitracin concentration would be 0.2119 units/mL. If we mix 50 mL of supernatant from the first extraction with 39 mL of supernatant from the second extract, bacitracin concentration would be 0.331 units/mL (total = 29.46 units). It would be an error to say that if the total units were theoretically present in 89 mL of extracting agent, the amount in 130 mL is 43.03 units, when in reality the theoretical concentration is 38.16 units. Consequently it would be an error to consider the dilution factor for this type of extraction similar to single extraction because we do not know exactly how much bacitracin was extracted in each extraction step. Furthermore, the bacitracin concentration in the combined supernatant is not the same as the concentration in the liquid trapped by the feed particles. This calculation then would lead to a positive bias of about 13%. To remedy this situation the Associate Referee suggests that after the addition of the second extraction volume (40 mL) and shaking, the supernatant from the first extraction step (50 mL) should be added back to the container (centrifuge bottle). By combining the extracts and re-slurring, the bacitracin concentration in the solid and solution will be the same. The dilution factor would, therefore, resemble the single extraction procedure.

The Associate Referee plans a collaborative study using this modified double extraction on poultry and swine mash. At random blind duplicates analysis will be performed. It is expected that the study will be reported at the AOAC International meeting in August 1991.

*Chlortetracycline in Feeds.*—Associate Referee Mary Lee Hasselberger of Nebraska Department of Agriculture suggested several modifications to the microbiological analysis of CTC–HCl in feeds (Ragheb, H.S. (1991) *J. Assoc. Off. Anal. Chem.* **74**, 163). The changes involve adjusting pH < 1.2 for section **967.39C(a)**; substitution of a pH range of 4.0–4.5 for the plating solution for feeds containing >50 ppm CTC–HCl instead of pH adjustment to 4.5 as in **967.39C(a)**; the use of 4-fold standard range instead of 16-fold (**967.39A**(b)) and plating media as mono-layer instead of double-layers (**967.39B**). Preliminary approval was given to these changes as editorial in nature. The Associate Referee investigation was reported (Hasselberger, M.L. (1991) *J. Assoc. Off. Anal. Chem.* **74**, 790–792). The Associate Referee is also planning studies on the effect of pelleting temperatures, feed matrix effect, and sample homogeneity on CTC–HCl potency in feeds.

*Neomycin in Feeds.*—Associate Referee G. Stahl, Upjohn Company, has designed a validation study of neomycin assay in feeds by NaCl-*tris* buffer method using *S. epidermidis* (Stahl & Kratzer (1984) *J. Assoc. Off. Anal. Chem.* **67**, 863). The study involves 2 complete feeds (swine and cattle, each at neomycin potency of 140 and 280 g/ton) and 2 premixes at neomycin potency of 1.4 and 7.0 g/lb. Samples will be assayed in duplicate on 6 different days. Three laboratories were selected for the study. The main objective is to ascertain repeatability within each laboratory. It is expected that results and statistical analysis will be completed and reported at the AOAC International meeting in August 1991.

*Monensin, Narasin, and Tylosin in Feeds.*—Associate Referee Mark Coleman of Eli Lilly Greenfield Laboratories will conduct an AOAC collaborative study of tylosin in premix and animal rations (swine, chicken, and cattle). The study will incorporate both the plate and turbidimetric assays. The Associate Referee is also planning an AOAC study of monensin and narasin by postcolumn derivatization liquid chromatography method as well as microbiological comparison. These studies are in the initial planning stages.

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## Drugs in Feeds

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No collaborative studies have been attempted in this topic area during the past year. There is 1 new topic area and Associate Referee (AR), and 2 AR changes for the coming year. The most common reason given by ARs for not completing method development or conducting collaborative studies is the current workload does not allow time for methods work. Associate Referees may be contacted as resources for their topic areas.

A list of the *Drugs in Feeds* topics for which there is an assigned Associate Referee follows. Included is a discussion of the status of each subject. Any references to the numbers of Official Methods are from the 15th Edition of the AOAC *Official Methods of Analysis*.

*Amprolium.*—Elzbieta J. Kentzer, Associate Referee. Kentzer has resigned her Associate Referee position because she is no longer working with medicated feeds and because of a lack of time to conduct a collaborative study. The General Referee has volunteered to take on the assignment to collaborate the method for the analysis of amprolium in medicated feeds that was developed when Kentzer and the GR worked together [*J. Assoc. Off. Anal. Chem.* (1988) **71**, 251–255].

*Carbadox.*—Alicia Henk, Associate Referee. The Associate Referee's laboratory has contracted with a private laboratory to develop methodology for the analysis of carbadox. When this is satisfactorily completed the AR will set up a collaborative study.

*Ethopabate.*—Joseph Hillebrandt, Associate Referee, has not had the time to initiate a collaborative study during the past year because of a job change. He wants to initiate a study during the coming year.

*Furazolidone and Nitrofurazone.*—Lori L. Rhodig, Associate Referee. The official HPLC method (**985.51**) continues to give higher results on some pelleted feed samples at concentrations of 330 g/ton and lower (probably due to the continuous extraction technique). The Associate Referee suggests use of the official HPLC method on problem pelleted feed samples that consistently test low via other methods (e.g. the official colorimetric method, **960.63**). Continued study on pelleted feeds containing furazolidone will be conducted through 1991.

*Lasalocid (Liquid Chromatography (LC) Method).*—Edward Waysek, Associate Referee. Waysek has resigned as AR because, with a laboratory reorganization, he no longer has responsibility for this drug. Alexander MacDonald has volunteered to become Associate Referee for Lasalocid by LC. MacDonald reports that neither the microbiological method (**975.60**) nor the spectrofluorometric method (**975.61**) are adequate for the medicated feeds currently found in the market place. MacDonald will be presenting an overview of available methods for lasalocid during the Antibiotics and Drugs Workshop preceding the 105th International AOAC in Phoenix, AZ on August 11, 1991.

*Melengestrol Acetate (MGA).*—Raymond Davis, Associate Referee. Davis reports that solid phase extraction has not proved beneficial in simplifying the cleanup of feed extracts containing MGA. He also feels that LC lacks the necessary sensitivity for the analysis of MGA in feeds. He hopes that lessons learned from current studies aimed at cleaning up tissue extracts for MGA analysis can be applied to feed.

*Monensin and Narasin (LC Method).*—Mark R. Coleman, Associate Referee. It is recommended that this be made a topic in the *Drugs in Feeds* topic area. Coleman has recently volunteered to be Associate Referee for this topic area and has plans for proceeding with a collaborative study. The proposed LC method makes use of postcolumn derivatization with vanillin as the reagent for the analysis of medicated feeds containing these materials.

*Morantel Tartrate.*—Linda Werner, Associate Referee. An LC method developed for the analysis of this drug has been submitted to U.S. Food and Drug Administration (FDA) for approval. Linda and her co-workers are currently evaluating questions raised by the FDA review and hope to have these resolved before initiating a collaborative study.

*Oxytetracycline (LC Method).*—Mary Lee Hasselberger, Associate Referee. Ms. Hasselberger reports that a lack of time to devote to method development during the past year has kept her from making progress, but she hopes that the coming year will prove more productive.

*Pyrantel Tartrate.*—Joyce Konrardy, Associate Referee. The Associate Referee's laboratory has contracted with a private laboratory to develop methodology for the analysis of pyrantel tartrate. When this is satisfactorily completed, the AR will set up a collaborative study.

*Sampling.*—John H. Gallagher, Associate Referee. No Report.

*Sulfadimethoxine and Ormetoprim.*—Edward H. Waysek, Associate Referee. Waysek has resigned as AR for these drugs. Alex MacDonald from the same laboratory has volunteered to assume the position vacated by Waysek. MacDonald will be making a poster presentation of an LC method for the simultaneous analysis of these drugs at the AOAC meeting in Phoenix AZ in August of 1991. His future plans are to publish and to get the method accepted by the U.S. Federal Department of Agriculture and Agricultural Canada; then he will organize an AOAC collaborative study. It is recommended that the topic name be changed from "Sulfadimethoxine" to "Sulfadimethoxine and Ormetoprim" to reflect the fact that the proposed method is for the simultaneous analysis of both drugs.

*Sulfa Drug Residues.*—Valerie Reeves, Associate Referee. The AR is waiting the completion of the LC/postcolumn derivatization collaborative for medicated feed level sulfamethazine before proceeding with this study (on the advice of the GR). See below.

*Sulfamethazine and Sulfathiazole in Feeds and Premixes.*—Dwight M. Lowie, Associate Referee. Lowie reports that efforts to develop an LC/UV method for the analysis of medicated feed level sulfamethazine and sulfathiazole continue to be unproductive. Like many with interests in developing methods for drugs in feeds, he finds a lack of methods development time a problem.

The General Referee's laboratory has completed precollaborative testing of the postcolumn derivatization method [*J. Assoc. Off. Anal. Chem.* (1988) **71**, 710–417] for sulfamethazine in medicated complete feeds and hopes to initiate a collaborative study of this method by the fall of 1991.

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*.

***Recommendations***

(1) *Amprolium.*—Continue study. New AR appointed.
(2) *Carbadox.*—Continue study.
(3) *Ethopabate.*—Continue study.
(4) *Furazolidone and Nitrofurazone.*—Continue study.
(5) *Lasalocid (LC Method).*—Continue study. New AR appointed.
(6) *Melengestrol Acetate.*—Continue study.
(7) *Monensin and Narasin (LC Method).*—Initiate topic area. Appoint AR.
(8) *Morantel Tartrate.*—Continue study.
(9) *Oxytetracycline (LC Method).*—Continue study.
(10) *Pyrantel Tartrate.*—Continue study.
(11) *Sampling.*—Continue study.
(12) *Sulfadimethoxine and Ormetoprim.*—Change name of topic to include ormetoprim. Continue study. New AR appointed.
(13) *Sulfa Drug Residues.*—Continue study.
(14) *Sulfamethazine and Sulfathiazole.*—Continue study.

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## Fertilizers and Agricultural Liming Materials

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There are several areas of fertilizer chemistry needing attention. Micronutrients by ICP is an area receiving more and more interest, but it remains untouched in AOAC circles. Method **980.02** for sulfur by a gravimetric technique is not a desirable method. There is potential that this element could better be analyzed using the combustion instrumentation now becoming popular for nitrogen. The AOAC approach to slow release fertilizer products in general needs attention. The technology in that area is running ahead of the testing methods. Melamine was collaborated several years ago, and is not currently an active topic. However, several people (the general referee included) have questioned several of the details of how that study was conducted. It should perhaps have another look. Finally, the true plant availability of $P_2O_5$ from various types of fertilizer materials has not been looked at for some time. Such an undertaking would be a very substantial project, but would deal with products not available when previous studies were done.

*Free and Total Water.*—Associate Referee James A. Farley conducted a comparison of the AOAC and International Organization for Standardization (ISO) vacuum desiccation and oven drying procedures for the ISO.

*Phosphorus.*—Joe R. Gliksman has been appointed Associate Referee. He is conducting a collaborative study of the Joe Trimm alternate extraction procedure for available $P_2O_5$. The outcome of the study is pending as of this report. It is the Associate Referee's opinion that available phosphorus analysis is not in a good state, because the existing methodology is difficult and often not followed properly. The GR agrees.

The AR plans to conduct a second collaborative study to validate new autoanalyzer instruments following the conclusion of the first study.

*Nitrogen by Combustion.*—Donald Tate has been appointed Associate Referee. He has developed a method using combustion instruments in the analysis of total nitrogen in fertilizers. The method is instrument manufacturer neutral in that it incorporates performance criteria to validate various instruments. A collaborative study is underway, and the outcome is pending as of this report.

*Urea and Methylene Ureas.*—Thomas Parham has been appointed the Associate Referee. Unfortunately there have been some changes in the structure of his laboratory, making it difficult for him to do the work he intended. He may still find it possible to at least outline the problems in the area in a journal paper, as he had hoped.

*Sampling.*—George Latimer, working with Associate Referee Doug Caine, has been conducting a collaborative study to evaluate the Texas fluid sampling tube. As of this report,

George is encouraged by the quality of the data, and is waiting for a response from 1 more lab to complete the study.

*Sample Preparation.*—Bob Beine has been appointed Associate Referee. He is working on a generalized approach to splitting and grinding samples, so that sample preparation procedures could be adjusted contingent on the capabilities of the grinder. He is also working on PC based statistical modeling of sample reduction procedures, with the intention of making software available to interested users.

*Iron.*—Associate Referee James Silkey reports no progress in the area of chelated iron, but anticipates working during the coming year.

*Potassium.*—Associate Referee Peter Kane reports no activity in the past year. Two areas needing attention are $K_2O$ by ICP and adapting the automated flame photometric method to new instrumentation. It would be appropriate to pass on the associate referee's position to someone who could work in one or both of these areas.

### *Recommendations*

(1) Continue study on all topics.

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## Nutrients in Soils

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This has been an important year in the area of nutrients in soils. The first associate referee was appointed, and a protocol for development of methods was determined.

First, in the area of pH measurements in soils, Yash Kalra of Forestry Canada was appointed as the associate referee. His choice came at the recommendation of the Soil Science Society of America (SSSA) liaison with the AOAC committee. Kalra has been working with the SSSA and the General Referee in anticipation of running a collaborative study on pH measurements.

The second point concerns methods use. For the topic of pH measurement specifically, and generally for the whole soils area, the SSSA has been working to develop a manner of choosing the appropriate method(s) to collaboratively study. They have come up with a "consensus method" approach. This would be a method, or group of methods, which, after being agreed upon by the appropriate SSSA committee, would be submitted to collaborative study. This will create greater assurances for wide applicability of the methods to be studied.

This approach is currently being used in the pH measurement project. As of this writing, the Associate Referee is reviewing comments from the committee, and will be working on a study protocol using the consensus method agreed upon.

Also of interest in this area is the choice of samples to be used in collaborative studies. To make the method(s) as widely applicable as possible, one should use a wide range of soils. The GR has been in conversation with a source who has access to many types of soils, and the GR is developing a way in which these soils can be used effectively for collaborative studies in this area.

There are many other areas where associate referees are needed. The General Referee is working with the SSSA committee to prioritize the needs, and choose appropriate associate referees to meet those needs.

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## Veterinary Analytical Toxicology

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Since the 1990 version of this report, 2 methods have received first action approval: serum zinc (**991.11**), and whole blood cholinesterase (**991.10**). The collaborative study of the pH method for whole blood cholinesterase has been submitted and is currently being reviewed.

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 6, 1990, in Denver, CO. Its annual report has been published (1).

The Eighth Annual Workshop on Veterinary Analytical Toxicology was held at the Midwest Regional AOAC meeting, June 4–6, 1991, Sioux Falls, SD. Oral presentations and posters were given on the following topics: fumonisin methodology, normal components from gas chromatography/mass spectrometry (GC/MS) screening, methodology for moniliformin determination in feeds, an overview of veterinary analytical toxicology in the United Kingdom, an update on a fumonisin check-sample program, determination of zinc phosphide by GC, analysis of poultry feeds for biogenic amines, bioluminescence bacterial detection of mutagens, and evaluation of extraction methods for liver vitamin E. The Ninth Annual Workshop is planned for the Midwest Regional meeting, June 1992, in Urbana, IL. Scientists interested in participation should contact this author.

Committee and workshop activities have remained strong during the past year. As witnessed by the wide range of topics at the above mentioned meetings, support for continued improvement in methods and the corresponding data is still strong. Following the recent trend, interlaboratory communications and cooperation has remained strong during the past year. The continued support for travel and methods development/improvement is encouraged. The encouragement and support of laboratory directors in the past has played an important role in the success of the Veterinary Analytical Toxicology area. This author wishes to thank the Associate Referees and their associates for a job well done.

Activities of the General Referee for the past 2 years have been centered on developing methodology for the fumonisin mycotoxins. These recently characterized *Fusarium moniliforme* metabolites (2, 3) dominated the Veterinary Diagnostic Toxicology during 1990. Equine leukoencephalomalacia (ELEM), a neurological disease, has long been associated with consumption of *F. moniliforme*-infected feeds. The diagnosis of ELEM has historically been made from gross and/or histopathological lesions. With the evolution of analytical methods for the fumonisins, diagnostic capabilities for ELEM will significantly improve; fumonisin concentrations in feeds will now support the pathology results. Several publications have detailed analytical techniques for the fumonisins (4–7). In addition to ELEM, fumonisins have also been linked to a porcine pulmonary edema (PPE) syndrome. Recent work (8) describes reproduction of PPE with intravenous injections of fumonisin $B_1$ in pigs and associations of PPE with fumonisin-contaminated feeds.

Interest and activities in other areas have remained strong. Summaries of some topics are listed below:

*Antibiotic Screening Methods.*—Associate Referees Wynne Landgraf (National Veterinary Services Laboratories, Ames, IA) and Stephen C. Ross (Illinois Animal Disease Laboratory, Centralia, IL) report on continued investigation of a thin-layer chromatography (TLC) method for screening feeds for monensin. A previous interlaboratory study with 3 samples (2 containing monensin and 1 blank) were generally encouraging. However, further evaluation by the Associate Referees revealed recovery problems. Further developmental work is planned. The Associate Referees recommend continued study.

*Cholinesterase, Colorimetric Method.*—Associate Referee Karen Harlin (University of Illinois, Urbana, IL) reports that the method for colorimetric method for whole blood cholinesterase (**991.10**) received first action status at the 1990 annual meeting. 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 (9) has been submitted for consideration of approval. The Associate Referee recommends the method be adopted first action.

*Multielement Analysis by ICP.*—Associate Referee W. Emmett Braselton (Michigan State University, East Lansing, MI) reports on continued work with assimilation of database information on the frequency distribution of element concentrations in serum, liver, kidney, and other tissues of animal species. This work has included a number of exotic species.

As previously suggested by the Associate Referee, there is a possibility of a collaborative study in the next few years. Many diagnostic laboratories now have ICP equipment. The Associate Referee recommends continued study.

*Nitrates and Nitrites.*—Associate Referee Mike Carlson (University of Nebraska, Lincoln, NE) reports on work by Anant Jain, University of Georgia, Athens, GA, on the dip-stick forage nitrate method. Jain has completed a thorough evaluation of the technique and is proposing a collaborative study. Jain is currently working with the General Referee to develop a protocol for a collaborative study. The Associate Referee recommends continued study.

*Pesticides in Toxicological Samples.*—Associate Referee H. M. Stahr (Iowa State University, Ames, IA) reports on continued development of a rapid method for organochlorine, organophosphate, carbamate, triazine, and phenoxy-acid pesticides. Using a C18 solid phase cleanup combined with TLC, liquid chromatography, and GC, urine and milk have been analyzed. There is good agreement between laboratories. Currently, the Associate Referee is evaluating other matrixes. The Associate Referee recommends continued study.

*Selenium in Animal Tissue.*—Associate Referee Karen Harlin reports that a collaborative study of a GC procedure (10) has been proposed. A preliminary study involving 6 laboratories and 2 low level samples was conducted. Results of the study show excellent between-laboratory agreement. As proposed, the study would involve the analysis of Se in whole blood ranging from 0.05 to 1.0 ppm. The Associate Referee is working with the General Referee to develop a protocol for the collaborative study. The Associate Referee recommends continued study.

*Sodium Monofluoroacetate.*—Associate Referee H. M. Stahr reports that the fluoride electrode procedure for screening animal tissues for sodium monofluoroacetate (1080) is currently being used by 6 laboratories. Plans for the next year include finding 2 more laboratories to run the method so that a collaborative study can be conducted.

The plasma after-glow detector for GC monitoring of 1080 offers potential, but few laboratories are interested. The Associate Referee recommends continued study.

*Zinc in Animal Tissues.*—Dana Perry (University of Arizona, Tucson, AZ) reports that the serum zinc method (**991.11**) received first action at the 1990 annual meeting. The Associate Referee also reports that ruggedness testing of a method for zinc in liver has been completed and that a protocol for a collaborative study will be prepared. The Associate Referee recommends continued first action status.

### *Recommendations*

(1) *Antibiotic Screening Methods.*—Continue study.
(2) *Arsenic in Animal Tissue.*—Retire topic.
(3) *Atomic Absorption Methods.*—Retire topic.
(4) *Cholinesterase Colorimetric Method.*—Continue first action status of the colorimetric method for whole blood cholinesterase (**991.10**).
(5) *Cholinesterase pH Method.*—Adopt as first action the pH cholinesterase method as recommended by the Associate Referee.
(6) *Copper in Animal Tissue.*—Retire topic.
(7) *Cyanide.*—Retire topic.
(8) *GC/MS Methods.*—Continue study.
(9) *Fluoride in Animal Tissue.*—Continue study.
(10) *Lead in Animal Tissue.*—Continue study.
(11) *Multielement Analysis by ICP.*—Continue study.
(12) *Multiple Anticoagulant Screening.*—Continue study.
(13) *Natural Products.*—Continue study.
(14) *Nitrates and Nitrites.*—Submit protocol for a collaborative study on dip-stick method for forage nitrate.
(15) *Pesticides in Toxicological Samples.*—Continue study.
(16) *Selenium in Animal Tissue.*—Continue study.
(17) *Sodium Monofluoroacetate.*—Continue study.
(18) *Vitamins A and E.*—Continue study.
(19) *Vitamins D and K.*—Continue study.
(20) *Zinc in Animal Serum.*—Continue first action status of the method serum zinc (**991.11**).

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This report of the General Referee was presented at the 105th AOAC Annual International Meeting, August 12–15, 1991, at Phoenix, AZ. 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*, 2nd supplement, 1991.

GENERAL REFEREE REPORTS

# Committee on Environmental Quality

## Cooperative Studies

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Cooperative studies over the past year have involved joint U.S. Environmental Protection Agency (EPA) and AOAC interlaboratory studies of EPA methods developed for the National Pesticide Survey Program. These interlaboratory studies have been conducted following AOAC guidelines for collaborative method validations. These studies have been 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 (Midwest Research Institute, Mountain View, CA).

Progress on individual cooperative studies is summarized below.

*Determination of 29 Chlorinated Pesticides in Finished Drinking Water by Gas Chromatography/Electron Capture Detection.*—Viorica Lopez-Avila, Raymond Wesselman, and Kenneth W. Edgell. This method was approved for first action at the AOAC 103rd Annual International Meeting in St. Louis in 1989. This method was recommended for final action at the AOAC 105th Annual International Meeting in Phoenix, August 1991.

*Determination of Chlorinated Acids in Ground Water by Gas Chromatography/Electron Capture Detection.*—Kenneth W. Edgell, Elizabeth J. Erb, and James E. Longbottom. The collaborative study of this method was completed in September 1988. The method was rewritten in AOAC format. The final study report was converted into AOAC journal format. The work was presented at the AOAC 105th Annual International Meeting in Phoenix, August 1991.

*Determination of Pesticides in Ground Water by Liquid Chromatography with Ultraviolet Detection.*—Kenneth W. Edgell, Elizabeth J. Erb, Viorica Lopez-Avila, and James E. Longbottom. The collaborative study report has been submitted to AOAC for review. This work was presented at the AOAC 104th Annual International Meeting in New Orleans, September 1990. This method was recommended for first action at the AOAC 105th Annual International Meeting in Phoenix, August 1991.

*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 collaborative study report on this method has been submitted to AOAC for review. Study results were presented at the AOAC 105th Annual International Meeting in Phoenix, August 1991.

*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. Based upon EPA Method 504, this method has been reformatted to meet AOAC guidelines and has been submitted for AOAC review.

*Determination of Carbonyl Compounds by High Performance Liquid Chromatography.*—Kenneth W. Edgell and James E. Longbottom. Collaborative laboratories have been selected, having affiliations including AOAC, Alberta Water Analysts Committee, and American Society for Testing and Materials. The study results will be presented at the AOAC 106th Annual International Meeting.

### *Recommendations*

(1) Adopt as official final action the following method: *Determination of 29 Chlorinated Pesticides in Finished Drinking Water by Gas Chromatography/Electron Capture Detection.*

(2) Adopt as official first action the following method: *Determination of Pesticides in Ground Water by Liquid Chromatography with Ultraviolet Detection.*

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This report of the General Referee was presented at the 105th AOAC Annual International Meeting, August 12–15, 1991, at Phoenix, AZ. 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*, 2nd supplement, 1991.

## Inorganics in Water

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The AOAC and U.S. Environmental Protection Agency (EPA) carried out a joint collaborative study for Method 200.8, "Analysis of Trace Elements in Water by ICP/MS" in the spring of 1990. Initial results of the study were presented in a poster session at the 1990 national meeting in New Orleans. The draft journal article entitled "Determination of Trace Elements in Water by Inductively Coupled Plasma-Mass Spectrometry: Collaborative Study" was written by the Co-Associate Referee, J. Longbottom, and distributed for peer review April 30, 1991. All comments were submitted by May 20, 1991. It is Longbottom's intent to finalize the report by the end of June so that it can be submitted to the Office of Drinking Water in time for their rule making process beginning in July.

The reason for the delay in preparing the final report was that Longbottom subjected the collaborative study data to 3 different statistical treatments, which differ in the method used to determine outliers. The first treatment was EPA's IMVS program, which follows the American Society for Testing and Materials (ASTM) method 2777. This has been the treatment used thus far by the EPA in evaluating collaborative studies. It is also the statistical treatment used in the final report for this study. The other 2 treatments used followed the AOAC "Guidelines for Collaborative Study Procedure to Validation Characteristics of a Method of Analysis," 4th (final) draft, 1989.

The AOAC approach tends to be more conservative in the percent of data rejected. The consequent precision estimates were somewhat higher. A concern was raised that if the precision estimates from method collaborative studies are to be used to direct regulatory requirements, then one must be careful about stating precision based on statistics that may bias the results.

Although there was not a consensus within EPA/Cincinnati as to which treatment was statistically most correct, it has been the driving force for further investigation within the agency into more "appropriate statistical modeling for future work."

Official first action still needs to be taken on Method 200.8. It is recommended that this be done as soon as possible.

Co-Associate Referee Bruce Warden was asked to review the draft of an ASTM/EPA joint collaborative study on EPA Method 218.6, "Determination of Dissolved Hexavalent Chromium in Drinking Water, Ground Water, and Industrial Wastewater Effluents by Ion Chromatography." It is EPA's intent to secure AOAC approval of this method based on results of the collaborative study and publish the study in the AOAC *Journal*. Because Ion Chromatography is encompassed within the Inorganics in Water subject area, Warden recommends that we undergo a review of the work done and make a determination as to the validity of the method. If the method is acceptable, it should be adopted by AOAC and official first action taken.

This report of the General Referee was presented at the 105th AOAC Annual International Meeting, August 12–15, 1991, at Phoenix, AZ. The recommendations were reviewed by the Committee on Environmental Quality. See the report of the committee, this issue.

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## Organics in Water

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The General Referee has served as Co-Associate Referee on the collaborative studies described under the General Refereeship on Cooperative Studies.

Three Associate Referees have been appointed: Kenneth Edgell of Bionetics Corporation for study on *Determination of Carbonyl Compounds in Waters by High Performance Liquid Chromatography,* Tammy Jones of Environmental Protection Agency-Las Vegas for study on *Thermospray LC/MS Analysis of Carbamates*, and Robert Harrison of Immunosystems Inc. for study on *Immunoassay Method for Determination of Atrazine and Related Triazine Herbicides in Water.*

*Diquat and Paraquat.*—Associate Referee needs to be appointed.

*Determination of Carbonyl Compounds in Waters by High Performance Liquid Chromatography.*—Collaborative study has been initiated (see *Cooperative Studies*).

*Future Studies.*—General Referee is pursuing U.S. Environmental Protection Agency-Cincinnati to fund 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 less organic solvent than the conventional procedures. The list of analytes has not been defined yet. Suggestions have been received to pursue the organochlorine pesticides. Craig Markell of 3M expressed interest in serving as Co-Associate Referee.

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## RECOMMENDATIONS FOR OFFICIAL METHODS

# Committee on Pesticide Formulations and Disinfectants

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The Committee on Pesticide Formulations and Disinfectants met on August 11, 1991 during the AOAC Annual International Meeting held in Phoenix, AZ. Portions of the meeting were attended by several General and Associate Referees and visitors. Topics discussed included the following: the Volunteer Education Program, "Managing the Method's Approval System," modifications to the criteria for appointment and term of office for Associate Referees, participation of Methods Committee Members in approval of collaborative study protocols, policies on equivalency and proprietary technology based methods and inclusion of safety information in methods, the issues of how to address accuracy in methods, and the role of standard reference materials. It was determined that the Committee should investigate accuracy in methods and the role of standard reference materials in an attempt to improve methodology. Because substantially different approaches may be needed in the Committee's 2 major subject areas, the Chairman appointed 2 task forces, one on Accuracy in Disinfectant Methods, consisting of Fran Porter (chairman), Thad Czerkowicz, Jean Jenkins, Joe Rubino, Donna Suchmann, and Zig Vaituzis; and the other on Accuracy in Pesticide Formulations Methods, consisting of Art Hofberg (chairman), Warren Bontoyan, and Tom Jensen. The task forces are to present their recommendations to the Committee by November 1, 1991. A final report will be assembled for the OMB Task Force on Accuracy.

A decision was made to continue review of protocols by Committee members. The Committee searched for potential incentives to increase volunteer efforts on collaborative studies. Several members suggested that requiring Associate and General Referees, and Committee Members to pay membership fees is not conducive to participation. As an incentive to participation, the Committee recommends that the President provide a letter to the chief executive of each organization represented by existing volunteers. The letter would thank the organization for allowing the volunteer's participation, pointing out the value of their efforts, and stressing the advantages of collaboratively validated methods. A certificate of recognition to the organization could accompany such a letter.

The Committee recommends adoption of **989.02** Fenitrothion Gas Chromatographic Method for final action. The method has been in use in a number of laboratories for 2 years or more. No negative comments were received by the Associate or General Referee or in response to the recommendation announced in *The Referee*. The General Referee indicated that the gas chromatographic methods for parathion and methyl parathion (**978.06** and **977.04**, respectively) are likely to be recommended for final action next year. However, he recommended that the definition of packing material should be changed from a mixture of SE–30 and OV–210 to a single liquid phase, OV–17, which is used in the newer method for parathion in microencapsulated formulations (**980.11**). The Committee recommended that the General Referee investigate whether sufficient in-house data exists to support the proposed change and, if so, that the change be reworded to a more generic description of the column packing with an appropriate system suitability test.

Several potential improvements to the Hard Surface Carrier Test were suggested by Zig Vaituzis. Among the suggested improvements were a more definitive requirement for test organism confirmation and a more stringent dilution of the neutralizer. The Committee recommended that the suggested modifications be discussed with the Associate and General Referees for consideration.

Methods activities for the coming year are expected to include several methods from CIPAC and protocols for studies of disinfectant methods (tuberculocidal test and virucidal test). The disinfectant methods are being developed under cooperative agreements supported by the EPA. The EPA also plans to

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The recommendations submitted by the Committee on Pesticide Formulations and Disinfectants were adopted by the Association.

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issue a cooperative agreement to develop new or improved sporicidal test methodology within the next few months.

Alan Hanks, General Referee for CIPAC Methods, requested the Committee's suggestions for improving the timeliness of communication with CIPAC, particularly for announcements of collaborative studies to be initiated and for calls for collaborators. The Committee suggested use of electronic mail, sharing of mailing lists between CIPAC and AOAC to allow direct (air) mail, and use of the AOAC Europe office as a conduit for improving communication.

The Committee discussed a proposal to reorganize 9 pesticide formulation General Referee topics into 4: Fungicides and Rodenticides; Herbicides; Organophosphorus Insecticides; and Other Insecticides, Synergists, and Repellents. The Committee endorsed the reorganization and directed the Chairman to communicate the change to the appropriate AOAC staff, provided the affected General Referees agree to the changes.

### *Pesticide Formulations: Carbamate Insecticides and Substituted Urea Insecticides*

(1) *Carbaryl*: Continue study.
(2) *Carbosulfan*: Continue study.
(3) *Methomyl*: Continue study.
(4) *Oxamyl*: Continue study.

### *Pesticide Formulations: CIPAC Studies*

(1) *Bensultap*: Open topic.
(2) *Bentazon*: Continue study.
(3) *Carbetamide*: Open topic.
(4) *Cyanazine*: (a) Continue official first action status of Maneb with fentin. (b) Continue study.
(5) *Deltamethrin*: Continue study.
(6) *Dichlobenil*: Continue study.
(7) *Dimethoate*: Continue study.
(8) *Edifenphos*: Open topic.
(9) *λ-Cyhalothrin: Open topic.*
(10) *Malathion*: Open topic.
(11) *Maneb in Fentin Acetate and Fentin Hydroxide*: Continue study.
(12) *Methamidophos* (*Tamaron, Monitor*): Continue study.
(13) *Parathion and Methyl Parathion*: Open topic.
(14) *Pencycuron* (*Monceren*): Continue study.
(15) *Pirimiphos-Methyl*: (a) Continue official first action status method. (b) Continue study.
(16) *Temephos*: Continue study.
(17) *Triadimenol* (*Baytan*): Continue study.

### *Pesticide Formulations: Fungicides*

(1) *Benomyl*: (a) Continue official first action status of the liquid chromatographic method (**984.09**). (b) Continue study.
(2) *Carboxin and Oxycarboxin*: Continue study
(3) *Chlorothalonil*: Continue study
(4) *Dithiocarbamate Fungicides*: Continue study.
(5) *Tebuconazole* (*Folicur*): Continue study.
(6) *Triphenyltin* (*Fentin*): (a) Continue official first action status of the gas chromatographic method (**984.04**). (b) Continue study.

### *Pesticide Formulations: Herbicides I*

(1) *Acetochlor*: Establish new topic and appoint Associate Referee.
(2) *Alachlor, Butachlor, and Propachlor*: Continue study.
(3) *Alachlor/Atrazine Mixtures*: Continue study.
(4) *Bromoxynil*: Continue study.
(5) *Fomesafen*: Continue study.
(6) *Glyphosate*: Continue study.
(7) *Metolachlor*: Continue study.
(8) *Pesticides in Fertilizers*: Continue study.
(9) *Propanil*: Continue study.

### *Pesticide Formulations: Herbicides II*

(1) *Bromacil*: Continue study.
(2) *Chlorophenoxy Herbicides*: (a) Continue official first action status of the following liquid chromatographic 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**); and 2,4-dichlorophenoxyacetic acid esters and amine salts (**978.05**). (b) Continue study.
(3) *Chlorsulfuron* (*Glean*): Continue study.
(4) *Dicamba*: Continue study.
(5) *Ethafluralin and Pendimethalin*: Continue studies.
(6) *Fluometuron*: Continue study.
(7) *Methazole*: Continue study.
(8) *Metasulfuron-Methyl* (*Ally*): Continue study.
(9) *Oryzalin* (*Surflan*): Discontinue topic.
(10) *Pentachlorophenol*: Discontinue topic.
(11) *Sulfometuron-Methyl* (*Oust*): Continue study.

### *Pesticide Formulations: Microbial Pesticides*

(1) *Bacillus Thuringiensis* β-*Exotoxin*: Open topic.

### *Pesticide Formulations: Organohalogen Insecticides*

(1) *Benzene Hexachloride and Lindane*: Open topic.
(2) *DDT*: (a) Continue the official first action the official method for *p,p'*–DDT in technical and formulated products. (b) Continue study.

### *Pesticide Formulations: Organothiophosphorus Insecticides*

(1) Azinphos-Methyl (Guthion): Continue study.
(2) *Ethoprop*: Continue study.
*(3) *Fenitrothion*: (a) Recommend adoption of the gas chromatographic method as official final action (**989.02**). (b) Continue study.

(4) *Methidathion* (*Supracide*): Discontinue topic.
(5) *Methyl Parathion and Parathion*: Continue study.
(6) *Oxydemeton-Methyl* (*Metasystox–R*): (a) Continue official first action status the official first action liquid chromatographic method. (b) Continue study.
(7) *S,S,S–Tributyl Phosphorotrithioate* (*DEF*): Discontinue study.

***Pesticide Formulations: Other Insecticides, Synergists, and Insect Repellents***

(1) *d–trans Allethrin*: Continue study.
(2) *Cyfluthrin*: Continue study.
(3) *Cyhexatin* (*Plictran*): (a) Continue official first action status of the CIPAC-AOAC liquid chromatographic method (**988.02**). (b) Continue study.
(4) *Cyromazine* (*Larvadex*): Continue study.
(5) *Dipropyl Isocinchomeronate* (*MGK Repellent 326*): Continue study.
(6) *Resmethrin*: Discontinue topic.
(7) *Rotenone and Other Rotenoids*: Continue study.

***Pesticide Formulations: Other Organophosphorus Insecticides***

(1) *Crotoxyphos*: Discontinue topic.
(2) *Dichlorvos*: (a) Continue official first action status of the IR methods (**964.04** and **966.07**). (b) Open topic.
(3) *Fenamiphos* (*Nemacur*): Continue study.

***Pesticide Formulations: Rodenticides and Miscellaneous Pesticides***

(1) *Brodifacoum* (*Talon*): Continue study.
(2) *Sampling*: (a) Continue official first action status of the sampling procedures for fertilizers (**984.03**) as applied to pesticide-fertilizer mixtures. (b) Continue study.
(3) *Strychnine*: Discontinue topic.

***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) Continue official first action status of Use-Dilution method. (b) Continue study.

# Committee on Drugs and Related Topics

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The Committee met July 26, 1991, in Arlington, VA. The Committee discussed its membership and terms of reference, its general referees, the status of new methods and study reports, the safety check list, the methods equivalency policy, policy and procedures for interlaboratory collaborative study, review, and approval, administrative guidelines for collaborative study, checklist for protocol design, guidelines for writing an official AOAC method, and general referee reports.

Actions taken and items discussed by the Committee are presented below.

1. Recognized the appointment of Donna Bush as a member of the Committee for 1992–1994, replacing Ronald Backer, who resigned.
2. Recognized the appointment of Mark Torchia as a member of the Committee for 1992–1994, filling the place vacated by Charles C. Clark.
3. Recognized that this was the last Committee meeting for Charles C. Clark, who had completed 6 years as a member of the Committee, and thanked him for his contributions.
4. Voted to recommend reappointment of Thomas Doyle to a second 3-year term as a member of the Committee.
5. Voted to recommend the reappointment of Charlie Barnes (General Referee, Drug Residues in Animal Tissues) for 3 years.
6. Voted to recommend that the appointment of Rita Jhangiani (General Referee, Diagnostics and Test Kits) not be renewed.
7. Voted not to renew the appointments of Salvatore Marchese (General Referee, Drugs I) and Martin Finkelson (General Referee, Drugs III) due to the lack of active topics in these General Refereeships.
8. Indicated that John O'Rangers would be recommended to be Chairman of the Committee for 1993–1995.
9. Noted that as a result of the telephone voting the Committee had nominated Eileen Bargo as its Associate Referee of the Year.
10. With the aid of a 5-year tabulation of the Committee's actions prepared by Chairman Montgomery, the Committee discussed certain topics under various general referees, notably those items presented below.

### *Drugs I*

The General Referee's report had not been received. In the absence of a report and in view of a lack of activity on the assigned topics, the Committee voted to recommend continued study on the topics for which there were first action methods and to discontinue all other topics.

### *Drugs II*

a. General Referee Edward Smith recommended that Associate Referees should be sought for Rauwolfia Alkaloids and Rauwolfia Serpentina, topics that were discontinued last year.
b. The Committee voted to recommend acceptance of the General Referee's report, as modified.

### *Drugs III*

The General Referee's report had not been received. In the absence of a report, the Committee voted to recommend contin-

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The recommendations submitted by the Committee on Drugs and Related Topics were adopted by the Association.

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ued study on topics for which there were first action methods and to discontinue study on all other topics.

*Drugs IV*

a. General Referee Linda Ng reported that the new protocol for the collaborative study of Associate Referee Charles Clark's method for heroin had been approved and that the collaborative study was in progress.
b. The Committee voted to recommend acceptance of the General Referee's report.

*Drugs V*

a. The Committee voted to recommend acceptance of the report of General Referee Thomas Alexander.

*Cosmetics*

a. Because the General Refereeship on Cosmetics is still vacant, Chairman Montgomery recommended that the methods on Eye Irritants in Cosmetic Constituents, Water and Ethyl Alcohol in Cosmetics, and Zirconium should be continued in first action status.
b. The Committee voted to recommend that the specified methods be continued in first action status.

*Diagnostics and Test Kits*

The General Referee's report had not been received. In the absence of a report, the Committee agreed that continued study should be recommended on all topics.

*Drug Residues in Animal Tissues*

a. The Committee suggested that the General Referee's report should be revised to indicate that study on the topic *Chloramphenicol in Milk* should be discontinued.
b. The Committee discussed the status of the collaborative study protocol prepared by the Associate Referee on *Sulfonamides in Milk.* One aspect of the actions taken so far has been the provision of "practice samples" to prospective collaborators before the collaborative study in order to obtain their comments and suggestions. The Committee considered the position that this is part of method development and refinement, not collaborative study of a specified method. Barnes informed that it is desirable to provide training for those laboratories that seem to have trouble with the practice samples.
c. The Committee voted to recommend acceptance of the General Referee's report, as revised.

*Forensic Sciences*

a. General Referee Stanley Cichowicz emphasized that there was a critical need for standardized forensic methods.
b. The General Referee informed that the topic on Gunshot Residues had been reinitiated and R.D. Koons appointed as Associate Referee. A collaborative study on Gunshot Residues, which had not been accepted for publication in *J. Assoc. Off. Anal. Chem.*, was being rewritten for consideration for first action status.
c. The Committee voted to recommend acceptance of the General Referee's report.

11. Chairman Montgomery presented for discussion some items from the May 1991 meeting of the OMB:
    a. General Referee Reports must be presented at a poster session at the AIM. General Referees not able to attend should arrange for someone else to present their reports.
    b. AOAC Safety Check List. It was stressed that the check list must be followed when developing a new method and when preparing the collaborative study protocol. The completed check list must be submitted with the request for protocol approval.
    c. *Official Methods of Analysis* Chapter review. General Referees should be reviewing their respective chapters of *Official Methods of Analysis* for action, equivalency statements, safety, and editorial changes. Recommendations should be made to the Committee for the disposition of first action methods to final action, modification, surplus, or repeal. The question was raised as to whether recommended surplus or repeal of first action methods needs to be announced in *The Referee.* Chairman Montgomery said he would get an answer. Such recommendations must be voted on by the entire AOAC Membership. O'Rangers asked whether an accounting of the votes will be given to indicate how many voted, how many approved, and how many disapproved.
    d. Associate Referee appointments. The Committee discussed the OMB decision that the appointment of an Associate Referee should be made only after a protocol for a collaborative study of a method has been prepared. Some Committee members felt strongly that appointment of an Associate Referee is needed to develop the method.
    Some agencies require that an analyst must be an Associate Referee before they will budget time for the analyst to do the developmental work.
    e. Committee Review of Methods and Collaborative Study Reports. This review is most important. It constitutes the only peer review the reports will receive.
    f. Final action recommendation. The Committee discussed the question of whether positive data on the successful use of a first action method is necessary before recommending final action status.
12. Discussed the draft AOAC Policy on Equivalency of Products named in *Official Methods of Analysis*. General Referees should keep in mind during review of *Official Methods of Analysis* that methods should be as generic as possible. They should take steps to remove references to specific equipment model numbers where possible,

and generic descriptions and performance requirements of the equipment should be developed. If editorial changes are not possible, a collaborative study can be suggested to the appropriate Associate Referee. Any such changes must be recommended to the Committee; it would be helpful if both the old and new versions are shown. The Committee discussed the possibility of listing within a method known sources of reagents that may be difficult to obtain, or of giving reagent specifications at the end of the chapter.

13. Discussed the Policy and Procedures on Interlaboratory Collaborative Study, Review, and Method Approval and Action Process, and noted the procedures for adopting methods as first action and final action.
14. Discussed the Guidelines for Collaborative Study Procedure to Validate the Characteristics of a Method of Analysis (*J. Assoc. Off. Anal. Chem.* (1989) **72**, 694–704). Of particular interest in the discussion were the minimum numbers of materials, laboratories, and replicates recommended, the need to include the method in AOAC style and format, and the requirement that the collaborators be instructed to follow the method exactly as written.
15. Discussed the Checklist for Protocol Design. Chairman Montgomery stated that if the actions in the checklist are thoroughly performed before the collaborative study is initiated, the preparation of the final report of the study would not involve much more than summarizing the results.
16. Discussed the instructions on How to Write an Official AOAC Method, and noted that if many of the practices recommended in other checklists are followed, a separate checklist on elements of a study report would not be needed. It was decided, however, that such a checklist should be developed for studies already in progress.
17. Mark Presser, the Committee Statistician, requested that, in addition to the report and data from a collaborative study, supporting statistical data be provided to him. He will not repeat the statistical calculations. He will determine if the statistical methods used were implemented in a valid manner. Print-outs and documentation of calculations would allow him to see what was done and to confirm that the data were handled correctly. He needs to know why outliers were rejected, and what statistical test was used. This would make it easier for him to provide a quick evaluation. He emphasized that the Associate Referee is responsible for the statistical analysis of results. AOAC provides a statistical checklist as part of its volunteer package. Several Committee members and General Referees asked how they could obtain a copy of the recommended statistical procedure. Presser said he would be glad to send copies to those interested.
18. Discussed what appeared to be a noticeable decrease in the participation of FDA laboratories in AOAC collaborative studies of drug methods. It was recognized that this may be due to changing priorities and reprogramming of agency resources.
19. Discussed its Terms of Reference:
    a. Agreed on a shorter Mission statement.
    b. Agreed on a new statement of Responsibilities.
    c. Agreed that the topic areas of the Committee should be simplified by stating "Drugs," instead of Drugs I, Drugs II, Drugs III, Drugs IV, and Drugs V.
    d. Agreed that as short-term goals during the coming year there should be:
        i. A thorough review of current topics with a view to discontinuing any that have not shown any activity for greater than 3 years.
        ii. A review of *Official Methods of Analysis* chapters and recommendations made to move all first action methods either to final action or to surplus or repeal them.
    e. Agreed that its long-term goals and activities should be the same as listed in last year's plan, with the following exceptions:
        i. Help improve the efficiency of the methods development efforts of the Associate Referees.
        ii. Assist General Referees in developing Associate Referee topic areas.
        iii. Review topics for pertinence.
20. The following recommendations for official methods will be reported at the Annual Meeting on August 15, 1991:

***Drugs I***

(1) *Acetaminophen in Drug Mixtures*: (a) Continue first action status of the liquid chromatographic method for acetaminophen in tablets, **987.12**. (b) Continue study.
(2) *Acetaminophen with Codeine Phosphate*: Discontinue topic.
(3) *p–Aminobenzoic Acid and Salicylic Acids in Pharmaceuticals*: Discontinue topic.
(4) *Diethylpropion Hydrochloride*: (a) Continue first action status of the method for the determination of diethylpropion hydrochloride in drug substance and in tablets, **988.23**. (b) Continue study.
(5) *Phenothiazine and Related Drugs*: Discontinue topic.
(6) *Salicylic Acid in Acetylsalicylic Acid Preparations*: 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) *Curare Alkaloids*: Discontinue topic.
(6) *Dicyclomine*: Continue study.
(7) *Epinephrine and Related Compounds by LC-Electrochemical Detectors*: Discontinue topic.
(8) *Ergot Alkaloids*: Continue study.
*(9) *Morphine Sulfate in Morphine Injection*: (a) Adopt as final action the first action liquid chromatographic

method for the determination of morphine sulfate in drug substance and injections, **989.08**. (b) Discontinue topic.

(10) *Physostigmine and Its Salts*: Continue study.

### *Drugs III*

(1) *Halogenated Hydroxyquinoline Drugs*: (a) Continue first action status of the method for the liquid chromatographic determination of clioquinol in cream and ointment preparations, **990.14**. (b) Continue study.

(2) *Haloperidol*: Discontinue topic.

(3) *Hydralazine*: (a) Continue first action status of the method for the ultraviolet spectrophotometric determination of hydralazine hydrochloride in tablets, **989.07**. (b) Continue study.

(4) *Medicinal Gases*: Discontinue topic.

(5) *Metals in Drug Bulk Powders*: Discontinue topic.

(6) *Penicillins*: (a) Continue first action status of the method for the liquid chromatographic determination of penicillin V in tablets, **990.15**. (b) Continue study.

(7) *Salts of Organic Nitrogenous Bases*: Discontinue topic.

### *Drugs IV*

(1) *D- and L-Amphetamines—LC Separations*: (a) Continue first action status of the method for the liquid chromatographic determination of enantiomers of amphetamine in bulk drug, syrup, and capsules, **988.28**. (b) Continue study.

(2) *Benzodiazepines*: (a) Continue first action status of the liquid chromatographic method for oxazepam capsules and tablets, **983.30**. (b) Continue study.

(3) *Diazepam*: Discontinue topic.

(4) *Dicloxacillin*: Continue study.

*(5) *Flurazepam*: (a) Adopt as first action the liquid chromatographic method for flurazepam hydrochloride in bulk drug and dosage forms studied collaboratively (*J. Assoc. Off. Anal. Chem.* (1991) **74**). (b) Continue study.

(6) *Fumagillin*: Discontinue topic.

(7) *Heroin*: Continue study.

(8) *Miconazole Nitrate*: Continue study.

### *Drugs V*

(1) *Anabolic Steroids—LC Screening*: Continue study.

*(2) *Chlorpropamide*: (a) Adopt as final action the first action method for the liquid chromatographic determination of chlorpropamide in tablets, **986.37**. (b) Discontinue topic.

(3) *Conjugated Estrogens by LC*: Continue study.

(4) *Pentaerythritol Tetranitrate*: (a) Continue first action status of the method for liquid chromatographic determination of pentaerythritol tetranitrate in pharmaceuticals. (b) Continue study.

(5) *Progestins in Tablets—Automated Methods*: Discontinue topic.

*(6) *Steroid Acetates*: (a) Adopt as final action the first action method for the liquid chromatographic determination of cortisone acetate in drug substance and in dosage forms, **988.25.** (b) Adopt as final action the first action method for the liquid chromatographic determination of dexamethasone acetate in drug substance and in suspensions, **988.26.** (c) Discontinue topic.

*(7) *Steroid Phosphates*: (a) Change title of topic to Steroids. (b) Adopt as final action the first action methods for the liquid chromatographic determination of dexamethasone in drug substance and elixirs, **988.27A–F**, the identification of dexamethasone in drug substance by thin-layer chromatography, infrared spectroscopy, and relative retention times, **988.27G–J**, and the gas chromatographic determination of alcohol in elixirs, **988.27K–N**. (c) Discontinue topic.

### *Cosmetics*

(1) *Water and Alcohol*: Continue first action status of the method for water and alcohol, **966.22.**

(2) *Zirconium*: Continue first action status of the method for soluble zirconium, **976.24.**

(3) *Eye Irritants in Cosmetic Constituents*: Continue first action status of the method for eye irritants in cosmetic constituents, **973.59.**

### *Diagnostics and Test Kits*

(1) *Automated Microbial Identification Systems—VITEK*: Continue study.

(2) *Automated Microbial Identification Systems—HP5898A*: Continue study.

(3) *Immunological and Diagnostic Assay of Peptides, Hormones, and Enzymes*: Continue study.

(4) *Multicomponent Analysis of Clinical Specimens*: Continue study.

(5) *Tuberculosis and Enteric Infections by Gene Probe*: Continue study.

(6) *Release Rates of Drugs from Transdermal Patches*: Continue study.

### *Drug Residues in Animal Tissues*

(1) *Benzimidazoles in Cattle Tissues*: (a) Continue first action status of the liquid chromatographic method for benzimidazoles in beef liver and muscle. (b) Continue study.

(2) *β-Lactam Antibiotics in Tissues (Chromatographic Methods): (a) Change title of topic to 3 topics:* β-Lactam Antibiotics in Milk (DELVO test), β-Lactam Antibiotics in Milk (ELISA test), and β-Lactam Antibiotics in Milk (Qualitative). (b) Continue study.

(3) *Chloramphenicol in Milk*: Discontinue topic.

(4) *Clopidol in Chicken Tissues*: Discontinue topic.

(5) *Enzyme Immunoassays for Antimicrobial Compounds*: Continue study.

(6) *Sulfamethazine in Milk (Chromatographic Methods)*: Continue study.

(7) *Sulfonamides in Milk (Chromatographic Methods)*: Continue study.
(8) *Tetracyclines in Tissues (Chromatographic methods)*: Continue study.
(9) *Tetracyclines in Tissues (Microbiological methods)*: Discontinue topic.

*Forensic Sciences*

(1) *Gunshot Residues*: Continue study.

**RECOMMENDATIONS FOR OFFICIAL METHODS**

# Committee on Foods I

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*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) *Color in Other Foods and Drugs*: Discontinue topic.
(7) *Synthetic Additives in Colors in Foods by Quantitative TLC Method*: Discontinue topic.
(8) *Trace Organic Constituents of Certifiable Color Additives*: Continue study.
(9) *Uncombined Intermediates and Subsidiary Colors in Certifiable Colors*: Continue study.
(10) *Other Topics*: Establish a new topic, Isolation of Colors from Complex Food Matrixes, and appoint an Associate Referee.

*Dairy Chemistry*

(1) *Alkaline Phosphatase*: Continue study.
(2) *Babcock Test and Babcock Glassware*: Continue study.
(3) *Calcium, Phosphorus, and Magnesium in Cheese*: Continue study.
(4) *Cholesterol in Eggs*: Transfer topic to Meat, Poultry, and Meat and Poultry Products General Referee, continue study.
(5) *Composition of Fluid Milk*: Adopt as official final action modified Mojonnier ether extraction method **989.05**; editorially revise method **989.05(B)**(a) to remove the parenthetical statement identifying the manufacturer; continue study.
(6) *Cryoscopy of Milk*: Continue study.
(7) *Fat in Milk (Gerber test)*: Continue study.
(8) *Iodine*: Continue study.
(9) *Lactose in Dairy Products (Euzymatic 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 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,* 2nd supplement (1991).

(10) *Mid-Infrared Instrumentation*: Adopt as official final action mid-infrared spectroseopic methods for fat, lactose, protein, and solids in milk **972.16**, and for protein in milk **975.18**; continue study.
(11) *(Robotic) Mojonnier Method*: Continue study.
(12) *Moisture in Cheese*: Continue study.
(13) *Nitrates in Cheese*: Continue study.
(14) *Raw Milk Sampling*: Continue study.
(15) *Tyramine*: Continue study.
(16) *Other Topics*: Adopt the IDF standard for calibration of Babcock glassware as an official procedure to be published in *Official Methods of Analysis*.

### *Flavors*

(1) *Additives in Vanilla*: Continue study.
(2) *Adulteration of Vanilla*: Discontinue topic.
(3) *C14 in Flavoring Materials*: Continue study.
(4) *Citral*: Discontinue topic.
(5) *Deuterium NMR Method for Detection of Adulteration of Flavors*: Continue study.
(6) *Essential Oils*: Discontinue topic.
(7) *Glycyrrhizic Acid Salts in Licorice–Derived Products*: Adopt as official final action HPLC method for determination of glycyrrhizic acid and its salts in licorice products **982.19**; discontinue topic.
(8) *Imitation Maple Flavor, Identification and Characterization*: Discontinue topic.
(9) *Licorice Products*: Continue study.
(10) *Moisture in Vanilla Beans*: Appoint an Associate Referee; continue study.
(11) *Vanillin and Ethyl Vanillin*: Continue study.
(12) *Other Topics*: Declare surplus the method for preparation of pure vanilla extract **960.37**(C)(b); adopt as official final action method for carmel color in vanilla extracts **920.133**; adopt as official final action chromatographic method for nonvanilla resins **960.36**; adopt as official final action the HPLC method for determination of sugar in licorice extracts **984.17**; adopt as official final action various methods for alcohol in flavoring materials **973.23, 975.24, 950.44c, 920.147c, 920.148A, 950.45c,** and **975.25**.

### *Food Additives*

(1) *Antioxidants*: Continue study.
(2) *Brominated Vegetable Oils*: Continue study.
(3) *Indirect Additives from Food Packages*: Continue study.
(4) *Monier-Williams Modification*: Continue study.
(5) *Nitrates and Nitrites*: Continue study.
(6) *Nitrosamines in Foods*: Continue study.
(7) *Nitrosamines in Food Contact Items*: Adopt as official final action the GC method for *N*-nitrosodibutylamine in latex infant pacifiers **986.01**; continue study.
(8) *Nonvolatile Nitro Compounds*: Continue study.
(9) *Polycyclic Aromatic Hydrocarbons*: Continue study.
(10) *Polydimethylsiloxane (PDMS)*: Continue study.
(11) *Sulfiting Agents in Foods*: Continue study.
(12) *Urethane in Foods*: Continue study.
(13) *Other Topics*: Adopt as official final action the methods for the determination of NDMA in beer and ale **982.12**, and NDMA in nonfat dry milk powder **984.16.**

### *Meat, Poultry, and Meat and Poultry Products*

(1) *Bouillon and Consommes*: Discontinue topic.
(2) *Creatinine in Soups and Bouillons*: Continue study.
(3) *Glutamic Acid and Monosodium Glutamate*: Continue study.
(4) *Heme, Nonheme, and Total Iron in Meat, Fish, and Poultry Products*: Continue study.
(5) *Hydroxyproline in Meat*: Discontinue topic.
(6) *Immunological Methods in Meat and Poultry Products*: Discontinue topic.
(7) *Ion Chromatographic Methods in Meat and Poultry Products*: Continue study.
(8) *LaBudde Rapid Microwave Moisture Analysis of Meat Products*: Discontinue topic.
(9) *LC Methods for Meat and Poultry Products*: Continue study.
(10) *Microwave Techniques for Meat Analysis*: Continue study.
(11) *Protein Crude*: Discontinue topic.
(12) *Crude Protein in Meat and Meat Products (Combustion Method)*: Continue study.
(13) *Proteins in Meat and Meat Products*: Appoint an Associate Referee; continue study.
(14) *Proximate Analysis of Meat Product by Near Infrared*: Continue study.
(15) *Robotic Methods for Meat and Poultry Products*: Continue study.
(16) *Specific Ion Electrode Applications*: Continue study.
(17) *Temperature, Minimum Processing*: Continue study.
(18) *Total Fat*: Adopt as official first action solvent extraction (submersion) method for crude fat in meat and meat products; continue study.
(19) *Volatiles in Meat and Poultry, and Meat and Poultry Products*: Continue study.

### *Mycotoxins*

(1) *Aflatoxin M*: Appoint an Associate Referee; continue study.
(2) *Aflatoxin Methods*: Editorially revise **989.06**, inserting "Absorbance measurement (Optical density)" after "Method Performance"; adopt as official first action the ELISA method for aflatoxins $B_1$, $B_2$, $G_1$, and $G_2$ in peanut butter; 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*: Adopt as official first action the LC method for ochratoxin in A in corn and barley. Continue study.
(10) *Trichothecenes*: Continue study.
(11) *Zearalenone*: Continue study.

***Plant Toxins***

(1) *Cyanogenic Glycosides*: Appoint an Associate Referee; continue study.
(2) *Glucosinolates*: Continue study.
(3) *Hydrazines*: Continue study.
(4) *Hypoglycine in Ackee Fruit*: Continue study.
(5) *Phytoestrogens*: Continue study.
(6) *Pyrrolizidine Alkaloids*: Continue study.
(7) *Steroidal Alkaloids*: Continue study.

***Preservatives and Artificial Sweeteners***

(1) *Sulfites (Flow Injection Analysis)*: Continue study.
(2) *Sulfites (Ion-Chromatographic Methods)*: Continue study.
(3) *Sulfites (Polarographic Methods)*: Continue study.
(4) *Sulfites in Shrimp (Screening Methods)*: Continue study.
(5) *Other Topics*: Combine the General Referee topic areas for Preservatives and Artificial Sweeteners and for Food Additives under the current General Referee for Food Additives.

***Seafood Products***

(1) *Decomposition by Gas and Liquid Chromatography*: Continue study.
(2) *Drained Weight of Block Frozen, Raw, Peeled Shrimp*: Continue study.
(3) *Flow Injection Analysis for Decomposition in Seafood Products*: Continue study.
(4) *Minced Fish in Fillet Blocks*: Continue study.
(5) *Nitrites in Smoked Fish*: Continue study.
(6) *Rancidity in Fishery Products*: Continue study.
(7) *Shellfish Decomposition*: Continue study.
(8) *Solids (Total) in Seafood*: Appoint an Associate Referee; continue study.
(9) *Volatile Amines–TMA and DMA by GC*: Continue study.
(10) *Ciguatoxins (LC Methods)*: Continue study.
(11) *Ciguatoxins (Biochemical Methods)*: Continue study.
(12) *Cyanobacterial Peptide Toxins*: Continue study.
(13) *Diarrhetic Shellfish Poisons*: Continue study.
(14) *Domoic Acid*: Continue study.
(15) *Neurotoxic Shellfish Poisons*: Continue study.
(16) *Paralytic Shellfish Poisons (Electrochemical Methods)*: Continue study.
(17) *Paralytic Shellfish Poison–Immunoassay Method*: Continue study.
(18) *Paralytic Shellfish Poison by HPLC*: Continue study.
(19) *Tetrodotoxins*: Appoint an Associate Referee; continue study.
(20) *Other Topics*: Initiate new topics Cell Bioassays for Detection of Seafood Toxins, and Enzymatic Detection of Seafood Toxins.

RECOMMENDATIONS FOR OFFICIAL METHODS

# Committee on Foods II

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The performance of 6 General Referees was reviewed by the Committee, based on their performance of the duties of the General Referee. The Committee recommends the following:

1. *Alcoholic Beverages*: Randy Dyer—Reappoint for 3 years.
2. *Cereals and Cereal Products*: Ralph Lane—Reappoint for 3 years.
3. *Fats and Oils*: Dave Firestone—Reappoint for 3 years.
4. *Nonalcoholic Beverages*: John Newton—Reappoint for 3 years.
5. *Processed Vegetable Products*: Tom Mulvaney—Reappoint for 2 years.
6. *Spices and Other Condiments*: James Woodbury—Reappoint for 3 years.

### *Alcoholic Beverages*

(1) *Alcohol Content*: Continue study.
(2) *Ascorbic Acid in Wine by HPLC*: Initiated as new topic.
(3) *Carbon Dioxide in Wine*: Continue study.
(4) *Ethanol in Wine by GLC*: Continue study.
(5) *Ethyl Carbamate in Alcoholic Beverages*: Continue study.
Discussed the GR's recommendation to adopt following changes for *Official Methods of Analysis*:
First Action on "Gas Chromatographic Determination of Ethyl Carbamate in Distilled Wine and Spirits Using Capillary GC equipped with a Thermal Energy Detector."
(6) *Glycerol in Wine*: Continue study.
(7) *Glycerol Monooleate in Wine*: Initiated as a new topic.
(8) *Malic Acid in Wine*: Continue study.
(9) *Malt Beverages and Brewing Materials*: Continue study.
(10) *Polydimethylsiloxane in Wine*: Initiated as a new topic.
(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) Mark Schwiesow will be the new AOAC-ASBC (American Society of Brewing Chemists) liaison.
*(18) The following corrections be made to the *Official Methods of Analysis*, 15th Ed. Sections **942.06** and **983.12** should read: Density Meter—Mettler/Paar DMA 55D, or equivalent Section **988.06** should read: Density Meter—Mettler/Paar DMA 46, or equivalent.
*(19) Recommend the establishment of the topic "Lead in Alcoholic Beverages" and the appointment of an Associate Referee.

### *Cereals and Cereal Products*

(1) *β-Glucan Methodology: Continue study.*
(2) *Crude Protein by Combustion Methods*: Continue study.
(3) *Fat Acidity*: Continue study.
(4) *Gliadin in Gluten-Free Products*: 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 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,* 2nd supplement (1991).

(5) *Gluten in Foods*: Continue study.
(6) *Iron*: Continue study.
(7) *Mineral Analysis*: Continue study.
(8) *Near Infrared Analysis of Cereal Products*: Topic initiated and AR appointed. Commence study.
(9) *Phytates*: Continue study.
*(10) Adopt following changes for *Official Methods of Analysis* in line with the policies of the editorial board regarding cross listing and joint methods:
List method **985.29** (Total Dietary Fiber) as an AOAC/AACC Method (AACC 32.05)
List method **984.13** (Crude Protein, Cu Catalyst...) as an AOAC/AACC Method (AACC 46-11a). Cross list in cereals chapter.
List method **950.03** (Neutralizing Value of Acid-Reacting...) as an AOAC/AACC Method (AACC 02-32a). Cross list in cereals chapter.
List method **976.22** (Zearalenone(ol) in Corn) as an AOAC/AACC Method (AACC 45-21). Cross list in cereals chapter.
List method **960.52** (Crude Protein, Micro-Kjeldahl) as an AOAC/AACC Method (AACC 46-13). Cross list in cereals chapter.
List method **960.04** (Crude Protein, 5 min Biuret) as an AOAC/AACC Method (AACC 46-15). Cross list in cereals chapter.
List method **948.05** (Residual $CO_2$ in Baking Powder) as an AOAC/AACC Method (AACC 12-10). Cross list in cereals chapter.
List method **965.09** (Elements by AAS) as an AOAC/AACC Method (AACC 40-70). Cross list in cereals chapter.
List method **986.17** (Deoxynivalenol in Wheat, TLC Method) as an AOAC/AACC Method (AACC 45-41). Cross list in cereals chapter.
List method **988.05** (Crude Protein, Kjeldahl/Cu $TiO_2$) as an AOAC/AACC Method (AACC 46-16). Cross list in cereals chapter.
List method **988.05** (Benzoyl Peroxide in Flour) as an AOAC/AACC Method (AACC 61-01). Cross list in cereals chapter.

### *Chocolate and Cacao Products*

(1) *Carbohydrates in Chocolate Products*: Continue study.
(2) *Alternate Fats in Chocolate*: Appoint new AR. Commence study.
(3) *Shell in Cacao Products*: Continue study.
(4) *Total and Solid Fat Content in Chocolate Products by NMR*: Appoint new AR. Commence study.

### *Dietary Fiber*

(1) *Determination by UED*: Continue study.
(2) *Dietary Fiber-Modified Englyst Method*: Roger Wood will be contact person on this submitted collaborative study. Continue study.
(3) *Dietary Fiber-Uppsala Method*: Continue study.
(4) *Enzymatic Gravimetric Methods-MES/TRIS*: Continue study.
During the past year, the Committee recommended the OMB adopt the following changes for *Official Methods of Analysis*:
First Action on "Determination of Soluble, Insoluble, and Total Dietary Fiber-MES/TRIS Method."
(5) *Enzymatic Gravimetric Methods-Phosphate*: Continue study.
During the past year, the Committee recommended the OMB adopt the following changes for *Official Methods of Analysis*:
First Action on "Determination of Insoluble Dietary Fiber-Phosphate Buffer Method."

### *Fats and Oils*

No report submitted by GR
(1) *Emulsifiers*: Continue study.
(2) *Hydrogenated Fats*: Continue study.
(3) *Lower Fatty Acids*: Continue study.
(4) *Marine Oils*: Continue study.
(5) *Olive Oil Adulteration*: Continue study.
(6) *Oxidized Fats*: Continue study.
(7) *Sterols and Tocopherols*: Continue study.

### *Fruit and Fruit Products*

(1) *Apple Juice Adulteration*: Appoint new AR. Continue study.
During the past year, the Committee recommended the OMB adopt the following changes for *Official Methods of Analysis*:
First Action on "Determination of L-Malic/Total Malic Acid Ratio in Apple Juice."
(2) *Fruit Acids*: Continue study.
(3) *Geographic Origin of Orange Juice*: Continue study.
(4) *Identification and Characterization of Fruit Juices*: Continue study.
(5) *Moisture in Dried Fruits*: Continue study.
(6) *Naringin and Neohesperidine in Orange Juice*: Collaborative study in progress. Continue study.
(7) *Orange Juice Adulteration with Pulpwash*: Continue study.
(8) *Sodium Benzoate in Orange Juice*: Continue study.
(9) *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) *Decaffinated Coffee and Tea-Solvent Residues*: Continue study.
(5) *Moisture*: Continue study.

*(6) *Pyrrolizidine Alkaloids in Herbal Tea*: Initiate topic and appoint AR.
(7) *Quinine*: Continue study.

### *Processed Vegetable Products*

*(1) *Aseptic Processing*: Initiate topic, appoint AR: Commence study.
(1) *LC Determination of Sugar*: Continue study.
*(2) *pH Determination*: Appoint new AR. Continue study.
*(3) *Sodium Chloride*: Reestablish topic. Appoint J. Anderson Williams as AR.
*(4) *Total Solids by Microwave Moisture Analyzer*: Continue study.
The followir.g corrections should be made to the *Official Methods of Analysis*, 15th Ed.
Add the required calibration and warm up procedures to method **985.26**. Details and wording to be worked out by the AR and GR.
(5) *Water Activity in Foods*: 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.
Discussed the GR's recommendation to adopt following changes for *Official Methods of Analysis*:
First Action on "Stable Carbon Isotope Ratio Method for Determining Corn-Derived Acetic Acid in Apple Cider Vinegar."
(9) *Water Activity of Spices*: Continue study.

### *Sugars and Sugar Products*

(1) *Amyloglucosidase Enzyme Activity*: Topic initiated and AR appointed. Commence study.
(2) *Corn Syrup and Sugar Products*: Continue study.
(3) *Enzymatic Methods*: Continue study.
*(4) *Gas Chromatographic Methods*: Discontinue topic.
(5) *Honey*: Continue study.
Adopt following changes for *Official Methods of Analysis*:
Method **978.17**: Add the performance parameters for the determination of stable carbon isotope ratio of honey to the current description of the method.
Method **978.17**: Add a suitable description of the Sofer combustion procedure as an alternate combustion method. GR will submit recommended wording for change.
During the past year, the Committee recommended the OMB adopt the following changes for *Official Methods of Analysis*:
First Action on "Apparent Corn or Cane Syrup in Honey."
(6) *Lactose*: Continue study.
(7) *Liquid Chromatographic methods*: Continue study.
*(8) *Maple Sap, Maple Syrup, and Maple Syrup Products*: Appoint a new referee. Continue study.
(9) *Methods Standardization*: Continue study.
(10) *Oligosaccharides*: Continue study.
(11) *Polarimetric Methods for Measurement of Sugars*: Continue study.
(12) *Stable Carbon Isotope Ratio Analysis*: Continue study.
Discussed the recommendation of the GR to adopt following changes for *Official Methods of Analysis*:
First Action on "Detecting Adulteration of Frozen Concentrated Orange Juice with Sugar Beet-derived Syrups on the Basis of $^{18}O/^{16}O$ Measurements in Water."
(13) *Sugars in Cereals*: Continue study.
(14) *Sugars in Syrups*: Continue study.
Discussed the recommendation of the GR to adopt following changes for *Official Methods of Analysis*:
First Action on "Sugars in Syrups-HPLC Method."
(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.
(2) *Automated Methods*: Continue study.
(3) *Biotin*: New AR appointed. Commence study.
(4) *Carotenoids*: Continue study.
(5) *Cholesterol*: Continue study.
(6) *Folic Acid*: Appoint AR. Commence study.
(7) *Infant Formula Nutrient Assay*: Study phases IV and V submitted. Continue study.
(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*: Appoint new AR. Commence 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.

# Committee on Residues

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### *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 in cadmium 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) *Hydride Generating Techniques*: Discontinue topic.

(5) *Lead in Calcium Supplements*: Draft, in AOAC official method format, the method developed for the determination of lead in calcium supplements that was adapted from *J. Assoc. Off. Anal. Chem.* (1979) **62**, 1054–1061, 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) *Methyl Mercury in Fish and Shellfish*: Adopt as official final action the official first action method, Mercury (Methyl) in Fish and Shellfish, **988.11**. Discontinue topic.

(7) *Neutron Activation Analysis*: Submit protocol for collaborative study of the method for the determination of sodium in foods 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 liquid chromatographic/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*: Continue official first action status of the graphite furnace atomic absorption spectrometry method for copper, iron, and nickel in edible oils and copper and iron in edible fats, **990.05.**

*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,* 2nd supplement (1991).

***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*-methyl carbamates 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) *Gel Permeation Chromatography (GPC) Cleanup*: Discontinue topic.

(4) *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 have been evaluated for review and comment by the General Referee. If satisfactory perform interlaboratory trial.

(5) *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.

(6) *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.

(7) *Sweep Codistillation*: Obtain results and design of the latest Australian National Pesticide Residue Proficiency Testing Program (NPRPTP) for review and comment by the General Referee and Committee Statistician. Continue study and, if possible, assist in the design of future NPRPTP sample studies that could serve both the AOAC and the NPRPTP.

(8) *Synthetic Pyrethroids*: Conduct interlaboratory trial of the Associate Referee's method for using the mixed solvent elution system and the wide bore capillary EC chromatographic system. 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. (b) Review the National Council of the Paper Industry for Air and Stream Improvement method for dioxins and furans in paper products and guide the organization in their decisions about AOAC collaborative study of the method.

(2) *Chlorophenoxy Alkyl Acids and Pentachlorophenol*: Continue as official first action the GC method for pentachlorophenol in gelatin, **985.24**. Draft, in AOAC Official Method format, a specific method for chlorophenoxy alkyl acids based on methodology of Hopper (*J. Agric. Food Chem.* (1987) **35**, 265–269) for review and comment by the General Referee and Committee on Residues. Recovery and method performance data concerning all chemicals tested must be included with the proposed method. If satisfactory, perform efficiency studies of extracting field incurred residues from samples. If satisfactory, perform interlaboratory trial and prepare protocol for collaborative study on commodities likeliest to be contaminated with residues for approval by General Referee, Committee Statistician, and Committee on Residues; initiate collaborative study.

(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, 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*: Adopt as official final action the official first action method, Polychlorinated Biphenyls (as Aroclor 1254) in Serum, **990.07**. 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-benzimidazolecarbamate (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, 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)*: Evaluate data from an interlaboratory study of the method of Conditt et al. (*J. Assoc. Off. Anal. Chem.* (1988) **71**, 735–739) conducted by the National Food Processors Association, submit report and recommendation to Committee on Residues for possible interim action.

(9) *Diquat and Paraquat*: Complete revision of manuscript on the collaborative study of the method for diquat and paraquat in potatoes, submit report and recommendation to General Referee and Committee on Residues for possible interim action. 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*: Appoint an Associate Referee to evaluate multiresidue methods for nitro- and dinitro-substituted pesticides and to collaboratively study method selected.

(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 evaluate methods for determining residues of atrazine, simazine, and cyanazine in agricultural products and to collaboratively study method selected.

### *Organophosphorus Pesticides*

(1) *Extraction Procedures*: Appoint an Associate Referee to study the efficiency of procedures for extracting field-incurred residues of organophosphorus pesticides from crops and to develop improved extraction procedures for incorporation into multiresidue methods.

(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).

(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**.

### *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 lower levels 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 General Referee and Committee on Residues 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*

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*Manual* (1982) pp. E-Pu-01-01) and related procedures; design and conduct collaborative study of selected method.

(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 and Committee Statistician, 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

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*An asterisk before a recommendation indicates that official action on that recommendation resulted in its appearance in "Changes in Official Methods."

The recommendations submitted by the Committee on 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*, 2nd supplement (1991).

### *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) *Alkaline Phosphatase in Foods, Electrophoresis Detection Method*: New topic.

(2) *Baked Goods with Fruit and Nut Tissues, Light Filth by Flotation Method*: Continue study.

(3) *Basil (Ground), Light Filth by Flotation Method*: Continue study.

(4) *Bean Paste, Light Filth by Flotation Method*: New topic.

(5) *Chocolate and Chocolate Products, Light Filth by Flotation Method*: Continue study.

(6) *Coffee (Ground), Light Filth by Flotation Method*: Continue study.

(7) *Condimental Hot Sauces, Light Filth by Flotation Method*: Continue Study.

(8) *Condimental Sauces Containing Soy Sauce, Thickeners, and Spices, Light Filth by Flotation Method*: New topic.

(9) *Crabmeat, Shrimp, and Tuna (Canned), Light Filth by Brine Flotation Method*: Continue study.

*(10) *Extraneous Materials (Foreign Matter) in Products*: In **945.75B**, add the following as (t) Sieve handle.— For holding 8" diameter sieves during wet sieving. See Fig. **945.7D**. Constructed from 1" o.d. hard aluminum tube, 14" long; 1/4"-28, 2" brass bolt; 1/4"-28 brass wingnut; and 2 1/4" brass washers. Cross drill 1/4" hole. Saw tube

lengthwise perpendicular to 1/4" hole. Cut off 2" of upper half of slit tube. Spread split tube ca 2" and flatten ca 2" of top and bottom halves. Bend 1/4" right angle lip on each flattened end. In method **970.66B**(a), after the sentence "Hold sieve under aerator...at approx. 30° angle...." add, "Use of a sieve handle, **945.75**(t), or similar device helps in maintaining the proper angle of the sieve."

(11) *Fish (Canned) and Fish Products, Light Filth by 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*: Continue study.
(16) *Grains (Whole), Internal Insect Infestation by Cracking Flotation Method*: Continue study.
(17) *Grains (Whole), Internal Infestation by ELISA Method*: New topic.
(18) *Grains and Seeds (Whole), External Light Filth by Flotation Method*: New topic.
(19) *Mammalian Feces, Coprostanol by Thin Layer Chromatography*: Discontinue topic.
(20) *Onion and Garlic Powders and Granules, Filth by Sedimentation Method*: Discontinue topic.
(21) *Rodent Gnawing of Packaging Materials and Foods, Salivary Amylase Test*: Continue study.
(22) *Soybean Curd, Light Filth by Flotation Method*: Continue study.
(23) *Spices, Mammalian Feces by Chemical Detection Method*: Continue study.
(24) *Spirulina, Light Filth by Flotation Method*: Discontinue topic.
(25) *Urine Stains on Foods and Containers, Chemical Detection Methods*: Continue study.
(26) *Vegetable Products (Dehydrated), Light Filth by Flotation Method*: Continue study.

### *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) *Bacterial and Coliform Counts in Dairy Products, Petrifilm Methods*: Continue Study.
(2) *Bactoscan Methods*: Continue study.
(3) *Coliform in Milk, Impedance Detection by Vitek Systems*: New topic.
(4) *Coliform in Milk, Impedance Detection by Radiometer America*: New topic.
(5) *Coliforms in Dairy Products, Pectin Gel Method*: Continue study.
(6) *Listeria monocytogenes in Dairy Products, Detection by Culture Methods*: Continue study.
(7) *Listeria monocytogenes in Dairy Products, Detection by DNA Probe*: Continue study.
(8) *Raw Milk in Cheese, Alkaline Phosphatase Test*: Continue study.
(9) *Somatic Cells, Automated Optical Methods*: Continue study.
(10) *Somatic Cells, Fossomatic Method*: Continue study.
(11) *Salmonella detection from Dairy Products by Motility Enrichment on Modified Semisolid Rappaport-Vassiliadis Medium*: New topic.

### *Drug and Device Related Microbiology*

(1) *Biological Indicators, Testing and Standardization*: Continue study.
(2) *Biological Sterility Indicators*: Discontinue topic.
(3) *Chemical Indicators*: Continue study.
(4) *Endotoxins, Limulus Amoebocyte Lysate Test*: Continue study.
(5) *Medical Devices, Packaging Integrity*: Continue study.
(6) *Medical Devices, Sterility Testing*: Discontinue topic.
(7) *Sporicidal Testing of Disinfectants/Sterilants*: Continue study.

### *Environmental Sanitation Microbiology*

(1) *Total Coliforms and Escherichia coli on Surfaces, Defined Substrate Technology*: New topic.
(2) *Cleanliness of Surfaces, Using an ATP-Based System*: New topic.

### *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*: New topic.
(4) *Bacillus cereus Enterotoxin, Microslide Gel Double Diffusion Test*: Continue study.
(5) *Bacillus cereus, Enzyme Immunoassay for Enterotoxins*: New topic.
(6) *Bacillus cereus, Isolation and Enumeration*: Discontinue topic.
(7) *Bactoscan Methods*: Continue study.
(8) *Campylobacter Species*: Discontinue topic.
(9) *Clostridium perfringens, Recovery from Marine Environment by Iron Milk Test*: Continue study.
(10) *Enterotoxigenic Escherichia coli, Detection by DNA Hybridization Method*: Discontinue topic.

(11) *Escherichia coli in Chilled and Frozen Foods, MUG Test*: Continue study.
(12) *Escherichia coli in Shellfish, MUG Test*: Continue study.
(13) *Listeria, DNA Probe*: Continue study.
(14) *Listeria, Listeria-Tek Assay*: Continue study.
(15) *Listeria, MICRO-ID System*: Continue study.
(16) *Listeria, Vitek AutoMicrobic System*: Continue study.
(17) *Listeria, Assurance Enzyme Immunoassay*: New topic.
(18) *Listeria, GENE-TRAK Colorimetric DNA Hybridization Method*: New topic.
(19) *Listeria, Identification by Gas Chromatography of Cellular Fatty Acids*: New topic.
(20) *Listeria, Tecra Enzyme Immunoassay*: New topic.
(21) *Salmonella, Assurance Enzyme Immunoassay*: Continue study.
(22) *Salmonella, Bio-EnzaBead Enzyme Immunoassay Screening Method*: Discontinue topic.
*(23) *Salmonella, GENE-TRAK DNA Hybridization Screening Method*: Adopt as official first action the modified colorimetric DNA hybridization procedure to replace the current official first action method, *Salmonella* in Foods, Colorimetric Deoxyribonucleic Acid Hybridization (GENE-TRAK) *Salmonella* Assay Screening Method. **990.13**.
(24) *Salmonella, ImmunoBand Screening Method*: Continue study.
*(25) *Salmonella, ISO-GRID Screening Method*: Repeal the official first action method, *Salmonella* in Foods, Hydrophobic Grid Membrane Filter Screening Method, **985.42**.
(26) *Salmonella, Malthus Automated Conductance Method*: Continue study.
(27) *Salmonella, Modified Semisolid Rappaport-Vassiliadis Method for Cocoa and Chocolate*: New topic.
(28) *Salmonella, Oxoid Method*: New topic.
(29) *Salmonella, Q-TROL Enzyme Immunoassay Screening Method*: Continue study.
(30) *Salmonella, Salmonella-Tek Enzyme Immunoassay*: New topic.
*(31) *Salmonella, TECRA Enzyme Immunoassay Screening Method*: In method **989.14C**, add trade names "Report" and "BioPro" to sentence "Items (a)–(m) are available as TECRA *Salmonella* Visual Immunoassay...or from Distributors as TECRA (Vitek Systems, Inc., Hazelwood, MO), Report (3M Microbiology Products, St. Paul, MN) and BioPro (International BioProducts, Inc., Redmond, WA)."
(32) *Salmonella, Escherichia coli, and Other Enterobacteriaceae, Identification by Micro ID Diagnostic Kit*: Continue study.
(33) *Salmonella, Escherichia coli, and Other Enterobacteriaceae, Identification by the Vitek Gram Negative Identification Card*: Continue study.
(34) *Salmonella in Chocolate*: Continue study.
(35) *Staphylococcal Enterotoxin, TECRA Enzyme Immunoassay*: New topic.
(36) *Staphylococcus aureus, GENE-TRAK Colorimetric DNA Hybridization Method*: New topic.
(37) *Staphylococcus Isolation and Enumeration by Most Probable Number Technique*: Discontinue topic.
(38) *Total Coliforms and Escherichia coli, Petrifilm Methods*: Continue study.
(39) *Total Coliforms and Escherichia coli, ColiComplete Discs*: New topic.
(40) *Vibrio cholerae, Elevated Temperature Enrichment Method*: Continue study.
(41) *Vibrio vulnificus, Identification by Gas Chromatography of Cellular Fatty Acids*: New topic.
(42) *Yeasts and Molds, Mycological Media for Isolation*: Transfer topic to Analytical Mycology and Microscopy.
(43) *Yersinia enterocolitica*: Discontinue topic.

### *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.

# Committee on Feeds, Fertilizers, and Related Topics

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### *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) *Microbiological Assays*: Discontinue topic.
(7) *Monensin Microbiological Method*: Continue study.
(8) *Narasin Microbiological Method*: Continue study.
(9) *Neomycin*: Continue study.
(10) *Oxytetracycline in Feeds by Microbiological Methods*: Continue study.
(11) *Tylosin*: Continue study.
(12) *Virginiamycin*: Continue study.
(13) *Virginiamycin with Other Drugs*: Continue study.

### *Drugs in Feeds*

(1) *Amprolium*: Continue study.
(2) *Carbadox*: Continue study.
(3) *Ethopabate*: Continue study.
(4) *Furazolidone and Nitrofurazone*: Continue study.
(5) *Lasalocid (LC Method)*: Continue study.
(6) *Melengestrol Acetate*: Continue study.
(7) *Monensin and Narasin (LC Method)*: Continue study.
(8) *Morantel Tartrate*: Continue study.
(9) *Oxytetracycline (LC Method)*: Continue study.
(10) *Pyrantel Tartrate*: Continue study.
(11) *Sampling*: Continue study.
(12) *Sulfadimethoxine and Ormetoprim*: Continue study.
(13) *Sulfa Drug Residues*: Continue study.
(14) *Sulfamethazine and Sulfathiazole*: Continue study.

### *Feeds*

(1) *Amino Acids*: Continue study.
(2) *Atomic Absorption Spectrophotometry*: Continue study.
(3) *Calcium Salts of Isobutyric and Mixed 5-Carbon Volatile Fatty Acids*: Discontinue topic.
(4) *Carotenoids*: Continue study.
(5) *Crude Protein*: Continue study.
(6) *Emission Spectroscopy*: Continue study.
(7) *Fat*: Continue study.
(8) *Fiber*: Continue study.
(9) *Iodine and EDDI in Feeds*: Continue study.
(10) *Microscopy*: Continue study.
(11) *Minerals*: Continue study.
(12) *Mixed Feeds-Infrared Reflectance Tech*: Continue first action status of the method for fiber (acid detergent) and protein (crude) in forage, near infrared spectroscopic method (**989.03**).
(13) *Moisture in Mixed Feeds and Forages*: Continue study and first action status of moisture in forage near-infrared reflectance spectroscopy (**991.01**).
(14) *Moisture in Pet Foods*: Continue first action status of Karl Fisher method for determination of moisture in sort-moist pet foods.

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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,* 2nd supplement (1991).

(15) *Sampling*: Continue study.
(16) *Vitamins*: Continue study.

***Fertilizers***

(1) *Nitrogen by Combustion*: Continue study.
(2) *Iron*: Continue study.
(3) *Manganese in Feed Ingredients*: Continue study.
(4) *Phosphorus*: Continue study.
(5) *Potassium*: Continue study.
(6) *Sample Preparation*: Continue study.
(7) *Sampling*: Continue study.
(8) *Slow-Release Mixed Fertilizers*: Continue study.
(9) *Urea and Methyleneureas in the Fertilizer and Agricultural Liming Materials*: Continue study.
(10) *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) *Arsenic in Animal Tissue*: Discontinue topic.
(3) *Atomic Absorption Methods*: Discontinue topic.
(4) *Cholinesterase Colorimetric Method*: Continue first action status of the colorimetric method for whole blood cholinesterase (**991.10**).
(5) *Cholinesterase pH Method*: Continue study.
(6) *Copper in Animal Tissue*: Discontinue topic.
(7) *Cyanide*: Discontinue topic.
(8) *GC/MS Methods*: Continue study.
(9) *Fluoride in Animal Tissue*: Continue study.
(10) *Lead in Animal Tissue*: Continue study.
(11) *Multielement Analysis by ICP*: Continue study.
(12) *Multiple Anticoagulant Screening*: Continue study.
(13) *Natural Products*: Continue study.
(14) *Nitrates and Nitrites*: Continue study.
(15) *Pesticides in Toxicological Samples*: Continue study.
(16) *Selenium in Animal Tissue*: Continue study.
(17) *Sodium Monofluoroacetate*: Continue study.
(18) *Vitamins A and E*: Continue study.
(19) *Vitamins D and K*: Continue study.
(20) *Zinc in Animal Serum*: Continue first action status of the method for serum zinc (**991.11**)

# Committee on Environmental Quality

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The Committee recommended that the Official Methods Board move adoption of the revised terms of reference and the Committee activities for 1991.

The Committee recommends that a member of the Laboratory Quality Assurance Committee be appointed as an advisor to each methods committee and that quality assurance requirements be added to the protocol checklist.

Both the terms of reference and Committee activities have been revised to reflect the Committee's priorities for General Referee topics.

Quality assurance needs to be given the same status as statistics and safety in the collaborative study process.

### *Cooperative Studies*

(1) *Nitrogen- and Phosphorus-Containing Pesticides in Groundwater*: Continue first action status 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*: Adcpt 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*: Continue first action status 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*: Continue first action status for Digestion of Solid Wastes for Determination of 23 Elements by Inductively Coupled Plasma Atomic Emission Spectroscopy.

### *Inorganic Methods*

(1) *Inductively Coupled Plasma/MS*: Finalize study report.
(2) *Ion Chromatographic Methods for Groundwater*: Continue topic.

### *Organic Methods*

(1) *Diquat and Paraquat*: Appoint an Associate Referee.
(2) *Herbicide Residues in Environmental Waters*: Continue first action status 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.

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(4) *Explosive Residues*: Continue first action status for Determination of Munition Residues in Soil by Liquid Chromatography.
(5) *Triazine Herbicides*: Continue Topic.
(6) *Carbonyl Compounds in Water*: Collaborative study underway.
(7) *Carbamates in Water*: Associate Referee has been appointed.
(8) *Immunoassay for Atrazine*: Associate Referee has been appointed.

# EXECUTIVE, OFFICER, AND COMMITTEE REPORTS

## Executive Director's Report

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### *Preface*

This has been an extraordinary year in the history of AOAC—a year in which the recent examination of our future direction began to take shape in a number of ways. As President Michael Wehr reported in his opening address, the Association will have a new name, a revised mission, and new challenges at the conclusion of this meeting.

Henceforth, we will be known as "AOAC International"—"The scientific association dedicated to analytical excellence." In reality, this "change" is not a change at all, but rather a culminating recognition of where the Association already is and where it plans to go—both in international activity and in promoting excellence in analytical laboratory measurements.

The Association has been international since its founding 107 years ago, but the international scope of our activities, and influence, have expanded greatly in recent years, and they will continue to do so.

Similarly, we have always been dedicated to analytical excellence through our core purpose of collaboratively studying, adopting, and publishing analytical methods used to protect the health, welfare, and safety of people all around the world. However, those methods, no matter how well studied and refined, need high quality samples, high quality laboratories, and, most importantly, high quality analysts to produce quality measurements. Our new name and purpose affirm the fact that AOAC has been and will continue to be a world leader in promoting and bringing about total quality management in the analytical laboratory.

### *Introduction*

Changing topics, my main purpose is to report to you on activities of the AOAC staff in support of the AOAC volunteers and programs over the past year. Others will report to you on the financial health of the Association, the status of the AOAC official methods activities, the work of the Editorial Board, and the work of the many AOAC committees.

As usual, the work of AOAC staff 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 AOAC headquarter's departments.

### *Executive Director's Office*

Beginning with the Executive Director's Office, the major responsibilities of this department include 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. During the past year, the department has arranged and coordinated 5 Board of Directors meetings.

Early in 1991, a major effort was devoted to preparing for and coordinating the Board of Directors planning retreat, which was conducted in conjunction with the March 1991 Board meeting. A significant item that was carried out in preparation to the retreat was a 4-phase DELPHI project to arrive at prior Board consensus on the mission of AOAC and the major components of the mission. A direct result of this project was the proposal approved by the recent vote of the membership to amend the AOAC mission statement.

Late in 1990, the department completed the first comprehensive compilation of past Board of Directors policy statements. The first update of that policy compendium, incorporating decisions of the Board for the past year, has just been completed.

In another area, late in 1990, in conjunction with the AOAC Europe Section meeting held in Brussels, Belgium, visits were made to the offices and representatives of The Netherlands Inspectorate of Health, the International Dairy Federation (IDF), the Commission on European Normalization (CEN), the International Standards Organization (ISO), the International Union of Pure and Applied Chemistry (IUPAC), and the Royal Society of Chemistry.

Early in 1991, working with AOAC's European Representative, Margreet Lauwaars, AOAC sought and achieved liaison status with the newly formed CEN technical committee on the horizontal aspects of food analysis (CEN/TC 275). The Executive Director's Office also represented the Association, with Lauwaars and several volunteers, at the Interagency meeting and Codex Committee on Methods of Analysis and Sampling meeting held in Budapest, Hungary, in April 1991.

Liaison with other organizations included the signing of a new cooperative agreement with the American Association of Cereal Chemists (AACC) in areas of joint methods development and approval and the continuing development of closer relations with the Association of Food and Drug Officials (AFDO) and the Technical Committee for Juice and Juice Products.

### *Finance and Data Processing*

Switching to the AOAC headquarter's department for finance and data processing, this has been a year of change and ad-

vancement, as well. The Finance and Data Processing Department has responsibility for 3 major areas of the Association: financial and accounting services, data processing, and publications fulfillment services.

A major initiative affecting all these areas was the department's management of the acquisition and installation of a 26 station local area computer network and associated software in mid-1990. This project was designed to be phased in over a 2 to 3 year period and result in a total revamping of our data processing, accounting, reporting, communicating, and data base management capabilities.

Unfortunately, late 1990 and early 1991 were devoted to "debugging" a complex combination of hardware and software problems that caused the system to "crash" numerous times each day—setting the total project back nearly a year. The good news is the problem was solved, and plans for a major conversion of the Association's membership and marketing data bases, the next major phase, are progressing well.

In the meantime, a new system of receipts processing has been implemented, a new accounting and financial reporting package has been installed and has been operating since the first of 1991, and a new meetings registration system has been installed and has been put in operation. These systems augment the internal electronic mail system and advanced word processing, calendar, and scheduling capabilities included in the initial installation. In the near future, we plan to improve our external communications system by installing electronic FAX capability on the network.

### *Marketing and Membership*

Turning to the Marketing and Membership Department, this AOAC department has overall responsibility for promoting the Association and its various products and services. Coupled with this is overall responsibility for managing the individual membership, sustaining membership, U.S. representative, and AOAC regional section programs.

In the membership area, AOAC headquarters maintains member records and oversees recruitment and retention of members. At the first of August, individual membership stood at 3594, almost the same as last year at the same time. Sustaining member organization membership at the first of August was 206, down 13 from 1990. Although neither figure has shown growth during 1991, due primarily to weak worldwide economies and corporate mergers and downsizing, both figures are generally better than membership decreases being experienced by similar associations in the same and other fields.

Our U.S. Representative, Howard Moore, who concentrates mainly on sustaining member issues represented us at the Pittsburgh Conference, the American Association of Feed Control Officials meeting, the Northeast North America AOAC Regional Section meeting, and the Northeast (North America) Association of Food and Drug Officials meeting early in 1991. In addition, he has visited 39 sustaining members and sustaining member prospects in the course of traveling to and from these meetings.

Throughout the first part of 1991, the membership office has been assisting a group of volunteers in the organization of a proposed Mid-Atlantic U.S. Regional Section. Right now, there are 11 sections: 6 wholly within the United States, 2 spanning the United States and Canada, 2 wholly in Canada, and one in Europe. The proposed Mid-Atlantic U.S. Regional Section plans to hold its organizational meeting in October 1991 and should be operating in 1992.

In the marketing area, the department carried on significant operations in promoting the 1991 and 1992 annual meetings, coordinating a new short course catalog and promoting the short course program, updating the publications catalog and promoting the Association's publications, exhibiting at the 1991 Pittsburgh Conference, and issuing several press releases. In addition, the department prepared the 1991 annual membership directory for publication and is jointly working with the data processing office to redesign and convert our membership and marketing records.

### *Administration and Meetings*

In a different area, the Administration and Meetings Department at AOAC headquarters is responsible for 2 very dissimilar functions of the Association. The first is office administration, including personnel, 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.

In addition to overseeing the total organization and arrangements for the 1991 AOAC annual meeting, work progressed on planning for the 1992, 1993, and 1994 annual meetings. The 1992 AOAC annual meeting is scheduled in Cincinnati, OH, on August 31–September 4. In 1993, the Association will meet in Washington, DC, on July 26–29. Current plans are to return to the West Coast of the United States in 1994 for a September meeting. West Coast sites currently being considered include San Diego, CA; San Jose, CA; Portland, OR; and Seattle, WA.

Attendance at the 1991 AOAC annual meeting slightly exceeded 750 paid delegates and exhibitors, accompanied by approximately 350 spouses and guests, for a total of 1100 people—slightly better than what was budgeted for a year ago and significantly exceeding recent expectations resulting from the poor economic conditions.

Significant work went on in 1991 in the continuing improvement of the AOAC short course program. An advanced 1991 schedule was developed, planned, and promoted. A series of courses was held early in 1991 in Toronto, Canada, with reasonably good success. Although slow to develop, attendance at the short courses held at the 1991 AOAC annual meeting has turned out to be good. Two additional series of the courses are scheduled late in 1991 in San Diego, CA, and Durham, NC. The schedule for 1992 is currently being developed and should be available soon.

In the administration area, a not so visible but very important part of a smooth running organization, advances were made on a new employee orientation program. Employee health and

benefits costs continue to increase, but, through selection of new providers a year and one-half ago, have been kept at levels less than others are experiencing in the Washington, DC, labor market.

A major undertaking for the administration office early in the year included arranging for and overseeing the construction and outfitting of internal space to accommodate new employees and future expansion. As a result, part of our internal storage space was lost and arrangements for build-out and lease of storage space in the basement of the building was also undertaken and completed.

### *Technical Services*

Switching to the AOAC Technical Services Department, the primary responsibilities of this department are managing and coordinating the scientific and technical affairs of the Association. This includes administering the collaborative 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 harmonizing relations and activities with other international organizations.

Major accomplishments in 1991 included improving the methods tracking and volunteer tracking systems, assessing and documenting methods currently in the system, compiling the Official Methods Board operating policies, improving communication between the AOAC headquarters and our international representatives, serving as AOAC staff liaison to 2 new task forces involved in the development of the test kit recognition program and the intermediately validated methods of analysis, negotiating new AOAC-AACC and AOAC-USFDA cooperative agreements, and coordinating AOAC representation and response at the quality assurance harmonization conference held early in 1991 in Geneva, Switzerland.

In addition, the department prepared and electronically submitted *Changes in Official Methods of Analysis of the AOAC*, the second supplement to the 15th edition of the *Official Methods of Analysis of the AOAC*, for publication. While doing this, the department developed a new process for editing proposed official methods in final format, prior to submission to the Official Methods Board, to ensure that the Official Methods Board reviews and approves first action methods as they will actually appear in published form. Another advance has been the development of a method notification plan for *The Referee*, including announcements of new protocols received, completed collaborative studies received, committee approved methods being submitted to the Official Methods Board for adoption first action, methods to be submitted to the membership for final action approval, and methods recommended for changes in status such as surplussing or repeal.

Internationally, in addition to meetings mentioned elsewhere in this report, the Association was represented by its International Representatives, Lauwaars and Derek Abbott, at meetings of the AOAC Europe Executive Committee, the Codex Committee on Food Additives, the Codex Committee on Pesticide Residues, and the IDF Tripartite meeting. Lauwaars will be the AOAC keynote speaker at the Nordic Committee on Food Analysis' annual meeting scheduled on August 27–30, 1991, in Bergen, Norway, where she will be making a requested presentation on the AOAC test kit recognition program. George R. Heavner, the department's technical coordinator, will make a similar presentation to an industrial group in Finland late in 1991.

### *Publications*

Lastly, a major contributor to the success of AOAC is the Publications Department. This department has responsibility for coordination of peer review, editorial review, and production of the *Journal of the AOAC*; management and production of the monthly newsletter *The Referee*; editorial control and publication of *Official Methods of Analysis of the AOAC* and the *Changes in Official Methods of Analysis of the AOAC*; and acquisition, development, review, and production of miscellaneous books and manuals of the Association.

One of the major efforts for the department in 1991 has been to increase *Journal* manuscript submissions through a more aggressive approach to obtaining manuscripts. Submissions have been declining in recent years, but, although too early to tell definitively, the efforts of the department appear to be having an impact.

A major accomplishment has been the fact that 60 to 70 percent of all manuscripts now accepted for publication are submitted electronically and edited directly on computer, greatly increasing the efficiency of the editorial process and the accuracy of the final product.

Another major accomplishment was the acquisition and staffing of an in-house desktop publishing system early in 1991. Since May 1991, *The Referee* has been produced completely in-house at a cost savings of some $1,000 per month in typesetting charges and an additional savings of $667 per month for outside editorial services. Starting with a portion of the July/August 1991 issue of the *Journal*, the *Journal* is now being typeset in-house, as well. Total real savings as a result of this capability are expected to be on the order of $40,000 per year when fully implemented.

In another area, preliminary studies have begun to convert the electronic, typeset/code-embedded version of the 15th edition of *Official Methods of Analysis* to a standard ASCII file. This will greatly facilitate preparation of the 16th edition of *Official Methods of Analysis* scheduled for publication in 1995 and also facilitate potential electronic editions of *Official Methods of Analysis*.

In new areas, the Publications Department oversaw the preparation and publication in 1991 of a new edition of *Quality Assurance Principles for Analytical Laboratories*, and the major new work titled *Ecology and Management of Food Industry-Pests*. Both are currently available and a must for most reference shelves. In addition, three-quarters of the work on revision of the *U.S. EPA Manual of Chemical Methods for Pesticides and Devices* has been completed and the "1990 FDA Pesticide Residues Report" was published. While this was on-

going, the department was coordinating an advisory group to work on the development of a series of monographs on sampling. These will eventually be bound into a comprehensive manual on field and laboratory sampling for analytical purposes.

### *Conclusion*

In closing, it should be noted that this report is only a summary of some significant activities and accomplishments at the AOAC staff level. Many significant and day-to-day accomplishments have been left out. It should also be noted that it reports nothing about the many, many accomplishments of the volunteers serving AOAC. For those good works, you will have to 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 AOAC 1990 fiscal year was completed as of December 31, 1990. The accounting records of the Association were audited by the certified public accounting firm of Harab, Kamerow, and Associates of Rockville, MD. The results of that audit were reported to the Board of Directors. (The amounts reported on in this report are from the AOAC General Fund and do not include Restricted Fund Balances.)

The AOAC operations for the 1990 fiscal year produced a surplus of revenue over expenditures in the amount of $1,063,707. This surplus exceeded even our budget expectations of an $880,000 surplus. Total FUND BALANCE or NET WORTH of AOAC at the end of the 1990 fiscal year rose to $2,594,000.

At the completion of the audit, the auditors, in addition to providing the financial report to the Board of Directors, provide a management letter. This management letter outlines suggestions for improvements in our accounting systems. This fiscal year's reportable conditions and suggestions for improvements contained only a few minor items, all of which have been addressed in an appropriate manner by AOAC staff.

The fiscal position of AOAC is very solid. Our balance sheet, which represents our ASSETS, our LIABILITIES, and our NET WORTH, is very strong indeed. At the end of 1990, we had $3,479,000 in ASSETS and only $885,000 in LIABILITIES. That means that we had more than $3.00 in assets for every $1.00 that was owed to creditors. Further, the bulk of our assets ($2.8 million) are liquid and are invested in federally insured certificates of deposit.

The fiscal health of our Association is very strong and viable. The prognosis is excellent, dependent mostly upon continuing wise and prudent management of our enterprise.

***Statement of Financial Condition***
***December 31, 1990***
***Total All Funds***

| | |
|---|---|
| ***Assets:*** | |
| Cash | 405,762 |
| Accounts receivable (net of allowance of $4,088) | 67,025 |
| Contracts and grants receivable | 94,919 |
| Accrued interest receivable | 65,509 |
| Prepaid insurance | 1,951 |
| Deferred expenditures | 17,726 |
| Inventory | 339,658 |
| Advances | 2,433 |
| *Total Current Assets:* | $ 994,983 |
| Investments | 2,468,263 |
| Deposits | 15,829 |
| Furniture and equipment (net of accumulated depreciation of $394,126) | 165,986 |
| *Total Assets:* | $ 3,645,061 |
| *Liabilities:* | |
| Accounts payable | $ 84,234 |
| Accrued leave | 40,434 |
| Accrued salaries | 17,364 |
| Payroll withholding | 615 |
| Deferred income | 511,425 |
| Lessor incentive—current portion | 5,871 |
| Deferred rent obligation—current portion | 29,466 |
| *Total Current Liabilities:* | $ 689,409 |
| Lessor incentive—long-term portion | 51,501 |
| Deferred rent obligation—long-term portion | 235,728 |
| *Total Liabilities:* | $ 976,638 |
| ***Fund Balances:*** | |
| Unrestricted fund balance | 1,993,923 |
| Reserve—16th Edition | 600,000 |
| Harvey Wiley | 50,604 |
| FAAM | 14,676 |
| ADAM | 6,895 |
| MAM | 2,325 |
| *Total Fund Balances:* | $ 2,668,423 |
| *Total Liabilities and Fund Balances:* | $ 3,645,061 |

### *Sustaining Members and Supporting Government Agencies*

*November 5, 1991 (205)*

Advanced Instruments, Inc.
Agriculture Canada
Agway Technical Center
Alabama Department of Agriculture and Industries
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
Arizona State Agriculture Laboratory
Arkansas State Plant Board
Association of American Feed Control Officials, Inc.
Association of American Pesticide Control Officials, Inc.
Association of American Plant Food Control Officials
Association of Public Analysts (UK)
Atochem North America, Inc.
J.T. Baker Inc.
BASF Corporation Chemicals Division
Beckman Instruments, Inc.
Bio-Rad Laboratories
Blue Diamond Growers
Boehringer Mannheim
Borden, Inc.
Bristol-Myers-Squibb
U.S. Pharmaceutical & Nutritional Group
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
Charm Sciences (formerly Pencillian Assays, Inc)
Chemical Waste Management, Inc.
Chesebrough-Pond's, Inc.
Chevron Chemical Company
CIBA-GEIGY Corporation
Agricultural Division
The Coca-Cola Company
Colorado Department of Agriculture
CompuChem Corporation
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
Duphar BV
E.I. DuPont 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
GAF Corporation
Galbraith Laboratories, Inc.
E & J Gallo Winery
General Foods Corporation
General Mills, Inc.
GENE-TRAK Systems
Georgia Department of Agriculture
Gerber Products Company
Good Housekeeping Institute
Grand Metropolitan Laboratories
Gujarat State Fertilizers Company Limited
Haarman & Reimer Corporation
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.
Iowa Department of Agriculture
Iowa State Veterinary Diagnostic Laboratory
JEM Laboratory Services
S.C. Johnson & Son, Inc.
Kansas State Board of Agriculture
Kellogg Company
Kemira Oy
Kentucky Agricultural Experiment Station
Division of Regulatory Services
Kraft General Foods
The Kroger Company
Kuwait Institute for Scientific Research
Laboratorio di Chimica Analitica Applicata SNC (Italy)
Laboratorio Technologio del Uruguay
Laboratory of the Government Chemist (UK)
Lancaster Laboratories, Inc.
Land O'Lakes, Inc.
Lehn & Fink Products Company
Libra Laboratories
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
Ministry of Agriculture, Fisheries and Food (UK)
Minnesota Department of Agriculture
3M Company
Mississippi State Chemical Laboratory
Mobay Corporation
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 Jersey Department of Agriculture
New Mexico Department of Agriculture
New York Department of Agriculture and Markets
Nicolet Instrument Corporation
NIR Systems
North Carolina Department of Agriculture
North Dakota State Laboratories Department
Northeast Laboratories, Inc.
Novo Biochemicals
The NutraSweet Company
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
Oxoid USA, Inc.
Pennsylvania Department of Agriculture
Pfizer Inc.
Pharmacia AB
Philip Morris USA
Pitman-Moore, Inc.
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 Crop Protection Corporation
Saskatchewan Soil Testing Laboratory
Savannah Cocoa, Inc.
Joseph E. Seagram & Sons, Inc.
Shaklee Corporation
ShriRam Institute for Industrial Research
Silliker Laboratories, Inc.
SmithKline Beecham Animal Health Products
South Carolina Department of Agriculture
South Dakota State Chemical Laboratories
Spencer Laboratories
E.R. Squibb & Sons, Inc.
A.E. Staley Manufacturing Company
State Laboratory (Ireland)
Swift-Eckrich, Inc.
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
Utah State Department of Agriculture
Vitek Systems
Wallace Laboratories
Division of Carter-Wallace, Inc.
W.M. Ward
Warner Lambert Company
Washington State Public Health Laboratory
Waters, Division of Millipore
Webb Technical Group, Inc.
Welch Foods, Inc.
Westreco, Inc. (formerly Calreco)
Wisconsin Department of Agriculture
Trade/Consumer Affairs
Wyoming Department of Agriculture
Zoecon Corporation

## Editorial Board



### *Recommendations for Board of Directors*

The Editorial Board recommends that the Board of Directors approve the "Policy on Submission of Material to *The Referee*." This policy states that material submitted for publication in *The Referee*, whether a commentary, a letter to the editor, or other correspondence, shall be subject to the following: *(1)* AOAC reserves the right to publish or to edit submitted material for the purposes of length, form, grammar, and appropriateness; *(2)* Prior to publication, AOAC will, in good faith, attempt to resolve with the author any questions of content and style; *(3)* Submission of material for publication in *The Referee* does not automatically ensure publication. Final selection of materials submitted for publication rests solely with the editor of *The Referee*.

### *General Report*

The Editorial Board activities for 1991 were highlighted by the publication of 2 books and *Changes in Official Methods of Analysis of the AOAC*, Second Supplement, 1991. The books included the publication of the second edition of *Quality Assurance Principles for Analytical Laboratories* by Frederick M.

Garfield and *Ecology and Management of Food-Industry Pests*, produced under the direction of the U.S. Food and Drug Administration, J. Richard Gorham, Editor.

The Editorial Board meetings of May 17 and August 10, 1991, focused on developing an aggressive strategy for addressing the critical decline in the number of manuscripts submitted to the *Journal of AOAC International* and on putting in place the necessary guidelines and policies. In the Editorial Board's view, there are several levels at which these initiatives should proceed, and these were discussed at the fall meeting. Short-term strategies include: *(1)* Establishing contacts in Europe and at universities. Terms of reference for the Academic Liaison Committee were approved. *(2)* Broadening the scope of the *Journal* through the addition of new sections. *(3)* Increasing awareness of the advantages of publishing in the *Journal*. Letters were sent to all symposia and poster presenters at the 1991 AOAC annual meeting inviting them to submit their material for consideration of publication in the *Journal*. In addition, letters of invitation were also sent to authors who have published papers in other journals that would be suitable for publication in the *Journal*. *(4)* Streamlining the manuscript review process. To ensure the quality and timeliness with which manuscripts are reviewed for publication in the *Journal*, the Reviewer Checklist Form has been simplified and an "Instructions to Reviewers" sheet has been drafted outlining basic criteria by which reviewers judge manuscripts submitted for *Journal* publication. Longer term strategies include modifying the rigid style of manuscript presentation that is currently demanded for all *Journal* manuscripts to allow the use of more common scientific writing styles for papers. This approach could be considered for manuscripts that do not deal with collaborative methods. This initiative will be more fully developed at the spring meeting. An updating of the cover, format, and style of the *Journal* to reflect the name change to AOAC International was discussed, and examples will be presented at the spring meeting.

Additional Editorial Board activities included the review and approval of the Editorial Board's revised "Terms of Reference," and a review and revision of its short- and long-term goals, which are now separate from the "Terms of Reference" and will be reviewed prior to each meeting to more closely monitor the completion of Editorial Board activities. The Board of Directors approved the revised Editorial Board "Terms of Reference" but amended the terms of service for members to a maximum of 2 consecutive 3-year terms, instead of the previous one 6-year term.

Joseph Sherma, one of the Section Editors, has been asked to become a member of the Editorial Board. His participation, and the more active input of the other Section Editors, will greatly assist the Editorial Board in its drive to increase the number of manuscripts submitted to the *Journal*. Richard Durst is a new Editorial Board member, and it is anticipated that because of his position as Professor of Chemistry at Cornell University and Director of Analytical Chemistry Laboratories at the New York State Agricultural Experiment Station, he will be able to assist the Editorial Board in developing the essential liaison between AOAC and universities.

Other Editorial Board activities included the approval of "Performance Review Criteria for AOAC *Journal* Editors." The "Policy of Submission of Material to *The Referee*" was approved by the Editorial Board and will be submitted to the Board of Directors for approval. The Editorial Board is finalizing a booklet to assist potential authors of books to develop their proposals for consideration by AOAC. The booklet outlines the various categories of AOAC publications and provides specific details on the way the authors should develop their proposals.

### *Objectives for the Coming Year*

The Editorial Board will continue its on-going goals of increasing *Journal* manuscript submissions, promoting *Journal* visibility, and identifying and overseeing the development of publications.

Specific objectives for the coming year include overseeing the development of a compendium on sampling, finalizing a "Book Publications Guidelines and Publishing Procedures," and forming an Editorial Advisory Committee for Europe and academia to increase *Journal* manuscript submissions from Europe and universities.

The *Journal* Section Editors Performance Committee will review the performance of the Section Editors using the performance review criteria approved by the Editorial Board at its Spring 1991 meeting.

Finally, the Editorial Board will take a more aggressive approach to publishing AOAC sponsored symposia, short courses, or topical conferences in the *Journal* or as separate monographs by ensuring that symposia organizers encourage presenters to prepare their presentations for AOAC publication.

# Official Methods Board

MICHAEL H. BRODSKY, CHAIRMAN
Chief, Environmental Bacteriology & Microbiological Support Services, Ontario Ministry of Health, Laboratory Services Branch, Etobicoke "B," ON, Canada

Other Members: R.H. Bowers; H.H. Casper; R.H. Collier; P.E. Corneliussen; R.A. Martin; D.A. Mastrorocco; R.M. Montgomery; A.B. Strong

## *Recommendations for Board of Directors*

The Official Methods Board requests that the Board of Directors move adoption of the following recommendations:

A. Accept the report of the Task Force on Equivalency and adopt as AOAC policy.

B. Accept the recommendations of the Task Force on Methods Adoption as current AOAC policy with the understanding that streamlining the system will be a continuous challenge for the Official Methods Board.

C. Discontinue the *Handbook for AOAC Members* as a separate publication and incorporate the pertinent information into the AOAC Membership Directory so that it can be updated annually.

## *Basis for Recommendations*

A. The report of the Task Force on Equivalency was presented to the Board of Directors for consideration at its meeting in December 1990. At that time, the Board of Directors expressed some concerns with respect to the practical implementation and interpretation of the policy. Written comments were referred to the Task Force chairman, Richard M. Montgomery. A reviewed report was prepared and submitted to the Official Methods Board at its May 1991 meeting in Boston, MA. The Official Methods Board unanimously agreed that all issues of concern raised by various members of the Board of Directors had been adequately addressed and approved the Task Force's recommendations.

B. The procedures for method adoption by the Official Methods Board, under the revised AOAC Constitution, were presented to the Board of Directors for consideration at its meeting in December 1990. At that time, the Board of Directors expressed some concerns with the time frames and the apparent detailed bureaucracy of the approvals process. Subsequently, the approvals process was simplified and the time frames significantly modified. This revised protocol was presented to the Official Methods Board at its May 1991 meeting and approved by 7 of the 8 Official Methods Board members. The concerns of the dissenting member were considered at the August 10, 1991, meeting and were resolved to the satisfaction of the entire Official Methods Board. These procedures formed the basis for the educational symposium "Managing the Method Validation Process" presented to methods volunteers at the 1991 AOAC annual meeting.

C. The *Handbook for AOAC Members* is outdated and would require annual updating to ensure that the membership is receiving current information. Too many documents are floating around purporting to be operational Official Methods Board policies and guidelines. We need a consistent source of information to improve the education of AOAC members and volunteers.

## *Meetings*

The Official Methods Board met 4 times during the year to fulfill its primary responsibility for administering the AOAC collaborative study and approval process: September 13, 1990, at the 1990 AOAC annual meeting in New Orleans, LA; January 10–12, 1991, in St. Petersburg, FL; May 30–June 1, 1991, in Boston, MA; and August 10, 1991, at the 1991 AOAC annual meeting in Phoenix, AZ. A report from the fall and winter meetings was published in *The Referee* to inform the membership of the Official Methods Board activities and outline key dates for methods volunteers.

## *Awards*

The Official Methods Board annually coordinates the selection process for recognition of excellence in performance by Associate and General Referees:

A. In 1991, the Official Methods Committee nominees for General Referee of the Year were: Committee on Pesticide Formulations: Alan R. Hanks (CIPAC Studies); Committee on Drugs and Related Topics: Linda Ng (Drugs IV); Committee on Foods I: Margaret A. Clarke (Sugars); Committee on Microbiology and Extraneous Materials: Wallace H. Andrews (Foods Microbiology-Nondairy); and Committee on Feeds, Fertilizers, and Related Materials: P. Frank Ross (Veterinary Analytical Toxicology). During the May 1991 meeting, the Official Methods Board voted to select Andrews as the winner of the General Referee of the Year Award for 1991.

B. One of the Associate Referee awards recognizes the single best collaborative study (approved interim official first action prior to the AOAC annual meeting) as the Collaborative Study of the Year. The selection is based on scientific innovation, practical application, and the overall merit of the collaborative study report. The Official Methods Board selected "Fatty Acids in Encapsulated Fish Oils and Ethyl Esters, GC Method" by Jeanne Joseph as the winner of the Collaborative Study of the Year Award for 1991.

C. The recipients of the Methods Committees Associate Referee of the Year Awards for 1991 were: Eileen Bargo, Associate Referee for Benzodiazepines, by the Committee on Drugs and Related Topics; Sung Soo Lee, Associate Referee for Enzymatic Gravimetric Method for Dietary Fiber, by the Committee on Foods II; Phyllis Entis, Associate Referee for Aerobic Plate Count and Colliforms-HGMF Method, by the Committee on Microbiology and Extraneous Materials;

Michael Olden, Associate Referee for Nicotine in Environmental Tobacco Smoke by the Committee on Feeds, Fertilizers, and Related Materials; and Kenneth Edgell, Associate Referee for Pesticides in Groundwater, by the Committee on Environmental Quality.

### *Task Forces*

Three task forces were working this year to provide solutions and guidance for dealing with or managing various problem areas.

A. The Task Force in Equivalency was created in 1989 as an expansion of the Instrumental Methods Task Force and was chaired by Montgomery. The Task Force's final report was accepted by the Official Methods Board at its May 1991 meeting, and the Task Force was dissolved with the thanks of the Official Methods Board chairman.

B. The Task Force on Proprietary Methods was created in 1990 as an extension of the mandate accorded to its predecessor, the Task Force on Test Kits. This Task Force was cochaired by Raymond H. Bowers and Robert A. Martin. The Task Force's recommended "Policy Guidelines for Collaborative Study of Proprietary Technology Based Methods" was approved by the Official Methods Board at its January 1991 meeting in St. Petersburg, FL, and accepted by the Board of Directors in March 1991. The Task Force was dissolved with the thanks of the Official Methods Board chairman.

C. The Task Force on Methods Adoption was created in 1989 and chaired in 1990–1991 by Michael H. Brodsky. The recommendations of this Task Force continue to evolve. The adoption process recommended by the Task Force at the May 1991 meeting of the Official Methods Board was adopted by the Official Methods Board and is recommended for approval by the Board of Directors.

### *Other Actions, Activities, and Decisions*

In addition to establishing special task forces to address specific issues, the Official Methods Board also took other actions in keeping with its objectives and mission statement. A number of these decisions were or will be published in various issues of *The Referee*, including:

A. Ten collaboratively studied methods were reviewed and approved as AOAC First Action at the May 1991 meeting of the Official Methods Board.

B. Nine collaboratively studied methods were reviewed and approved as AOAC First Action at the August 1991 meeting of the Official Methods Board.

C. Software for calculating collaborative study statistics using Lotus 1-2-3 has been developed, and the Statistics Committee is currently validating its performance. Appropriate mechanisms for distribution to method volunteers is under consideration.

D. Definitions of and requirements for various types of changes, i.e., editorial, minor change, method modification (including extension to other matrixes), and substantive revision, to previously adopted methods were adopted.

Other actions and decision taken in 1991 were:

E. The appointment of members of the Safety Committee to each of the methods committees to ensure that safety concerns are addressed in all collaboratively studied methods.

F. A revised checklist, to ensure the completeness of protocol designs for collaborative studies, was developed and implemented by all method committees as part of the method validation process.

G. Several short- and long-term projects for 1990–1991 were completed: *(1)* A stronger liaison with the Laboratory Quality Assurance Committee was developed by having the Official Methods Board chairman appointed as an ex-officio member. *(2)* The Official Methods Board adopted the practice of requiring the inclusion of Safety Data Sheets in AOAC collaboratively study protocols. *(3)* The status of methods committees and topic alignments continues as an ongoing project. *(4)* A completely revised and updated Official Methods Board Policy and Procedures Manual was developed and distributed to each Official Methods Board member with the understanding that it would be passed on to their successor following the completion of the term of service.

H. The volunteer education session at the 1990 AOAC annual meeting covered the following topics: *(1)* Brodsky briefly reviewed the history and structure of AOAC with particular attention to the evolution of the methods validation process. *(2)* Nancy Palmer discussed the role of the AOAC headquarters and, in particular, the Methods Coordinator. *(3)* Richard H. Collier reviewed the procedures for appointment and role of the Associate Referee. *(4)* Bowers reviewed the Checklist for Protocol Design. *(5)* Montgomery reviewed the Official Methods Board operational policy on Equivalency. *(6)* Donald A. Mastrorocco reviewed the Official Methods Board policy on proprietary methods. *(7)* Howard H. Casper reviewed the procedures for appointment and role of the General Referee. *(8)* Terry Nelson, substituting for John G. Phillips, reviewed the role of the Committee Statisticians and the statistical parameters for both in-house validation and interlaboratory collaborative studies, illustrating the different requirements for qualitative and quantitative methods. *(9)* In the absence of Mark G. Torchia, Brodsky reviewed the role of the Committee Safety Advisors and the Safety Checklist. *(10)* Martin discussed the structure and role of the methods committees and the responsibilities of the methods committee chairmen. *(11)* Ann B. Strong reviewed the Official Methods Board Checklist for Approval of Collaborative Studies. *(12)* Paul E. Corneliussen reviewed how collaborative studies conducted outside AOAC are brought on-line and the requirements for compliance with AOAC specifications. *(13)* Brodsky summarized by reviewing the role of the Official Methods Board with respect to the approvals process, including First Action, Final Action, Appeals, Repeals, Surplussing, and requests for method action. *(14)* Hanks discussed current thinking of the AOAC Task Force on Validated Methods regarding the status of alternate pathways for achieving "lesser validated status." *(15)* Frank Thomas concluded the session by reviewing the activities of the AOAC Task Force on Test Kits and the $R^2$ process.

The Official Methods Board thanks all of the volunteers involved in the methods validation, review, and approval process for their contributions to a successful year. It is with a tinge of sadness that we bid adieu to those methods committee chairmen who are completing their terms of service: Raymond H. Bowers, Committee on Foods I; Robert A. Martin, Committee on Foods II; Donald A. Mastrorocco, Committee on Microbiology and Extraneous Materials; and Howard H. Casper, Committee on Feeds, Fertilizers and Related Topics.

The conduct of the duties and responsibilities of the Official Methods Board requires considerable AOAC staff support. Even with the turmoil of changing the basis of the methods approval process, the Official Methods Board managed to accomplish all the short-term tasks defined in its 1990–1991 Activities List and made significant progress in both its short- and long-term goals for 1991–1992. For these achievements, under adverse conditions, we salute all of our AOAC staff.

## Archives Committee

CHARLOTTE A. BRUNNER, CHAIRMAN
U.S. Food and Drug Administration, HFD-473, 200 C St, SW, Washington, DC 20204

Other Members: T. Alexander; S. Barkan; P. Bulhack; W.V. Eisenberg; W. Landgraf; H.L. Reynolds; E. Sarnoff; H.M. Stahr

The revised Terms of Reference for the Archives Committee were submitted to the Committee for comments. Additional revisions were incorporated, and the newly revised Terms of Reference were approved.

Funds ($500) have been sent to the Park Library at Iowa State University for the restoration of archival photographs maintained there.

A permanent display cabinet has been purchased and will be installed shortly in AOAC headquarters. When the display cabinet has been installed, a notice will be placed in *The Referee* to request donations of small pieces of old laboratory equipment for a permanent display. It was decided that the theme for the Committee's exhibition at the 1992 AOAC annual meeting would be the history of AOAC name changes, from the Association of Official Agricultural Chemists to the Association of Official Analytical Chemists to AOAC International. Thomas Alexander has volunteered to work on this project. The display for 1992 will also show the chronological development of particular instruments (i.e., the pH meter). The discussion was extended to include possible themes for the Committee's exhibition at the 1993 AOAC annual meeting in Washington, DC. It was suggested that an interesting subject would be the history of the Harvey Wiley Award.

Helen L. Reynolds reported that she transcribed the oral history that Alexander obtained from AOAC Past President James Minyard. Minyard corrected the transcription, and the tape and transcription will be deposited in the AOAC archives. A copy was given to each member of the Committee. It was agreed that excerpts from the oral history will be published in *The Referee* when space is available.

Reynolds volunteered to prepare the obituary for Joseph Levine, who was very active in AOAC, particularly with the Committee on Drugs (formerly Subcommittee B). The necessary information will be supplied by Charlotte A. Brunner.

Members of the Committee have agreed to interview Reynolds, AOAC past president, to obtain her oral history.

A lengthy discussion ensued on the compilation and publication of 2 books: a set of photographs and biographical sketches of AOAC past presidents, and the history/records of AOAC Bylaws. The Committee concluded that neither the AOAC staff nor the Committee members had the necessary resources to develop either book theme in the near future. However, Alexander volunteered to investigate the feasibility of obtaining the necessary information on the past presidents from available materials. A decision on the project was deferred until 1992, pending Alexander's report on the results of his research.

A notice will be placed in *The Referee* to solicit new members for the Archives Committee.

## 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 Board of Directors*

The Bylaws Committee recommends that the Board of Directors authorize the Committee to undertake a comprehensive review of the AOAC Bylaws and recommend to the Board any changes necessary for clarity, internal consistency, or improved functioning of AOAC International.

### *General Report*

The Committee noted that since the last major review and revision of the Bylaws, more than 15 amendments have been voted by the AOAC membership. In addition to a review of the AOAC Bylaws, the Committee would also review the Model Bylaws for Regional Sections to ensure their compatibility.

If the Committee recommendation is approved by the AOAC Board of Directors, the Committee chairman will send each member a plan for accomplishing the review. The chair-

man hopes to distribute this plan by October 15, 1991. Although the Committee would work primarily by mail and telephone, it would be necessary to schedule a meeting in late March or early April 1992 to produce a finished draft of the revised Bylaws. This would allow sufficient time for the necessary legal review prior to placing any amendments on the 1992 ballot.

The Committee noted that since it presently has no charges from the Board, it will become dormant until the Board issues new charges or approves the charge recommended above.

# AOAC INTERNATIONAL BYLAWS

## Revised September 15, 1991

### 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.

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 regional 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 Past Presidents of the Association, provided that, if Past Presidents are not available to serve, other Members shall be appointed to the extent necessary to constitute the three member committee.

*Section 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 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 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, 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 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 regional sections, and approve bylaws adopted by the regions
- consider recommendations of the Standing and Special Committees
- review all proposed amendments to the bylaws which are received by the Board and submit the proposals, with Board recommendations, to the membership in accordance with procedures in these bylaws
- upon dissolution of the Association, distribute remaining assets in accordance with these bylaws and other applicable authorities.

*Section 3. Meetings*

A. Regular meetings shall be held at the call of the President, or as ordered by the Board.

B. Special meetings of the Board may be called by the President.

C. The President shall preside at all meetings except as otherwise provided in these bylaws.

*Section 4. Voting*

A mail ballot may be authorized by the President.

## ARTICLE VII
**Appointed Officers**

*Section 1. Appointed Officers*

The Board of Directors may appoint such individuals as are necessary to carry out the following functions: execute the directives and policies of the Board; supervise the Business Office and perform any duties necessary in the day-to-day management of the Association; keep the minutes of the meetings of the Members and the Board of Directors; see that all notices are duly given in accordance with the provisions of the bylaws; keep a register of membership of the Association and their addresses; edit and produce the publications of the Association.

An appointed officer with concurrence of the President shall appoint General Referees from the Members of the Association, one for each of the general subjects designated by the Official Methods Board.

An appointed officer with concurrence of the President shall appoint Associate Referees on subjects complementary to the general subjects assigned to the General Referees. In lieu of an Associate Referee, the appointed officers with concurrence of the President may appoint an Associate Referee Committee of two or more persons, designating one as Chairman. Appointment of General Referees, Associate Referees, and members of an Associate Referee Committee must have the approval of each appointee's appropriate supervisor.

*Section 2. Liaison Officers*

The Liaison Officers shall coordinate the development and adoption of uniform analytical methods between the Association and related interested organizations.

## ARTICLE VIII
### Editorial Board and Standing Committees

*Section 1. Editorial Board*

The Editorial Board shall be responsible for developing, editing, and publishing the publications of the Association. The appointed officer responsible for editing and producing the publications of the Association shall be a member *ex officio*.

*Section 2. Committee on the Wiley Award*

The Committee shall consist of six (6) members with 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 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 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. No waiver of membership dues shall be granted except that members serving as Associate Referees at the close of the 1989 Business Meeting of the Association may continue to receive waivers, upon written request, for any membership year in which their service as Associate Referee remains essentially unbroken.

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 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
**Regional Sections**

*Section 1. Regional Sections*

The Board of Directors shall set geographic limits and grant authority to groups of Members of the Association residing or working in the same geographical regions for the establishment of regional sections.

*Section 2. Purpose of Regional Sections*

The purpose of regional sections shall be to promote and sponsor the purpose of the Association.

*Section 3. Membership in Regional Sections*

Membership in a regional section shall consist of Members and Honorary Members of the Association residing and working within the geographical boundaries of the section. Other individuals interested in the purpose of the regional section and residing and working within the boundaries of the section shall also be eligible for section membership but shall not be eligible for election to the Executive Committee for the section. No person shall be a member of more than one regional section.

*Section 4. Bylaws of Regional Sections*

Each regional section shall adopt for its own government, subject to approval of the Board of Directors, bylaws not inconsistent with these bylaws.

*Section 5. Dissolution of Regional Sections*

When any regional section shall cease to function as a section for a period of more than one year, or if its membership shall be less than 10 Members of the Association for a period of one year, the Board of Directors may terminate the existence of such regional section.

*Section 6. Actions of Regional Sections*

No act of a regional 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.

## Fellows Committee

THOMAS P. LAYLOFF, CHAIRMAN
U.S. Food and Drug Administration, Division of Drug Analysis, 1114 Market St, Rm 1002, St. Louis, MO 63101

Other Members: E.A. Bunch; T. Fazio; E.R. Jackson; F.J. King; J.E. McNeal; A.E. Waltking

The Committee recommended and the Board of Directors approved the following candidates for the 1991 Fellow of the AOAC Award:

Ruth Bandler, Food and Drug Administration, Washington, DC; Reginald W. Bennett, Food and Drug Administration, Washington, DC; Stephen G. Capar, Food and Drug Administration, Washington, DC; Lester Hankin, Connecticut Agricultural Experiment Station, Hartford, CT; Mary Lee Hasselberger, Nebraska Department of Agriculture, Lincoln, NE; Dean Kassera, McLaughlin Gormley King Co., Minneapolis, MN; James F. Lawrence, Health and Welfare Canada, Ottawa, ON, Canada; and John O'Rangers, Food and Drug Administration, Rockville, MD.

## Interlaboratory Studies Committee

RICHARD ALBERT, CHAIRMAN
U.S. Food and Drug Administration, HFF-116, 200 C St, SW, Washington, DC 20204

Other Members: M.H. Brodsky; D.W. Fink; W. Horwitz; P.C. Kelly; K.A. McCully; J. O'Rangers; R.C. Rund; L.R. Williams; R. Wood

### *Recommendations for Board of Directors*

The Committee, which is now terminating, recommends that the Board of Directors present the following problems and concerns to a future focused task force or subcommittee:

A. The recommendation by Dennis Pocklington of the United Kingdom that collaborative studies have a fixed and rigid format for reporting method performance parameters has been favorably received.

B. The collaborative study (eventually to be called "interlaboratory method performance studies") of automated methods has been a general concern.

C. The collaborative study of qualitative methods has also been a general concern, especially in view of the "Reviewed and Recognized" $R^2$ procedure being developed.

D. The software being used and developed for computing precision parameters needs on-going validation.

E. Recoveries and limits of detection are parameters for collaborative studies that may need to be determined more often.

F. A "Dear Abacus" column containing questions and authoritative answers would be of value to those undertaking collaborative studies.

G. The recommendation from the Statistics Committee that "sensitivity rate" and "specificity rate" be among the parameters evaluated in collaborative studies of qualitative methods has been favorably received.

### *Basis for Recommendations*

A. A fixed and rigid format for reporting method performance parameters in the *Journal of the AOAC* will benefit information retrieval. But, will one format work for the collaborative study of all quantitative methods? The Editorial Board and other committees need to comment before the format is adopted.

B. Automated methods may not have repeatability (i.e., within laboratory) standard deviation. Equivalency can also be questioned because the devices are heavily dependent on the manufacturer. The fate of the method may depend on the fate of the manufacturer.

C. The estimate or verification of a rate (e.g., sensitivity rate and specificity rate) requires a minimum of 15 independent estimates as now required for collaborative studies for qualitative methods.

D. The Committee, and other sources, now provide computer programs to implement the IUPAC-87/AOAC adopted algorithms for estimating the measures of precision from a quantitative collaborative study. The Committee hopes that these programs have been adequately validated.

E. Materials containing known concentrations of the analyte of interest are often not available. Therefore, collaborative studies rarely address the issue of recovery. Limits of detection may be determined by the preliminary development of a method by a single laboratory, but a collaborative study may yield verification or refinement of these estimates.

F. Key questions are regularly asked. It would be convenient to have these questions and their authoritative answers available in a readily accessible format such as *The Referee.*

G. The Committee has agreed that "sensitivity rate" is the percent of truly positive samples that are found by the method to be positive; "selectivity rate" is the percent of truly negative samples that are found by the method to be negative. "Rate" was appended to avoid confusion with "sensitivity" as used by many chemists. These rates can be expressed in terms of the statistician's alpha (false positive rate) and beta (false negative rate).

## International Coordination Committee

ROGER WOOD, CHAIRMAN
Ministry of Agriculture, Fisheries and Food, Food Science Laboratory, 65 Romney St, London SW1P 3RD, United Kingdom

Other Members: C.Y. Ang; R.A. Case; L.H. Chen; A.R. Hanks; A.J. Harrison; G. Henniger; E. Hopkin; W. Horwitz; M. Ihnat; J.R. Iturbe; K. Naguib; D.L. Park; A.E. Pohland; R.C. Rund; M. Santoro; M. Smith; A. Williams

### *Recommendations for Board of Directors*

The International Coordination Committee (ICC) recommends that the Board of Directors consider abolishing and replacing the Committee by a strengthened liaison program and, thus, heighten the Association's awareness of international activities and developments.

### *Basis for Recommendations*

The Committee applauds the name change of the Association and the appointment of George R. Heavner as an AOAC staff member to specifically maintain international liaison. It is appreciated that the Association must maintain, and, indeed, increase its awareness of activities taking place in its national and international sister organizations. To achieve that, the Association should: *(1)* Maintain the coordination exercise that has been undertaken over the past 3 years. *(2)* Institute a strengthened liaison program with other national and international organizations. These liaison officers should maintain contact with Heavner on a regular basis, not on an annual basis. Such liaison officers should be Association members who are active in other organizations and, thus, able and willing to report back to the AOAC staff member who should advise the Board of Directors and the Official Methods Board of both "scientific" and "administrative" activities in other such organizations. It is further suggested that the invited (not appointed) liaison officers discuss their activities in an informal awareness meeting to be held during the AOAC annual meetings.

Now that an AOAC staff member has been identified to handle international affairs, ICC considers it essential that the Association's activities be focused through that member to avoid duplication and misunderstandings.

### *General Report*

Reports from a number of international standardization organizations were reviewed and discussed by the Committee. The main points of note arising from the discussion were: *(1)* Most of the Association's sister organizations have adopted the Harmonization Protocol for the validation of analytical methods. *(2)* The IUPAC/ISO/AOAC joint protocol on the Harmonization of Laboratory Proficiency Testing Schemes will be discussed and approved in a further meeting of the "Harmonization Working Group" planned in 1992. *(3)* The activity of the European Committee for Standardization (CEN) has increased the development and adoption of methods of analysis for foodstuffs.

It was noted that the 1990 reports had been abstracted by the chairman of ICC to form an agenda item at the April 1991 session of the Codex Committee on Methods of Analysis and Sampling (CCMAS).

It was anticipated that the chairman would be asked to prepare a similar paper for the 1992 session of CCMAS. The information contained in that paper would be made available to the Association's membership.

In addition, the complete collated report would be circulated to members of the Official Methods Board for their information. It was agreed that the "coordination" exercise should continue because it is proving to be particularly valuable.

## Laboratory Quality Assurance Committee

EUGENE J. KLESTA, CHAIRMAN
Quality Assurance Programs, 4300 W. 123rd St, Alsip, IL 60658

Other Members: R. Alvarez; M.H. Brodsky; P.O. Campbell; J.F. Cavins; B. Cottingham; E. Easterly; L.L. Gough; J. Hardy; J.H. Hirsch; J.E. Longbottom; K.A. McCully; R.J. Noel; C. Weaver

### *Recommendations for Board of Directors*

A. The Committee recommends that the Board of Directors include a library of reference materials (analyte, matrix, and source) on an electronic bulletin board.

B. The Committee recommends that the Board of Directors include a reference in the Associate Referee guideline package to documents on the production of natural and synthetic reference materials and check samples.

### *Basis for Recommendations*

A. Communication needs to be improved on the availability of reference materials. AOAC members are not always aware of materials that could be used in collaborative studies. The development of an electronic bulletin board is being surveyed during the 1991 AOAC annual meeting. The addition of reference material information would allow users to search for appropriate materials by analyte and/or matrix, which would be included in collaborative studies or used in their own laboratory.

B. The U.S. Environmental Protection Agency has developed 3 documents to be used by producers of reference materials and check samples. The Associate Referees should be directed in their guidance package to the existence of these documents. The AOAC headquarters could supply the entire set of documents when requested. The documents should not be included entirely in the interest of reducing the amount of paper an Associate Referee needs to review. A single page will be developed to be included. When Associate Referees need to develop materials for collaborative studies they will be able to acquire guidance on how to accomplish that goal.

### *General Report*

*Old Business.*—A summary of past activities regarding check samples was given by Eugene J. Klesta. A survey of AOAC members had been conducted, and a report submitted to the Board of Directors. A task group was asked to investigate probable costs to produce a check sample. No information on this issue was received during the past year. A discussion occurred, which included sources of information on check samples, suggestions to review the official methods to determine needs, and ways to communicate information. It was agreed that a recommendation to the Board of Directors be made to include this topic on the electronic bulletin board.

James Ault suggested that the protocol checklist should be reviewed by the Committee for quality assurance issues. Keith A. McCully suggested that a reference material should be included in the collaborative study and that the use of reference materials should be included in official methods. It was agreed that the latest version of the protocol checklist would be obtained, distributed at the 1991 AOAC annual meeting, and reviewed by October 1, 1991. Any suggestions would be forwarded to Jerry Hirsch for consolidation.

The 3 documents submitted by James E. Longbottom from the U.S. Environmental Protection Agency were reviewed by the Committee. No substantive changes were suggested. To keep the Associate Referees informed, it was suggested that a one-page summary of the documents should be included in the Associate Referee package. It was agreed that Klesta and the AOAC staff liaison would work on the development of the page. The Committee agreed to recommend to the Board of Directors that this be accomplished.

Klesta summarized for the Committee the meeting accomplishments held in Geneva, Switzerland, in May 1991. The separation of accreditation from the document was critical in its further development. A long discussion on the determination of the "true" value took place in Geneva. It was resolved that the mechanism for determining the "true" value must be clearly documented to the participants. The meeting attendees agreed that the Z-score is to be used for reporting a participant's results. The Z-score is the difference of the laboratory's results from the "true" value divided by the standard deviation. A draft document will be circulated to the committee when it is received.

The second edition of Frederick M. Garfield's book has recently become available. The review and comments produced by the Committee members was publicly acknowledged. The project would not have been completed without the efforts of the Committee members.

*New Business.*—Committee members are reviewing a draft version of a quality assurance checklist for small laboratories in conjunction with a series of checklists submitted by L. Gough. Comments should be forwarded to Hirsch. The intent is to develop a checklist that small laboratories can use to maintain their quality assurance program. The final product may be included in the quality assurance short course or sold directly or some other arrangement to be determined. The ISO 25 document was distributed to all members to use when reviewing the quality assurance checklist. This project is expected to be completed by the 1992 AOAC annual meeting.

Wayne R. Wolf distributed brochures on BERM 5 to the Committee asking for submission of papers and general involvement of AOAC. It was suggested that he talk to Nancy Palmer regarding sponsorship. The symposium focuses on reference materials and was appropriate for this Committee to discuss.

Hirsch asked for an update on the Quality Assurance short course. Klesta responded that the course had been conducted in Sweden by Robert C. Rund and James P. Dux, and in Denmark by Klesta and Dux. It will be conducted on Thursday and Friday at the 1991 AOAC annual meeting and is scheduled for San Diego, CA, and Durham, NC, later in 1991. Attendance has been good. Some discussion ensued on how to select the attendees and whether different levels of short courses are needed. This topic will be discussed at future meetings. Ault asked for participation at the Midwest Regional Section meeting and quality assurance training sessions.

Hirsch has volunteered to serve as chairman of the Laboratory Quality Assurance Committee.

# 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; J.B. Bourke; M. Brodsky; M. Cerny; T. Jensen; J. Karr; E.J. Klesta; G. Lancette; M. Lauwaars; S. Lee; E. Meier; J. Michelson; D. Osheim; M. Siewierski; R. Stubblefield; H. Thompson; A. Viscido; R. Wood

### *Recommendations for Board of Directors*

A. The Committee recommends the following changes in the opening session of the AOAC annual meeting program: *(1)* the opening session should be no more than 2 hours long; *(2)* in place of a formal Wiley Award Address, a special exhibit booth should be developed to highlight the accomplishments of the awardee; *(3)* the opening session should consist of opening remarks; award presentations, with a brief acceptance from the Wiley Award winner; presidential remarks; keynote address; and, immediately following the keynote address, the exhibits should be opened by the President and the Wiley Award winner. (During the following 2 hours, the Wiley Award winner would be at her/his booth to receive personal congratulations, and the exhibitors would have the "undivided attention" of the meeting attendees.)

B. The Committee recommends that the Board of Directors increase the budget allocation for support of invited speaker travel and per diem. Each symposium chairman will be asked to submit requests for consideration by this Committee, with subsequent submission of a final budget to the Board of Directors for consideration. This Committee will consider these additional requests on a case by case basis. The Committee proposes that this additional cost be covered by increased meeting sponsorship recruitment.

C. The Committee recommends that, contingent on an acceptable cost analysis by AOAC staff, the Association adopt the criteria and guidelines of the Council on the Education Unit for all AOAC short courses. This will allow AOAC to issue Continuing Education Unit (CEU) credits for all AOAC short courses.

### *Basis for Recommendations*

A. Historically, Wiley Award Addresses have not sufficiently reflected all the accomplishments of the winners, nor has the current format permitted sufficient opportunity for personal interactions of the winner with other meeting attendees. The many concurrent activities during the meetings have contributed to vendor dissatisfaction and loss of vendor participation. The current length of the opening session has resulted in considerable dissatisfaction among many attendees.

B. The increased emphasis on international participation at the AOAC annual meetings and the resulting additional costs of meeting travel for invited symposia speakers from outside the United States and Canada necessitates that additional funding be available to address these requests. The Committee recognizes the budgetary considerations and the fact that these costs will fluctuate. The Committee will work with AOAC staff to facilitate additional sponsor participation, particularly with regard to increasing international sponsorship.

C. CEU is an internationally recognized set of guidelines and criteria for continuing education programs. "The CEU concept informs regulatory, accrediting, and licensing agencies about acceptable practices in continuing education programs and activities, and may be used as a measure for developing standards for continuing education." There will be some administrative costs involved, primarily for additional record keeping.

### *General Report*

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, but some vendor dissatisfaction in regard to lack of attendee traffic. The situation in regard to alternative lodging at the AOAC annual meetings was discussed. The general consensus of the Committee was that current AOAC staff policy of referral to external travel organizations was necessary and appropriate.

The Committee requested that, due to numerous complaints, AOAC staff review the feasibility of increased scheduling of committee and task force meetings for the weekend prior to the AOAC annual meeting to facilitate increased participation of committee members in the technical program. It was recognized that the functions of this Committee may require scheduling toward the end of the meeting.

Ridgell briefly discussed the "internationalization" of AOAC. A summary of the resulting Committee "brainstorming" session will be submitted as a separate report. The chairman will appoint a task group to facilitate internationalization initiatives. Where possible, all future symposia will have co-chairmen, one from America and one from outside America. The need for additional speaker support was discussed as proposed.

The 1992 meeting plan was reviewed. Roger Wood and Margreet Lauwaars agreed to prepare a list of recommendations for a keynote speaker to address a "global" perspective to EC 92.

In addition to the workshops on juice adulteration and mass spectrometry, workshops on supercritical fluid extraction and on antibiotics and drugs in feeds will be developed. Further discussions are necessary before any final decision on an additional workshop on immunoassays. The finalization of the program chairmen is scheduled for October 1, 1991. The

Committee suggested that AOAC have an exhibit booth, separate from the usual AOAC booth, with information on the test kit evaluation process.

Ridgell and the AOAC Meetings and Education Coordinator, Carol L Rouse, gave a review of the status of the short courses that are currently being offered. Eugene J. Klesta reported that the Quality Assurance Committee does not recommend any changes in the quality assurance short courses at this time. John B. Bourke reported that the Advisory Group on Sampling was developing a series of monographs on sampling. The Committee recommended that the sampling short course be deferred until the completion of the basic monographs and several commodity specific monographs so that the sampling short course be revised around these monographs. The Committee requested that AOAC staff contact Carol Henderson Garcia, regarding her development of a short course on "How to Testify as an Expert Witness." R. Beatty of Telecations Associates inquired through AOAC staff regarding the development of a LIMS short course. He will be requested to submit a formal proposal.

Rouse discussed information on the CEU process. The Committee requested that AOAC staff obtain the necessary cost information for this process. If these costs are reasonable, the Committee recommends that this process be approved as stated above.

The chairman requested that Klesta consult with the Task Force on Analyst Training and Certification with regard to Committee activities.

The chairman will appoint a working group to develop specific guidelines for presentations that contain commercial information. The Committee recommended that all submitted papers be reviewed by the session chairmen for adherence to these guidelines and general appropriateness with regard to excessively commercial presentations.

Several proposals for the 1993 AOAC annual meeting, including a symposium of chemical methods in human toxicology, were briefly considered. These were tabled for inclusion in subsequent planning of the 1993 AOAC annual meeting program.

## Membership Committee

DAVID OSHEIM, CHAIRMAN
USDA/NVSL, Chemistry Section, Box 844, Ames, IA 50010

Other Members: C.T. Bell; R.H. Bowers; S. Cannon; W.F. Carey; A.S. Carmen; B.L. Funk; D.C. Montague; M. Morrison; A.A. Peake; N. Thiex; J.T. Sabater; S.M. Walters

### *Recommendations for Board of Directors*

A. Recommend to the Board of Directors that unemployed members be allowed to waive their membership dues for 1 year, by written request and with the approval of the Executive Director or his designate.

B. Recommend to the Board of Directors that the AOAC staff send unemployed members a packet of available AOAC services.

C. Recommend to the Board of Directors that a certificate of membership be issued to all members.

D. Recommend to the Board of Directors that Article V, Section 2 of the AOAC Bylaws be amended to read that the Nominating Committee be made up of 3 past presidents and at least 2 members at large.

E. Recommend to the Board of Directors that the nominating committee recommend at least 2 names for each elected position.

F. Recommend that the Board of Directors reinstate the Governance Council to be made up of the chairmen of all nonmethods committees.

### *Basis for Recommendations*

A. Members who are unemployed may not be able to pay for membership. Member appreciation in this situation should produce a loyal and a more active member.

B. These are services that AOAC already offers, but this recommendation would set up a process that would get the information to the members who need it.

C. This service would improve member retention, increase the visibility of the Association, and help in the recruitment of new members.

D. This would improve membership involvement in the AOAC election process.

E. This recommendation would also improve the involvement of membership in the election process.

F. This recommendation would provide exchange of information between committees and would help prevent cross purposes in committee.

### *General Report*

The following were discussed: *(1)* different recruitment and retention programs for members, and the development of member services; *(2)* the need to continue to encourage the regional sections to contact key persons located at universities in their area; *(3)* ways of getting professors to post *The Referee* in their departments; *(4)* the need for AOAC to publish a list of alternative and less expensive accommodations at the AOAC annual meetings; and *(5)* the need for a liaison between the Membership and Regional Section Committees.

## Nominating Committee

ROBERT C. RUND, CHAIRMAN
Purdue University, Dept of Biochemistry, West Lafayette, IN 47907

Other Members: T.P. Layloff; O.L. Shotwell

The Committee recommended the following slate of officers for 1991–1992:

**President-Elect:** Henry B.S. Conacher, Health and Welfare Canada, Health Protection Branch, Food Research Division, Tunney's Pasture, Ottawa, ON, Canada

**Secretary-Treasurer:** Arvid Munson, Phoenix Regulatory Associates, Inc., Sterling, VA

**Directors:** Alan R. Hanks, Dept of Biochemistry, Office of the Indiana State Chemist, West Lafayette, IN

Nicole Hardin, U.S. Food and Drug Administration, New Orleans, LA

Albert E. Pohland, U.S. Food and Drug Administration, Washington, DC

P. Frank Ross, U.S. Dept of Agriculture, National Veterinary Services Laboratories, Ames, IA

Alex Williams, Laboratory of the Government Chemist, U.K. (retired)

The current President-Elect, Edgar R. Elkins, will assume the office of President; the current President, H. Michael Wehr, will serve on the Board as Immediate Past President.

## Regional Section Committee

LINDA L. MURRAY, CHAIRMAN
MRC, Food Technology Service, 810 Phillips St, Portage La Prairie, MB, R1N 3J9, Canada

Other Members: P.R. Beljaars; T.C. Cronau; A. Gardner; J. Hirsh; E.I. Ibrahim; G.L. Latimer; G.A. Parker; J.M. Singer; H.M. Stahr; G.R. Tichelaar

### *Recommendations for Board of Directors*

The Committee recommends that the Board of Directors change Article II of the Regional Section Model Bylaws to read, "The name of the regional section shall be known as the _____ of AOAC International."

### *Basis for Recommendations*

The present wording is not responsive to the new name of AOAC or the make up of the sections in general.

### *General Report*

The section representatives reported on the activities of their sections for the past year.

The AOAC Director of Finance and Data Processing, Wilson Korpi, discussed the regional sections insurance coverage and the process for the sections to apply for their Federal Employee Identification Number.

The need for a liaison between the Regional Section and Membership Committees was also discussed. The Committee decided that the chairman of the Regional Section Committee would attend the Membership Committee meetings.

## Safety Committee

MARK G. TORCHIA, CHAIRMAN
Saint Boniface General Hospital, Dept of Surgery, 409 Tache Ave, Winnipeg, MB, R2H 2A6, Canada

Other Members: C. Abeyta; F.G.Burton; M.S. Cerny; D. Egelhofer; R.J. Everson; R. Nelson; H. Ostapenko; S. Pfeiffer; G. Schwartzman; D. Shoemaker; M.C. Walsh

### *Recommendations for Board of Directors*

The Safety Committee recommends that the Board of Directors move adoption of the Committee's revised terms of reference, which now reflect the new role of the Committee and the method committee safety advisors.

### *Basis for Recommendations*

The terms of reference did not refer to, or carry explanation of, the new role of the Committee as it pertains to the review of methods in process for safety considerations.

### *General Report*

The Committee reviewed the current understanding of liability issues surrounding the approval of an official method as "safe."

The Committee will prepare a "layman's" version of a release statement for OMS, relating to the inherent dangers associated with the profession of chemistry and substance analysis.

The Committee will develop a plan for an anonymous reporting mechanism to allow for review of an official method that has been involved or implicated in a laboratory accident.

The Committee will investigate holding a half-day symposium on current issues in laboratory safety at the 1992 AOAC annual meeting.

## Statistics Committee

J.L. CAWLEY, ACTING CHAIRMAN
Northwest Analytical Inc., 520 NW Davis St,
Portland, OR 97209

Other Members: R.H. Albert; S. Anderson; F. Garner; H. Marks; F.D. McClure; D.H. Mowrey; T. Nelson; M.A. Nemeth; J.T. Peeler; J. Phillips; M. Presser; R.C. Rund

### *Recommendations for Board of Directors*

A. The Statistics Committee recommends that the Official Methods Board and the Editorial Board refer documents that deal with the development and/or modification of policy in statistical matters to the Committee chairman for review and comment.

B. The Statistics Committee recommends that the reportable parameters from collaborative studies of qualitative methods should include the sensitivity rate and specificity rate as defined by Joseph L. Fleiss in *Statistical Analysis of Rates and Properties* (1973) John Wiley & Sons, New York, NY, pp. 3–7

### *Basis for Recommendations*

A. The Committee is concerned that novel or nonstandard applications or definitions of statistical concepts and methods can be used in Association documents without appropriate review. These concerns include: *(1)* statistical concepts developed in different disciplines be properly adapted to AOAC applications; *(2)* nonstandard definitions or use of terms. The intent is to provide for harmonization of definitions and applications.

B. The Committee is concerned by the variety of different definitions used for reportable test parameters. The Committee suggests reporting basic parameters that have an accepted definition. Additional parameters can be reported and should be explicitly defined by the author. Different application areas need to report sufficient information so that all disciplines can retrieve the necessary parameters.

### *General Report*

The Committee has decided to produce a handbook to support Associate Referee efforts in collaborative studies. The handbook will include: *(1)* commentary on establishing and managing a collaborative study; *(2)* the currently existing computational worksheets; *(3)* spreadsheet macros for calculating the worksheets.

Sallee Anderson and Daniel H. Mowrey will provide the document for review at the January 1992 Official Methods Board meeting.

To further support the Associate Referee training effort, the Committee proposes an Associate Referee training session at the 1992 AOAC annual meeting. The Committee proposes to videotape the session for possible use as a training aide. The Committee will evaluate the need for a larger scale statistical symposium after 2 years of training sessions.

Mowrey and John G. Phillips will evaluate the need for a new AOAC statistics handbook and report on the suitability of such a project to the January 1992 Official Methods Board meeting. If the decision is made that such a book is useful and necessary, Mowrey and Phillips will provide a topic outline to the Official Methods Board.

If the Association decides to produce such a book, the Committee recommends that the Editorial Board contract with an author for the project.

The Committee has decided in the interest of harmonization to make no official statement on definitions of limits of detection and quantification until IUPAC publishes its position.

The Committee calls for greater intra- and intercommittee communication during the year. This covers both definitions of policy and the specifics of current statistical issues.

## Harvey W. Wiley Committee

H. MICHAEL WEHR, CHAIRMAN
Oregon Dept of Agriculture, Export Service Center,
Albers Mill Bldg, Suite 320, 1200 NW Front Ave, Portland,
OR 97209-2898

Other Members: W.P. Cochrane; L.W. Doner; F.J. Johnson; R.C. Rund; P.M. Scott; O.L. Shotwell

The Committee selected Reginald W. Bennett, U.S. Food and Drug Administration, Washington, DC, to receive the 1991 AOAC Harvey W. Wiley Award. Bennett was one of the 8 outstanding scientists nominated and eligible for the 1991 award.

## Harvey W. Wiley Scholarship Award Committee

MARIETTA SUE BRADY, CHAIRMAN
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. Pfeifer

The Committee received applications from 7 eligible candidates for the 1991 Harvey W. Wiley Scholarship Award.

The Committee, voting by mail, selected Pamela Lynn Cooper of Ball State University, Muncie, IN, as the winner of the 1991 AOAC Harvey W. Wiley Scholarship Award.

## Joint Mycotoxin Committee

PETER M. SCOTT, CHAIRMAN
Health and Welfare Canada, Health Protection Branch, Ottawa, Ontario, K1A 0L2, Canada

Other Members: D.J. Bark (AACC); R. Bernetti (AACC); H. Casper (AOAC); J.C. Henderson (AOCS); D.L. Park (IUPAC); A.E. Pohland (IUPAC); R.D. Stubblefield (AOCS); S.N. Tanner (Secretary) (AACC); M.W. Trucksess (AOAC); A.E. Waltking (AOCS)

The following reports were presented at the Committee's annual meeting held in Phoenix, AZ, August 13, 1991.

### *AOAC*

Peter M. Scott gave a report on recent issues relating to AOAC and of interest to the Committee, including the subject of Associate Referee appointments. He also reviewed the General Referee report on mycotoxins, with the following recommendations: Adopt as official first action the method for determination of total aflatoxin levels in peanut butter by ELISA (Biokits); make an editorial clarification in **989.06**—insert Absorbance Measurement (optical density) after Method Performance; find a replacement for the Associate Referee on Aflatoxin M; perform a revised collaborative study of an ELISA method for aflatoxins in corn (now completed); perform a collaborative study of the Mycosep #224 column method for aflatoxins after protocol approval by AOAC; complete the collaborative study on ergot alkaloids in grains; and continue study on all other topics.

Glenn Bennett gave a synopsis of work on fumonisins presented at the AOAC Midwest Regional Meeting held June 3–5, 1991, in Sioux Falls, SD. It was furthermore stated the National Toxicology Program (National Institutes of Health) was seriously considering testing fumonisin $B_1$.

The Committee discussed at some length the program for review and recognition of test kits being developed by AOAC.

### *AOCS*

James C. Henderson highlighted the report of the AOCS Mycotoxin Committee. AOCS has taken over statistical reporting for the Smalley Check Sample Program, which includes aflatoxin $M_1$ as well as $B_1$; mean results for the newly introduced immunochemical methods program were slightly higher than for LC and TLC methods. The previously rejected ELISA method for aflatoxin $B_1$ in cottonseed products and mixed feeds will not be rewritten for the AOCS *Book of Methods and Recommended Practices*, but the AOCS Committee is considering incorporation of the AOAC immunoaffinity column method for determination of aflatoxins in corn, peanuts, and peanut butter as a "recommended practice."

Future AOCS Mycotoxin Committee meetings will be held jointly with the AOCS Seed and Meal Analysis Committee.

There was considerable discussion on different approaches to grinding and sampling, including the feasibility of taking more samples, of smaller size, when rapid methods of analysis are used. Collaborative study on grinding procedures and incorporation of sampling and sample preparation procedures into one volume were other suggestions.

### *AACC*

Steven N. Tanner summarized the report of the AACC Mycotoxin Committee. This Committee has accepted the report and generic sampling guidelines of its Subcommittee on Sampling for Mycotoxins. It has also decided to incorporate the AOAC method for determination of aflatoxins in corn by LC into AACC *Approved Methods of Analysis*. However, it was noted that the black light procedure was not a suitable procedure for the detection of aflatoxin contaminated corn with the availability of newer and rapid test kit technologies, although some Committee members believed that the black light should remain as an AACC approved method.

Vomitoxin and fumonisins are other AACC mycotoxin concerns.

### *IUPAC*

There was no new information on mycotoxin projects from the International Union of Pure and Applied Chemistry.

### *IDF*

Peter Scott gave a brief report on the activities of Group E33-Mycotoxins of the International Dairy Federation. An IDF collaborative study of an immunoaffinity column method for aflatoxin $M_1$ in milk is being performed using naturally contaminated samples. It was decided to add IDF to the membership of the Joint Mycotoxin Committee. Revised Terms of

Reference were adopted to reflect this and the Committee name has been officially changed to the Joint Mycotoxin Committee. The next meeting of IDF Group E33 has been proposed for March 1992 in Brussels, Belgium.

### *Other Business*

There was some discussion on the regulatory levels for aflatoxins being examined by the Codex Alimentarius Commission. The Codex Committee on Food Additives and Contaminants had proposed that a 10 ppb total aflatoxin level be established for all foods in international trade. However, Steve Tanner noted that this would not be acceptable on a world-wide basis. Codex is now exploring the establishment of levels based on the type of food product and how that food product is sampled.

Future conferences relating to mycotoxins that were brought to the attention of the Joint Mycotoxin Committee are: International Symposium on Stored Grain Ecosystems, June 7–10, 1992, Winnipeg, MB; AOAC Midwest Regional Meeting, June 8–10, 1992, Champaign, IL; 106th AOAC International Annual Meeting, August 31–September 3, 1992, Cincinnati, OH; Fusarium-Mycotoxins, Taxonomy, Pathogenicity and Host Resistance, September 22–24, 1992, Radzikow, Poland; and the 8th International IUPAC Symposium on Mycotoxins and Phycotoxins, November 8–12, 1992, Mexico City, Mexico.

Publication of Joint Mycotoxin Committee minutes in the *Journal of AOAC International* will be continued to provide a general summary of the meeting without going into too much detail.

The following Committee members have stepped down: Howard Casper (AOAC), Odette Shotwell (AACC), Art Waltking (AOCS), and Bob Stubblefield (AOCS). Douglas Bark is the new AACC representative.

## International Organization for Standardization (ISO)

ROBERT C. RUND
Purdue University, Dept of Biochemistry, W. Lafayette, IN 47907

### *ISO/TC 134*

This Technical Committee (TC) issues a report of activities and structural organization no later than January 31 of each year. This Committee makes final proposals on Draft International Standards (DIS), which are then transmitted to the International Organization for Standardization (ISO) Council for review, acceptance, and publishing as International Standards used for trade and by regulatory agencies in the movement of fertilizers and soil conditioners regarding Terminology and Labeling (Subcommittee (SC) 1), Sampling (SC 2), Physical Properties (SC 3), and Chemical Analysis (SC 4).

The structure of TC 134 at the beginning of 1991 was composed of the 4 SCs noted above and 4 working groups (WG). The working groups are composed of interested parties who collectively study draft proposals to determine reliability, in accord with ISO procedures. The working group then submits a proposal to the related subcommittee for review, and through that subcommittee to the technical committee.

At the beginning of the year, TC 134 had 17 participating (P) members (those national standard bodies actually engaged in studying proposals and voting on acceptance of proposals as standards), as well as 37 observers (O) (national standard bodies wishing to be informed of proceedings but who do not actively participate).

TC 134 has internal liaisons with 4 other ISO technical committees: TC 24, Sieves, Sieving, and Other Sizing Methods; TC 47, Chemistry; TC 69, Application of Statistical Methods; and TC 190, title unknown.

TC 134 has formal liaisons with 10 other international organizations, 3 of which are Category "A" liaisons. The Category "A" liaisons are with AOAC International, the World Phosphate Institute (IMPHOS), and the European Free Trade Association. Category "B" liaisons are maintained with the European Nitrogen Producers Association, the Customs Cooperation Council (CCC), the Centre International d'Information et de Documentation des Producteurs de Scories Thomas, the Council for Mutual Economic Assurance, the Food and Agriculture Organization of the United Nations (FAO), the International Potash Institute, the International Superphosphate and Compound Manufacturer's Association (ISMA), the Organization for Economic Co-operation and Development, the Sulfur Institute (TSI), and the Commission of the European Communities (CCE).

Category "A" liaisons are established with those international organizations that make "*an effective contribution to the*

*work of the Technical Committee or Sub-Committee for most of the questions dealt with by the Technical Committee or Sub-Committee.*" Category "A" liaison organizations are invited to all meetings of the Committee in which the liaison applies; they are invited to nominate an expert to participate in the work of pertinent WGs; they may submit papers; and they may send observers to participate at meetings, but have no voting rights. Category "A" organizations receive all reports and working documents and all DISs of the relevant Committees. Such liaison organizations may submit observations, but they have no voting rights on any DISs.

Category "B" liaison organizations are not invited to meetings nor do they receive the documents noted above unless specifically requested by the organization. They do receive reports on the work of relevant Committees.

### *6th Meeting of ISO/TC 134, London, November 16, 1990*

This was a full day meeting attended by 18 individuals representing 9 countries with full participation rights. I was the AOAC representative with category "A" liaison privileges.

Reports (to be discussed later in this paper) were received from secretariats of SC 2, SC 3, and SC 4, and adopted.

At this meeting the Association Française de Normalisation (AFNOR) delegation announced its desire to be relieved of the TC secretariat. The Central Secretariat will be notified of the need to seek a replacement for AFNOR in this capacity. If no candidate is apparent, AFNOR will continue to serve only as a contact point for the next 12 months. AFNOR was requested to reconsider this action.

With the establishment of European Committee for Standardization (CEN)/TC 260 "Fertilizers and Inorganic Soil Conditioners," AFNOR prefers to dedicate its activities in that direction.

Future meetings of TC 134 will need to await the assignment of a secretariat and the outcome of meetings of SC 3 and working groups.

### *ISO/TC 134/SC 1, Terminology and Labeling*

At the London meeting of the technical committee the TC Secretariat reminded those in attendance that SC 1 has been without a secretariat since 1989, when AFNOR asked to be relieved of that obligation. For matters of record, it was pointed out that ISO Draft International Standard 8157, page 7, Formula 2.24.1 is incorrect. The last bracket of this formula should be (CO), not (CH). It was agreed that SC 1 shall remain in existence but without a secretariat for the present. Because there were no volunteers to serve as secretariat, the Central Secretariat at Geneva will be notified.

This subcommittee has 12 "P" members, 15 "O" members, and 4 liaisons with other organizations. Because this subject is beyond the normal scope of AOAC, a liaison relationship with this subcommittee has not been sought.

### *ISO/TC 134/SC 2, Sampling*

This subcommittee has 13 "P" members, 13 "O" members, and 7 liaisons with other organizations, 2 of which are of Category "A." AOAC holds one of the Category "A" liaisons, and the other is held by the United Nations Economic Commission for Europe (UNECE). Category "B" liaisons are with FAO, IMPHOS, ISMA, TSI, and CCE. This subcommittee maintains internal ISO liaison with TC 69, Applications of Statistical Methods.

The ninth meeting of this subcommittee was held November 14–15, 1990, in London, in conjunction with TC 134 and TC 134/SC 4. The eighth meeting of this subcommittee had been held in 1984 in Arlington, VA, just before the 100th meeting of AOAC. Fifteen delegates representing 9 "P" member countries and the AOAC liaison (category "A") were present at the London meeting. Countries represented were France, Germany, Hungary, The Netherlands, Norway, Portugal, United Kingdom, United States, and Soviet Union.

Working Group 2 of SC 3 proposed a committee draft (CD 10696 Fluid Fertilizers—Methods of Sampling), which had been developed over a period of 3 years using principles and equipment inherent in AOAC methods **959.01** and **969.01**. In addition, the working group proposed that the aperture of the sampling device for slurries and suspensions be at least 50 mm. AOAC method **969.01(b)** requires only a 10 mm aperture. Further, the gross sample obtained is recommended to be upward of 20 times the amount taken under AOAC methods when product stored is heterogeneous. Also, the "Texas Tube" was proposed as an alternate to the "Indiana Bottle." Incidentally, the "Texas Tube" is currently under review within a collaborative study. The AOAC Liaison Officer was requested to provide a line drawing of the modified Indiana Bottle now in use for sampling by means of AOAC method **969.01(b)** so that the latest device can be included in the committee draft. The drawing has been made and will be supplied to the secretariat of SC 2 at an early date. With these noted revisions, SC 2 agreed to submit CD 10696 to ISO Central Secretariat as a DIS. The AOAC Liaison Officer recommends that this ISO proposal be referred to the AOAC General Referee for consideration relative to revision or expansion of current AOAC sampling methods.

Working Group 2 of SC 3 also proposed a committee draft (CD 10978 Solid Fertilizers—Sampling from Bulk Flowing Streams) for obtaining the gross sample from a vertical free falling stream of fertilizer, or from a horizontal conveyer belt or other means of horizontal fertilizer movement. These methods are useful for sampling loads that are loaded onto ocean going barges and ships where the rate of loading is approximately 200 metric tons/h. This is beyond the scope of AOAC work, but the principles still hold and would be satisfactory for AOAC investigation. This procedure would be mechanically continuous. Therefore, sample reductions would also be necessary, and some are presented. It was agreed that the draft submitted is to be rewritten by the United States to eliminate much of the theoretical statements and reduce it to a practical documentation.

SC 2 Draft Proposal 5308 Solid Fertilizers—Method of Checking Performance of Mechanical Devices for the Sampling of Fertilizers Moving in Bulk: This proposal provides a method of checking mechanical devices by comparison of particle size distribution with that obtained by a reference method. Many constructive comments have been received and the proposal is to undergo revision and recirculated as a DIS.

Future work of this subcommittee agreed upon includes the following:

(*1*) A revision of ISO International Standard 5306 Fertilizers—Presentation of Sampling Reports. This legally required report of sampling procedure was not designed for fluid fertilizers. The report is to be revised to incorporate this information. Mr. Palgrave of the United Kingdom has been assigned to proceed with the revision and to submit the report for comment.

(*2*) Responding to a question raised by the United States regarding a critical appraisal of documentation and interface aspects involved in sampling, the Secretariat was requested to investigate the application of quality criteria as set forth in ISO 9000 series to sampling activities. This was done to advise SC 2 whether or not amendments to existing SC 2 documents are in order.

(*3*) Because of concern that CEN/TC 260—Fertilizers and Inorganic Soil Conditioners, a new European based body, may attempt to include sampling in its work program and upon the invitation of the French delegation, the SC 2 Secretariat was requested to compile a list of published SC 2 standards and current work items and to forward these to the Secretariat of CEN/TC 260. CEN was asked to consider them for implementation as European Standards, as applicable, in accordance with CEN common rules. This action was taken to try to achieve worldwide acceptability of ISO standards.

The chair of this subcommittee emphasized that 6 years had elapsed since the last meeting in Arlington, VA. It was agreed that, if possible, SC 2 should meet again in conjunction with SC 4, possibly in the United States in the second half of 1992. This arrangement is subject to consultations and confirmation.

### *ISO/TC 134/SC 3, Physical Properties*

This subcommittee did not meet in London but did present its annual report.

(*1*) International Standard 8398 "Measurement of Static Angle of Repose" was published July 1, 1989.

(*2*) The following methods have been circulated as committee drafts to members of SC 3: (*a*) ISO/CD 10188 "Fluid Fertilizers–Determination of Density"; (*b*) ISO/CD 10189 "Solution Fertilizers–Determination of Saturation Temperature"; (c) ISO/CD 10248 "Fluid Fertilizers–De-aeration of Samples by Film Disentrainment"; and (*d*) ISO/CD 10249 "Fluid Fertilizers–Preliminary Visual Examination and Preparation of Samples for Physical Testing."

(*3*) Revised international standards of the following documents have been circulated: (*a*) "Fertilizers–Determination of Bulk Density (Loose)"; (*b*) "Fertilizers–Determination of Bulk Density (Tapped)"; and (*c*) "Fertilizers–Determination of Bulk Density (Loose) of Fine-grained Fertilizers."

(*4*) The following have been circulated as committee drafts to members of SC 3: (*a*) "Fluid Fertilizers–Preliminary Sedimentation Test for Suspensions"; (*b*) "Assessment of Stability in Suspension Fertilizers"; and (*c*) "Fluid Fertilizers–Determination of Gel Strength in Suspension Fertilizers by Gun Rheometry."

### *ISO/TC 134/SC 4, Chemical Analysis*

This subcommittee has 14 "P" members, 11 "O" members, and 6 liaisons with other organizations, 5 of which are of Category "A," and 1 Category "B." AOAC holds one of the Category "A" liaisons, and the others are held by UNECE, FAO, TSI, and CCE. A Category "B" liaison is held with the CCC. This subcommittee maintains internal ISO liaison with TC 47 Chemistry.

(*1*) The ninth meeting of this subcommittee was held November 12–13, 1990, in London in conjunction with TC 134 and TC 134/SC 2. The eighth meeting of this subcommittee had been held in 1984 in Arlington, VA, just before the 100th meeting of AOAC. Seventeen delegates representing 9 "P" member countries and the AOAC liaison (category "A") were present at the London meeting. Countries represented were France, Germany, Hungary, The Netherlands, Norway, Portugal, United Kingdom, United States, and Soviet Union.

(*2*) The work on slow release nitrogen continues, but the direction is to be concentrated on the determination of named constituents in fertilizer products. The CEE has legislation describing different sources of slow release phosphorus. F.N. Wilson (United Kingdom) suggests that efforts be directed toward the development of a method to measure the *rate* of mineralization of nitrogen slow release products. Germany has proposed a procedure whereby all forms of nitrogen are determined separately and then summed to provide the total nitrogen content. The term "slow release nitrogen" is perhaps not appropriate here and another term is being sought. In the meantime, WG 1 will undertake a study of the German proposal.

(*3*) Total nitrogen methods: Earlier work reported by the United Kingdom in 1988 indicated the chromium powder method had lower repeatability and reproducibility than the Devarda method. Certified reference standards were not used, so the question is raised as to how a determination can be made concerning which was more accurate. However, because the chromium is more toxic than the Devarda alloy (Al + Cu), the Devarda method is preferred. The AOAC liaison asked to review AOAC literature regarding Devarda vs iron on certified reference standards. The AOAC liaison is to obtain data on $R$ and $r$ values and send them to SC 4 Secretariat. The Secretariat was requested to edit the method using the Devarda alloy and revise the title to "Determination of the Sum of Ammoniacal and Nitrate Nitrogen." The scope is to be changed to exclude products containing all other forms of nitrogen. Potassium nitrate is to be used as a reference material in place of ammonium nitrate.

(*4*) Urease method and Xanthydrol method: A DIS has been accepted for the determination of ureic nitrogen. This is based on

the selective precipitation of urea with xanthydrol. Several collaborative tests over a period of 7 years involved more than 23 laboratories, 3 different methods, and 4 different types of fertilizers. This may be one that AOAC could well consider as a satisfactory method. The latest report may be found in Doc. N 129 DP 8603. The subcommittee requested that the Secretariat amend the scope of the xanthydrol method for urea to exclude urea condensates. Work on the urease method is to continue.

(*5*) Moisture by Karl Fischer: Continued study underway, assuming sufficient interest is expressed for good participation in collaborative studies. The Secretariat was requested to determine if sufficient laboratories are available and willing to undertake the work to improve the Karl Fischer Method using methyl alcohol.

(*6*) SC 4 has accepted Model "A" of the IUPAC/ISO/ AOAC "Harmonized Protocols for the Adoption of Standard Methods," for presentation of all precision data.

(*7*) The SC 4 Secretariat has been requested to circulate a new work proposal for the determination of cadmium, for water soluble sulfate, and for total sulfur in all its forms.

### *ISO/TC 134/SC 4/WG 5, Calcium, Magnesium, and Sulfur*

This working group met in Lisbon, Portugal, June 6–7, 1991. Sixteen delegates, including this AOAC liaison officer, representing 10 countries were in attendance. Because this was a working group the protocol allowed the liaison officer full membership with voting privilege within the group.

The following actions were taken:

(*1*) A collaborative study by 23 laboratories using 6 different samples of calcium–magnesium containing fertilizer materials was presented. The results were treated according to ISO 5725. Calcium was determined by oxalate precipitation followed by permanganate titration and atomic absorption spectrophotometry. Magnesium was determined only by atomic absorption spectrophotometry. All methods are similar to AOAC methods. Results were excellent. With some slight modifications, the final report of the test will be submitted to the Secretariat of SC 4 as a committee draft.

(*2*) To retain the experience and data resulting from the investigations into the complexometric methods, an official document is to be drafted containing the results and conclusions. These methods were not promising in the hands of the working group.

## IDF/ISO/AOAC Tripartite

MARGREET LAUWAARS
AOAC International, PO Box 153, 6720 AD Bennekom, The Netherlands

The International Dairy Federation (IDF), the International Organization for Standardization (ISO)/TC 34/SC 5-Milk and Milk Products, and AOAC have been cooperating over 25 years since the Food and Agriculture Organization/World Health Organization (FAO/WHO) Milk Committee requested that the 3 bodies reach agreement on methods of analysis, rather that submitting different texts for the same purpose.

At the meeting of the FAO/WHO Committee at Rome, Italy, November 1990, AOAC was represented by IDF Liaison Officer Ronald Case (USA) and European Representative Margreet Lauwaars. As chair of the preceding IDF/ISO/AOAC meeting, Lauwaars presented the IDF/ISO/AOAC report on methods of analysis submitted to the Milk Committee.

Of the 54 methods previously adopted by the Committee, 25 have yet to be adopted by AOAC. Many of these methods have undergone full interlaboratory study. The General Referee in Dairy Chemistry is now considering these studies, which have been published in IDF Bulletins 207 and 235.

Seven new methods have been submitted to the Milk Committee; 11 revised methods were resubmitted. The methods required by FAO/WHO Standards, for which no methodology exists, were submitted to the Chairman of the Joint IDF/ISO/AOAC Groups of Experts, and replies have been received for most of the methods. The Tripartite Group will consider these replies.

The Milk Committee agreed to retain the "B" series of references for the standard methods of analysis, even though FAO will no longer publish them. This means that IDF, ISO, and AOAC must decide how to incorporate an appropriate reference to FAO/WHO in the texts they publish.

AOAC actively participates in the more than 40 Joint IDF/ISO/AOAC Groups of Experts, which meet at least once a year. AOAC has full membership in these groups.

The IDF/ISO/AOAC Group meets twice a year to review progress; in 1991 the group met in May at Ede, The Netherlands, and in October at Poligny, France.

AOAC INTERNATIONAL

# AOAC Officers and Committees: 1992

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## Committees

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*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 established policy; and to oversee the activities of the Safety and Statistics Committees.

***Committee on Safety:*** Mark G. Torchia (St. Boniface General Hospital, Dept of Surgery, 409 Tache Ave, Winnipeg, Manitoba R2H 2A6, Canada), *Chairman;* Sandra Pfeiffer; David Egelhofer; Robert Nelson; Dirk Shoemaker; Milan Cerny; Maire C. Walsh; Harry Ostapenko; Carlos Abeyta

*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.

***Committee on Statistics:*** John G. Phillips (U.S. Dept of Agriculture, Agriculture Research Station, Eastern Regional Research Center, 600 E. Mermaid Ln, Philadelphia, PA 19118), *Chairman;* Robert C. Rund; Jeffrey L. Cawley; Margaret A. Nemeth; Terry C. Nelsen; Harold Marks; Daniel Mowrey; J. Timothy Peeler; Sallee M.

Anderson; Mark Presser; William Horwitz

*Mission:* To establish statistical guidelines for collaborative studies, and to encourage greater use of statistical techniques.

***Editorial Board:*** Claire Franklin (Acting Director, Bureau of Human Prescription Drugs, Health & Welfare Canada, Place Vanier, 355 River Rd, Second Floor, Vanier, Ontario K1A 1B8, Canada), *Chairman*; Jerry A. Burke; John K. Taylor; Franklin E. Barton, II; James Lichtenberg; Anant V. Jain; W. Harvey Newsome; David L. Terry; Arpad Somogyi; Joseph Sherma; Richard A. Durst

*Mission*: To oversee the development, editing, and publishing of all Association publications; to provide for long and short range publication planning.

## Standing Committees

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*Mission*: To collect and preserve the history of AOAC.

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*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.

***Committee on Finance:*** 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.

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*Mission*: To plan programs for the Annual International Meeting, topical conferences, etc.; and to oversee short courses and other educational programs.

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*Mission*: To make recommendations to the Board of Directors on policies and procedures on all types of AOAC memberships.

***Committee on Regional Sections:*** Linda L. Murray (MRC, Food Technology Service, 810 Phillips St, Portage la Prairie, Manitoba R1N 3J9, Canada), *Chairman*; Keith A. McCully; George R. Tichelaar; Paul R. Beljaars; Jeffrey M. Singer; Gail Parker; Ezzat Ibrahim; Audrey Gardner; Jerry Hirsch; George W. Latimer, Jr; Henry M. Stahr; Tom C. Cronau

*Mission*: To make recommendations to the Board of Directors on policies and procedures pertaining to Regional Sections.

***Laboratory Quality Assurance Committee:*** Jerry H. Hirsch (Health & Welfare Canada, Health Protection Branch, Food & Drug Laboratory Division, 3155 Willingdon Green, Burnaby British Columbia V5G 4P2, Canada), *Chairman*; Carlton Weaver; Rodney J. Noel; Bruce Cottingham; Keith A. McCully; Paul O. Cambell; Eugene Easterly; John Hardy; James Longbottom; Wayne R. Wolf; Joseph Polywacz; Ian Borst; Ellen Koenig; James P. Dux

*Mission*: To provide advise and make recommendations for adoption and application of quality assurance principles toward the improvement of analytical laboratory operations.

***Long Range Planning Committee:*** Henry B.S. Conacher (Health & Welfare Canada, Health Protection Branch, Food Research Division, Tunney's Pasture, Ottawa, Ontario K1A 0L2, Canada), *Chairman*; Edgar R. Elkins; H. Michael Wehr; Ronald A. Case; Ross Elliot; Fred R. Shank; Damien A. Gabis; Roy Dagnall; Ronald R. Christensen

*Mission:* To gather and analyze information in order to identify opportunities to serve the collective interests of members and constituent groups. To recommend long term goals and activities that will best position the Association in the future.

***Harvey W. Wiley Award Committee:*** Edgar R. Elkins (National Food Processors Association, 1401 New York Ave, NW, Suite 400, Washington, DC 20005), *Chairman*; Robert C. Rund; Odette L. Shotwell; Frank J. Johnson; Peter M. Scott; William P. Cochrane; Landis W. Doner

*Mission:* To select the recipient of the annual AOAC Harvey W. Wiley Award.

***Harvey W. Wiley Scholarship Award Committee:*** Marietta Sue Brady (Rutgers University, Cook College, Dept of Biochemistry, Lipman Hall, New Brunswick, NJ 08903), *Chairman*; Joel Padmore; Patricia Bulhack; James W. Fitzgerald; Adeline Peake; Rae Gabrielle Krueger; Thomas Romer

*Mission:* To select the recipient of the annual AOAC Harvey W. Wiley Scholarship.

***Committee on Fellows:*** H. Michael Wehr (Oregon Dept of Agriculture, Export Service Center, Albers Mill Bldg, Suite 320, 1200 NW Front Ave, Portland, OR 97209-2898), *Chairman*; Thomas Fazio; Edwin Jackson; Frederick J. King; Jon E. McNeal; Arthur Waltking; Elaine A. Bunch

*Mission:* To select and recommend to the AOAC Board of Directors candidates for the "Fellow of the AOAC" awards.

***Nominating Committee:*** Odette L. Shotwell (5517 N. James, Peoria, IL 61615), *Chairman*; Thomas P. Layloff; H. Michael Wehr

*Mission:* To select a slate of candidates for election of AOAC Officers and Directors.

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***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 Bemetti; Steven N. Tanner: *Representing AOCS:* Arthur E. Waltking; Robert D. Stubblefield; James C. Henderson: *Representing IUPAC:* Albert E. Pohland; Douglas L. Park

***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 1488, Tuscaloosa, AL 35487-1488)

***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., 607 Park Ave, PO Box 1849, Wilson, NC 27893)

***American Oil Chemists' Society:*** David Firestone (Food & Drug Administration, Division of Contaminants Chemistry, 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:** Russell Bishop (Food Science & Technology, Virginia Polytechnic & State University, Blacksburg, VA 24061)

***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 (Cal-Compack Foods, Inc., PO Box 265, Santa Ana, CA 92702)

***California Department of Food & Agriculture; Science Advisory Committee:*** George R. Tichelaar (4341 Jan Dr, Carmichal, CA 95608)

***Codex Alimentarius Commission:*** Derek Abbott (33 Agates Ln, Ashtead, Surrey KT21 2ND, United Kingdom); Margreet Lauwaars (PO Box 153, 6720 AD Bennekom, The Netherlands)

***Collaborative International Pesticides Analytical Council:*** Alan R. Hanks (Dept of Biochemistry, Office of the Indiana State Chemist, Purdue University, West Lafayette, IN 47907)

***Cosmetics, Toiletries, & Fragrances Association:*** Open

***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 and Technology:*** Ralph H. Lane (University of Alabama, Dept of Food, Nutrition, & Institutional Management, PO Box 1488, Tuscaloosa, AL 35487-1488)

***International Atomic Energy Agency:*** Leslie G. Ladomery (FAO/IAEA Div. of Nuclear Techniques in Food and Agriculture, PO Box 200, A-1400 Vienna, Austria)

***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)

*Dried Milk (E-2):* Open

*Water Content (E-5):* Robert Bradley (University of Wisconsin-Madison, Dept of Food Science, 1605 Linden Dr, Madison, WI 53706); Graham McEachern (Agriculture Canada, Plant Products Building, Ottawa, Ontario K1A 0C5, Canada)

*Lactose (E-6):* Dick H. Kleyn (Rutgers University, Dept of Food Science, Dudley Rd & College Farm Rd, New Brunswick, NJ 08903); Leslie West (Kraft General Foods, Tech Center, 801 Waukegan Rd, Glenview, IL 60025); John W. Sherbon (Cornell University, Dept of Dairy & Food Science, Stocking Hall, Ithaca, NY 14853)

*$NO_2$, $NO_3$, P, Ca (E-8):* Thomas Fazio (Food & Drug Administration, Office of Physical Sciences, 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, ARS ERRC, 600 E. Mermaid Ln, Philadelphia, PA 19118)

*Lactic Acid (E-9):* Open

*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 (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 (Food & Drug Administration, Division of Microbiology, 200 C St, SW, Washington, DC 20204); Larry J. Maturin (Food & Drug Administration, Laboratory for Quality Assurance Br, 6502 S. Archer Rd, Summit Argo, IL 60501-1399)

*Nonpathogenic Contaminants (E-22):* Larry J. Maturin (Food & Drug Administration, Laboratory for Quality Assurance Br, 6502 S. Archer Rd, Summit Argo, IL 60501-1399)

*Staphylococcus (E-24):* Gayle A. Lancette (Food & Drug Administration, 60 Eighth St, NE, Atlanta, GA 30309); George Jackson (Food & Drug Administration, Division of Microbiology, 200 C St, SW, Washington, DC 20204)

*Numerical Selection of Samples (E-26):* William Horwitz (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 (Food & Drug Administration, 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 (Food & Drug Administration, Division of Microbiology, 200 C St, SW, Washington, DC 20204); Larry J. Maturin (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 (Food & Drug Administration, Division of Contaminants Chemistry, HFF 423, Washington, DC 20204)

*Yeasts & Molds (E-34):* Open

*Free Fatty Acids (E-39):* Robert Bradley ( University of WI Madison, Dept of Food Science, 1605 Linden Dr, Madison, WI 53706); Graham McEachern (Agriculture Canada, Plant Products Bldg, Ottawa, Ontario K1A 0C5, Canada)

*Fat (E-40):* Dick H. Kleyn (Rutgers University, Dept of Food Science, Dudley Rd & College Farm Rd, New Brunswick, NJ 08903)

*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)

*Yogurt Microorganisms (E-44):* Open

*Vitamin A (E-46):* Ellen J. DeVries (Duphar BV, PO Box 2, 1380 AA Weesp, The Netherlands); Shu-Guang Greg Cheng (Kraft General Foods, Tech Center, 801 Waukegan Rd, Glenview, IL 60025)

*Antibiotics (E-47):* Stanley E. Charm (Penicillin Assays, 36 Franklin St, Malden, MA 02148); Larry J. Maturin (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):* Graham McEachern (Agriculture Canada, Plant

Products Bldg, Ottawa, Ontario K1A 0C5, Canada); 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 (Food & Drug Administration, Division of Microbiology, 200 C St, SW, Washington, DC 20204); Larry J. Maturin (Food & Drug Administration, Laboratory for 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, Dudley Rd & College Farm Rd, New Brunswick, NJ 08903); Gopala Murthy (1615 Northwood Dr, Cincinnati, OH 45237)

*Listeria (E-64):* Robert M. Twedt (Food & Drug Administration, Division of Microbiology, HFF 236, 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):***

*Agricultural Food Products (ISO/TC34):* George R. Heavner (AOAC International, 2200 Wilson Blvd, Suite 400, Arlington, VA 22201), Liaison Coordinator

*Oleaginous Seeds & Fruits (ISO/TC34/SC2):* Gary R. List (U.S. Department 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 1488, Tuscaloosa, AL 35487-1488); 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 & Insepction Service, Annex Bldg, Rm 519, Washington, DC 20250)

*Spices & Condiments (ISO/TC34/SC7):* James E. Woodbury (CalCompack Foods, Inc., Technical Director, PO Box 265, Santa Ana, CA 92702)

*Microbiology (ISO/TC34/SC9):* Wallace H. Andrews (Food & Drug Administration, Division of Microbiology, HFF 234, 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 (Food & Drug Administration, Division of Contaminants Chemistry, 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 Agriculture, Marketing Research Branch, PO Box 96456, Washington, DC 20090-6456)

*Starch (ISO/TC93):* Open

*Fertilizers (ISO/TC134):* Robert C. Rund (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 (Food & Drug Administration, Division of Contaminants Chemistry, HFF-423, 200 C St, SW, Washington, DC 20204)

***Nordic Committee on Foods Analysis:*** Margreet Lauwaars (PO Box 153, 6720 AD Bennekom, The Netherlands)

***Organization International du Cacao et du Chocolat:*** Michael E. Hillyer (Ambrosia Chocolate Co., 1133 N. Fifth St, Milwaukee, WI 53203)

***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 (Food & Drug Administration, Division of Nutrition, HFF-268. Washington, DC 20204)

***U.S. Pharmacopeial Convention:*** Thomas P. Layloff (Food & Drug Administration, National Center for Drug Analysis, 1114 Market St, St. Louis, MO 63101)

## Committee on Pesticide Formulations and Disinfectants

**Richard H. Collier** (Purdue University, 1158 Entomology Hall, West Lafayette, IN 47907), *Chairman*; **Fran Porter** (Florida Dept of Agriculture & Consumer Services, 3125 Conner Ave, Tallahassee, FL 32301); **Arthur H. Hofberg, Jr** (Ciba Geigy Corp., Analytical Section, Production Technology Dept, PO Box 18300, Greensboro, NC 27419); **Thaddeus J. Czerkowicz** (U.S. Environmental Protection Agency, Office of Pesticide Programs, BARC E, Bldg 306, Rm 113, Beltsville, MD 20705); **Warren Bontoyan** (Maryland Dept of Agriculture State Chemist, 50 Harry S. Truman Pkwy, Annapolis, MD 21401); **Peter D. Bland** (ICI Americas Inc., Western Research Center, 1200 S. 47th St, PO Box 4023, Richmond, CA 94804-0023); **Stephen C. Slahck** (Mobay Corp., Hawthorn Rd, PO Box 4913, Kansas City, MO 64120), *Secretary*; **Margaret A. Nemeth** (Monsanto T2A, 800 N. Lindbergh Blvd, St. Louis, MO 63167), *Committee Statistician*; **Harry Ostapenko** (Heinz USA, PO Box 57, Pittsburgh, PA 15230-0057), *Safety Advisor*

### CIPAC Studies

*Referee:* Alan R. Hanks, Dept of Biochemistry, Office of the Indiana State Chemist, Purdue University, West Lafayette, IN 47909

***Bentazon (Basagran)*** Thomas M. Schmitt, BASF Corp., Analytical Research, 1419 Biddle Ave, Wyandotte, MI 48192-3736

***Dichlobenil*** A. A. De Reyke, Duphar B.V., PO Box 2, 1380 AA Weesp, The Netherlands

***Dimethoate*** Richard S. Wayne, American Cyanamid Co., Agriculture Division, Box 400, Princeton, NJ 08540

***Methamidophos (Monitor)*** James W. Baird, Mobay Corp., Hawthorn Rd, PO Box 4913, Kansas City, MO 64120

### Disinfectants

*Referee:* Aram Beloian, U.S. Environmental Protection Agency, H7503W, 401 M St, SW, Washington, DC 20460

***AOAC Use Dilution Tests*** Joseph R. Rubino, Lehn & Fink Produce 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 Division, 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 State Chemist, 50 Harry S. Truman Pkwy, Annapolis, MD 21401

***Tebuconazole (Folicur)*** James W. Baird, Mobay Corp., Hawthorn Rd, PO Box 4913, Kansas City, MO 64120

### Pesticide Formulations: Herbicides

*Referee:* Open

***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

***Chlorosulfuron*** Glenn A. Sherwood, Jr, E.I. du Pont de Nemours & Co., Biochemicals Dept, Du Pont Experiment Station, Wilmington, DE 19898

***Dicamba*** Benjamin A. Belkind, Sandoz Crop Protection Corp., 1300 E. Touhy Ave, Des Plaines, IL 60018

***Fluometuron*** Arthur H. Hofberg, Jr, Ciba-Geigy Corp., Analytical Section, Production Technology Dept, PO Box 18300, Greensboro, NC 27419

***Glyphosate*** Lynn W. Morlier, Monsanto Co., Central Laboratory, PO Box 174, Luling, LA 70070

***Metasulfuron-Methyl*** Glenn A. Sherwood, Jr, E.I. du Pont de Nemours & Co., Biochemicals Dept, Du Pont Experimental Station, Wilmington, DE 19898

***Methazole*** Benjamin A. Belkind, Sandoz Crop Protection Corp., 1300 E. Touhy Ave, Des Plaines, IL 60018

***Sulfometuron-Methyl*** Glenn A. Sherwood, Jr, E.I. du Pont de Nemours & Co., Biochemicals Dept, Du Pont

Experimental Station, Wilmington, DE 19898

### Pesticide Formulations: Organophosphorus Insecticides

*Referee:* William Betker, Mobay Corp., Hawthorn Rd, PO Box 4913, Kansas City, MO 64120

***Azinphos-Methyl (Guthion)*** Stephen C. Slahck, Mobay Corp., Hawthorn Rd, PO Box 4913, Kansas City, MO 64120

***Ethoprop*** Richard W. Smith, Rhone Poulenc, Inc., Box 352, Mt. Pleasant, TN 38474

***Fenamiphos (Nemacur)*** Carl Gregg, Mobay Corp., Hawthorn Rd, PO Box 4913, Kansas City, MO 64120

***Oxydemeton-Methyl (Metasystox-R)*** Stephen C. Slahck, Mobay Corp., Hawthorn Rd, PO Box 4913, Kansas City, MO 64120

### Pesticide Formulations: Other Insecticides, Synergists, & Repellents

*Referee:* David F. Tomkins, Monsanto Co., PO Box 473, Muscatine, IA 52761

***Allethrin*** Dean Kassera, McLaughlin Gormley King Co., 8810 Tenth Ave, N., Minneapolis, MN 55427

***Carbaryl*** Karin A. Mede, Rhone Poulenc Ag Co., PO Box 12014, Research Triangle Park, NC 27709

***Carbosulfan*** John Shuttleworth, FMC Corp., 100 Niagara St, Middleport, NY 14105

***Cyfluthrin*** Stephen C. Slahck, Mobay Corp., Hawthorn Rd, Box 4913, Kansas City, MO 64120

***Cyromazine (Larvadex)*** Arthur H. Hofberg, Jr, Ciba-Geigy Corp., Analytical Section, Production Technology Dept, PO Box 18300, Greensboro, NC 27419

***DDT*** Frederick C. Churchill, Health & Human Services, Centers for Disease Control, CTB DPD CID, Bldg 23, Atlanta, GA 30333

***Dipropyl Isocinchomeronate (MGK Repellant 326)*** Dave Carlson, McLaughlin Gormley King Co., 8810 Tenth Ave, N., Minneapolis, MN 55427

***Methomyl*** James E. Conaway, Jr, E.I. du Pont de Nemours & Co., Du Pont Experimental Station, Bldg 402, PO Box 80402, Wilmington, DE 19880-0402

***Oxamyl*** Glenn A. Sherwood, Jr, E.I. du Pont de Nemours & Co., Biochemicals Dept, Du Pont Experimental Station, Wilmington, DE 19898

***Rotenone & Other Rotenoids*** Rodney J. Bushway, University of Maine at Orono, Dept of Food Science, 102 B Holmes Hall, Orono, ME 04469

## Committee on Drugs and Related Topics

**Richard M. Montgomery** (Unilever Research US, 45 River Rd, Edgewater, NJ 07020), *Chairman*; **G.D. Anthony** (Parke-Davis Division, Warner-Lambert Co., 170 Tabor Rd, Morris Plains, NJ 07950); **Donna M. Bush** (National Institute on Drug Abuse, Division of Applied Research, 5600 Fishers Ln, Rm 9A-53, Rockville, MD 20857); **Ted M. Hopes** (19 Tennyson Pl, Greenlawn, NY 11740); **Kenneth Manning** (The Upjohn Co., 7000 Portage Rd, 4820-259-12, Kalamazoo, MI 49001); **John O'Rangers** (Food & Drug Administration, Center for Veterinary Medicine, HFV-140, 750 Standish Pl, Rockville, MD 20857); **Thomas Doyle** (Food & Drug Administration, Division of Research & Testing, HFD-473, 200 C St, SW, Washington, DC 20204); **William W. Wright** (US Pharmacopeia, 12601 Twinbrook Pkwy, Rockville, MD 20852), *Secretary*; **Mark Presser** (Unilever Research US, 45 River Rd, Edgewater, NJ 07020), *Committee Statistician*; **Mark G. Torchia** (St. Boniface General Hospital, Dept of Surgery, 409 Tache Ave, Winnipeg, Manitoba P2H 2A6, Canada), *Safety Advisor*

### Drugs II

*Referee:* Edward Smith, 14203 Castaway Dr, Rockville, MD 20853

***Aminacrine*** Elaine A. Bunch, Food & Drug Administration, 22201 23rd Dr, SE, PO Box 3012, Bothell, WA 98021

***Antihistamines in Combination with Decongestants by HPLC*** Raja Achari, Bristol Myers Products, 1350 Liberty Ave, Hillside, NJ 07207

***Colchicine in Tablets*** Richard D. Thompson, Food & Drug Administration, 240 Hennepin Ave, Minneapolis, MN 55401

***Physostigmine & Its Salts*** Norlin W. Tymes, Food & Drug Administration, HFR-MA260, 900 Madison Ave, Baltimore, MD 21201

### Drugs III

*Referee:* Open

***Halogenated Hydroxyquinoline Drugs*** Edward J. Wojtowicz, Food & Drug Administration, Buffalo District, 599 Delaware Ave, Buffalo, NY 14202

***Hydralazine*** Barry Mopper, Food & Drug Administration, New York Regional Laboratory, HFF-2660, 850 Third Ave, Brooklyn, NY 11232

***Metals in Bulk Drug Powders*** Walter Holak, Food & Drug Administration, New York Regional Laboratory, HFF-2660, 850 Third Ave, Brooklyn, NY 11232

***Penicillins*** Barry Mopper, Food & Drug Administration, New York Regional Laboratory, HFF-2660, 850 Third Ave, Brooklyn, NY 11232

### Drugs IV

*Referee:* Linda Ng, Food & Drug Administration, HFD-150, 5600 Fishers Ln, Rockville, MD 20857

***Benzodiazepines*** Eileen Bargo, Food & Drug Administration, 900 Madison Ave, Baltimore, MD 21201

***D- & L-Amphetamines—HPLC Separation*** Thomas D. Doyle, Food & Drug Administration, Division of Research & Testing, HFD-473, 200 C St, SW, Washington, DC 20204

***Dicloxacillin Bulk Drug & Its Preparations*** Mei Chich Hsu, Food & Drug Bureau, Dept of Health, 161 Kuen Yang St, Nankang, Taipai 11513, Taiwan, ROC

***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

### Drugs V

*Referee:* Thomas G. Alexander, 16716 Huron St, Accokeek, MD 20607

***Aminobenzoic Acid and Salicylic Acid Salts in Pharmaceuticals*** Richard D. Thompson, Food & Drug Administration, 240 Hennepin Ave, Minneapolis, MN 55401

***HPLC Screening for Anabolic Steroids*** Milda Walters, Food & Drug Administration, 1560 E. Jefferson Ave, Detroit, MI 48207

***Pentaerythritol Tetranitrate*** Marvin Carlson, Food & Drug Administration, 240 Hennepin Ave, Minneapolis, MN 55401

***Steroids in Tablets*** Elaine A. Bunch, Food & Drug Administration, 22201 23rd Dr, SE, PO Box 3012, Bothell, WA 98021

### Diagnostics & Test Kits

*Referee:* Open

***Analytical Release Rate of Drugs from Transdermal Patches*** Norlin W. Tymes, Food & Drug Administration, HFR-MA260, 900 Madison Ave, Baltimore, MD 21201

***Automated Microbial Identification System (Hewlett Packard 5898A)*** Linda English, Food & Drug Administration, OHHS PHS, Baltimore District Office, Science Branch, HFR-MA260, 900 Madison Ave, Baltimore, MD 21201

***Immunological and Diagnostic Assay of Peptides, Hormones, and Enzymes*** John Dyminski, PO Box 2021, Saratoga, CA 95070

***Multicomponent Analysis of Clinical Specimens*** Uday J. Mehta, Food & Drug Administration, OMHS PHS, Baltimore District, Science Branch, 900 Madison Ave, Baltimore, MD 21201

***TB & Enteric Infections by Gene Probe*** Harvey George, Commonwealth of Massachusetts Center for Laboratory & Communicable Disease Control, 305 South St, Boston, MA 02130

### Drug Residues in Animal Tissues

*Referee:* Charlie J. Barnes, Food & Drug Administration, Office of Science, HFV-501, Bldg 328A, BARC-E, Beltsville, MD 20705

***β-Lactam Antibiotics in Milk by LC (Qualitative)*** William A. Moats, U.S. Dept of Agriculture, ARS, Meat Science Research Laboratory, Bldg 201, BARC-E, Beltsville, MD 20705

***β-Lactam Residues in Milk by Delvotest*** Wesley N. Kelley, 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, Food & Drug Administrtion, Division of Veterinary Medical Research, HFV-501, Center Rd, Bldg 328A, BARC-E, 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:* Open

*Water & Alcohol in Cosmetics* Mohinder Singh, Blistex, Inc., 1800 Swift Dr, Oak Brook, IL 60521

### Forensic Sciences

*Referee:* Stanley M. Cichowicz, Food & Drug Administration, Division of Microbiology, HFF-237, 200 C St, SW, Washington, DC 20204

*Gunshot Residues* Robert D. Koons, Forensic Science Research & Training Center, FBI Academy, Quantico, VA 22135

## Committee on Foods I

**James F. Lawrence** (Health & Welfare Canada, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario K1A OL2, Canada), *Chairman*; **David B. Berkowitz** (5360 Racegate Run, Columbia, MD 21045); **Douglas R. Engebretson** (Land O'Lakes, Inc., 4001 Lexington Ave N., PO Box 116, Arden Hills, MN 55126); **Max L. Foster**, Kansas State Board of Agriculture, 2524 Sixth Street, Topeka, KS 66606; **Harriet Wallin**, VTT Technical Research Center, Food Research Laboratory, Biologinkufa 1, SF-02150; **Donald E. Carpenter** (Kraft General Foods, Inc., 801 Waukegan Rd, Glenview, IL 60025); **Marleen M. Wekell** (Food & Drug Administration, 22201 23rd Dr, SE, PO Box 3012, Bothell, WA 98041-3012); **John Gilbert** (Ministry of Agriculture, Fisheries & Food, Food Safety Directorate, Food Science Laboratory, Colney Ln, Norwich, NR4 7UQ, United Kingdom); **Douglas Park** (University of Arizona, Dept of Nutrition & Food Science, 309 Schantz Bldg, Tucson, AZ 85721), *Secretary*; **Terry Nelsen** (U.S. Dept of Agriculture, Northern Regional Research Center, Agriculture Research Service, 1815 N. University St, Peoria, IL 61604), *Committee Statistician*; **Sandra Pfeiffer** (Gerber Products Co., 445 State St; Fremont, MI 49412), *Safety Advisor*

### 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, Food & Drug Administration, Division of Colors & Cosmetics, HFF-445, 200 C St, SW, Washington, DC 20204

***Colors in Candy & Beverages*** Mary Young, Food & Drug Administration, New York Regional Laboratory, HFF-2660, 850 Third Ave, Brooklyn, NY 11232

***Uncombined Intermediates & Subsidiary Colors in Certifiable Colors*** Alan Scher, Food & Drug Administration, Division of Colors & Cosmetics, HFF-445, 200 C St, SW, Washington, DC 20204

### 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, Centre for Food and Animal Research, Agriculture Canada, 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, Advanced Instruments, Inc.,1000 Highland Ave, Needham Heights, MA 02194

***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

***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, Dept of Food Science and Agricultural Chemistry, McGill University, 111 Lakeshore Dr, Ste. Anne de Bellevue, Quebec HPX 1C0, Canada

*Gerber Test* Dick H. Kleyn, Rutgers University, Dept of Food Science, Dudley Rd, New Brunswick, NJ 08903

*Iodine in Milk* David Sertl, Russ 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, 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

## 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

## Food Additives

*Referee:* Thomas Fazio, Food & Drug Administration, Office of Physical Sciences, HFF-400, 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

*Identification of Irradiated Foods* Leslie G. Ladomery, FAO/IAEA Division of Nuclear Techniques in Food & Agriculture, Wagramerstrasse 5, PO Box 200, A-1400 Vienna, Austria

*Indirect Additives from Food Packages* Henry Hollifield, Food & Drug Administration, Division of Food Chemistry & Technology, HFF-413, 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, Food & Drug Administration, Division of Chemistry & Physics, HFF-459, 200 C St, SW, Washington, DC 20204

*Polydimethylsiloxane* R. Firmin, 70 Rue St Georges, 1050 Brussels, Belgium

*Sulfites—Flow Injection Analysis* Marleen M. Wekell, Food & Drug Administration, 22201 23rd Dr, SE, PO Box 3012, Bothell, WA 98041-3012

*Sulfites in Foods—Ion-Chromatographic Methods* Darryl M. Sullivan, Hazleton Laboratories America, Inc., 3301 Kinsman Blvd, PO Box 7545, Madison, WI 53707; Hie Joon Kim, Kim & Associates, 33 Pleasant St, Wayland, MA 01778

*Sulfites—Polarographic Methods* Walter Holak, Food & Drug Administration, New York Regional Laboratory, HFF-2660, 850 Third Ave, Brooklyn, NY 11232

## Meat, Poultry, & Meat & Poultry Products

*Referee:* David L. Soderberg, Food Safety and Inspection Service, 300 Twelfth St, SW, Washington, DC 20250

*Cholesterol in Eggs* Daniel G. Lebryk, KGF Technology Center, 801 Waukegan Rd, Glenview, IL 60025

*Gluten in Meat* John H. Skerritt, Csiro Wheat Research Unit, Division of Plant Industry, PO Box 7, North Ryde, New South Wales 2113, Australia

*Heme, Nonheme, and Total Iron in Meat, Fish, and Poultry Products* Arthur W. Mahoney, Utah State University, Dept of Nutrition & Food Sciences, College of Agriculture, Logan, Utah 84322-2379

*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

*Potentiometric Determination of Sodium and Salt* Rob W. Maeijer, Nestle Nederland B.V., c/o Walstraat 17, 8011 NR Zwolle, The Netherlands

***Microwave Techniques*** David L. Fish, CEM Corp., PO Box 200, Matthews, NC 28105

***Minimum Processing Temperature for Meats*** Grover Pickel, U.S. Dept of Agriculture, FSIS Science Eastern Laboratory, Russell Research Center, PO Box 6085, Athens, GA 30604

***Non Meat Protein in Meat and Poultry Products*** Philip R. Goodwin, Cortecs Diagnostics Ltd, Techbase 1, Newtech Square, Deeside, Clwyd, CHS 2NT, United Kingdom

***Protein Determination in Meat by Combustion Method*** Joseph G. Sebranek, Iowa State University, Dept of Animal Science, 215 Meat Laboratory, Ames, IA 50011

***Total Fat*** Max L. Foster, Kansas State Board of Agriculture, Division 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

## Mycotoxins

*Referee:* Peter M. Scott, Health & Welfare Canada, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario K1A 0L2, Canada

***Aflatoxin Methods*** Mary W. Trucksess, Food & Drug Administration, HFF-423, 200 C St, SW, Washington, DC 20204

***Alternaria Toxins*** Angelo Visconti, Instituto 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 Labortory, 1011 Forrester Dr, SE, Dawson, GA 31742

***Ergot Alkaloids*** George Ware, Food & Drug 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, Food & Drug Administration, Division of Contaminants Chemistry, HFF-423, 200 C St, SW, Washington, DC 20204

***Trichothecenes*** Robert M. Eppley, Food & Drug Administration, Division of Chemistry & Physics, HFF-423, 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:* Samuel W. Page, Food & Drug Administration, Natural Products & Instrumentation Branch, HFF-423, 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, Food & Drug Administration, Division of Contaminants Chemistry, HFF-423, 200 C St, SW, Washington, DC 20204

***Phytoestrogens*** Shia S. Kuan, Food & Drug Administration, 4298 Elysian Fields Ave, New Orleans, LA 70122

***Pyrrolizidine Alkaloids*** Robert M. Eppley, Food & Drug Administration, Division of Chemistry & Physics, HFF-423, 200 C St, SW, Washington, DC 20204

***Steroidal Alkaloids*** Allen S. Carman, Food & Drug Administration, 4298 Elysian Fields Ave, New Orleans, LA 70122

## Seafood Products

*Referee:* James M. Hungerford, 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, Food & Drug Administration, Fishery Research Branch, #1 Iberville, PO Box 158, Dauphin Island, AL 36528

***Conjugated Dienes as Indicators of Decomposition*** Judith Kryznowek, National Marine Fisheries Service, 30 Emerson Ave, Gloucester, MA 01930

***Cyanobacterial Peptide Toxins*** Judith Pace, U.S. Army Med Res 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 W. Gilgan, Dept of Fisheries & Oceans, Regional Inspection Laboratory, PO Box 550, Halifax, Nova Scotia B3J 2S7, Canada

***Flow Injection Analysis*** James M. Hungerford, 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*** James M. Hungerford, 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

## Committee on Foods II

**Jonathan W. DeVries** (General Mills Inc., 9000 Plymouth Ave N., Minneapolis, MN 55427), *Chairman*; **Benjamin Krinitz** (Whitestone Laboratories, 147–27 Seventh Ave, Whitestone, NY 11357-1620); **Joseph C. Brucciani** (Food & Drug Administration, 60 Eighth St, NE, Atlanta, GA 30309); **Adeline Peake** (Agriculture Canada, Laboratory Division Service West, 3650 36 St NW, Calgary, Alberta T2L 2L1, Canada); **Roger Wood** (Ministry of Agriculture, Fisheries & Food, Food Science Laboratory, Colney Ln, Norwich NR4 7UQ, United Kingdom); **Randall Smith** (Hazleton Laboratories America, Inc., 3301 Kinsman Blvd, PO Box 7545, Madison, WI 53707); **Douglas J. Bark** (Woodson-Tenent Laboratories, Inc., PO Box 2135, 345 Adams Ave, Memphis, TN 38103) *Secretary*; **John G. Phillips** (U.S. Dept of Agriculture, Eastern Regional Research Center, 600 E. Mermaid Ln, Philadelphia, PA 19118), *Committee Statistician*; **David Egelhofer** (National Food Processors Association, 1401 New York Ave, NW, Suite 400, Washington, DC 20005), *Safety Advisor*

### Alcoholic Beverages

*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*** Randolph H. Dyer, Bureau of Alcohol, Tobacco & Firearms, 1401 Research Blvd, Rockville, MD 20850

***Glycerol Monooleates in Wine*** Tony Ribeiro, E & J Gallo Winery, PO Box 1130, Modesto, CA 95353

***Lead in Wine*** Alan L. Reisig, Bureau of Alcohol, Tobacco, and Firearms, 1401 Research Blvd, Rockville, MD 20850

***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 OBB 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

## Cereals & Cereal Products

*Referee:* Ralph H. Lane, University of Alabama, Dept of Food & Nutrition, 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, Leiden University, Wassendarseweg 62, PO Box 9604, 2300 RC Leiden, The Netherlands

***Gluten in Foods*** John H. Skerritt, Csiro Wheat Research Unit, Division of Plant Industry, PO Box 7, North Ryde, New South Wales 2113, Australia

***Iron*** James I. Martin, 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:* Michael E. Hillyer, Ambrosia Chocolate Co., 1133 N. Fifth St, Milwaukee, WI 53203

***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

## Dietary Fiber

*Referee:* Leon Prosky, Food & Drug Administration, Division of Nutrition, HFF-268, 200 C St, SW, Washington, DC 20204

***Detection by UED*** Joseph L. Jeraci, Syracuse Research Corp., Merrill Ln, Syracuse, NY 13210

***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

***Nonenzymatic Method for Products with Little or No Starch*** Betty Li, U.S. Dept of Agriculture, Agricultural Research Service, Beltsville, MD 20705

## Fats & Oils

*Referee:* David Firestone, Food & Drug Administration, Division of Contaminants Chemistry, HFF-426, 200 C St, SW, Washington, DC 20204

***Emulsifiers*** Theresa W. Lee, Ross Laboratories, Analytical Research & Services, 625 Cleveland Ave, Columbus, OH 43216

***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, Food & Drug Administration, 1 Montvale Ave, Stoneham, MA 02180-3542

## Fruits & Fruit Products

*Referee:* Frederick E. Boland, Food & Drug Administration, Division of Food Chemistry & Technology, HFF-414, 200 C St, SW, Washington, DC 20204

***Added Invert Sugars by LC/Pulsed Amperometric Detection*** Nicholas Low, Dept of Applied Microbiology and Food Science, University of Saskatchewan, Saskatoon, Saskatchewan S7N 0W0, Canada

***Fruit Acids*** Elia D. Coppola, Ocean Spray Cranberries, One Ocean Spray Dr, Lakeville, Middleboro, MA 02349

***Geographic Origin of Orange Juice*** Seifollah Nikdel, Florida Dept of Citrus, 700 Experiment Station Rd, Lake Alfred, FL 33850

***Identification & Characterization of Fruit Juices*** Ronald E. Wrolstad, Oregon State University, Dept of Food Science & Technology, Corvallis, OR 97331

***Naringin & Neopheoperidin in Orange Juice*** Wilbur Widmer, Florida Dept of Citrus, 700 Experiment Station Rd, Lake Alfred, FL 33850

***Orange Juice Adulteration with Pulpwash*** Donald Petrus, 2218 Port St, NW, Winter Haven, FL 33881

*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

*Stable Carbon Isotope Ratio Analysis* Rae Gabrielle Krueger, Krueger Food Laboratories, Inc., 24 Blackstone St, Cambridge, MA 02139

### Nonalcoholic Beverages

*Referee:* John M. Newton, 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, Food & Drug Administration, 240 Hennepin Ave, Minneapolis, MN 55401

*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, Food & Drug Administration, HFR-9160, 50 Fulton St, San Francisco, CA 94102

*Safrole in Sassafras Root* Marvin Carlson, Food & Drug Administration, 240 Hennepin Ave, Minneapolis, MN 55401

### Processed Vegetable Products

*Referee:* Thomas R. Mulvaney, Food & Drug Administration, Center for Food Safety & Applied Nutrition, HFF-412, 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

*Sodium Determination in Processed Vegetable Products* J. Anderson Williams, Woodson-Tenent Laboratories, Inc., PO Box 2135, 345 Adams Ave, Memphis, TN 38101

*Sugars in Processed Vegetables by LC* 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 Division, Western Research Laboratory, 6363 Clark Ave, Dublin, CA 94568-3001

*Water Activity in Foods* William H. Stroup, Food & Drug Administration, Rm 5C31, IITRI Research Tower, 10 W. 35th St, Chicago, IL 60616

### Spices & Other Condiments

*Referee:* James E. Woodbury, Cal Compack Food, Inc., PO Box 265, 4906 First St, Santa Ana, CA 92702

*Capsicum Spices & Oleo Resins—Extractable Color* James E. Woodbury, Cal Compack Food, Inc., PO Box 265, 4906 First St, Santa Ana, CA 92702

*Capsicum Spices & Oleo Resins—Pungency* Mark Parrish, McCormick & Co., Inc., 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

### Sugars & Sugar Products

*Referee:* Margaret A. Clarke, Sugar Processing Research, Inc., 1100 Robert E. Lee Blvd, New Orleans, LA 70124

*Corn Syrup & Sugar Products* Raffaele Bernetti, CPC International, Inc., Moffett Technical Center, PO Box 345, Argo, IL 60501

*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

*Liquid Chromatographic Methods* Charles W. Tsang, Sugar Processing Research, Inc., 1100 Robert E. Lee Blvd, PO Box 19687, New Orleans, LA 70179

*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, United Kingdom

***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, Food & Drug Administration, Division of Nutrition, HFF-264, 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

***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

***Infant Formula Nutrient Assay*** James T. Tanner, Food & Drug Administration, HFF-266, 200 C St, SW, Washington, DC 20204

***Iodine*** Walter Holak, 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 B.V., 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, Food & Drug Administration, Division of Nutrition, HFF-268, 200 C St, SW, Washington, DC 20204

***Vitamins A, D, E, & K by Gel Permeation & HPLC*** Abdel-Gawad Soliman, Food & Drug Administration, 60 Eighth St, NE, Atlanta, GA 30309

## Committee on Residues

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### Metals & Other Elements

*Referee:* Stephen G. Capar, Food & Drug Administration, Division of Contaminants Chemistry, HFF-426, 200 C St, SW, Washington, DC 20204

***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, Tunney's Pasture, Ottawa, Ontario K1A 0L2, Canada

*Graphite Furnace—Atomic Absorption Spectrophotometry* Robert W. Dabeka, Health & Welfare Canada, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario K1A 0L2, Canada

*Lead in Calcium Supplements* Paul H. Sitonen, Food & Drug Administration, National Center for Toxicological Research, Jefferson, AR 72079-9502

*Neutron Activation Analysis* William C. Cunningham, Food & Drug Administration, NIST, Bldg 235, Rm B108, Gaithersburg, MD 20899

*Organometallics in Fish* Walter Holak, 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, Food & Drug Administration, Division of Contaminants Chemistry, HFF-426, 200 C St, SW, Washington, DC 20204

*Comprehensive Multiresidue Methodology* S. Mark Lee, California Dept of Food & Agriculture, Division of Inspection Services, Chemical Laboratory Services, 3292 Meadowview Rd, Sacramento, CA 95832

*Fumigants* James L. Daft, 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, Food & Drug Administration, Division of Contaminants Chemistry, HFF-426, 200 C St, SW, Washington, DC 20204

*Miniaturized Multiresidue Methods for Fat Containing Foods* D. Ronald Erney, Food & Drug Administration, HFR-5360, 1560 E. Jefferson Ave, Detroit, MI 48207

### Organohalogen Pesticides

*Referee:* Bernadette McMahon, Food & Drug Administration, Division of Contaminants Chemistry, HFF-426, 200 C St, SW, Washington, DC 20204

*Chlorinated Dioxins* David Firestone, Food & Drug Administration, Division of Contaminants Chemistry, HFF-426, 200 C St, SW, Washington, DC 20204

*Chlorophenoxy Alkyl Acids* Marvin L. Hopper, Food & Drug Administration, HFR-SW360, 1009 Cherry St, Kansas City, MO 64106

*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 Division, 1600 Clifton Rd, Atlanta, GA 30333

### Organonitrogen Pesticides

*Referee:* W. Harvey Newsome, Health & Welfare Canada, Health Protection Branch, Food Research Division, Tunney's Pasture, Ottawa, Ontario K1A 0L2, Canada

*Captan & Related Fungicides* Dalia M. Gilvydis, Food & Drug Administration, 1560 E. Jefferson Ave, Detroit, MI 48207

*Carbamate Insecticides* Richard Krause, Food & Drug Administration, Division of Chemical Technology, HFF-246, 200 C St, SW, Washington, DC 20204

*Diquat & Paraguat* Brian L. Worobey, Health & Welfare Canada, Bureau of Chemical Safety, Tunney's Pasture, Ottawa, Ontario K1A 0L2, Canada

*Substituted Ureas* Ronald Luchtefeld, Food & Drug Administration, 1009 Cherry St, Kansas City, MO 64106

### Organophosphorus Pesticides

*Referee:* Gail Abbott Parker, Florida Dept of Agriculture & Consumer Services, 3125 Conner Blvd, Tallahassee, FL 32399-1650

*Organophosphorus Pesticide Residues* Ronald R. Laski, Food & Drug Administration, HFR-2260, 599 Delaware Ave, Buffalo, NY 14202

### Radioactivity

*Referee:* Edmond J. Baratta, 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

## Committee on Microbiology and Extraneous Materials

**Gayle A. Lancette** (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** (Food & Drug Administration, Division of Microbiology, HFF-237, 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, ARS, Russell Research Center, PO Box 5677, Athens, GA 30613), *Secretary*; **Foster D. McClure** (Food & Drug Administration, Division of Mathematics, HFF-116, 200 C St, SW, Washington, DC 20204), *Committee Statistician*; **Carlos Abeyta** (Food & Drug Administration, 22201 23rd Dr, SE, PO Box 3012, Bothell, WA 98041-3012), *Safety Advisor*

### Analytical Mycology & Microscopy

*Referee:* Ruth Bandler, Food & Drug Administration, Division of Microbiology, HFF-237, 200 C St, SW, Washington, DC 20204

***Geotrichum Mold in Canned and Frozen Fruits, Vegetables, and Fruit Juices*** Stanley M. Cichowicz, Food & Drug Administration, Division of Microbiology, HFF-237, 200 C St, SW, Washington, DC 20204

***Mold by Chemical Detection*** Ruth Bandler & George C. Ziobro, Food & Drug Administration, Division of Microbiology, HFF-237, 200 C St, SW, Washington, DC 20204

***Standardized Plant Tissue Concentrations for Mold Counting*** Raymond Galacci, Food & Drug Administration, 900 Madison Ave, Baltimore, MD 21201

***Vegetable Substitutes in Horseradish*** Raymond Galacci, Food & Drug Administration, 900 Madison Ave, Baltimore, MD 21201

***Yeasts & Molds—Mycological Media for Isolation*** Philip B. Mislivec, Food & Drug Administration, Division of Microbiology, HFF-237, 200 C St, SW, Washington, DC 20204

### 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, Food & Drug Administration, HFF-234, 200 C St, SW, Washington, DC 20204

### Drug- & Device-Related Microbiology

*Referee:* Open

***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, Food & Drug Administration, MLMI, 240 Hennepin Ave, Minneapolis, MN 55401

***Packaging Integrity of Medical Devices*** Ana M. Placencia, Food & Drug Administration, Sterility Research Center, 240 Hennepin Ave, Minneapolis, MN 55401

***Sporicidal Testing of Disinfectants/Sterilants*** James Danielson, 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

***Bactoscan Methods*** J. D. Cunningham, University of Guelph, Environmental Biology, Guelph, Ontario N1G 2W1, Canada; Robert Maxey, U.S. Environmental Protection Agency, 1105 NASA NTSL Bldg, Stennis Space Center, MS 39529-6000

***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, Food & Drug Administration, Division of Microbiology, HFF-234, 200 C St, SW, Washington, DC 20204

***Aerobic Plate Counts & Coliforms—HGMF Methods*** Phyllis Entis, QA Laboratories, Ltd, 135 West

Mall #2, Toronto, Ontario M9C 1C2, Canada

***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, Food & Drug Administration, Division of Microbiology, HFF-234, 200 C St, SW, Washington, DC 20204

***Clostridium perfringens—Iron Milk Test for Recovery from Marine Environment*** Carlos Abeyta, 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

***E. coli—Chilled & Frozen Foods, MUG Test*** Lloyd Moberg, General Mills, Inc., PO Box 1113, Minneapolis, MN 55440

***E. coli—Shellfish, MUG Test*** William Watkins, Food & Drug Administration, Bldg S 26, Construction Battalion Center, N. Kingstown, RI 02852

***Listeria—Gas Chromatography of Cellular Fatty Acids for Species Identification*** Linda English, Food & Drug Administration, OHHS PHS, Balltimore District Office, Science Branch, HFR-MA260, 900 Madison Ave, Baltimore, MD 21201

***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—Tecra System*** Judy Heisick, Food & Drug Administration, MW Laboratory for Microbiology Investigations, 240 Hennepin Ave, Minneapolis, MN 55401

***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—Automated Conductance Methods*** Donald Gibson, Ministry of Agriculture, Fisheries & Food, Torry Research Station, 135 Abbey Rd, PO Box 31, Aberdene ABG BDG, United Kingdom

***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 Laboratories, Ltd, 135 West Mall #2, Toronto, Ontario M9C 1C2, Canada

***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—Non-Isotopic DNA-RNA Hybridization Probe 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, Food & Drug Administration, Division of Microbiology, HFF-234, 200 C St, SW, Washington, DC 20204

***Vibrio cholerae in Oysters—Elevated Temperature Enrichment Method*** Angelo DePaolo, RR 1, Box 980, Coden, AL 36523

***Vibrio vulnificus—Identification by GC of Cellular Fatty Acids*** Warren Landry, Food & Drug Administration, 3032 Bryan St, Dallas, TX 75204

## Filth & Extraneous Materials in Foods & Drugs

*Referee:* Jack L. Boese, Food & Drug Administration, Division of Microbiology, HFF-237, 200 C St, SW, Washington, DC 20204

***Detection of Alkaline Phosphatase in Foods by Electrophoresis*** George C. Ziobro, Food & Drug Administration, Division of Microbiology, HFF-237, 200 C St, SW, Washington, DC 20204

***Fecal Contamination in Grain Products by GC*** George C. Ziobro, Food & Drug Administration, Division of Microbiology, HFF-237, 200 C St, SW, Washington, DC 20204

***Grains (Whole), Internal Insect Infestation by ELISA Method*** G. Barrie Kitto, Center for Biotechnology, Welch Hall 4.260C, University of Texas at Austin, Austin, TX 78712

***Internal Insect Infestation of Whole Grains—Cracking Flotation Methods*** Richard Trauba, Food & Drug Administration, 240 Hennepin Ave, Minneapolis, MN 55401

***Light Filth in Baked Goods with Fruit & Nut Tissues—Flotation*** Joseph K. Nagy, Food & Drug Administration, Second & Chestnut Sts, Rm 900, Philadelphia, PA 19106

***Light Filth in Bean Paste by Flotation*** John R. Bryce, Food & Drug Administration, Division of Microbiology, HFF-237, 200 C St, SW, Washington, DC 20204

***Light Filth (External) in Grains and Seeds by Flotation Method*** James Gallman, 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, Food & Drug Administration, Division of Microbiology, HFF-237, 200 C St, SW, Washington, DC 20204

***Light Filth in Unground Basil—Flotation*** Beverly Kent, Food & Drug Administration, Buffalo District, 599 Delaware Ave, Buffalo, NY 14202

***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, Food & Drug Administration, Division of Microbiology, HFF-237, 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, Food & Drug Administration, Division of Microbiology, HFF-237, 200 C St, SW, Washington, DC 20204

***Light Filth in Grain Products—Flotation Methods*** John R. Bryce, Food & Drug Administration, Division of Microbiology, HFF-237, 200 C St, SW, Washington, DC 20204

***Light Filth in Ground Coffee—Flotation Methods*** Gerald E. Russell, Food & Drug Administration, 1560 E. Jefferson Ave, Detroit, MI 48207

***Light Filth in Soybean Curd—Flotation Methods*** Marvin Nakashima, Food & Drug Administration, Division of Microbiology, HFF-237, 200 C St, SW, Washington, DC 20204

***Mammalian Feces in Grain Products—Alkaline Phosphatase Detection*** Harriet R. Gerber, Food & Drug Administration, HFC-141, Division of Field Science, 5600 Fishers Ln, Rockville, MD 20857

***Rodent Gnawing*** Jack L. Boese, Food & Drug Administration, Division of Microbiology, HFF-237, 200 C St, SW, Washington, DC 20204

***Urine Stains—Chemical Methods*** Robert S. Ferrera, Food & Drug Administration, Division of Microbiology, HFF-237, 200 C St, SW, Washington, DC 20204

***Mammalian Feces in Spices—Chemical Detection*** Harriet R. Gerber, Food & Drug Administration, HFC-141, Division 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** (Food & Drug Administration, National Center for Toxicological Research, Jefferson, AR 72079), *Chairman*; **Lori Rhodig** (Smithkline Animal Health Products, 4444 S. 76th Circle, Omaha, NE 68127); **Carolyn A. Geisler** (Food & Drug Administration, Denver Federal Center, HFR SW260, Bldg 20, PO Box 25087, Denver, CO 80225-0087); **Stanley E. Katz** (Rutgers University-Cook College, Dept of Biochemistry & Microbiology, New Brunswick, NJ 08903); **George W. Latimer, Jr** (Office of the Texas State Chemist, PO Box 3160, College Station, TX 77841-3160); **Thomas M. Parham, Jr** (Arcadian Corp., Triazone Division, PO Box 307, Geismar, LA 70734); **Lars Reimann** (Woodson-Tenent Laboratories, Inc., PO Box 2135, 345 Adams Ave, Memphis, TN 38101); **W. Emmett Braselton, Jr** (Michigan State University, Dept of Pharmacology & Toxicology, East Lansing, MI 48824), *Secretary*; **Daniel H. Mowrey** (Lilly Research, Laboratories Division 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 in Feeds*** Anil D. Desai, A.L. Laboratories, Inc., 400 State St, Chicago Heights, IL 60411

***Chlortetracycline in Feeds*** Mary Lee Hasselberger, Nebraska Dept of Agriculture, Laboratory Division, 3703 S. 14th St, Lincoln, NE 68502

***Direct-Fed Microbiological Products & Silage Inoculants*** William Aimutis, Hansen's Biosystems, 9015 W. Maple St, Milwaukee, WI 53214-4213

***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

***Narasin Microbiological Method*** Mark R. Coleman, Lilly Research Laboratories, PO Box 708, Greenfield, IN 46140

***Neomycin*** Gerald L. Stahl, The Upjohn Co., Downtown Complex, 9760 209 5, Kalamazoo, MI 49001

***Oxytetracycline in Feeds by Microbiological Methods*** Mary Lee Hasselberger, Nebraska Dept of Agriculture, Laboratory Division, 3703 S. 14th St, Lincoln, NE 68502

***Tylosin*** Mark R. Coleman, Lilly Research Laboratories, PO Box 708, Greenfield, IN 46140

***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 in Feeds*** 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 Division, 3703 S. 14th St, Lincoln, NE 68502

***Ethopabate*** Joseph Hillebrandt, Polysey Corp., PO Box 111, Upper North Main, Mechanicville, NY 12118

***Furazolidone & Nitrofurazone*** Lori L. Rhodig, Smithkline Animal Health Products, 4444 S. 76th Circle, Omaha, NE 68127

***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 & Narasin Chromatographic Method*** Mark R. Coleman, Lilly Research Laboratories, PO Box 708, Greenfield, IN 46140

***Morantel Tartrate*** Linda D. Werner, Pfizer, Inc., 1107 South Missouri 291, Lee's Summit, MO 64081

***Oxytetracycline in Feeds by Liquid Chromatographic Methods*** Mary Lee Hasselberger, Nebraska Dept of Agriculture, Laboratory Division, 3703 S. 14th St, Lincoln, NE 68502

***Pyrantel Tartrate*** Joyce Konrardy, Pfizer, Inc., 1107 South Missouri 291, Lee's Summit, MO 64063

***Sampling*** John B. Gallagher, Pitman Moore Inc., PO Box 207, Terre Haute, IN 47808

***Sulfadimethoxine and Ormetoprim*** Alexander MacDonald, Hoffmann-La Roche, Inc., Food & Agricultural

Products, 340 Kingsland St, Nutley, NJ 07110

***Sulfa Drug Residues in Feeds*** Valerie Reeves, Food & Drug Administration, HFV-502, Bldg 328A, BARC-E, Beltsville, MD 20705

***Sulfamethazine & Sulfathiazole in Premixes & Finished Feeds*** 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

***Fat*** George Wilkens, Agway Technical Center, 777 Warren Rd, Ithaca, NY 14850

***Fiber*** David R. Mertens, U.S. Dairy Forage Research Center, 1925 Linden Dr, W., Madison, WI 53706

***Inorganic Elemental Constituents of Meat Samples, Microwave Digestion Method*** David Miller, DANR Analytical Laboratory, University of California, 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, U.S. Dept of Agriculture, ARS, Russell Research Center, 950 College Station Rd, PO Box 5677, Athens, GA 30604

***Moisture in Mixed Feeds & Forages*** William R. Windham, U.S. Dept of Agriculture, ARS, PO Box 5677, Athens, GA 30677

***Moisture in Pet Foods*** Roy E. Schulze, Ralston Purina Co., Checkerboard Square, St. Louis, MO 63164

***Sampling 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 Nitrogen by Combustion Techniques*** Donald F. Tate, Illinois Dept of Agriculture, Chemistry Laboratory, PO Box 19281, Springfield, IL 62794

***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

***Phosphorus*** Joe Gliksman, ICM Fertilizer, Inc. PO Box 1035, Mulberry, FL 33860

***Potassium*** Natalie Newlon, Purdue University, Dept of Biochemistry, West Lafayette, IN 47907-1154

***Sample Preparation*** Robert L. Beine, Kentucky Agricultural Experiment Station, Division 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

***Slow-Release Mixed Fertilizers*** Stanley E. Katz, Rutgers University-Cook College, Dept of Biochemistry & Microbiology, New Brunswick, NJ 08903

***Urea & Methlyeneuraes in the Fertilizer and Agricultural Liming Materials*** Thomas M. Parham, Jr, Arcadian Corp., Triazone Division, PO Box 307, Geismar, LA 70734

***Water in Fertilizers*** James A. Farley, Tennessee Valley Authority, NFE 2J-M, Muscle Shoals, AL 35660

### Nutrients in Soils

*Referee:* Thomas L. Jensen, State Dept of Agriculture, 3703 S. 14th St, Lincoln, NE 68502

***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. Eversan, 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, Department 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

*Selenium* Karen S. Harlin, University of Illinois, Dept of Veterinary Biosciences, 2001 S. Lincoln, Urbana, IL 61807

*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

*Vitamins D & K* Karen S. Harlin, University of Illinois, Dept of Veterinary Biosciences, 2001 S. Lincoln, Urbana, IL 61801

*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, Analytical Laboratory Section, 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 NW, North Canton, OH 44720); **Larry Lane** (Mississippi State Chemistry Laboratory, PO Box CR, Mississippi State, MS 39762); **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** (Quality Assurance Program, 4300 W. 123rd Street, Alsip, IL 60658), *Secretary*; **Paul Britton** (U.S. Environmental Protection Agency, EMSL Cincinnati QARD PMB, 26 W. Martin Luther King Dr, Cincinnati, OH 45268), *Committee Statistician*; **Dirk Shoemaker** (Nebraska Dept of Agriculture, 3703 S. 14th St, Lincoln, NE 68502), *Safety Advisor*

### 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, DANR Analytical Laboratory, University of California, 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:* Kenneth P. Stoub, Waste Management, Inc., Environmental Monitoring Laboratories, 2100 Cleanwater Dr, Geneva, IL 60134

*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

## 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

*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 on May 30–June 1, August 10, and August 15, 1991, the following recommendations on specific methods were adopted, i.e., 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, May 30, 1991, adoption of first action methods and changes or revisions in first action methods become official immediately upon vote by the Official Methods Board. No adoption of first action methods as final action or changes or revisions in final action methods are listed here because these actions require vote by the Association membership, which has not occurred by this publication date.

Chapter and method numbers refer to *Official Methods of Analysis* (1990) 15th edition and "Changes in Official Methods of Analysis," first supplement (1990) and second supplement (1991).

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," third supplement (1992), 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

No additions, deletions, or other changes.

### Ch. 3. Plants

(1) A change was made in first action method **990.01**, nicotine in environmental tobacco smoke, gas chromatographic method.

### Ch. 4. Animal Feed

No additions, deletions, or other changes.

### Ch. 5. Drugs in Feeds

No additions, deletions, or other changes.

### Ch. 6. Disinfectants

(1) The hard surface carrier test method for testing disinfectants against *Salmonella choleraesuis* was adopted first action, **991.47**.

(2) The hard surface carrier test method for testing disinfectants against *Staphylococcus aureus* was adopted first action, **991.48.**

(3) The hard surface carrier test method for testing disinfectants against *Pseudomonas aeruginosa* was adopted first action, **991.49**.

### Ch. 7. Pesticide Formulations

(1) The liquid chromatographic method for determination of cyanazine in technical products and pesticide formulations was adopted first action as a CIPAC-AOAC method, **991.32**.

(2) The carbon disulfide evolution method for determination of maneb in pesticide formulations containing fentin acetate or fentin hydroxide was adopted first action as a CIPAC-AOAC method, **991.33**.

(3) The gas chromatographic method for determination of pirimiphos-methyl in technical products and pesticide formulations was adopted first action as a CIPAC-AOAC method, **991.34**.

### Ch. 8. Hazardous Substances

No additions, deletions, or other changes.

### Ch. 9. Metals and Other Elements at Trace Levels in Foods

No additions, deletions, or other changes.

### Ch. 10. Pesticide and Industrial Chemical Residues

No additions, deletions, or other changes.

### 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

No additions, deletions, or other changes.

### Ch. 15. Cosmetics
No additions, deletions, or other changes.

### Ch. 16. Extraneous Materials: Isolation
(1) The flotation method for determination of light filth from fish paste and sauce (Bagoong) not containing spice was adopted first action, **991.37**.
(2) The flotation method for determination of light filth in dried bean curd was adopted first action, **991.40**.
(3) The isolation techniques for extraneous material, **945.75B**, was revised.
(4) The general method for light and heavy filth, **970.66B**, was revised.

### Ch. 17. Microbiological Methods
(1) The automated conductance method for determination of *Salmonella* in food was adopted first action, **991.38**.
(2) The first action colorimetric DNA hybridization method for determination of *Salmonella* in food, **990.13**, was revised.
(3) The first action hydrophobic grid membrane filter screening method for detection of *Salmonella* in foods, **985.42**, was repealed.
(4) The final action colorimetric polyclonal enzyme immunoassay screening method for detection of *Salmonella* in foods, **989.14C**, was editorially revised.

### Ch. 18. Drugs: Part I
No additions, deletions, or other changes.

### Ch. 19. Drugs: Part II
No additions, deletions, or other changes.

### Ch. 20. Drugs: Part III
No additions, deletions, or other changes.

### Ch. 21. Drugs: Part IV
No additions, deletions, or other changes.

### Ch. 22. Drugs: Part V
(1) The liquid chromatographic method for determination of flurazepam hydrochloride in bulk drug and capsules was adopted first action, **991.35**.

### Ch. 23. Drugs and Feed Additives in Animal Tissues
No additions, deletions, or other changes.

### Ch. 24. Forensic Sciences
No additions, deletions, or other changes.

### Ch. 25. Baking Powders and Baking Chemicals
No additions, deletions, or other changes.

### Ch. 26. Distilled Liquors
(1) The final action method for determination of alcohol by volume in distilled liquors, **982.10**, was editorially revised.
(2) The final action method for determination of alcohol by volume in liqueurs (dairy products), **983.12A(b)**, was editorially revised.

### Ch. 27. Malt Beverages and Brewing Materials
(1) The first action method for determination of specific gravity of beer and wort **988.06B(a)** was editorially revised.

### Ch. 28. Wines
(1) The liquid chromatographic method for determination of glycerol in wine and grape juice was adopted first action, **991.46**.

### 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 enzymatic-gravimetric method (phosphate buffer) for the determination of insoluble dietary fiber in food and food products was adopted first action, **991.42**.
(2) The enzymatic-gravimetric method (MES-TRIS buffer) for the determination of total, soluble, and insoluble dietary fiber in foods was adopted first action, **991.43**.
(3) The first action method for total dietary fiber in foods **985.2** was mutually adopted as an AOAC-AACC Method.
(4) The first action method for crude protein in animal feed, **984.13**, was mutually adopted as an AOAC-AACC Method, and cross referenced in the Cereal Foods chapter.
(5) The final action method for neutralizing value of baking chemicals, **950.03**, was mutually adopted as an AOAC-AACC Method and cross referenced in Cereal Foods chapter.
(6) The final action method for zearalenone in corn, **976.22**, was cross referenced in the Cereal Foods chapter.
(7) The final action method for microchemical determination of nitrogen, **960.52**, was mutually adopted as an AOAC-AACC Method and cross referenced in the Cereal Foods chapter.
(8) The final action method for biuret in fertilizers, **960.04**, was mutually adopted as an AOAC-AACC method and cross referenced in the Cereal Foods chapter.
(9) The final action method for residual carbon dioxide in baking powder, **948.05**, was mutually adopted as an AOAC-AACC Method and cross referenced in Cereal Foods chapter.
(10) The final action AAS method for minor nutrients in fertilizers, **965.09**, was mutually adopted as an AOAC-AACC Method and cross referenced in Cereal Foods chapter.

(11) The first action TLC method for deoxynivalenol in wheat, **986.17**, was mutually adopted as an AOAC-AACC Method and cross referenced in Cereal Foods chapter.
(12) The first action mixed catalyst method for crude protein in animal feeds, **988.05**, was mutually adopted as an AOAC-AACC Method and cross referenced in Cereal Foods chapter.
(13) The final action photometric method for benzoyl peroxide bleach in flour, **935.34**, was mutually adopted as an AOAC-AACC Method and cross referenced in Cereal Foods chapter.

**Ch. 33. Dairy Products**
No additions, deletions, or other changes.

**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**
No additions, deletions, or other changes.

**Ch. 37. Fruits and Fruit Products**
No additions, deletions, or other changes.

**Ch. 38. Gelatin, Dessert Preparations, and Mixes**
No additions, deletions, or other changes.

**Ch. 39. Meat and Meat Products**
(1) The solvent extraction (submersion) method for determination of fat (crude) in meat and meat products was adopted first action, **991.36**.

**Ch. 40. Nuts and Nut Products**
No additions, deletions, or other changes.

**Ch. 41. Oils and Fats**
(1) The gas chromatographic method for determination of fatty acids in encapsulated fish oils and fish oil methyl and ethyl esters was adopted first action as an AOCS-AOAC method, **991.39**.

**Ch. 42. Vegetable Products: Processed**
The first action microwave oven drying method for total solids in processed tomato products, **985.26**, was revised.

**Ch. 43. Spices and Other Condiments**
No additions, deletions, or other changes.

**Ch. 44. Sugars and Sugar Products**
(1) The internal standard stable carbon isotope ratio method for determination of C-4 plant sugars in honey was adopted first action, **991.41**.
(2) The final action carbon ratio mass spectrometric method for com syrup products in honey, **978.17**, was editorially revised.

**Ch. 45. Vitamins and Other Nutrients**
No additions, deletions, or other changes.

**Ch. 46. Color Additives**
No additions, deletions, or other changes.

**Ch. 47. Food Additives: Direct**
No additions, deletions, or other changes.

**Ch. 48. Food Additives: Indirect**
No additions, deletions, or other changes.

**Ch. 49. Natural Toxins**
(1) The liquid chromatographic method for determination of ochratoxin A in corn and barley was adopted first action, **991.44**.
(2) The enzyme-linked immunosorbent assay method (Biokits) for determination of total aflatoxin levels in peanut butter was adopted first action, **991.45**.
(3) The first action ELISA method for determination of aflatoxin $B_1$ in cottonseed and mixed feeds, **989.06**, was editorially revised.

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