1884-1984

AOAC

a century of analytical excellence

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ISSN 0004-5756

NOW SAFETY IS CONVENIENT

New 4-liter B-Jacket® Safety Bottle Cover protects laboratory personnel against flying glass and spilled solvents in case of bottle breakage. The bottle-hugging cover remains *on* the bottle throughout use and does not interfere with pouring from the bottle. If bottle breakage occurs, the durable twist-on two-part jacket contains the broken bottle and contents, minimizing splashing of liquid, and makes disposal convenient. Full details are provided in our new bulletin. Write for your copy today.

BURDICK & JACKSON LABORATORIES, INC.

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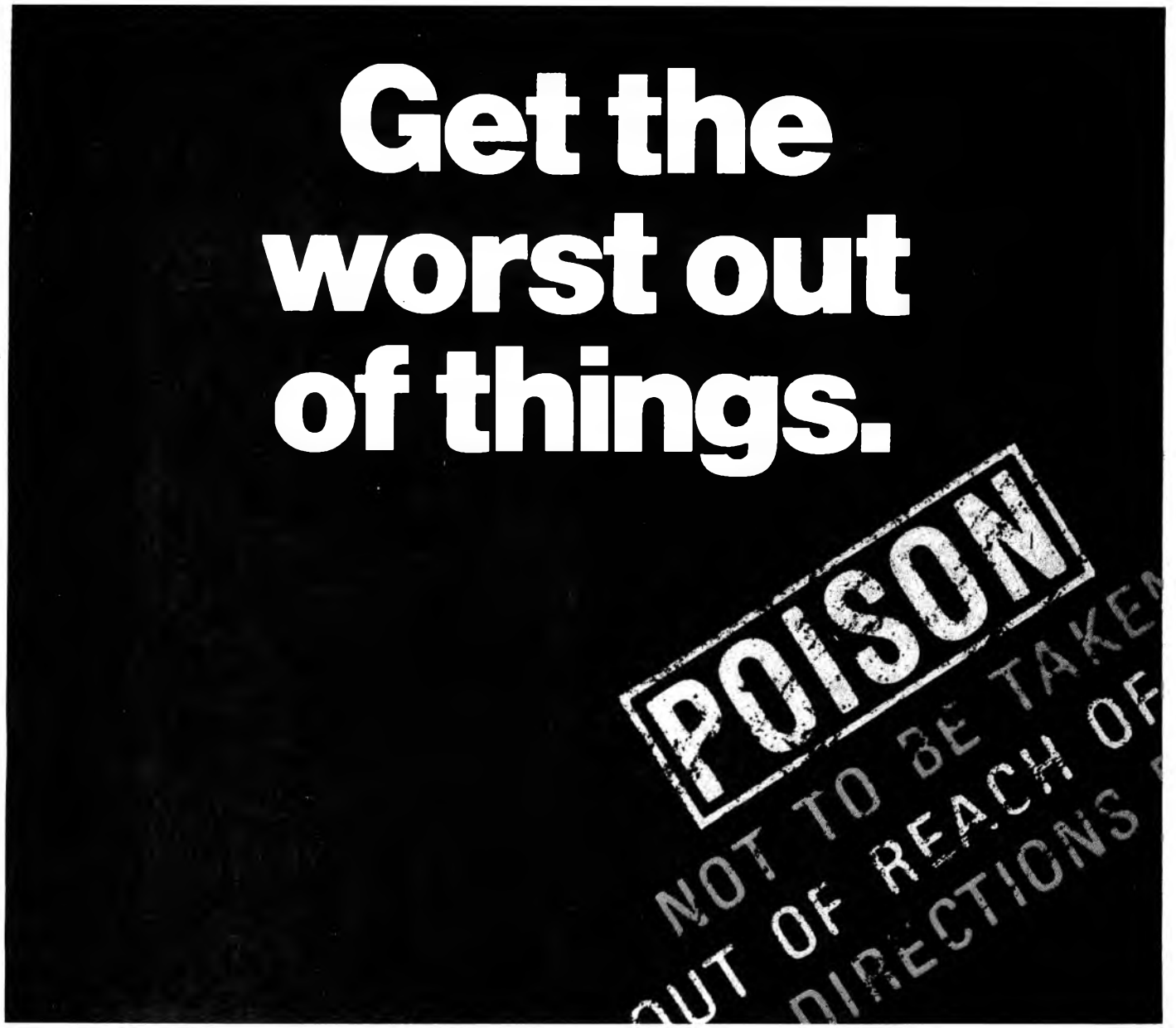
Get the worst out of things.

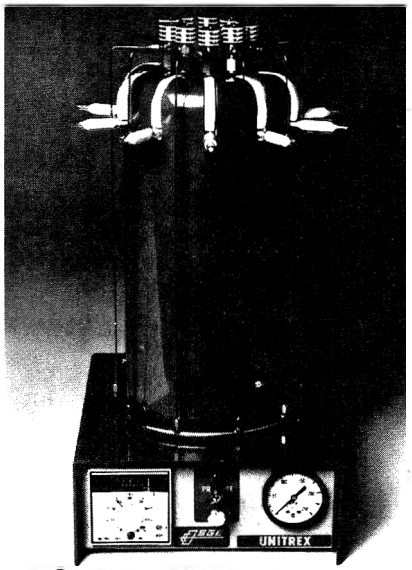


UNITREX
Universal Trace Residue Extractor.

SGE's Universal Trace Residue Extractor - Unitrex - gets the worst out of your samples. Unitrex has been developed as an efficient and economical clean-up system for the recovery of pesticides and organic residues from meat fats, butter, cheese, vegetable oils and a wide range of samples.

Operating and maintenance costs have been kept to a minimum. Unitrex can process 10 samples simultaneously, and only small amounts of solvent and gas are required. The processing is fast and reliable - and Unitrex has rapid warm-up and ± 1°C temperature stability.

Performance is excellent. Recoveries for a range of pesticides, both organo-chlorine and organo-phosphorous compounds, are better than 90% for most samples at ppb levels, and the extract is clean enough for direct chromatographic injection into capillary columns or packed columns, even with highly sensitive detectors such as ECD, FDP, and NPD.



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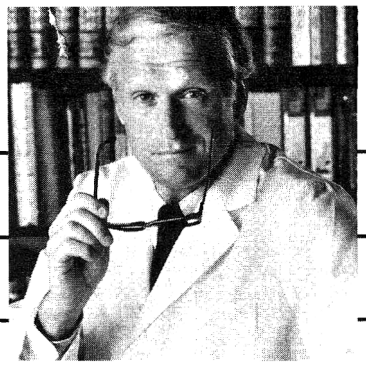
SGE

When HPLC, GC and AA don't.
When wet chemistry can't.
When electrochemistry won't.



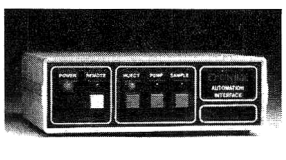
Dionex Ion Chromatography will!

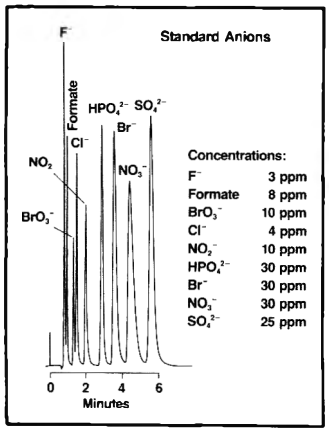
Dionex offers you a series of modular Ion Chromatography systems and high performance columns and accessories to meet your ion analysis needs—and your budget—from dedicated routine analysis to the most sophisticated research. In some cases, a Dionex Ion Chromatograph is the only system you need to perform analyses on hundreds of organic and inorganic ions in almost *any* sample matrix. And, thanks to the extraordinary selectivity of our advanced membrane based post column chemistries, usually all you have to do is dilute your sample and inject it.

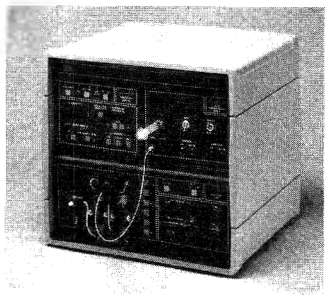
Introducing QIC—the Low Cost, Dedicated Performer

The QIC IonChrom Analyzer™ is the newest member in the complete line of Dionex Ion Chromatographs. QIC is an inexpensive, single channel system specifically developed for routine analysis of a broad range of commonly occurring anions, cations, and organic acids.

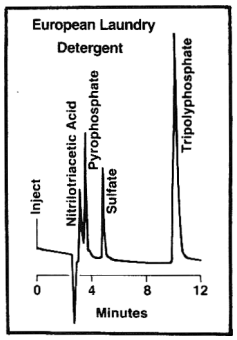
Simple design and ease of operation make QIC ideal for dedicated ion analysis applications where high sample throughput, sensitivity and rugged reliability are primary considerations. Standard features include a chemically-inert flow system for reduced contamination and corrosion, mechanical pulse damping for enhanced signal to noise, a new non-metallic pump with adjustable flowrates from 0.53 to 5.3 ml/min, and an easy to read conductivity display. In addition, QIC can be easily upgraded with a temperature selectable column heater for increased chromatographic selectivity and a controller for automatic sampling and analysis.



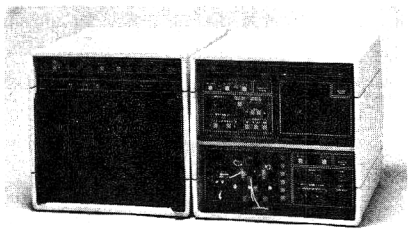




Dionex 2000i Series Ion Chromatographs—the Versatile Performers



The modular Dionex 2000i Series Ion Chromatographs offer outstanding versatility and performance for the analysis of ions including transition metals, organic acids, amino acids, amines, sulfonates, polyvalent ions, carbohydrates, and alcohols, as well as simple inorganic anions and cations. You can start with the basic 2000i single channel system and add more capability as your needs demand it such as dual channel analysis, remote control, and automatic operation.



The basic system comes equipped with a bipolar pulsed conductivity detector. In addition, Dionex offers a UV-Vis detector, a fluorescence detector, an electrochemical detector, and a new pulsed amperometric detector for increased sensitivity and analysis of a wide range of ionic and polar compounds. And of course, all 2000i Chromatographs are compatible with Dionex high performance Ion Chromatography columns and accessories for optimum ion separation and detection.

Find out for yourself

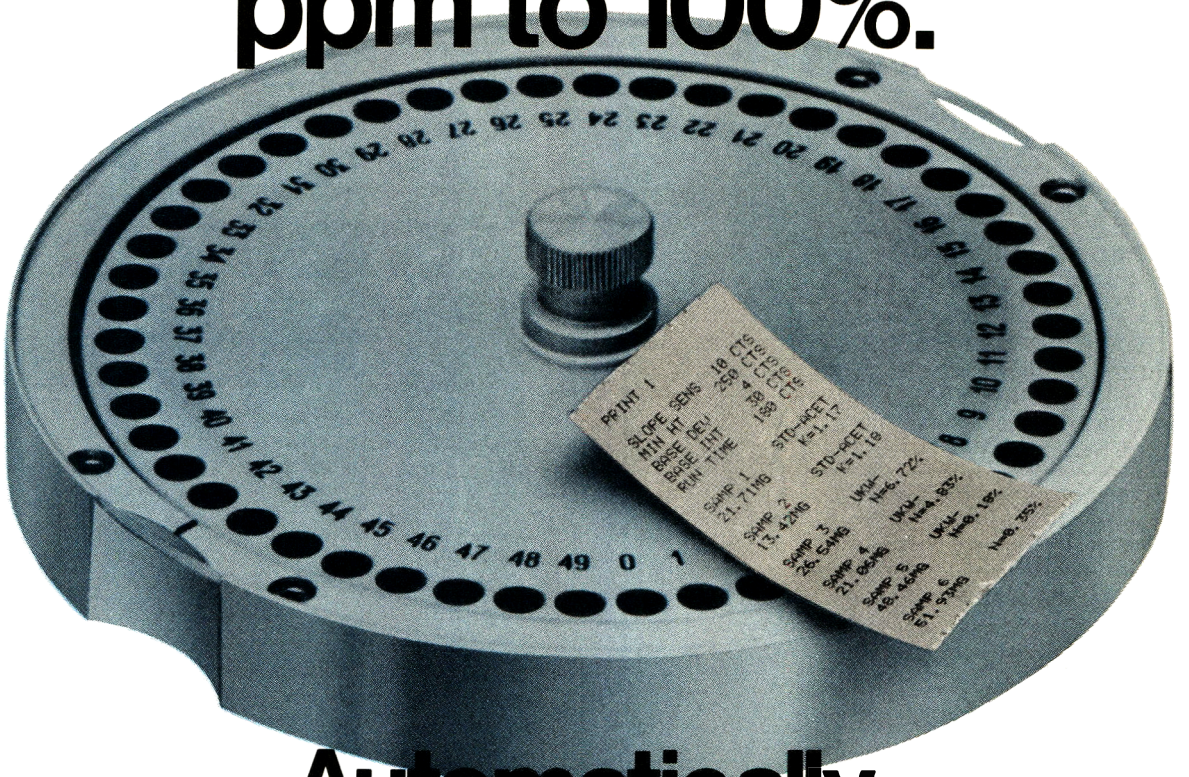
Contact the Dionex representative nearest you for complete information on Dionex Ion Chromatographs. Tell us about your ion analysis needs and we'll help you configure the right system and accessories for your applications. Plus, we'll give you all the details about our comprehensive customer support program.

When it comes to ion analysis, look to the leader. Look to Dionex—the first name in Ion Chromatography.

DIONEX™
The Ion Experts

Dionex Corporation, 1228 Titan Way, Sunnyvale, CA 94086. (408) 737-0700. Telex: 348347
US Regional Offices: Marlton, NJ (609) 596-0600; Chicago, IL (312) 860-1030; Houston, TX (713) 847-5652.
In Europe: Dionex (UK) Ltd., Camberley, England (0276) 29771
Dionex GmbH, Darmstadt, W. Germany 06150-12258;
Dionex SRL, Roma, Italy (06)536-130.

Nitrogen/Protein Analysis: ppm to 100%.



Automatically. Every 3 minutes. Day and night.

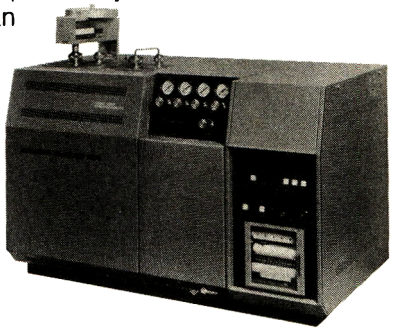
With the new ANA 1500 from Carlo Erba—the leader in microanalysis.

When productivity, cost per analysis, and accuracy are important factors in your micro and macro Nitrogen or Nitrogen and Carbon analysis, here is a system that will deliver unequalled performance in all three areas.

Higher Productivity. The ANA 1500 is a fully automatic system with a 50-sample carousel, designed for unattended, continuous operation. It will analyze a sample every three minutes—24 hours a day. An optional data system will reduce your calculations and record keeping.

Lower Cost Per Analysis. Rapid, pollution free analysis, built-in reliability and an inherent high uptime all contribute to a very low cost per analysis, especially in view of the very reasonable investment.

Improved Accuracy. The highly stable TC detector and automation deliver the previously unattainable accuracy of better than ±0.2%.



Write for a full list of applications and details on our ANA 1500 or the CHN-O/S Analyzer, or call us for a demonstration in your area.

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NEW PRODUCTS

Hexanary Solvent Selection and Column Selection Accessories

Varian has developed these 2 accessory packages for Model 5500 Series liquid chromatographs. The Solvent Selection System permits automatic selection from 1 to 6 solvent reservoirs with simultaneous operation of any 3, while the Column Selection accessory allows automatic selection via BCD coding from 1 to 8 columns. For information, contact: Varian Instrument Group, Publication SEP2796, 220 Humboldt Ct, Sunnyvale, CA 94089, or call Ronald Majors, 415/939-2400.

Sample Digestor

Labconco offers the Rapid Digestor-4, designed for wet digestions for laboratories handling moderate sample loads. The unit can digest many types of samples in about 25 min, and includes 4 ceramic-embedded heaters, temperature-regulated from ambient to 450°C. For information, contact: Labconco Corp., Susan Gregory, 816/333-8811, or toll-free 800-821-5506.

CRT Display and GC/FTIR Software

IBM Instruments announces that a new color CRT display and expanded GC/FTIR software are available for the IR/80 and IR/90 Series FTIR spectrometers. The 14-in., high-resolution display can be used to compare spectra by overlaying or spectral subtraction techniques, while the software package permits handling complex separations of different samples. For information, contact: IBM Instruments, Inc., PO Box 332, Danbury, CT 06810. Phone toll-free: 800/243-7054 (in Connecticut 800/952-1073).

Sample Injection and Metering Pumps

LDC/Milton Roy is now offering the new Automatic Sample Injector, Model 713, and miniMetric Series metering pumps, Models I and II. Model 713 can inject 60 samples up to 3 times each, in volumes from 0.5 to 200 μL, reduces the amount of sample required for injection, and is microprocessor controlled. Model I and II metering pumps feature continuously adjustable flow rates from 50 μL/min to 5.0 mL/min, at pressures up to 600 psi, and a fast-refill cam, coupled with micro-electronic control, for pulse-free, constant velocity liquid discharge. For complete specifications, contact: LDC/Milton Roy, PO Box 10235, Riviera Beach, FL 33404. Phone toll-free: 800/327-6182, in Florida 305/844-5241.

Industrial Sampling System

Intersystems Industrial Products, Inc., announces the Model PS Sampling System that collects representative samples of liquids or solids from gravity or high pressure, positive or negative air conveying systems. System components include a controller, operating automatically or manually, and a sampling tube, extending and retracting under pneumatic power into the product stream. For complete specifications, contact: Intersystems Industrial Products, Inc., Preston Rd, Suite 105D LB 342, Dallas, TX 75252. Phone: 214/380-0791.

Electrophoretic Transfer System

Bio-Rad's new high field intensity Trans-Blot system performs rapid, quantitative, and efficient electophoretic transfers of even high molecular weight denatured (SDS) proteins and nucleic acids. The Trans-Blot cell in combination with the new Model 250/2.5 power supply and Zeta-Probe positively charged, nylon blotting membranes, provides good resolution and operating simplicity. For information, contact: Bio-Rad Laboratories, Chemical Division, 2200 Wright Ave, Richmond, CA 94804. Phone: 415/234-4130, Telex: 355-358.

Cryogenic Product Literature

A new brochure is available that describes Minnesota Valley Engineer's line of dewars and flasks for distribution, storage, and handling of cryogenic liquids. The literature contains application photos, accessory information, and complete specifications. Copies of the brochure may be obtained from Minnesota Valley Engineering, Inc., 407 Seventh St., NW, New Prague, MN 56071. Phone: 612/758-4484, Telex: 29-0571.

Autosampler

Spectra-Physics announces the availability of the SP8110 LC autosampler with variable injection volume. The optional variable injection volume kit allows the injection of small volumes with minimal sample usage, and provides a convenient way of injecting different volumes of a standard to develop multilevel calibration for improved accuracy in quantitation. For information, contact: Dennis Gillen, Spectra-Physics, 3333 N First St, San Jose, CA 95134. Phone: 408/946-6080.

Micropipettor

The new positive displacement Varimetric Micropipettor from Labindustries comes in 4 adjustable, color-coded models, covering the range from 5 to 1000 μL, and has accuracy and reproducibility within 1% at full volume. Each micropipet is precalibrated, but can be recalibrated, and sample volumes remain unaffected by surface tension, viscosity, vapor pressure, or density. For information, contact: Labindustries, Inc., 620 Hearst Ave, Berkeley, CA 94710. Phone: 415/843-0220, or toll-free (except California) 800/227-0128.

Chemistry Software

Dynamic Solutions Corp. is now offering its full line of "Appligration Chemistry" software packages for use with Cyborg's ISAAC data acquisition hardware for the Apple II microcomputer. "Appligration" is designed for testing and research laboratories using quantitative, analytical techniques such as chromatography, spectroscopy, colorimetry, thermal analysis, electrochemistry, and flow totalization. For more information, contact: David M. Strand, Marketing, or Debra Khanna, Sales Engineer, Dynamic Solutions Corp., 61 S Lake Ave, Suite 309, Pasadena, CA 91101. Phone: 213/577-2643.

pH Monitor

The recent "Precision Driven Shield" design for McNab's Aqua Purometer II pH monitor/controller eliminates the need for frequent restandardization, and provides permanent system stability and accurate, repeatable readings with low drift. For more information, contact: McNab, Inc., Mail Stop A81-37, 20 N McQuestion Parkway, Mount Vernon, NY 10550. Phone: 914/699-1616.

Production-Scale Liquid Chromatographic System Literature

Elf Aquitaine Development has published a free literature package on its new production-scale LC system. The system has a column efficiency exceeding 10 thousand theoretical plates per meter and a throughput of 1 kg product/h per injection. The literature pack can be obtained by contacting: J. Reed Margulis, Elf Aquitaine Development Division, Elf Technologies, Inc., 9 West 57th St, New York, NY 10019. Phone: 212/750-1140.

Laboratory Bench

Labconco Corp. introduces a new 4-ft horizontal laminar flow bench, providing an uncontaminated working environment. The latest in the SterilBench line has a Dacron prefilter to trap large particles to lengthen the life of the HEPA filter, which removes 99.97% of all particles of 0.3 μm size, and supplies class 100 air, therefore meeting Federal Standard 209b. For more information, contact: Labconco Corp., 8811 Prospect, Kansas City, MO 64132. Phone: 816/333-8811, Telex: 4-2568.

Phenol in Air Monitor

CEA Instruments TGM 555 phenol in air monitor continuously monitors phenol levels below 1 ppm. Typical full scale range is 0–2 ppm, adjustable from 0 to 100 ppb detecting as low as 2 ppb, up to 0–10 ppm full scale. The unit is applicable for the continuous monitoring of workplace atmospheres in the manufacture of phenolic resins, disinfectants, antiseptics, dyes, and others. For further information, contact: Martin H. Adelman, CEA Instruments, Inc., 15 Charles St, Westwood, NJ 07675. Phone: 201/664-2300.

Plotter

IBM Instruments, Inc. announces its XY/749 digital plotter, which features high precision, simplicity of use, high throughput, low noise operation, and 8 different colored pens to produce multi-colored charts. The XY/749 can also be set to monitor data streams, operate as a line printer, and execute a test program which lists interface parameters. For more information, contact IBM Instruments, Inc., PO Box 332, Danbury, CT 06810. Phone toll-free: 800/243-7054 (in Connecticut 800/952-1073).

Waveform Recorder

Bell & Howell now offers a 16K memory module for its Datalab DL 2000 Series multichannel waveform recorders. The DL 2020 module quadruples the memory by providing 16 kilowords of storage at sample rates as high as 2 MHz, while maintaining the 10-bit resolution capability. For more information, contact Ed Clarke, CEC Division/Bell & Howell, 360 Sierra Madre Villa, Pasadena, CA 91109. Phone: 213/796-9381.

Granule/Pellet Sampler

InterSystems Industrial Products, Inc. announces a new granule/pellet sampler, Model PT, that comes in a variety of sizes and can be mounted on positive or negative, horizontal or vertical, pneumatic conveying systems. Model PT is easily installed and comes in dust-tight, aluminum housing, although other material can be furnished. For more information, contact InterSystems Industrial Products, Inc., 17330 Preston Rd, LB 342, Suite 105D, Dallas, TX 75252. Phone: 214/380-0791.

Freeze Dry Glassware

Labconco has published a new free brochure, "Labconco Freeze Dry Glassware," highlighting features of Fast Freeze and Lyph-Lok flasks, as well as specifications on other freeze dry glassware. For information, call toll-free 800/821-5506, or contact Susan Gregory, 816/333-8811.

EC/LC Flow Cell

IBM Instruments, Inc. has developed a new EC/LC Flow Cell for amperometric detection. The cell features negligible dead volume, minimum (IR) drop, better peak shape, simplicity of operation, and allows the detection of new transmitters at the 4–6 pg levels with good resolution and stable, clean chromatographic background. For information, call toll-free 800/243-7054 (in Connecticut 800/952-1073).

Laboratory Supply Catalog

Interex Corp. announces its latest laboratory supplies catalog, 1983 issue. The 104 page catalog is illustrated, cross-indexed, and contains current prices. For a copy, write to: Interex Corp., 3 Strathmore Rd, Natick, MA 01760. Phone: 617/237-6650.

IS YOUR AOAC LIBRARY COMPLETE?

Optimizing Laboratory Performance Through the Application of Quality Assurance Principles—Proceedings of a Symposium. 1980. 160 pp. Softbound. ISBN 0-935584-19-6. Members: $25.50 in U.S., $28.50 outside U.S. Nonmembers: $28.00 in U.S., $31.00 outside U.S.
Eleven papers covering setting up and operating a quality assurance program.

FDA Training Manual for Analytical Entomology in the Food Industry. 1978. 184 pp. Looseleaf. ISBN 0-935584-11-0. Members: $13.25 in U.S., $14.25 outside U.S. Nonmembers: $14.50 in U.S., $15.50 outside U.S.
With the aid of this text, organizations can set up their own in-house training.

Infrared and Ultraviolet Spectra of Some Compounds of Pharmaceutical Interest. 1972. 278 pp. Softbound. ISBN 0-935584-04-8. Members: $12.80 in U.S., $13.80 outside U.S. Nonmembers: $14.00 in U.S., $15.00 outside U.S.
An expansion of an earlier compilation. More than 800 spectra.

Mycotoxins Methodology. 1980. 22 pp. Softbound. ISBN 0-935584-16-1. Members: $10.90 in U.S., $11.90 outside U.S. Nonmembers: $12.00 in U.S., $13.00 outside U.S.
Reprinted from Chapter 26 Official Methods of Analysis, 13th Edition. Approved methods for natural toxins in many commodities.

Micro-Analytical Entomology for Food Sanitation Control. 1962. 576 pp. Hardbound. 840 Illustrations. ISBN 0-935584-00-5. Members: $30.00 in U.S., $33.00 outside U.S. Nonmembers: $33.00 in U.S., $36.00 outside U.S.
A training and reference manual for identification of insect debris extracted from foods.

Test Protocols for the Environmental Fate and Movement of Toxicants—Symposium Proceedings. 1981. 336 pp. Softbound. ISBN 0-935584-20-X. Members: $27.30 in U.S., $30.30 outside U.S. Nonmembers: $30.00 in U.S., $33.00 outside U.S.
Chemical and biological tests plus methods for interpreting or predicting results through mathematical models.

FDA Bacteriological Analytical Manual (BAM) 5th Ed. 1978. 448 pp. Looseleaf. ISBN 0-935584-12-9. Members: $24.50 in U.S., $27.50 outside U.S. Nonmembers: $27.00 in U.S. $30.00 outside U.S.
Provides regulatory and industry laboratories with methods for detection of microorganisms. Updated by supplements.

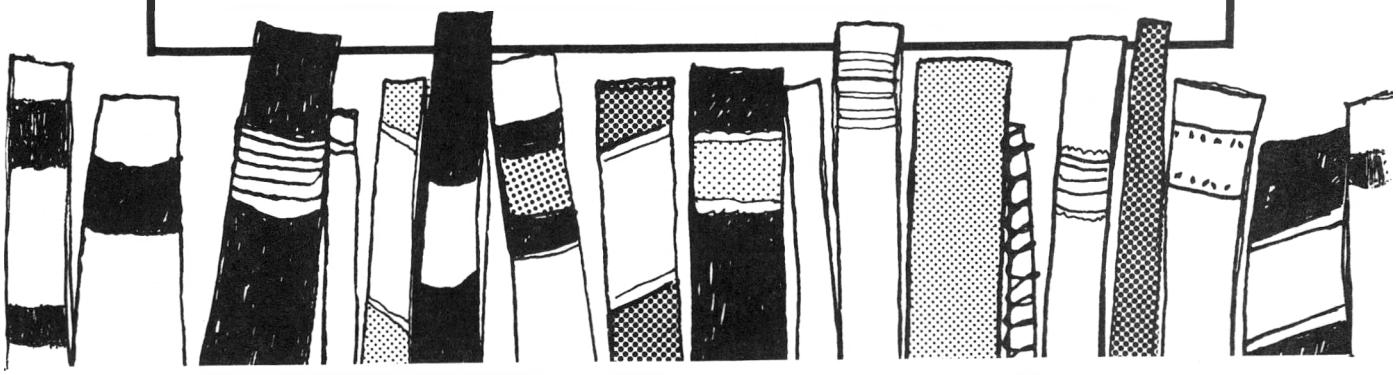
Mycotoxins Mass Spectral Data Bank. 1978. 60 pp. Softbound. ISBN 0-935584-13-7. Members: $12.80 in and outside U.S. Nonmembers: $14.00 in and outside U.S.
A computer-based compilation of 104 mass spectra with listing by molecular weight.

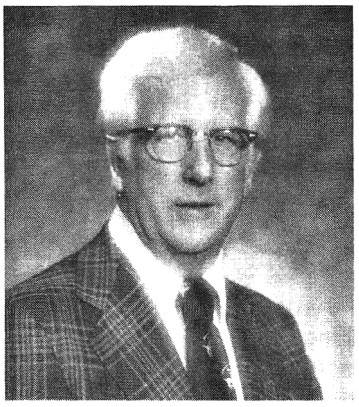
Newburger's Manual of Cosmetic Analysis 2nd Ed. 1977. 150 pp. Softbound. ISBN 0-935584-09-9. Members: $13.70 in U.S., $14.70 outside U.S. Nonmembers: $15.00 in U.S., $16.00 outside U.S.
Chromatographic techniques and spectroscopy with analyses for various specific cosmetics.

Statistical Manual of the AOAC. By W. J. Youden and E. H. Steiner. 1975. 96 pp. Softbound. With illustrations. ISBN 0-935584-15-3. Members $12.25 in U.S., $13.25 outside U.S. Nonmembers $13.50 in U.S., $14.50 outside U.S.
A do-it-yourself manual for statistical analysis of interlaboratory collaborative tests.

Send check to AOAC, Suite 210-J, 1111 N 19th St., Arlington, VA 22209, USA. 703/522-3032.

To qualify for member price, include Member Number with order.





Charles W. Gehrke to Serve as President During AOAC Centennial Year

Charles W. Gehrke has been named AOAC President for 1984. He is Professor of Biochemistry and Manager of the Experiment Station Chemical Laboratories at the University of Missouri-Columbia, and also serves as Missouri State Chemist.

Dr. Gehrke, who is author of over 200 scientific publications in analytical chemistry and biochemistry, has been an invited scientist lecturing on gas chromatography of amino acids in Japan, China, and many universities and institutes in the United States and Europe. He has been the recipient of numerous prestigious scientific awards including: the 1971 AOAC Harvey W. Wiley Award; the 1973 Senior Faculty Member Award of the College of Agriculture; the 1975 Faculty-Alumni Gold Metal Award; the 1979–80 Kenneth A. Spencer Award in the Kansas City Section of the American Chemical Society for meritorious achievement in agricultural and food chemistry; the 1980 "Chromatography Memorial Medal" from the Scientific Council on Chromatography of the USSR Academy of Sciences; and the 1980 Sigma Xi Research Award by the University of Missouri Chapter.

Dr. Gehrke has also been an invited lecturer in the Soviet Academy of Sciences, the Central American Research Institute for Industry, and other scientific institutes in Taiwan, the Phillipines, and Hong Kong.

He holds a Ph.D. from Ohio State University, and assumed his present position at the University of Missouri in 1949.

Dr. Gehrke is active in the American Chemical Society as well as AOAC. His research interests include: development of quantitative gas and high resolution liquid chromatographic methods for amino acids, purines, pyrimidines, major and modified nucleosides, fatty acids, and biological markers in the detection of cancer; characterization and interaction of proteins; chromatography of biologically important molecules; automation of analytical methods for nitrogen, phosphorus, and potassium in fertilizers; and automated spectrophotometric methods for lysine, methionine, and cystine.

1983 AOAC Scholarship Awarded to Laronna Colbert of Des Moines, IA

Due to the outstanding nature of her academic record and extra-curricular activities at Union College, Lincoln, NE, Laronna Colbert is another recipient of a 2-year, $1000 scholarship sponsored by AOAC.

As a chemistry major, Ms Colbert has maintained a 3.90 grade point average. In addition to her demonstrated ability in the sciences, Ms Colbert has worked as a tutor in the Union College Writing Program, a position often given to English majors. Working in this capacity, and as a representative for "Home Health Education Services," she is able to finance her education.

Ms Colbert will continue her studies at Union College as a chemistry major, with biochemical research as a future goal.

Each year, AOAC awards a 2-year scholarship to a college sophomore who is studying a subject important to public health and agriculture. To qualify, the student must be in need of financial aid, maintain at least a B average during the first 2 years of undergraduate study, and plan to do research, regulatory work, quality control, or teach in an area of interest to AOAC.

Nominations for the 1984 award must be received before May 1, 1984. Send 6 copies of a nomination letter and 2 supporting reference letters to AOAC, 1111 N 19th St, Arlington, VA 22209.

Meetings

April 8–13, 1984: ACS 187th National Meeting and Exposition, Cervantes Convention Center, St. Louis, MO. To obtain an application and contract for booth space for this event, contact: Chemical Expositions Dept., American Chemical Society, 1155 16th St, NW, Washington, DC 20036 (202/872-4485).

April 26–27, 1984: 15th Symposium on Advances in Applied Analytical Chemistry, Airport Hilton, New Orleans, LA. Contact: Dr. Linda Green, PO Box 1449, Kenner, LA 70063, 504/467-4100.

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

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

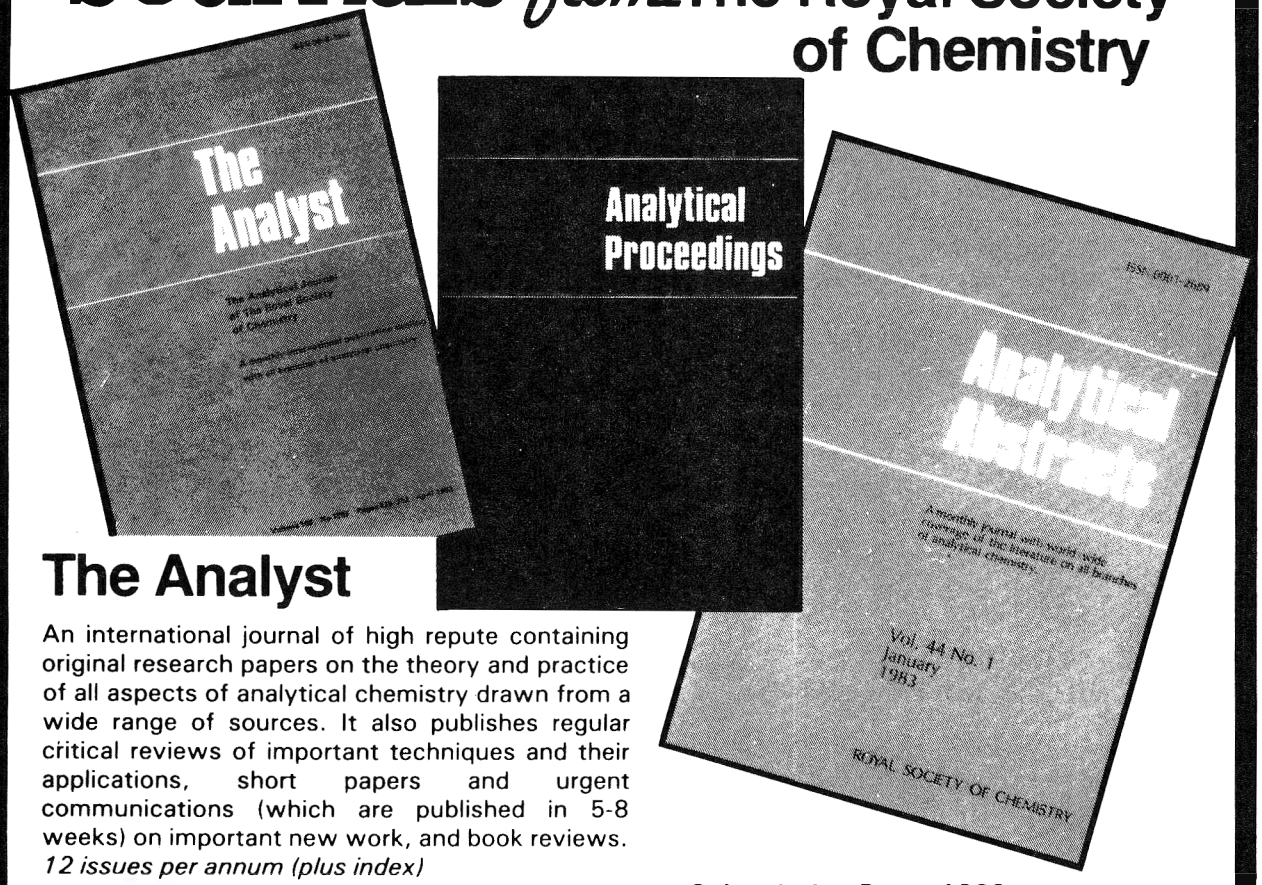
October 28–Nov 1, 1984: AOAC 98th Annual International Meeting, Shoreham Hotel, Washington, DC. Abstracts (on special forms) of papers and reports for the meeting must be received by the AOAC office by July 6, 1984. For information, please contact: Kathleen Fominaya, AOAC, 1111 N 19th St, Suite 210,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

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

AOAC Gains New Private Sustaining Members

AOAC welcomes 2 new additions to the growing list of companies aware of the need to support an independent meth-

Analytical Chemistry Journals *from* The Royal Society of Chemistry



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To calculate the ADI level, EPA uses no adverse effect level (NOEL), derived from chronic and subchronic tests on animals. NOEL is then extrapolated to humans, using a safety factor to allow for uncertainties in extrapolation and for differences in sensitivities in the human population.

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ISO Standards Published

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ISO 5986-1983	Animal feeding stuffs—Determination of diethyl ether extract	$11.00

Reminder of Deadlines for Nominations for Awards

The following deadlines have been set for nominations for awards: 1984 Fellow of the AOAC Award, March 1, 1984; 1984 Harvey W. Wiley Award, April 1, 1984; and 1984–1985 Scholarship Award, May 1, 1984.

The Fellow of the AOAC award was established in 1961 to recognize those persons giving meritorious service to the Association. Eligible candidates have performed notably for 10 years or more, usually as Officers, Referees, or Committee members. Fellows are selected by the Committee on Fellows and approved by the Board of Directors. Selection is made from a list which includes eligible candidates, their contributions, and the point count assigned to each candidate according to an established point system. The Committee is also informed of any nominations received.

CORRECTIONS

J. Assoc. Off. Anal. Chem. (1983) 66, 1257–1282,

"Detection and Determination of Error in Analytical Methodology. Part I. In the Method Verification program," by Mario J. Cardone, p. 1270, right column

Change expression to:

$$\bar{x}_w = \frac{n_1\bar{x}_1 + n_2\bar{x}_2 + \ldots . n_k\bar{x}_k}{n_1 + n_2 \ldots . n_k}$$

$$n_1 + n_2 \ldots . n_k = N$$

$$S_p = \sqrt{\frac{[(n_1 - 1)(s_1)^2 + (n_2 - 1)(s_2)^2 + \ldots . (n_k - 1)(s_k)^2]}{(n_1 - 1) + (n_2 - 1) \ldots . . (n_k - 1)}}$$

$$n_1 - 1 + n_2 - 1 + \ldots . n_k - 1 = N - k$$

J. Assoc. Off. Anal. Chem. (1983) 66, 1283–1294,

"Detection and Determination of Error in Analytical Methodology. Part II. Correction for Corrigible Systematic Error in the Course of Real Sample Analysis," by Mario J. Cardone, p. 1283, running head

Change NO. 2 to NO. 5

p. 1293, left column, numerator of fraction

Change (100) to (1000)

p. 1294, right column, reference 6

Change **62** to **52**

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A CENTURY OF ANALYTICAL EXCELLENCE

The Story of a Refereeship—Mycotoxins

The following three papers were presented at the 1982 Annual International Meeting of AOAC as part of a symposium honoring the memory of Alfred D. Campbell. They are reproduced in this issue commemorating the start of the AOAC centennial year because they describe the birth, development, and maturation of a General Referee topic. Although the events described may not be typical of most referee topics, these narratives could be useful because they are presented by people who, along with Dr. Campbell, were participants in these events, and the refereeship that evolved has been among the most active and successful in AOAC. Within 2 years of the inception of the refereeship, there were a sufficient number of papers on the topic offered for presentation at the AOAC annual meeting to constitute a symposium, a situation that has recurred each year since then; in the last several years, 3 technical sessions have been required to accommodate all the papers offered. The annual reports of the General Referee on this topic, starting with the one published in 1967, constitute a comprehensive review of the field each past year, a process in which the Associate Referees were intimately involved. Among those active in the refereeship are 3 recent winners of the AOAC Harvey W. Wiley Award (Walter A. Pons, 1976; Leonard Stoloff, 1981; Odette L. Shotwell, 1982).

Coping with the Aflatoxin Problem in the Early Years

HENRY FISCHBACH
Retired, Food and Drug Administration, Washington, DC

Alfred D. Campbell's sudden death left a legacy of devotion and dedication to an increasingly important field in human and animal health: protection of the food supplies from the mycotoxins. This symposium is a fitting tribute to Dr. Campbell who was instrumental in organizing international symposia on this very subject under the auspices of the International Union of Pure and Applied Chemistry, one in Kungalv, Sweden, in 1972 and another in Pulawy, Poland, in 1974. Throughout Al's FDA career, he was closely linked with the Administration's effort to eliminate mycotoxins, particularly the aflatoxins, from food channels, and in the process to elicit the cooperation of many individuals and numerous industrial, academic, and governmental groups. Hazy recollections of those early days of the aflatoxin problem, 20 years ago, permit mention of only a few of the many deserving individuals.

Sometime in the winter of 1961–62, just before Dr. Campbell joined FDA, E. S. Hiscocks, Scientific Attaché at the British Embassy, had made arrangements for Desmond Raymond of the Tropical Products Institute (TPI) in London to discuss with Food and Drug personnel their most recent findings associated with the turkey-X disease. That spring, on his return from a trip to study the Brazilian harvesting practices for groundnuts, Dr. Raymond met with members of the FDA Bureau of Biological and Physical Sciences. He recounted that, in 1960, the British Isles were confronted with a staggering loss of turkey poults as well as numerous calves and young cattle. The fungal toxin association was not immediately evident, but the Tropical Products Institute and the British Ministry of Agriculture, Fisheries and Food had quickly traced the cause to shipments of Brazilian groundnut meal. The British depend on groundnut meal as a protein supplement for animal feed. Dr. Raymond had observed that the harvested peanuts in Brazil were often left in piles exposed to the elements for widely varying periods. TPI was convinced that rain-soaked nuts exposed to the local high temperature and humidities were highly susceptible to mold growth and production of a fungal toxin. In those early days, some thought the problem was endemic to Brazil, a concept soon disproved. Given the appropriate substrate, and suitable conditions of temperature and humidity, various strains of *Aspergillus flavus*, the ultimately implicated mold, can produce the toxin almost anywhere. Some strains of *A. flavus* are much more abundant producers of aflatoxins than others. Of 145 strains of *A. flavus* tested by P. R. Austwick of the English Central Veterinary Laboratory, only 10% had produced significant amounts of toxin. Dr. Raymond indicated that toxicological studies on several warm-blooded animals were under way at the Ministry of Agriculture in Weybridge. He discussed the English extraction procedure and an awkward duckling bioassay. For monitoring incoming shipments of groundnut meal. TPI was recommending the following series of tests: a microscope screening for *A. flavus*, followed by a solvent extraction of the positive samples, and a paper chromatographic step. If a blue fluorescent spot was obtained on the chromatogram, a 2-week duckling assay was performed for final confirmation. The only reference standard available was the so-called "Rosetti Meal," a supply of highly contaminated groundnut meal from the 1960 Brazilian shipment on the freighter of that name. Under the appropriate conditions of temperature and humidity, TPI had produced the then unnamed toxin on such substrates as corn and wheat, besides groundnut meal.

Shortly after our discussion with Dr. Raymond, Dr. Munsey in the Food Research Branch of the FDA Division of Food was given the assignment to have his group become adept in performing the TPI tests and to search the literature for adequate methodology with which to conduct a field survey of peanut products and grains. For this goal, he selected Stanley Nesheim and Howard Smith to devote their full time

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to the physical and chemical aspects of the methodology. Bernard Armbrecht of our Division of Pharmacology was responsible for setting up and improving the 2-week duckling assay, and Allen Hodges, Division of Microbiology, was assigned to carry out the microscope screening as well as the growing of aflatoxin-containing cultures for chemical research.

In September 1962, Glenn Slocum, director of the Division of Microbiology, and I observed the activities of TPI in London and the Central Veterinary Laboratory in Weybridge, England. Our discussions with the English investigators left no doubt as to the hepatocarcinogenicity of aflatoxin. Liver cancer had developed in a number of warm-blooded animals under test in various European laboratories. Susceptibility to the toxic effects varied from very sensitive, e.g., ducklings, to apparent resistance, e.g., sheep. Pigs, calves, guinea pigs, turkey poults, and rats were sensitive; mice were less sensitive than rats. At TPI, some improvement had been made in the earlier chemical technique.

On our return, we conferred with Robert S. Roe, director of the Bureau of Biological and Physical Sciences, and Commissioner George P. Larrick. The potential for harmful contamination of such foods as peanuts and peanut products was evident. The aflatoxin problem was given top priority.

It was at this point, March 1963, that Dr. Campbell, who had been assistant director for research at Clinton Corn Products Processing Co., joined the Division of Food. He had been recruited because his expertise in processed foods, particularly modified starches and corn wet-milling products, was considered important for food additive evaluations, but instead he was put in charge of the aflatoxin work. He was ideally suited in temperament and experience to spearhead this effort, which required coordination of the toxicological, microbiological, and chemical groups involved.

Even though the British scientists had already uncovered valuable toxicological information with regard to aflatoxin, the methodology for identifying and measuring was deficient for purposes of mounting an investigative and regulatory program. Our scientists had familiarized themselves with the published and proposed methods and concluded that none or any combination of them was reliable enough to serve as unquestionable proof of aflatoxin contamination at levels around 0.1 ppm. Nevertheless, an investigation program was initiated to provide an insight into the occurrence of aflatoxin in food and feed. By the spring of 1963, 75 moldy peanut samples were collected by the field districts and examined in the Washington laboratories. Howard Smith had developed a thin layer chromatographic technique using microscope slides. This provided a semiquantitative value for aflatoxin which was essential for the proper dosing of the Peking white ducklings that Bernard Armbrecht found more suitable for the bioassay than the Khaki Campbell strain used in England. A few mold extracts caused yellow liver in ducklings, but the microscopic observation of the liver was negative; besides, the extract did not have the typical fluorescent bands after chromatography (1). These results, although negative, were not reassuring. The methodology, at best, offered only a qualitative interpretation of an aflatoxin contamination. Certainly the available analytical tools were not adequate for a regulatory program. The Food, Drug, and Cosmetic Act permits the seizure of adulterated foods. However, before FDA moves to institute action against an article as filthy, putrid, or decomposed, or containing a poisonous or deleterious substance, it must be satisfied that it can prove its case in court. In the absence of obvious mold, unambiguous biological and physicochemical tests are indispensable for such proofs. Not only should the tests be specific, they also must be sufficiently sensitive and accurate to measure minute, but significant quantities of deleterious substances. For practical reasons, the tests must be as simple and rapid as possible, without sacrificing reliability. In addition, they should yield consistent results in the hands of competent analysts so that the manufacturer's control group as well as government scientists can readily arrive at similar results.

At this point, FDA assigned even greater effort to improve the methodology. Even though budgeted positions were extremely scarce, 5 additional positions were allotted to the Division of Food. No space was available in Washington, but luckily we were able to acquire space recently released by our Boston District. Long before the concept of Program Manager became popular in FDA, Dr. Campbell assumed such a role for the expanded aflatoxin program. With his customary drive and enthusiasm, he interviewed several likely scientists, and then recruited Leonard Stoloff. Together, they recruited 3 additional scientists and a laboratory helper. A concerted effort, including a strong assist from the Boston District, quickly refurbished the space and the Mycotoxin Unit was under way. Dr. Campbell's energetic persistence was largely responsible for this operation becoming functional in a very short time. The Division of Microbiology stationed 3 bacteriologists alongside this unit in Boston. *A. flavus* was cultured on diverse substrates and under various conditions to study its range as a potential food contaminant. Our British colleagues of the Department of Scientific and Industrial Research had generously provided active strains of *A. flavus* together with detailed instructions for their care and nurture. The mold growths were harvested, and the aflatoxins were extracted and purified in an attempt to elucidate the molecular structures and the interrelationships among the toxic substances. These cultures were highly productive. With an innovative cleanup procedure, the Contaminants Branch produced large quantities of crude aflatoxins, part of which replaced the toxic peanut meal as a working reference standard. At that time, FDA did not possess a nuclear magnetic resonance (NMR) instrument. William Horwitz, Office of the Commissioner, offered 0.5 g of the crude aflatoxins to a former FDA colleague then at the Massachusetts Institute of Technology, Leo Friedman, Professor of Nutritional Toxicology. It was with this material that a graduate student under the leadership of Professor George Buchi elaborated, in 1963, the structure of the aflatoxins, in part by means of NMR spectra (2). In June 1963, Dr. Horwitz continued our active liaison with the scientists of the Ministry of Agriculture at Weybridge, England. On the same trip, he learned that John Collingwood of Unilever House was preparing to publish on the structure of the aflatoxins, also based on NMR spectra, and essentially corroborating the MIT work.

By 1964, our researchers on analytical methods had made significant gains. A lengthy overnight extraction procedure had been supplanted by a much shorter blender extraction and chromatographic column cleanup procedure. It was now possible for a single analyst to examine 8 samples conveniently in a single workday. Improvements in thin layer silica gel chromatographic techniques by Robert Eppley (3) and Stanley Nesheim et al. (4) provided a sharp separation of the 4 aflatoxins and replaced paper chromatography; a confirmation of identity test for aflatoxin B_1 had been devised by Andrellos and Reid (5); a new biological test based on the toxicity of aflatoxin to the fertile chick embryo was employed by Jacqueline Verrett et al. (6); and an additional biological test based on the inhibition of tissue culture cells was developed by Legator and Withrow (7). The development of this battery of tests in so short a time was a praiseworthy achieve-

ment for the scientists in FDA. It was due in large part to the uncommon managerial skill and sense of direction in this scientific endeavor displayed by Dr. Campbell.

Earlier, a joint FDA–USDA–industry task force had been formed to coordinate research efforts. Here, again, it was recognized that the most pressing need was for continued improvement in reliable, rapid methodology and adequate supplies of purified aflatoxins suitable for reference standards. The peanut growers and shellers worked directly with USDA on causes and prevention of aflatoxin contamination in peanuts. Dr. Campbell maintained a continual overview of the activities in this cooperative work, particularly with Leo Goldblatt and Walter Pons at the USDA Southern Regional Research Laboratory, as well as with Odette Shotwell and Robert Stubblefield at the USDA Northern Regional Research Laboratory. Through his numerous contacts with scientists in the food industries and universities, he stimulated an interest in mycotoxin research.

With intelligence and vigor, Dr. Campbell and the USDA–FDA–industry scientists applied their ingenuity to a noteworthy achievement in developing improved analytical tools for a more effective program to protect our food supplies from aflatoxin. We owe them much!

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The Aflatoxin Problem: Industry–FDA–USDA Cooperation

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It was our privilege to have been at the core of the aflatoxin problem, or challenge, as it was faced by industry and government in the early 1960s. Our companies, being leading producers of peanut butter, were at the forefront of industry's concern and we are privileged to have viewed the unparalleled cooperation between industry and government in dealing with a matter that had tremendous public health and safety aspects and threatened at least one industry.

The origin of the challenge is well known and has been aptly reviewed by Henry Fischbach in a preceding paper. We are referring, of course, to the contaminated Brazilian peanut meal imported into England in 1960, which when made into a feed for turkey poults, proceeded to kill at least 100,000. Our narrative picks up at that point, but first a quote:

"Even before FDA could formulate a policy with regard to aflatoxin, peanut growers and shellers and manufacturers of consumer peanut products recognized the potential for harm to the consuming public, and also to themselves from the very likely adverse publicity; they moved rapidly to develop a program for research into incidence, causes, and control. Acting through their trade association, the National Peanut Council, and its consultant professionals, Arthur D. Little & Company, the manufacturers arranged for a joint industry–USDA–FDA task force to coordinate the research efforts; growers and shellers worked directly with the Department of Agriculture on causes and prevention" (1).

That in essence is the story. The details are of interest and are essential to documenting Alfred D. Campbell's tremendous involvement. Concerning these details, several facts must be realized: compilation of records on this problem were not generally practiced by industry; industry generally does not keep active records for more than 1–2 years, let alone 2 decades; although we have been in touch with many individuals for their recall, we have undoubtedly overlooked others, unintentionally so; and, there are probably some errors in spite of our efforts to avoid them.

The preceding quotation noted that industry reacted promptly. That was true. As illustrations, we will summarize the research activities of Best Foods Division (CPC) and Procter and Gamble (P&G), the 2 companies with which we were then associated.

CPC's efforts were stimulated by a telephone call in late 1961 from Max Milner of UNICEF to Dan Melnick, at that time vice president in charge of research and quality control of Best Foods Division of CPC. Milner had learned of the problem from British workers in a meeting to discuss child feeding programs in developing nations. Melnick recognized the potential seriousness of the problem and transferred Wilbur Parker from the Portsmouth, VA, peanut butter plant to the Best Foods Central Research Laboratory to coordinate CPC's investigations. Initial efforts concentrated on the scope of the problem: Could U.S. peanuts, whose handling avoided mold growth, be involved? Could aflatoxins be found in other crops? Could changes be made in crop handling, such as rapid curing, to avoid mold development? Could the duckling bioassay be used to monitor crop toxicity? CPC showed that aflatoxins were indeed present in U.S. nuts—they found 2500 ppb in rejects or hand pickouts from U.S. No. 1 grade or better. They demonstrated that the problem was broader than peanuts. Aflatoxins were found in extracts from inoculated corn, wheat, oats, barley, rice, and even soybeans (2, 3). These discomforting first findings were alleviated considerably by other results. Best Foods demonstrated the elimination of aflatoxin during conventional processing of vegetable oils (3) and by the effective use of electronic sorters for the removal of contaminated peanuts during the manufacture of peanut butter.

P&G reacted to published reports (4, 5) and work started quickly in September 1961. P&G concentrated on demonstrating the presence of aflatoxins in domestic nuts; working on a more rapid analytical method; checking other commod-

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ities for the presence of aflatoxins; seeking better procedures for removal of contaminated nuts (P&G checked electronic sorting but concentrated on size/density differences using Hart-Carter separation); and investigating the chick embryo assay as an alternative to the duckling test.

The presence of aflatoxins in No. 1 grade peanuts was documented before the end of 1961, and soon afterward in commercial lots of copra and cottonseed. The latter finding led to the knowledge that fish hatchery rations could be contaminated and to the concern over hepatomas in trout. The slow, tedious Tropical Products Institute (TPI) paper chromatographic approach to chemical assay was quickly replaced by a thin layer chromatographic method. This unpublished method was the forerunner of the subsequent AOAC adopted methods, as details were disclosed at the earliest meeting between industry and FDA.

To their credit, CPC and P&G arranged a joint meeting in New York during February 1962 to discuss the problem; attending were Bob Ruark, Vice President Technical, and Aaron Yohalem, Senior Vice President of CPC, and Ed Korpi, Director, and John Brod, Associate Director of Food Product Development of P&G. It was John Brod who alerted P&G and involved Fred Baur, who at the time had responsibility for the analytical operations with the Food Division.

A second meeting was soon held at which other major peanut product manufacturers were also in attendance. Personnel at this meeting included W. Parker and D. Melnick of Best Foods, L. Brown and C. Mattil of Swift, L. Atkin and L. Kogan of Planters, and E. Korpi and J. Brod of P&G. This second meeting was followed by another at which the peanut industry, through the Peanut Butter Manufacturer's Association and the National Peanut Council, was represented. It was quickly decided to take the concerns of the industry to FDA and a meeting was arranged with Commissioner Larrick.

The peanut industry continued to move ahead through the efforts of George Hartnett, Chairman of the Research Committee of the National Peanut Council. Corporate members of the Research Committee included representatives of CPC, P&G, Standard Brands, Swift, General Foods, Hershey, and Gold Kist. The U.S. Department of Agriculture (USDA) was also represented.

A subcommittee of the Research Committee, composed of Bob Ruark and G. R. Johnson of CPC, John Brod of P&G, and John Maselli of Oz Foods (now with Standard Brands), was appointed by the Research Committee. This subcommittee was given the task of hiring Arthur D. Little (ADL) as consultants to coordinate industry's efforts. More about that later; chronology requires a review of FDA activities.

Was FDA sitting there quietly? No! Was FDA concerned? Yes! FDA recognized the seriousness of the potential contamination and that product seizures could be involved. They also realized that they needed biological and/or physical/chemical evidence for the presence of aflatoxins in interstate shipments of food products.

FDA first learned of the problem in early 1961 through a personal communication from E. S. Hiscocks, Director of TPI. Additional information came from a TPI and FDA meeting in the spring of 1962 and from an international meeting in London in September 1962, attended by H. Fischbach, Director of the Division of Food, and G. Slocum, Director of the Division of Microbiology. FDA's subsequent actions were covered in a preceding paper by Fischbach.

Following the initial industry meeting with Commissioner Larrick, a more broadly based meeting between industry and FDA took place in Washington sometime in 1963. The attendees were, in part, those representing industry on the Aflatoxins Methodology Working Group (AMWG) created by the ADL effort; both authors were present. FDA appeared quite satisfied with industry concern, actions, and progress. FDA confirmed its responsibility in the cooperative efforts to assess the nature and degree of risk and to provide guidelines to the total effort.

Back now briefly to ADL, the industry consultants. Charles Kenstler of that company does not recall when John Brod and Bob Ruark first visited him in Cambridge. Kenstler's first meeting with the National Peanut Council was on April 15, 1964. This was followed by a meeting of the AMWG on April 27, 1964. Attendees were L. Atkins of Standard Brands, F. Baur of P&G, E. Borker of General Foods, A. Campbell of FDA, J. Funkhouser of ADL, C. Jenest of the Canadian Food and Drug Directorate (FDD), L. Goldblatt of USDA, R. Gregory of Swift, C. Kenstler and L. Kogan of Standard Brands, and W. Parker of Best Foods. This working group met about a dozen times, the last time in 1966. The first meeting of the AMWG was the only one attended by Goldblatt.

Concurrent with the FDA activity, USDA became involved in the problem at the request of industry. During the 1963–1964 period, the USDA–industry activities were directed mainly through the Peanut Improvement Working Group with the cooperation of the USDA/ARS laboratory at New Orleans. Under the direction of the National Peanut Council, a meeting was held in August 1962 on aflatoxins in peanuts during the second National Peanut Research Conference at North Carolina State University. This meeting, chaired by Joe Sugg (Executive Secretary of the North Carolina Peanut Growers Association) and Astor Perry (North Carolina State Extension Peanut Specialist) included the following industry representatives: W. Bailey, USDA; J. Brod, P&G; M. Clark, USDA; G. Hartnett, National Peanut Council; G. Jenkins, Tom Houston; W. Livingston, Swift; W. Mills, Lilliston; S. Pace, S.E. Peanut Growers Association; W. Rawlings, Virginia Peanut Growers Association; S. Reagan, S.W. Peanut Growers Association; E. Sexton, CPC.

During the third National Peanut Research Conference in July 1964 at Auburn University, papers on aflatoxin were presented by Goldblatt (USDA), Hartnett (National Peanut Council), and Golumbic (USDA). The opening address by Nyle C. Brady, then USDA Director of Science and Education, also made reference to the potential problems from mold contamination of agricultural products.

Growers and shellers were becoming increasingly concerned. Then the news of the detention by the Canadian Government of a car of peanuts shipped by Stephens Industries circulated among industry leaders. Shellers, including Ray Singletary of Blakely Peanut, contacted Jim Thigpen of USDA. This was in the spring of 1963. Meetings in Washington followed. Representatives from Anderson (J. Anderson), Birdsong (H. Birdsong), Columbian (W. Woodley), Dothan (J. Roberts), and Gold Kist (D. Sands) were among the shellers present. The regional peanut associations were represented by D. Hardin (SE), Sidney Reagan (SW), and Joe Sugg (North Carolina). The National Peanut Council was represented by CPC and P&G.

As a result of numerous industry–government meetings, a cooperative marketing contract was developed for 1964 under which the shellers were assessed $2/ton on farmers' stock peanuts. In turn, the Commodity Credit Corp. of USDA agreed to buy back and reimburse the sheller for any peanuts that were rejected by the manufacturer because of aflatoxin contamination. Shellers were repaid on a formula based on 107% of the farmer's stock kernel content value. Also in 1964,

the National Peanut Council published the first edition of the Voluntary Code of Good Practices for Peanut Product Manufacturers. The emphasis of this publication was on the elimination of aflatoxin from peanut products.

In July 1965, a committee was formed which provided leadership for the formation of an autonomous group to administer the marketing agreement, the Peanut Administrative Committee (PAC). PAC was given official sanction by USDA, and the first sampling program for aflatoxin contamination went into effect with the 1966 peanut crop. The purpose of PAC was, and still is, to prevent lots of contaminated peanuts from entering commercial channels. Joe Genske was the USDA representative in the formation years of the PAC marketing programs.

An important technical development also occurred during this period that made it possible to deal with heavily contaminated lots. The mold damage associated with aflatoxin production is often apparent on the surface of peanuts after removal of the redskins. This blanching operation as practiced by the Seabrook Blanching Corp. in conjunction with color sorting of blanched kernels was shown to be effective in removing most of the aflatoxin-contaminated peanuts from even heavily contaminated peanut lots. As a result, peanut lots which failed to meet USDA–FDA guidelines could be brought into compliance through blanching and sorting to remove contaminated kernels.

Back briefly to another USDA action. Goldblatt of the USDA/ARS became involved in the summer of 1963. One of his first actions was to have Walter Pons transferred to his group to work an aflatoxin methodology. This was a very positive action.

The cooperation between industry and government did not develop overnight. When it did develop, it was open, candid, and complete. The public was the winner. The pattern of cooperation established with peanuts was repeated with brazil and pistachio nuts, with Al Campbell again playing the leading role.

What were some of the other areas of industry–government cooperation in which Al Campbell was involved? Certainly one of the more important has been the AOAC refereeships. Table 1, taken from the General Referee report for 1979 (6), depicts the efforts from inception in 1962 to 1980. Since 1978, Associate Refereeships on Altenaria Toxins and on Ergot Alkaloids have been added. Table 1 shows the 4-year evolution period before establishment of a separate general refereeship. The first Associate Refereeship was Mycotoxins in Moldy Nuts, an unfilled Associate Referee topic, under the General Referee on Nuts and Nut Products. In 1963, Mycotoxins as a topic was moved to Decomposition and Filth in Foods, and Stanley Nesheim was appointed Associate Referee. Al Campbell became involved in 1963 when he was appointed Associate Referee for Aflatoxin—Multiple Methods Study, still under Decomposition and Filth in Foods. Recognizing the growing importance of the need for methodology in the mycotoxins area, AOAC appointed a General Referee in November 1965. It was also in 1965 that the first method (7) was adopted official first action for aflatoxins in peanuts and peanut products, an Al Campbell effort. The very first published rapid method in 1964 by Nesheim resulted from work under the direction of the General Referee, Al Campbell. Al kept the assignment until 1969, when Leonard Stoloff took over. At that point, Al assumed the associate refereeship on Aflatoxin Methodology which he retained until his death. Stoloff resigned in September 1982, upon retiring from FDA. Peter Scott, Canadian Food and Drug Directorate, is the current General Referee.

Over these 20 years, there have been a total of 2 General Referees and 30 different individuals as Associate Referees (a few served in more than one capacity). Of the 30 Associate Referees, 13 have come from or are from FDA, 9 from industry, and 5 from USDA. Truly FDA, industry, and USDA cooperated in the development of reliable, validated methods. As a side note, the maximum number of Associate Referees was reached in 1974, a total of 21. There are now 14. A list of those who have served and are now serving is provided in Table 2.

A continuing cooperative effort to this date is the Joint Mycotoxins Committee. AOAC, since its inception in 1884, has been involved in methods of analysis. The American Oil Chemists' Society (AOCS) is also interested in food, the prevention of contamination, and methods for control. AOCS interest is greatest in vegetable oils and oil seed meals. The American Association of Cereal Chemists (AACC) has a similar interest but with grain crops such as corn, wheat, rye, and barley. AOAC and AOCS were the first professional groups responding to the aflatoxin problem revealed by the British workers in 1961. After all, peanuts and cottonseed are commodities about which oil chemists are more concerned than cereal chemists.

In October 1964, George Cavanagh of Ranchers Cotton Oil and Richard Phelps of National Cottonseed Processors Association arranged an informal session at the 38th fall AOCS meeting in Chicago to discuss aflatoxins in cottonseed. The suggestion was made that AOCS and AOAC form a joint committee to develop a standard procedure for the determination of aflatoxins. Industry members present at this session were from Anderson Clayton, Procter and Gamble, Swift, Ralston Purina, and Hunt Foods; USDA was represented by Leo Goldblatt, Walter Pons, and Marion Whitten. The primary purpose of the Joint Mycotoxins Committee is to coordinate and consolidate the methodology efforts of the involved organizations in the commodity areas of interest and to provide reliable, validated, approved methods to meet the needs that exist. In addition, it has become a forum for exchange of views on mycotoxin occurrences, control, and hazards.

A planning meeting was held in New Orleans on February 23, 1965, between representatives of AOAC and AOCS. A 6-member committee, 3 each from the 2 societies, was chosen. The first committee consisted of R. Duggan (FDA), H. Fischbach (FDA), and D. M. Smith (Canadian FDD) representing AOAC and F. Baur (P&G), L. Goldblatt (USDA), and W. Pons (USDA), representing AOCS. The first meeting of the Joint AOAC–AOCS Aflatoxins Committee was held in Houston on April 27, 1965, in conjunction with the spring AOCS meeting. Notable results from that first effort were election of H. Fischbach as chairman, a role he filled with distinction until his retirement; a definition of the aim of the committee, "to evaluate and recommend practical and uniform methodology to both AOCS and AOAC in the area of aflatoxin;" a decision to concentrate efforts on the peanut and cottonseed commodities; the delegation of peanuts to AOAC, recognizing Campbell's experience in that area as Associate Referee, and the cottonseed area to AOCS; and a decision to compare the Celite (FDA), Florisil (P&G), and aqueous acetone (USDA) procedures on peanut butter and peanut meal, thereby building on work done by the A. D. Little, National Peanut Council efforts.

Until 1969, the committee met semiannually in the spring at the AOCS meeting and in the fall at the AOAC meeting in Washington. In 1969, AACC joined the effort. Since that time, single annual meetings have been held in Washington at the fall AOAC meeting.

Table 1. Associate Refereeships on mycotoxin topics, including General Refereeships under which they functioned and meeting dates on which they were established and terminated (the General Refereeship on mycotoxins was established in 1966)

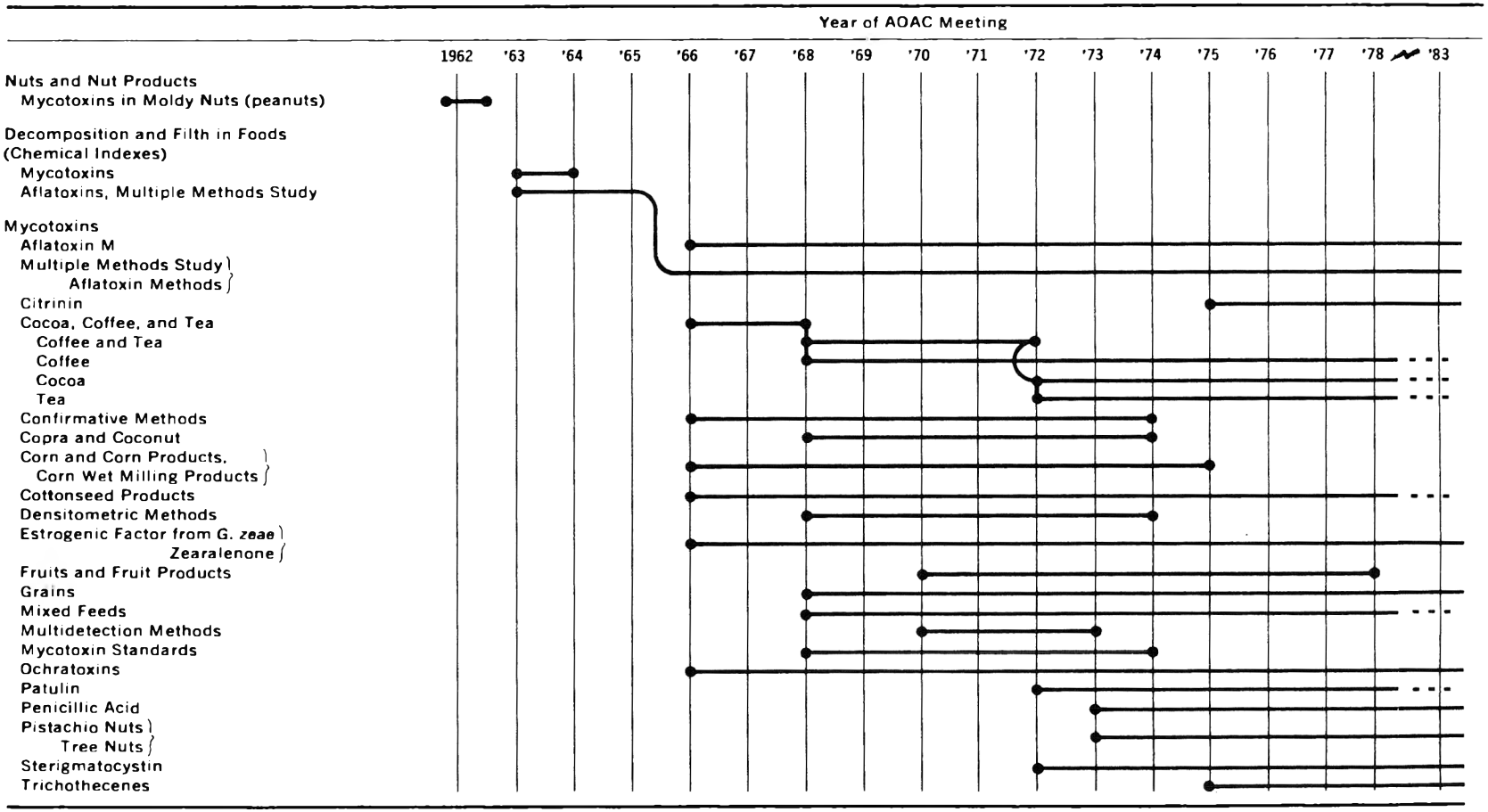

Table 2. Associate Referees for mycotoxins to 1983

From FDA	From Industry	From USDA
A. Beckwith	F. J. Baur	G. A. Bennett
A. D. Campbell	F. J. Farrell	D. King
V. P. DiProssimo	M. Formo	W. A. Pons
R. M. Eppley	V. B. Hill	O. L. Shotwell
O. J. Francis	C. Levi	R. D. Stubblefield
J. L. Moor	J. Peña	
S. Nesheim	T. Romer	Other Affiliations
D. L. Park	G. Reid	C. J. Mirocha (Univ. of Minn.)
A. E. Pohland	R. J. Smith	P. M. Scott (Canadian FDD)
J. V. Rodricks		D. M. Wilson (Univ. of Georgia)
L. Stoloff		
D. Takahashi		
C. W. Thorpe		
M. J. Verrett		

The committee became the Joint Mycotoxins Committee in 1979 when it added a representative from the International Union of Pure and Applied Chemistry, Al Campbell.

The present makeup of the Committee is Chairperson P. Scott (Canada FDD), D. Park (FDA), and John Wessel (FDA), all for AOAC; L. Goldblatt (FDA/USDA), R. Stubblefield (USDA), and A. Waltking (CPC), all for AOCS; and O. Shotwell (USDA), L. Stoloff (retired FDA), and T. Romer (Consultant, formerly Ralston Purina), all for AACC. Al Campbell, in his lifetime, was the committee's resource, its one and only consultant. His services, dedication, and knowledge were invaluable.

One of Al's larger accomplishments, one with which his name has received little publicity, was the International Aflatoxin Check Sample Series. The fact that aflatoxin contamination in food commodities crossed geographic boundaries was well recognized by industry and government. Peanuts and copra were the 2 early examples. The need existed for methods used throughout the world to give comparable results. Distribution of blank samples of known uniform aflatoxin levels permitted various laboratories to check the performance of their methods. No existing international organization could readily handle this need. A meeting, organized by Al Campbell, was held in Washington on February 9, 1971, to address the need and react to it. Representatives from industry, private laboratories, and Canadian and U.S. regulatory agencies participated. As a result of the meeting, a Committee on International Aflatoxin Check Samples was formed. The Committee was chaired by Frances Coon of the WARF Institute, Inc. (now Hazleton Raltech), assisted by F. Baur (P&G) and L. R. Symmes (Canada Packers). Under the auspices of the committee, check samples were distributed in 1971 (8) and in 1972 (9). The committee agreed at the start, as all good committees should, to work itself out of a job. To do this, a permanent home was needed for the effort. The finding of a home was strictly Al's doing, through Ernest Walker of the Unit of Environmental Carcinogens of the International Agency for Research on Cancer located in Lyon, France. That agency took over and has retained responsibility for this effort. The committee remained in a consulting role until 1980.

What impact has this monumental effort had on aflatoxin levels in peanuts and peanut products in the market place? This information is summarized in the paper on "Aflatoxin Control: Past and Present" (1) and in a more recent review (10). Despite some years, such as 1980, when a severe drought was present throughout the peanut belt, causing levels and incidence of aflatoxins in the raw nuts to be higher than normal, levels in finished peanut butter and confectionary products have been regularly low. Both shellers and processors adjust their controls to compensate for higher levels in the feed stream of raw peanuts.

We will close the historical portion of this review by touching briefly on some of the awards that have accrued to individuals resulting from their participation in helping "solve" the aflatoxin problem. D. Melnick in 1967 was the recipient of the Progress in Lipid Research Award from the American Oil Chemists' Society, North East Section, for his contributions. In 1971, the Golden Peanut Award was given to A. Waltking for his work on methodology and fate of aflatoxin in peanuts on roasting. This 11th award was the first given to a member of industry. The prior 10 individuals worked in academia, extension service, and the like. Three recent Wiley Award winners were W. Pons, USDA, in 1976; L. Stoloff, FDA, in 1981; and O. Shotwell, USDA, in 1982. These awards all reflect to a significant degree the awardees' efforts in assessing the practical significance of the aflatoxin problem and in developing aflatoxin methodology, and underscore their dedication, the importance of the effort, and the magnitude of the problem faced by government and industry in the early 1960s.

This is but a partial story of unparalleled cooperation between industry and government on a consumer problem, cooperation that fulfilled the responsibility of the regulatory agencies and concerned industries to protect the public, cooperation that kept the public safety, the economic concerns, the scientific aspects, the compliance needs, the significant breadth and depth of the problem all in proper perspective during a difficult learning period. Another unparalleled aspect of the aflatoxin problem was the contribution of Al Campbell. He had a vital role in all that went on.

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Past and Present Research on Aflatoxin in Peanut Products

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For many years, A. D. Campbell held positions of responsibility in FDA and AOAC concerned with the presence of aflatoxins in food products. Throughout this period, he had a profound influence on the speed with which new research developments from government and industry were disseminated and used.

One of the first analytical methods, proposed by Coomes and Sanders and used soon after the causative agent of turkey-X disease was identified, was based on Soxhlet extraction and paper chromatography (1). This method required almost 24 h of extractions and separations for the analysis of one sample. Even the so-called rapid procedures developed by FDA, the Celite (2) and CB methods (3), which use column chromatography and thin layer chromatography, required from 3 to 5 h for an analysis of 1–4 samples.

Since the hold time for peanut butter produced by a continuous processing system is generally about 1 h, it was clear that the available analytical methods were too time consuming for quality control in a processing plant. This and the fact that the distribution of aflatoxin in peanuts was extremely nonuniform, and that little was known concerning the effects of oil or peanut butter processing conditions on aflatoxin and its distribution in edible products, spurred research by industry in resolving these questions.

Waltking, Bleffert, and Kiernan extensively researched the component segments of the various analytical procedures and developed the BF method which permitted 1–4 samples to be analyzed within 1 h (4). This breakthrough permitted the analysis of finished peanut butters at a rate fast enough to monitor peanut butter production and ensure removal of significantly contaminated product.

A paper on this method was presented at the joint AOCS–AACC meeting in Washington, DC, in 1968. Al Campbell was in the audience and later urged the authors to run an AOAC collaborative study against the Celite and CB methods to validate the new procedure. Al's influence was a catalyst which helped move this development out of the developing laboratory and into industry-wide use.

The collaborative study of the 3 procedures among 15 laboratories in 1969 provided support for the adoption of the BF method as an official AOAC method (5). The shorter analysis time, along with the reduced volume of reagents used with the BF method, resulted in a wide industrial preference for this procedure for routine detection of the presence of aflatoxin in peanut products. Al Campbell was a great promoter of this method, personally training analysts in laboratories in the Middle East for analysis of aflatoxins in peanuts and pistachios.

After Cucullu and Lee (6) of the USDA Southern Regional Research Center demonstrated that aflatoxin was not uniformly distributed even within a single peanut, concern increased over the variability obtainable with chunk-style peanut butter analyses. Whereas a sample of 50 g taken from any jar of creamy peanut butter usually will be equal (for any aflatoxin contamination present) to another sample from the jar, this uniformity is not obtained with chunk-style product because the latter is a blend of large and small particles. The large chunk particles may be derived from a peanut which has been severed into fewer than 50 particles. If one piece of a contaminated peanut is present in a 12 oz jar of chunk peanut product, there is only a 1 out of 7 chance that an analysis of the jar would detect any aflatoxin.

Because Al Campbell decided it was time that all should become cognizant of the problems with commodity sampling, he organized a session on sampling at an AOAC Spring Workshop. It was here that Dickens discussed his extensive research on sampling of peanuts (7) and Waltking proposed the use of a slurry method for sample preparation of chunk peanut butter (8). The latter involved the comminution with hexane in a Waring blender of up to 54 oz of chunk style peanut butter to uniform particle size. An aliquot of the slurry could then be readily assayed and be representative of the sample.

Lee et al. of USDA (9) also studied the effect of roasting on peanuts containing aflatoxin and found that a reduction in aflatoxin concentration will occur. In 2 separate studies using samples of individual nuts and samples of 50 g each, 80% and 65% reductions of the original concentration of aflatoxins were obtained.

Because the analysis of a single peanut kernel and even 50 g whole peanuts results in a large analytical error, additional roasting studies were conducted by Waltking (10). A pilot plant roaster, modeled after the commercial roasters, was used to prepare 50 lb batches of contaminated peanuts. These along with matching batches of the same well mixed samples of unroasted nuts were made into peanut butter-type products. To a major degree, the nonuniformity of the distribution of aflatoxin was averaged out by the preparation of a product of peanut butter consistency of both the raw and roasted materials. This permitted ready statistical analysis of the multiple samples drawn for comparative study. After all data were averaged, 40–50% of the B_1 and G_1 aflatoxins and 20–40% of the B_2 and G_2 aflatoxins originally present were found to have been destroyed by the roasting process.

Following these studies, there was speculation that aflatoxin might still be biologically active although altered and not detected by the analytical procedure. Therefore, samples were submitted to an independent laboratory experienced in running the chick embryo test for aflatoxin confirmation. The concentration of the solution injected into the air sac of the egg was adjusted on the basis of the aflatoxin concentration obtained with the BF method and 100% mortality with 20 μL. Thus, the concentration of the aflatoxin extract used for the roasted samples was increased roughly by a factor of 2. The data presented in Table 1 show that the values obtained by the biological tests compare well with the chemical procedure. If roasting had not destroyed some of the aflatoxin in the peanuts, the mortality of a given injection of the roasted batches would have been closer to the mortality of the next higher concentration. Such results confirmed that roasting is a means of reducing the presence of aflatoxin.

With improvements in methodology, new mycotoxins were detected in the milk of cows which had eaten aflatoxin-containing feed. These were called M-toxins. Al Campbell noted during the 1970 AOAC meeting that he had been informed that M-toxins had been detected in peanuts, and he raised the concern that it would also be necessary to routinely analyze peanut products for M-toxins. A survey was made of all peanut shipments received by one company over a 2-year period. The extracts from any sample found to contain more than 30 ppb aflatoxin were separately assayed for M-toxins.

Table 1. Biological confirmation of chemical analyses for aflatoxins

No. of eggs	Injected, μL[a]	Mortality, Found, % 1	2	3	Av.	Theor.
		Raw Batches				
20	20	85	100	88	88	100
20	10	65	70	31	55	50
20	5	22	26	20	23	25
20	2	20	30	55	35	10
20	1	5	27	11	14	5
		Roasted Batches				
20	20	100	100	100	100	100
20	10	90	50	67	69	50
20	5	30	21	20	24	25
20	2	27	21	11	20	10
20	1	28	5	5	13	5

[a]Concentration adjusted to provide equal mortality based on difference in level of chemical analysis.

A summary of the 255 peanut samples assayed is shown in Table 2: 68% of the samples tested did not contain a detectable amount of M-toxins; 25% of the samples tested contained only a trace (less than 1 ppb M_1); 6% of the samples tested contained measurable amounts of M_1 with an average concentration relative to total aflatoxins present of about 3%. If the 2 samples which were determined to be statistical outliers at the 95% confidence level, by the Dixon method, are eliminated, the M_1 concentration averaged 2.29 ± 1.28% of the total aflatoxins and 3.65 ± 1.63% of the B_1 concentration of the same sample. Only 1.2% (3 samples) of the 255 samples contained M_1 at a concentration of 0.16% of the total aflatoxins present. Overall, the presence of M-toxins in peanuts was shown to be of little significance; even in the 6 of 100 samples in which it might be found, it is present in smaller concentrations relative to aflatoxin B_1 than the error in measurement of B_1 by the official procedure for peanuts.

Since most of the peanuts which are separated from the edible streams by the USDA inspectors or rejected by the processors are sent to oil crushers, it is logical to assume that most crude peanut oils should be contaminated. Corn and cottonseed are also known to be frequently invaded by *Aspergillus flavus;* therefore, there was deep concern that aflatoxin could also be present in these processed vegetable oils.

Table 2. Survey of M-toxins in 1971 and 1972 peanuts

Roasted & raw samples	Range of aflatoxins, ppb Total	B_1	M_1	M_2
172	30–1001+	12–1001+	neg.	neg.
64	30–1000	16–208	0–1	neg.
4	54–145	43–68	1	neg.
3	21–112	16–82	2	neg.
4	55–299	45–118	3	neg.
2	32–255	20–75	4	neg.
Raw pickouts				
6	45–4752	29–2996	2–120	0–7

Parker and Melnick (11) studied the pathway that aflatoxin followed during the alkali refining of peanut and corn oils. Through these studies, they proved that virtually all aflatoxin present in the crude oil was removed by the caustic wash and the remaining traces were removed by acid bleaching clay. Their summary of results for a cold-pressed peanut oil shows that less than 1 ppb was present in the refined oil from peanuts containing 5500 ppb. Recently, the analysis of aflatoxins in peanut products has begun to use liquid chromatographic and high performance thin layer chromatographic techniques. LC has had the advantage over TLC of being a more objective means of determining aflatoxins in peanut products because of removal of the subjective human visual evaluation of the plate. However, LC imposes time constraints because of late-eluting peaks that require considerable column equilibration between successive samples. The use of HPTLC for aflatoxins was reported years ago by Vitek et al. (12), but has only recently generated interest with improvements in the sensitivity of the measuring densitometer. A comparison of these 2 techniques is reported in a separate paper (13). In summary, the HPTLC method surpasses LC with respect to analysis time by permitting simultaneous multiple sample development, and the 2 techniques appear to be equivalent with respect to precision, accuracy, sensitivity, and reproducibility. The disposable nature of the stationary phase (TLC plates) eliminates the problem of loss of sensitivity due to possible contaminations that affect the life and performance of microparticulate silica gel LC columns. Also, the amount of solvent used is less for HPTLC. These points make the use of HPTLC a viable alternative technique in the determination of aflatoxins.

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PESTICIDE FORMULATIONS

Fluoride Ion-Selective Electrode Determination of Sodium Monofluoroacetate in Meat Baits and Formulations

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Hot aqueous alkaline conditions were used to defluorinate sodium monofluoroacetate (Compound 1080) with a mean efficiency of 98% and a coefficient of variation of 2%. The liberated fluoride was determined by ion-selective electrode. These procedures were used to analyze meat baits and commercial 1080 formulations. Compound 1080 (1–50 mg) was extracted from meat baits into 50% (v/v) acetone–water by blending, followed by ultrasonic agitation and digestion on a steam bath. The extract was filtered and the solids were agitated ultrasonically in more extracting solvent. The combined extracts were concentrated by evaporation prior to alkaline hydrolysis. This technique was well adapted to processing the large sample sizes encountered during analysis of whole baits (<400 g). After correction for co-extracted inorganic fluoride, the typical recovery was 86% with a coefficient of variation <7%. Proprietary 1080 formulations (>90% w/w powders) were simply dissolved in water and diluted before alkaline hydrolysis. Recoveries for added reagent grade material, after correction for free fluoride, averaged 97% with a coefficient of variation 2%.

Compound 1080 is a potent, broad spectrum toxicant which is widely used for the control of vertebrate pests. Because this practice places nontarget vertebrates at risk, it is strictly regulated by government authorities in most countries. However, this regulation has been hampered by the lack of a rapid, robust method to determine the minimum toxic dose of 1080 required in baits and, to a lesser extent, the concentration of 1080 in formulations from which they are prepared.

Gas chromatographic (GC) procedures for the determination of intact or derivatized fluoroacetate, with one exception (1), are not suitable for meat baits (2–4): They typically give low recoveries and variable results, largely due to interferences caused by the matrix. Similar considerations apply to liquid chromatographic techniques, unless the extracts are subject to elaborate cleanup procedures (5), or the baits contain 100–1000 mg 1080/kg (6)—a level in excess of that normally used in Australia (<30 mg/kg) for the control of wild dogs.

Methods based on the measurement of fluorine after cleavage from fluoroacetate have been described (7–10) but they are too lengthy, are not suited to large bait sizes, or are semiquantitative. However, it appeared likely that these drawbacks could be overcome by substituting aqueous hydrolysis (11) for the methods of defluorination previously used. Such defluorination reactions are not specific for monofluoroacetate (11); however, the identity of the parent fluorocarbon can be confirmed by another technique, ideally GC/MS (mass spectrometry) (3, 10).

An ion-selective electrode method for determining fluoride, following aqueous alkaline hydrolysis of fluoroacetate extracted from baits, had previously been investigated in this laboratory (A. Crocker and B. Coman, 1980, personal communication). Many aspects of this method were modified to improve its reproducibility and speed. In addition, a variation of the technique was developed for 1080 formulations. Recovery data are presented.

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METHOD

Materials and Apparatus

(a) *Reagents.*—Analytical grade.

(b) *Sodium monofluoroacetate.*—Reagent grade (E. Merck, Darmstadt, Germany).

(c) *Baits.*—Inject pieces of lean meat (ca 250 g each) with 1 mL 1080 solution (1–50 mg/mL). Prepare baits from raw beef, horse, or kangaroo meat.

(d) *Filter aid.*—Wash Celite 535 (Johns-Manville Products Corp., Lampoc, CA), with water until eluant contains <0.1 μg F/mL.

(e) *Buffer.*—TISAB III concentrate (Orion Research Inc., Cambridge, MA).

(f) *Fluoride standards.*—Dissolve NaF (1.105 g) in water (1 L) to produce stock solution containing 500 μg F/mL. Stored in plastic bottles, this solution is stable at least 3 months. Prepare working standards daily (0.50 and 5.0 μg F/mL) from stock solution so that each 50 mL contains 5 mL buffer and 0.75 g NaCl.

(g) *Blender.*—Sorvall Omni-Mixer fitted with 400 mL capacity stainless steel chamber.

(h) *Ultrasonic bath.*—1 L capacity, 80 watt power output (Varian Instrument Group, Walnut Creek, CA).

(i) *Fluoride measuring equipment.*—Use fluoride-selective electrode and double junction reference electrode ($Hg/Hg_2Cl_2/KCl_{(SATD)}$), with outer compartment filled with 10% (w/v) KNO_3 (Ionode Pty Ltd, Brisbane, Australia). Connect electrodes to high impedance digital mV meter with direct concentration readout (TPS, Brisbane, Australia).

Procedure

(a) *For baits.*—Freeze samples (below −12°C) if analysis is to be delayed for more than 24 h. Thaw, cut meat into cubes (ca 1 cu. cm), and use extracting solvent, acetone–water (1 + 1), to wash cutting utensils (scalpel and ceramic plate). Place cubes (<150 g at a time) into blender cup, and add washings and sufficient additional extracting solvent to just cover meat.

Blend at low speed until puree is obtained (2–5 min). Transfer mixture to 1 L conical flask, using more extracting solvent, to give at least 1.5 mL/g meat. Place in ultrasonic bath 10 min. Cover with watch glass and heat on steam bath 30 min. Cool and add sufficient acetone (usually 50–100 mL) to replace losses during digestion.

Vacuum-filter digest through bed of filter aid on coarse paper (e.g., Whatman No. 41). Wash with five 20 mL portions of extracting solvent. Transfer residual solids to 600 mL beaker, taking with it the minimum quantity of filter aid, and add sufficient extracting solvent to give 1 mL/g. Ultrasonicate 10 min and filter through same filter bed. Wash with three 20 mL portions of extracting solvent.

Combine filtrates, record total volume (V_1) and transfer aliquot (V_2) containing between 1 and 5 mg 1080 (3 mg optimum), to squat-form 1 L beaker. Add boiling chips and evap-

orate to ca 30 mL by heating on sand bath. Adjust volume to 50.0 mL with water (solution Y). Retain 10.0 mL solution Y in plastic bottle for background fluoride determination.

Transfer remaining 40 mL to platinum dish. Add 2 g NaOH and several boiling chips; then heat on sand bath (ca 200°C) to dryness. At this stage, fats and other co-extractives may cause frothing and caking on sides of dish, but heating should be continued until the mixture reaches solidification stage. Failure to do this will result in incomplete hydrolysis and low recoveries. Remove dish from bath, add 10 mL water, swirl to wet all material, and again reduce to dryness. While still warm, transfer contents of dish to 100 mL plastic beaker, using six 5 mL rinses of 1M HCl and several rinses of hot water. After cooling, adjust pH to 5–6 with 1M HCl. Dilute to exactly 200 mL with water (solution X).

Calibrate response of fluoride electrode as follows. Pour diluted, buffered standards (0.50 and 5.0 μg F/mL) into 100 mL plastic beakers. Insert electrodes and continuously stir solutions until stable response is obtained (usually within 3 min). Adjust concentration display to read 0.5 and 5.0 μg F/mL, respectively. Check calibration every 15 min to ensure its accuracy, and recalibrate as required (usually 3 times a day). Ensure that electrodes and all solutions with which they are in contact are maintained at almost constant temperature (± 1°C).

Determine concentration of fluoride in solutions Y and X as follows: To 10 mL solution Y, add 12.5 mL water, 2.5 mL buffer, and 0.38 g NaCl. Mix and thermally equilibrate in plastic beaker. Immerse calibrated electrodes in solution and stir continuously until stable reading is obtained (F_y μg F/mL). Similarly, to 45 mL solution X, add 5.0 mL buffer and measure fluoride concentration (F_x μg F/mL).

Calculate weight of 1080 in bait, using following equation:

$$1080, \text{mg} = (277.8\ F_x - 125\ F_y)\ V_1/190\ V_2R$$

where V_1 = total volume of filtered extract (mL); V_2 = volume of aliquot (mL) taken from V_1 for preparation of solutions Y and X; F_y = fluoride concentration (μg/mL) in solution Y, i.e., background fluoride concentration; F_x = fluoride concentration (μg/mL) in solution X, i.e., fluoride hydrolyzed from 1080, plus background; R = recovery factor determined for 1080 injected into meat (in our experiments, 86.1%).

Report calculated quantity of 1080 to nearest 0.1 mg.

(b) *For formulations.*—Into 200 mL volumetric flask, accurately weigh sufficient powder to contain ca 50 mg 1080. Dilute to volume with water and transfer 20.0 mL to platinum dish. Hydrolyze and determine fluoride (F_x) as for baits. Measure free fluoride (F_y) on another 20.0 mL aliquot, after addition of 2 mL buffer and 0.35 g NaCl.

$$1080, \%\ (w/w) = 115.8\ (10.1\ F_x - F_y)/WR$$

where F_x = total fluoride, i.e., fluoride hydrolyzed from 1080 plus free fluoride (μg/mL); F_y = fluoride concentration (μg/mL) in solution (free fluoride); W = weight (mg) of sample taken for determination; R = recovery factor determined for 1080 formulations (in our experiments, 97.3%).

Report calculated 1080 percentage (w/w) to nearest whole number.

Results and Discussion

Defluorination of Monofluoroacetate.—Known amounts of reagent grade sodium monofluoroacetate were subject to aqueous alkaline hydrolysis as described in our procedure and the released fluoride was measured. The amount of fluoride recovered was always between 94 and 101% of the theoretical value. Mean recovery was 97.9% with a coefficient of variation of 2.0%. In preliminary experiments, we found that concentrated alkali and strong heating were necessary to obtain quantitative hydrolysis. These findings are consistent with results reported previously (11). The strongly alkaline conditions required to rupture the C–F bond were achieved by reducing to dryness. The amount of base was kept to a minimum to avoid high total ionic concentrations in the final solutions and thereby avoid adverse effects on electrode sensitivity.

Extraction and Recovery.—The ideal solvent for extraction of fluoroacetate from meat should give quantitative recovery while dissolving and emulsifying a minimum quantity of the matrix. With this in mind, we tried dilute HCl, methanol, acetone, water, and mixtures of the latter 2 solvents. Of all these, the most successful was 1 + 1 acetone–water, a mixture similar to that used by Okuno et al. (1). This mixture could be evaporated with relative ease without risk of co-distilling fluoroacetic acid. Moreover, the amount of co-extracted fat generally did not affect the determination. Should such interference occur, the procedure could be extended by the addition of a final ashing step (7).

The extraction efficiency of the solvent system is illustrated in Figure 1, a plot of the recovery of 1080 (39.1 mg) injected into about 250 g meat. The procedure used was as described in the previous section, except that the digested material was extracted with 6 aliquots of solvent and the 1080 was determined in each. Almost 80% was recovered in the first extract but following extractions were less efficient: The cumulative recovery after 6 extractions was only 96%. Therefore, in the interest of speed, only 2 extractions are recommended. Using this procedure, the mean recovery of 1080 added at 10 levels in the range 1–50 mg/250 g meat was 86.1% with a coefficient of variation of 6.3%. The data are typically means for 4 replicated additions at each level of 1080. As indicated by the linearity of the plot (r^2>0.99), the efficiency of the extraction was independent of the level of addition over the entire range.

Recovery of 1080 was not affected by the type of meat used as carrier. To reduce the likelihood of enzymatic defluorination (12, 13), baits were frozen during storage. No breakdown was observed for up to 4 months—the longest storage period used.

Pure monofluoroacetate added to 1080 formulations was consistently recovered between 95 and 100%, with a mean of

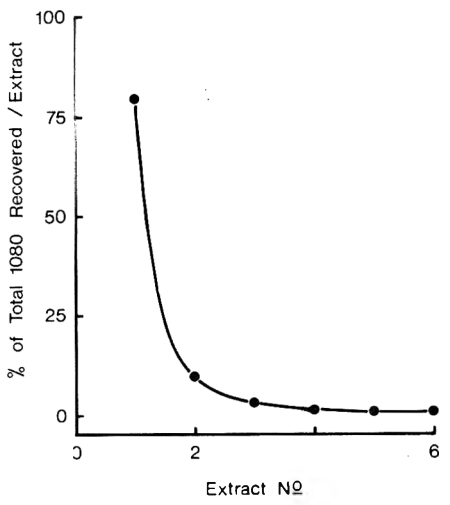

Figure 1. Percent 1080 recovered following each of 6 extractions from meat (250 g) spiked with 39.1 mg compound.

97.3% and a coefficient of variation of 2%, i.e., values similar to those obtained for hydrolysis of pure monofluoroacetate.

Interferences and Precautions—Correction for free fluoride content in extracts of meat and formulations was essential for the reliable quantitation of 1080. No attempt was made to ensure total recovery of free fluoride from meat or formulations; however, the amounts extracted, calculated as 1080, were up to 1 mg/bait and 2% (w/w) for formulations. Correction for fluorocarbons other than 1080 in meat was unnecessary in almost all cases examined, as levels rarely exceeded 0.05% (calculated as 1080). It is advisable, however, to conduct a blank determination on the meat used for each batch of baits and apply a correction factor if the levels of other organic fluorine compounds are significant.

It is also noteworthy that significant amounts of fluoride may be leached from unwashed filter aid during the extraction of baits. For this reason it should be thoroughly washed before use. Similarly, fluoride contamination was caused by using glass fiber filters.

Fluoride Measurement.—Standards and blanks were adjusted to a similar total ionic strength as the samples and all were pH-buffered to ensure uniform electrode response to changes in fluoride concentration (14). This is essential if one is to accurately read concentration directly using a meter which assumes a linear voltage response to a logarithmic change in analyte concentration (as most instruments do). We found this type of instrument particularly useful when analyzing large numbers of samples, all of which fell within a narrow concentration range. The precision achieved was about ±2% relative in this range (0.50–5.0 μg F/mL). Other measurement methods may be preferred under different circumstances, e.g., standard addition.

Extracts of a few baits adversely affected electrode response and stability. Response characteristics were restored by washing the electrodes in ethanol and then rinsing well with water, so it was concluded that the effects had been caused by deposition of fatty acids on the active surfaces of the electrodes. To ensure that this treatment is effective, it is advisable to recalibrate after washing.

The detection limit of the electrode (14) and the foregoing considerations limit the lower level of 1080 determination to 1 mg/bait (150–400 g meat).

Analysis of Commercial Baits and Formulations.—Forty baits, each with a nominal 1080 content of 6 mg, were prepared in a similar way to that used by field operators. Twenty-nine of these were dropped from an aircraft and retrieved the same day. Analyses were 5.28 ± 0.5 mg/bait (mean ± SD). For the 11 laboratory baits, analyses were 5.27 ± 0.9 (all results corrected for 86% recovery). The 1080 recovery was unaffected by the aerial drop ($P_{0.001}$). The discrepancy between nominal and actual 1080 contents, and the relatively high standard deviation compared with baits prepared in the laboratory, was attributed to dosing errors in the field. This source of variation can be large, as demonstrated by the data for another group of 49 baits, each nominally containing 4 mg active ingredient. The average content was 3 mg with a standard deviation of ± 1 mg. Of 36 commercial 1080 formulations tested, all but 2 were close to, or slightly above, specification (>90% w/w). The exceptions contained only 72 and 78% (w/w), respectively. Results such as these reflect the need for improved meat-spiking techniques and regular monitoring of fluoroacetate content in baits and formulations.

Over a 12 month period, 4 analysts used these procedures and the between-analyst variation was <5% relative. The average daily analysis rate was 3 baits or 12 formulations.

Acknowledgments

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COSMETICS

Liquid Chromatographic Separation and Fluorometric Determination of Quaternium-15 in Cosmetics

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A method is described for the determination of the cosmetic preservative quaternium-15 (*cis*-1-(3-chloroallyl)-3,5,7-triaza-1-azoniaadamantane chloride) in the presence of free formaldehyde, 2-bromo-2-nitro-1,3-propanediol, and imidazolidinyl urea. Quaterium-15 is separated from the other preservatives in cosmetic products by using liquid chromatography on a pellicular reverse phase C_{18} column. Formaldehyde released from the eluted quaternium-15 reacts with ammonium acetate-buffered 2,4-pentanedione reagent in a heated reaction coil to form the highly fluorescent post-column derivative 3,5-diacetyl-1,4-dihydrolutidine. A minimum of 1 μg quaternium-15/mL (0.001%) is detected by fluorescence. Recoveries of quaternium-15 from samples of a lotion, a body detergent, and a mascara spiked at 0.01, 0.1, and 0.2% ranged from 94 to 106% and averaged 100%.

Quaternium-15 (*cis*-1-(3-chloroallyl)-3,5,7-triaza-1-azoniaadamantane chloride) is a broad spectrum biocide widely used for the preservation of cosmetic products (1, 2). It is not inactivated by nonionic, anionic, or cationic ingredients and is highly soluble in water (3). This preservative is used either alone or in combination with other preservatives, including imidazolidinyl urea (methane bis-*N,N'*-(5-ureido-2,4-diketotetrahydroimidazole)-*N,N'*-dimethylol) and 2-bromo-2-nitro-1,3-propanediol.

Quaternium-15 is a derivative of 1*H*-imidazole-4-ethanamine (hexamine) quaternized with dichloropropene. In 1961, Scott and Wolf (4) reported that formaldehyde was released slowly from the hexamine moiety of this halohydrocarbon derivative. Storage of this material in aqueous solution at elevated temperatures reduces its stability, with a release of formaldehyde (4, 5).

The procedure used in our laboratory to determine formaldehyde is based on the work of earlier investigators. In 1953, Nash (6) developed a colorimetric procedure based on the Hantzsch reaction, in which formaldehyde reacts with 2,4-pentanedione in the presence of ammonium salts to form 3,5-diacetyl-1,4-dihydrolutidine, a yellow, highly fluorescent derivative. In 1963, Belman (7) showed that this derivative could be determined quantitatively using fluorometry; the sensitivity of the method was 0.01 μg formaldehyde. When the reaction was buffered to pH 6 with ammonium acetate and acetic acid, Wilson (8) found that formaldehyde was determined specifically and quantitatively whether present initially or released in situ in a cosmetic product. The fluorometric intensity of the lutidine derivative formed from formaldehyde was directly proportional to the amount of formaldehyde present over the 1–4 μg/mL concentration range. This procedure is limited, however, because it determines the total amount of formaldehyde present in cosmetic products irrespective of source.

Wisneski (9) showed that quaternium-15 quantitatively releases close to its theoretically expected 6 moles formaldehyde in the presence of the 2,4-pentanedione reagent. The fluorescence of the lutidine derivative formed from the released formaldehyde was measured by direct comparison to the fluorometric emission intensity of a standard of similar concentration. However, this procedure is limited in that it only can be applied to the determination of quaternium-15 in cosmetic products in which this preservative is the only source of formaldehyde.

The identification of quaternium-15 in cosmetics has been reported by Wisneski (9) and Liem (10). They used thin layer chromatography for separation and a developing spray containing the 2,4-pentanedione reagent for detection.

The method described here can be used to determine the amount of quaternium-15 in a cosmetic product and is applicable in the presence of either formaldehyde, 2-bromo-2-nitro-1,3-propanediol, imidazolidinyl urea, or a combination of these ingredients. The quaternium-15 is separated from the other components in a cosmetic product by liquid chromatography (LC) on a pellicular reverse phase C_{18} column. The column eluant containing the quaternium-15 is mixed with 2,4-pentanedione and ammonium acetate in a heated reaction coil, where the lutidine derivative is formed from formaldehyde released from the quaternium-15. The amount of quaternium-15 is determined by measuring the fluorometric intensity of the lutidine derivative.

Experimental

Apparatus

(a) *LC pumps.*—A and B (Model 8500, Varian Aerograph, Palo Alto, CA 94303).

(b) *Syringe-loading sample injector valve.*—Model 7125 (Rheodyne Inc., Berkeley, CA 94710), equipped with 10 μL sample loop.

(c) *LC analytical column.*—39 × 4.6 cm stainless steel, with 10 μm frits, packed with C_{18} reverse phase pellicular material, 37–44 μm particle size (Chromosorb LC-4, Johns-Manville, Denver, CO 80217).

(d) *Reaction coil.*—0.51 mm (0.02 in.) id × 91 m (300 ft) stainless steel capillary tubing immersed in temperature-controlled 66°C water bath.

(e) *Fluorescence detector.*—Models 420-E and 420-C (Waters Associates, Milford, MA 01757), equipped with 500 nm emission filter (No. 78232, Waters Associates) and ca 400 nm excitation filter (cut from cobalt glass).

(f) *Strip chart recorder.*—Model A-25 (Varian Aerograph). Connect in series LC pump A, injector valve, and LC column, using appropriate fittings and 1.6 mm (1/16 in.) stainless steel tubing. Connect tubing from LC pump B to tubing at end of column with "tee" fitting. Attach outlet from tee to reaction coil. Attach outlet of reaction coil to fluorescence detector.

Operating conditions: LC eluant in pump A: 0.03M ammonium acetate buffered acetonitrile–ethanol–water at 60 mL/h. Derivatizing reagent in pump B: 2M ammonium acetate-buffered 2,4-pentanedione at 40 mL/h. Strip chart recorder speed 2.54 mm/min.

(g) *Filtering apparatus.*—Micro-syringe Luer insert, 25 mm diameter, and 30 mL glass syringe, equipped with Type EG 0.2 μm cellulose acetate filters (Millipore Corp., Bedford, MA 01730).

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(h) *Syringes.*—25 μL (Model 702, Hamilton Co., Reno, NV 89510).

Reagents

(a) *Acetonitrile.*—Distilled-in-glass (Burdick & Jackson Laboratories, Inc., Muskegon, MI 49442).

(b) *Water.*—LC grade.

(c) *Eluting solvent (pump A).*—0.03M ammonium acetate-buffered acetonitrile–water–ethanol. Mix 50 mL water with 400 mL acetonitrile. Add 3 mL glacial acetic acid and neutralize solution to pH 6.5–6.8 with ammonium hydroxide. Mix 200 mL buffered acetonitrile and water solution with 500 mL ethanol, degas, and filter solvent through fine sintered glass funnel.

(d) *Dowicil 200 (quaternium-15).*—Dow Chemical Co., Midland, MI 48640.

(e) *Standard solutions.—Stock solution 1.*—2 mg/mL. Accurately weigh 50 mg quaternium-15 into 25 mL volumetric flask and dilute to volume with water. *Stock solution 2.*—200 μg/mL. Pipet 1 mL stock solution 1 into 10 mL volumetric flask and dilute to volume with water. *Working solutions.*–13.3, 80, and 160 μg/mL. Pipet 1 mL stock solution 2 into 15 mL volumetric flask and dilute to volume with water. Pipet 1 and 2 mL stock solution 1 into separate 25 mL volumetric flasks and dilute to volume with water. Prepare fresh solutions daily. Store all solutions in refrigerator.

(f) *2,4-Pentanedione (acetylacetone).*—Aldrich Chemical Co., Milwaukee, WI 53233.

(g) *Derivatizing reagent.*—Weigh 46 g ammonium acetate in 250 mL beaker and dissolve in ca 150 mL water. Pour into 500 mL graduated cylinder, add 1 mL glacial acetic acid and 0.8 mL 2,4-pentanedione, and dilute to 300 mL with water. Filter and degas solution through fine sintered glass funnel.

Preparation of Sample

Accurately weigh ca 2 g sample into tared 30 mL beaker. Dilute sample with 10–15 mL water and quantitatively transfer to 25 mL volumetric flask. Dilute to volume and mix. Filter sample solution through Millipore filtering apparatus to remove sediment, if necessary. Verify approximate concentration of quaternium-15 in prepared sample by injecting aliquot into analytical LC system; concentration of 0.02–0.2% quaternium-15 in product falls within concentration range of standards. Peak height of lutidine derivative in sample should closely correspond to one of peak heights of lutidine derivative in 3 standard solutions. If sample peak height is greater than that of 160 μg/mL quaternium-15 standard solution, dilute measured aliquot of sample accordingly and note final volume for calculations. If concentration of quaternium-15 is <13 μg/mL, adjust sample weight to fall within range of standards and proceed with dilution to 10–15 mL as above. Under above conditions, retention time of formaldehyde was 14 min and retention time of quaternium-15 was 17 min. Retention time may vary from column to column.

Determination

Operate chromatograph, hot water bath, and fluorometric detector following manufacturers' instructions and conditions outlined under *Apparatus*. Place eluting solvent in pump A and derivatizing reagent in pump B.

When baseline has stabilized, inject two 25 μL aliquots of standard solution into LC valve to rinse and completely fill sample loop. Set attenuation to give maximum peak height. Alternately, inject two 25 mL aliquots of each sample and standard solution into LC system until at least 3 responses for each solution have been obtained.

Figure 1. Representative LC chromatogram of 20 μL injection from 100 ppm quaternium-15 fortified shampoo sample. Peak height of peak B is 50% full-scale deflection at an attenuation setting of 0.01 absorbance unit full scale. Peak A, formaldehyde; peak B, quaternium-15.

Table 1. Recoveries of quaternium-15 added to lotion, body detergent, and mascara[a]

Concn, %	Amt added, mg	Amt recd, mg	Rec., %
	Lotion[b]		
0.01	0.256	0.264	103
	0.256	0.260	102
	0.256	0.252	98
0.1	2.21	2.32	105
	2.21	2.21	100
	2.21	2.21	100
0.2	3.99	4.20	105
	3.99	3.92	98
	3.99	3.85	96
	Body Detergent[c,d]		
0.01	0.232	0.232	100
	0.232	0.232	100
	0.232	0.232	100
0.1	2.22	2.34	105
	2.22	2.25	101
	2.22	2.31	104
0.2	4.29	4.24	99
	4.29	4.07	95
	4.29	4.07	95
	Mascara[e]		
0.01	0.308	0.317	103
	0.308	0.317	103
	0.308	0.293	95
0.1	1.91	1.88	98
	1.91	2.03	106
	1.91	1.81	95
0.2	4.46	4.20	94
	4.83	4.54	94
	4.20	4.08	97

[a]Approximately 2 g sample used. Triplicate determinations.
[b]Average recovery 101%, standard deviation (SD) 3.19, % SD 3.17.
[c]Contained a small amount of formaldehyde.
[d]Average recovery 100%, SD 3.41, % SD 3.41.
[e]Average recovery 98%, SD 4.53, % SD 4.60.

Calculation

For each set of data, measure peak heights of standards and samples and calculate average for each sample and standard. Calculate percentage of quaternium-15 in sample as follows:

$$\% \text{ Quaternium-15} = (PH_x \times C_s \times 100) / (PH_s \times C_x)$$

where PH_x = peak height of sample; C_s = concentration of standard quaternium-15, mg/mL; PH_s = peak height of standard; and C_x = concentration of sample, mg/mL.

Results and Discussion

Using the method described, recovery studies were conducted on a mascara, a lotion, and a body detergent. Commercially available products which did not contain quaternium-15 were used. Quaternium-15 was added at concentrations of 0.01, 0.1, and 0.2%. The overall average recovery of quaternium-15 from the 3 products was 100% (Table 1).

Preliminary investigations indicated that the pellicular reverse phase C_{18} column separates quaternium-15 from formaldehyde (see Figure 1), imidazolidinyl urea, and 2-bromo-2-nitro-1,3-propanediol. However, the latter 3 ingredients coelute, and thus cannot be determined by this method.

Quaternium-15 readily hydrolyzes in the presence of water at room temperature to release formaldehyde. Small amounts of formaldehyde were detected in solutions of quaternium-15 almost immediately after preparation. When a solution containing 0.1 mg quaternium-15/mL was stored at room temperature (27°C) for 2 weeks, almost half of the quaternium-15 decomposed, with the release of formaldehyde. To reduce decomposition, all solutions were stored in the refrigerator, where they were stable for up to 2 days.

Because the formation of the lutidine derivative is a temperature- and time-dependent reaction, certain variables in this procedure, such as the temperature of the hot water bath, the length of the tubing in the mixing chamber, and the rate of flow of solvent in the system, greatly influence the amount of quaternium-15 detected. Under the conditions described in this procedure, the limit of detection is 1 μg/mL (0.001%).

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MEAT, POULTRY, AND MEAT AND POULTRY PRODUCTS

Histologic Detection of Cardiac Musculature, Soy Flour, and Partially Defatted Tissue in Ground Beef: Interlaboratory Study

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A collaborative study was designed and conducted to evaluate the accuracy of a procedure for the histologic detection of cardiac muscle, soy flour, and partially defatted tissue that may occur as adulterants in ground beef. Ground beef samples were prepared containing 0, 3, 5, 10, and 15% of each of the 3 adulterants. Five samples of each composition at each of the 5 dilutions, for a total of 75 unknown samples, were analyzed at each of 5 participating laboratories. The study revealed that this technique is reliable for the detection of these adulterants in ground beef.

Historically, cardiac musculature, soy flour, and partially defatted tissue have been used as normal ingredients in comminuted meat products (when there has been no regulatory prohibition against their use and when the product is properly labeled). The use of these materials, however, does constitute an adulteration if there has been a regulatory prohibition against their use or if they are not properly listed on the ingredient statement. Histologic examination of a comminuted product is a method of detecting the presence of adulterants. A study was designed to evaluate the accuracy of this procedure for the identification of cardiac musculature, soy flour, and partially defatted tissue when used as adulterants in ground beef.

Collaborative Study

Five pathology laboratories participated in the collaborative study. This study was designed so that each of the participating laboratories would receive 75 unknown samples. The samples consisted of 5 samples of 15%, 5 samples of 10%, 5 samples of 5%, 5 samples of 3%, and 5 samples of 0% of each of the 3 adulterants (cardiac musculature, soy flour, and partially defatted tissue). Ground beef with no adulterant (0%) served as the control.

Sample Formulation and Distribution

A total of 441 lb ground beef was ground through a ½ in. breaker plate and stored in meat trucks. Cardiac muscle, soy flour, and partially defatted tissue were individually ground through a ½ in. breaker plate and stored in meat trucks. Each additive was incorporated with ground beef in proper proportions to make 30 lb batches of each percentage level of adulterant (Table 1). Each batch, including the 0% level, was then ground through a ⅛ in. plate. Between grindings, the meat grinder was washed with hot and then cold water to clean and cool the grinder. Each batch was divided into 5 samples (6 lb each) and placed in numbered bags.

Five sub-samples were selected from each of the numbered bags. Each sample consisted of 4 disposable circular tissue cassettes (measuring 38 × 10 mm) filled with sample material. The cassettes were filled with the aid of a spatula, covered, and placed in plastic specimen vials containing 85 mL 10% buffered formalin. Thus, each laboratory received 75 samples as shown in Table 2. Each of the 75 samples was re-coded with a number from a table of random numbers. The samples were mailed to each laboratory sequentially, according to the coded numbers. Ten samples were mailed each week for 8 weeks until all 5 participating laboratories each received all 75 samples.

The formulation and distribution of the samples was done by the Meat Science Research Laboratory, Agricultural Research Service.

METHOD

Apparatus

(a) *Tissue processor.*—Autotechnicon Duo (Technicon Corp., Tarrytown, NY).

(b) *Vacuum infiltrator.*—Tissue-Tek II (Lab-Tek Products Division, Miles Laboratories, Inc., Naperville, IL).

(c) *Tissue embedding center.*—Tissue-Tek II (Lab-Tek Products Division, Miles Laboratories).

(d) *Microtome-"820".*—American Optical Corp., Scientific Instrument Division, Buffalo, NY.

(e) *Tissue floatation bath.*—Water bath (Technicon Corp.).

(f) *Automatic slide stainer.*—Histo-Tek slide stainer (Lab-Tek Products Division, Miles Laboratories).

Sample Preparation

On arrival at each laboratory, the formalin-fixed samples were removed from the cassettes and cut in half. One of the halves was again sectioned to obtain one semicircular specimen from each cassette, making a total of 4 specimens from each sample for microscopical examination. The fixed specimens were dehydrated, cleared with xylene, and infiltrated with paraffin. The specimens were imbedded in paraffin, sectioned at 6 μm, placed on microscopic slides, and stained with haematoxylin and eosin (per standard pathology laboratory procedure). The slides were coverslipped and then examined microscopically for the presence of adulterants.

Identification

Cardiac Musculature

Histologic descriptions of cardiac musculature have been well documented in the literature (1, 2). The histological features which differentiate cardiac musculature from skeletal musculature include the following: *(1)* the presence of centrally placed nuclei in fibers; *(2)* the branching and anastomosing arrangement of the muscle fibers.

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Mention of trade names or commercial products does not constitute endorsement or recommendation by the U.S. Department of Agriculture.

Table 1. Formulation of samples

Ground beef, lb	+	Additive, lb	=	Total, lb	Additive, %
30.0		0		30	0
29.1		0.9		30	3
28.5		1.5		30	5
27.0		3.0		30	10
25.5		4.5		30	15

Table 2. Distribution of treatment numbers within treatments[a]

Additive	0%	3%	5%	10%	15%
Cardiac musculature	1–5	6–10	11–15	16–20	21–25
Soy flour	26–30	31–35	36–40	41–45	46–50
Partially defatted tissue	51–55	56–60	61–65	66–70	71–75

[a]Each of the 5 laboratories received each of the 75 samples. Ground beef (cow—23% fat) was ground through a ½ in. breaker plate to produce 441 lb of course ground beef. Additives were ground separately through the ½ in. (1.27 cm) breaker plate and incorporated in proper proportions, mixed 3 min, and ground through a ⅛ in. (0.32 cm) plate. Samples were re-coded using a table of random numbers and a double blind procedure of distribution.

Soy Flour

The wall of the seed of soya is composed of 4 histologically recognizable layers. The 4 layers, starting from the periphery of the seed toward the center, are as follows (3):

(1) The palisading layer.—The palisading layer is made up of columnar cells measuring approximately 50 × 10 μm. The outer surface of the cell is thickened. The palisading layer is strongly birefringent between crossed polarizers.

(2) The supporting layer.—The supporting layer, depending on the plane of sectioning, appears as hourglass or round, hollow configurations. The configurations are approximately 45–70 μm long and 45 μm wide. The elements of the supporting layer are also birefringent and may take a purplish hue between crossed polarizers. The elements of the supporting layer are often disassociated from their normal location and can be observed singly within the mass of comminuted product.

(3) The parenchymal layer.—The parenchymal layer is ca 6 to 8 cells thick and may appear compressed. It has no significant distinguishing feature.

(4) The endosperm layer.—The endosperm layer appears to be composed of 2 zones: (a) the outer zone is thick walled and, because of its high protein content, stains strongly with eosin. It is made up of a single row of cells. (b) The inner zone is thin walled, appears compressed, and has no significant distinguishing feature.

The 4 layers described above, because of the grinding and subsequent mixing process, may not be observed together.

Partially Defatted Tissue

Partially defatted tissue is a product of the rendering process. The raw material is composed of fat trimmings which are subjected to a temperature of 120°F for ½ h. When the product of this process is mixed with ground beef, the dilution factor eliminates the possibility of detecting its presence by the standard chemical coagulation test.

The histologic features of partially defatted tissue had not been previously documented. A series of steps was taken to determine if the low temperature rendering process to which the tissues were subjected caused alterations that could be consistently recognized. As a first step, specimens of partially defatted tissues were obtained, routinely processed in the pathology laboratory, and examined microscopically. Histologic features were noted and compared with those of unadulterated ground beef. Then, specimens of ground beef mixed with 15 and 25% of partially defatted tissue were examined microscopically to ascertain whether the identifying histologic features were still demonstrable in the mixture. Finally, after determining that the features were still recognizable, a series of unknown specimens was formulated, examined, and successfully identified. The correct identification of the unknowns suggested the possibility that histological examination could be used in the detection of partially defatted tissue in ground beef.

A workshop was organized to acquaint the representatives of the 5 participating laboratories with the identifying characteristics of the adulterants used in the study.

The participants of the workshop concluded that the histologic features of tissues derived from low temperature rendering (partially defatted tissue) include: *(1)* effacement of the normal architecture of the connective tissue, accompanied by a clumping of nuclear material; *(2)* increase in nuclear pyknosis and karyorrhexis, accompanied by a diffuse sprinkling of nuclear fragments in the tissues; *(3)* increase in tissue basophilia.

Results

Cardiac Musculature

All participating laboratories correctly identified the 20 samples that contained cardiac musculature (Table 3).

Soy Flour

All participating laboratories correctly identified the 20 samples that contained soy flour. In one instance, a laboratory identified soy flour in one sample which was supposed to contain no adulterants (0% level control sample) (Table 4).

Partially Defatted Tissue

Four participating laboratories correctly identified the 20 samples that contained partially defatted tissue. The fifth laboratory correctly identified 17 of the 20 samples that contained partially defatted tissue. The 3 samples that were not correctly identified contained partially defatted tissue at the 3% level (Table 5).

One laboratory in 9 instances and another laboratory in one instance reported finding partially defatted tissue in samples that contained no adulterants (0% level).

All 5 participating laboratories were asked to re-examine all slides and indicate the number of positive/questionable slides, and negative slides in each sample.

Re-examination of the positive cases revealed that positive material was detected in all 4 slides in 90% of the cases, in 3 slides in 9% of the cases, but was undetected in 1% of the cases.

Re-examination of the negative cases revealed that in 80% of the cases all 4 slides were negative. In 18.7% of the cases, suspect material was present in only 1 of 4 slides examined and in 1.3% of the cases was present in 2 of 4 slides examined.

Discussion

The false positives reported were no doubt a normal professional response on the part of the examiners. The examiners were all pathologists who have been trained to examine tissue slides and note any deviations from the normal and record these alterations in their description and identification of the disease process(es) involved. Because these were test samples, there was a tendency to note all abnormalities, however slight, and possibly to call the conditions too closely.

Table 3. Collaborative results for cardiac musculature[a]

		0%				3%				5%				10%				15%			
		Slide				Slide				Slide				Slide				Slide			
Lab.	Sample	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1	1	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2	−	−	−	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	3	+	−	−	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	4	−	−	−	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+
	5	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	1	+	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	3	−	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	4	−	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	5	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	1	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	3	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	4	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	5	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	1	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2	−	−	−	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	3	−	−	−	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	4	−	−	−	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	5	−	−	−	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	1	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	3	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	4	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	5	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

[a]After decoding, it was found that some of the questionable material, suggestive of partially defatted tissue, was observed in some of the slides of the 0% level of cardiac musculature.

Table 4. Collaborative results for soy flour

		0%				3%				5%				10%				15%			
		Slide				Slide				Slide				Slide				Slide			
Lab.	Sample	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1	1	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2	−	−	−	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	3	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	4	−	−	−	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	5	−	−	−	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+
2	1	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	3	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	4	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	5	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	1	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	3	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	4	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	5	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	1	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2	−	−	−	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	3	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	4	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	5	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	1	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	3	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	4	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	5	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Instructions to the collaborators should have included guidelines to the effect that more than 50% of the 4 slides should show evidence of the adulterant for a case to be considered positive. Future instructions to examiners will include this guideline.

Inability to detect adulterants at the 3% level was not unexpected. The dynamics of the mixing process and the size of the sample were such that it was possible to take samples which may not have included any of the adulterant.

The results of the study indicate that the histologic examination of comminuted products is a valid method for the detection of cardiac musculature, soy flour, and partially defatted tissue when they are used as adulterants in ground beef at levels as low as 5%.

Table 5. Collaborative results for partially defatted tissue

		0%				3%				5%				10%				15%			
		Slide				Slide				Slide				Slide				Slide			
Lab.	Sample	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1	1	+	−	−	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+
	2	+	−	−	−	+	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+
	3	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	4	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	5	−	−	−	+	+	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+
2	1	−	−	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+
	2	−	+	−	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	3	−	+	−	−	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+
	4	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	5	−	−	−	+	+	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+
3	1	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	3	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	4	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	5	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	1	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
	2	−	−	+	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
	3	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	4	−	−	−	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	5	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
5	1	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	3	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	4	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	5	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

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FOOD ADDITIVES

Rapid Column Method for Determination of *N*-Nitrosodimethylamine in Malt

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A rapid column elution method has been developed for the determination of *N*-nitrosodimethylamine (NDMA) in malt. The average recovery of NDMA added to malt at the 10 ppb level was 87%. When a single malt sample was analyzed 10 times, the NDMA found averaged 8.1 ppb with a coefficient of variation of 3.93%. In a study of 15 malt samples, data obtained by the column elution method were in good agreement with those from a vacuum distillation method.

The occurrence of *N*-nitrosodimethylamine (NDMA), a potent animal carcinogen, in beer and other malt beverages has been the subject of numerous reports (1–7). Malted barley produced by direct-fire kilning is the source of the nitrosamine. A number of analytical methods for determining NDMA in malt have been described, including vacuum distillation (3, 8, 9), Soxhlet extraction (8), aqueous extraction (8, 10, 11), steam distillation (8), enzyme digestion (10), and Preptube extraction (11). Most methods incorporate the use of a thermal energy analyzer, a relatively specific detector for determining nitrosamines.

Recently, we reported the development of a rapid column elution method for the determination of NDMA in beer (12). This method has also been applied to nonfat dry milk (13) and soy protein isolates and concentrates (14) for volatile nitrosamine determination. In this report, we describe the application of the rapid column elution method to malt for the determination of NDMA.

Experimental

Apparatus and Reagents

Apparatus and reagents have been previously described (13). Water and methylene chloride were checked by gas chromatography/thermal energy analysis before use to ensure the absence of interfering peaks. An ammonium sulfamate-sulfuric acid solution was prepared by dissolving 1.0 g ammonium sulfamate in 100 mL 1N H_2SO_4.

Determination

Grind 26 g malt in Sorvall Omni-mixer (or equivalent) for 15 s at highest setting. Weigh 25.0 ± 0.1 g ground malt into tared 600 mL beaker along with 15 g Celite. Mix with spatula until homogeneous. Add *N*-nitrosodipropylamine (NDPA) in ethanol as internal standard and 25 mL ammonium sulfamate–sulfuric acid solution to beaker. Mix until homogeneous.

Place glass wool plug in bottom of chromatographic column (32 mm id × 400 mm) and cover with 20 g anhydrous sodium sulfate. Place tamping rod and powder funnel in column with end of tamping rod extending through funnel. With spatula, load malt-Celite-acid mixture into column. Gently tamp mixture, a little at a time, to 12–13 cm bed depth. Add 100 mL methylene chloride to beaker, stir with spatula, and pour onto chromatographic column before removing tamping rod. Adjust stopcock to obtain flow rate of ca 1–2 mL/min and collect eluate in Kuderna-Danish concentrator with 4 mL concentrator tube. Let column completely drain.

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Add 3 small boiling chips, connect Snyder column, and carefully concentrate methylene chloride eluate to 4 mL in 60°C water bath at rate of 1 mL/min. Let column drain, remove concentrator tube, and further concentrate to 1 mL under gentle stream of nitrogen. (Note: Final concentration to 1 mL under nitrogen for malt extracts containing significant quantities of lipid material is facilitated by immersing bottom of concentrator tube in warm water.) Analyze sample extract, using gas chromatograph coupled to thermal energy analyzer as previously described (13).

Results and Discussion

The method described was tested for nitrosamine artifact formation by spiking a malt-Celite mixture with sodium nitrite and morpholine at the 10 ppm level. Neither nitrosomorpholine nor an enhancement of the previously determined NDMA level was observed.

Recovery studies were conducted on one sample of malt found to contain a low level of NDMA (<0.5 ppb). Ten replicate analyses were performed, each spiked at the 10 ppb level with NDMA. NDPA was also added as an internal standard at the 10 ppb level. The results are shown in Table 1. An average of 87% NDMA was recovered with a coefficient of variation (CV) of 4.68%. Application of the Dixon test (15) showed that the NDMA datum obtained from Sample 1 was an outlier at the 95% confidence level. Excluding Sample 1, the average recovery of NDMA was 86% with a CV of 2.46%. NDPA, the internal standard, was recovered at an average of 93%. The internal standard was used as an indicator of the success or failure of the analysis, and not as a means of correcting the NDMA data for recovery.

The repeatability of the method was determined by analyzing 10 replicates of a single malt sample. NDMA was found at an average level of 8.1 ppb (range 7.6–8.6) with a CV of 3.93%. Recovery of NDPA averaged 92% (range 87–97%) with a CV of 3.50%. The method had a detection limit of 0.1 ppb.

A study was conducted to compare the column method with a vacuum distillation method used in previous studies (3). Fifteen malt samples known to contain a range of NDMA levels were analyzed by both methods. The results obtained both before and after correction for the average NDMA

Table 1. Recovery (%) of NDMA and NDPA from malt spiked at 10 ppb

Sample	NDMA	NDPA
1	97	98
2	89	97
3	84	92
4	85	92
5	82	90
6	87	93
7	86	94
8	88	91
9	86	93
10	85	93
Range	82–97	90–98
$\bar{X}$	87 (86)[a]	93
CV, %	4.68 (2.46)[a]	2.68

[a]Results with Sample 1 omitted.

recovery of each method are shown in Table 2. The data from the 2 methods were in good agreement with the exception of sample 14. We believe a sampling error must have been made in this sample.

The column method has several advantages over other methods used for malt analysis. In addition to providing good precision and recovery, the method is rapid and requires little operator attention. Many analyses can be performed in a single day, while a minimal amount of glassware and equipment is used. The malt extract obtained by this method is, however, unsuitable for confirmation of the nitrosamine by mass spectrometry. Those samples requiring confirmation may be analyzed by methods previously published (3).

Attempts to reduce NDMA levels in malt by adding sulfur dioxide during the drying process have been relatively successful (16, 17). The precursors of NDMA in malt have also been investigated (18). Despite considerable progress in these areas, low levels of NDMA are still present in malt, so that frequent monitoring will be required. The column method described here would be useful for this purpose as it provides for precision, good recovery, and rapid sample analysis.

Table 2. Determination of NDMA (ppb) in malt samples, by using vacuum distillation and column methods

Sample	Vac. distn	Column	Vac. distn[a]	Column[a]
1	12.9	16.0	14.3	18.6
2	6.6	6.5	7.3	7.6
3	6.7	6.8	7.4	7.9
4	1.2	1.4	1.3	1.6
5	2.8	3.4	3.1	4.0
6	2.9	3.2	3.2	3.7
7	24.4	32.9	27.1	38.3
8	7.5	8.9	8.3	10.3
9	0.2	0.2	0.2	0.2
10	13.6	15.7	15.1	18.3
11	7.4	8.7	8.2	10.1
12	4.8	5.4	5.3	6.3
13	10.6	12.5	11.8	14.5
14	105.1	144.9	116.8	168.5
15	7.7	9.1	8.6	10.6

[a]Corrected for average NDMA recovery.

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SUGARS AND SUGAR PRODUCTS

Mass Spectrometric Determination of Cane Sugar and Corn Syrup in Maple Syrup by Use of $^{13}C/^{12}C$ Ratio: Collaborative Study

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Use of C_3 carbon dioxide (CO_2) fixation cycle by the sugar maple (*Acer saccharum* Marsh.), a dicotyledenous plant, and C_4 carbon fixation cycle by 2 monocotyledenous plants, corn and cane, results in a physiological discrimination between ^{13}C and ^{12}C isotopes. Therefore, determination of $^{13}C/^{12}C$ ratio of maple syrup by mass spectrometry can be used to detect adulteration with cane and corn sugars. Four samples of pure maple syrup and 3 adulterated maple syrup samples were analyzed in a collaborative study. Results indicate that stable carbon isotope analysis can determine authenticity of maple products. Samples with $\delta^{13}C$ values less negative than $-23.49‰$ (parts per thousand) can, with a high degree of confidence (95%), be classified as adulterated. The method has been adopted official first action.

Maple syrup, the heat-concentrated product of pure xylem sap of sugar maple (*Acer saccharum* Marsh.), has become more expensive because of rising fuel and labor costs. Economic incentives exist to adulterate pure maple products by adding cane sugar to boiling sap, or by blending the syrup with corn syrup. Adulterated maple syrup is sold as pure maple syrup, misleading consumers and competing with pure maple syrup. Current methods of analysis (1) are inadequate to detect the adulteration of maple products with sugars. The $^{13}C/^{12}C$ isotopic composition of corn and cane plants is distinctly different from that of maple trees (2). Hence, analysis of carbon isotopic ratio could detect adulteration of maple products with corn syrup or cane sugar.

The $^{13}C/^{12}C$ isotopic composition of plants can be divided into 3 categories, depending on which pathway of photosynthetic carbon dioxide fixation the plant uses. All plants derive carbon from the isotopically homogeneous composition of atmospheric CO_2; then enzymatic reactions introduce isotopic fractionation. The 3 photosynthetic cycles are termed the Calvin cycle, Crassulacean acid metabolism, and the Hatch-Slack cycle.

The Calvin cycle is the most frequently used by dicotyledenous plants and produces synthesis products depleted in the heavier ^{13}C isotope (3). This cycle involves the fixation of CO_2 present as HCO_3^- by the acceptor, ribulose-1,5-diphosphate. A 6C intermediate is formed and decomposes into 2 molecules of 3-phosphoglyceric acid. The 3-phosphoglyceric acid is reduced to 3-phosphoglyceraldehyde by ATP and NADPH and H^+ formed in primary processes (4). 3-Phosphoglyceraldehyde exists in equilibrium with its isomer, dihydroxy acetone phosphate (4). Collectively, these 2 compounds are termed triose phosphates, hence the cycle is called C_3.

The Hatch-Slack C_4 cycle occurs in monocotyledenous plants such as sugar cane and corn and less frequently than the C_3 Calvin cycle (5). In the first carboxylation reaction, phosphoenolpyruvic acid acts as an acceptor for CO_2 from HCO_3^-, and is converted to oxaloacetic acid by phosphoenolpyruvate carboxylase (4). The oxaloacetic acid is reduced by malate dehydrogenase to malic acid, with NADPH and H^+ supplying the hydrogen (4). All the above reactions occur in the leaf mesophyll cells. The malic acid is translocated to the bundle sheath cells where it is degraded to CO_2 and pyruvic acid, which simultaneously results in the formation of NADPH and H^+ (4). The released CO_2 is bound to ribulose 1,5-diphosphate and is used for synthesis of hexose phosphate in the Calvin cycle (4). The pyruvic acid is returned to the mesophyll cells where it is converted to phosphoenolpyruvate with consumption of an ATP molecule (4). The role of oxaloacetic and malic acids lends the Hatch-Slack cycle its C_4 name.

The Crassulacean acid metabolism cycle of certain succulent and semisucculent plants leads to isotopic compositions with values intermediate between the Calvin and Hatch-Slack cycles (6). Calvin C_3 plants have $\delta^{13}C$ values of -24 to $-34‰$, and Hatch-Slack plants from -7 to $-19‰$ (2). Crassulacean values range from -14 to $-31‰$ (6).

Carro et al. tested the $^{13}C/^{12}C$ isotopic composition of hundreds of maple syrups from different provenances (7). They found that $\delta^{13}C$ values of pure maple syrup range from -22.37 to $-24.81‰$ with slight differences within provenances; values of cane sugar range from -10.50 to $-12.20‰$ and values of corn syrup from -10.40 to $-12.40‰$ (7). They concluded that the isotopic difference between the products is significant and detects adulteration of maple syrup at the 12% level. This method of isotopic analysis has been introduced to officially detect high fructose corn syrup adulteration of apple juice and honey (8, 9), and is also used to diagnose the origin of secondary products of carbohydrate fermentation, such as alcohol and vinegar (10).

This paper reports the results of a collaborative study to detect adulteration of maple syrup with cane and corn sugars.

Collaborative Study

Materials

All samples were prepared in the Maple Research Laboratory, Botany Department, Vermont Agricultural Experiment Station, University of Vermont. Adulteration of pure maple syrup with 20% corn syrup and cane sugar was chosen as the lowest threshold because Carro et al. calculated a 12% detection level (7). Seven samples were sent to each of 6 collaborating laboratories in Canada, France, and the United States. The identities of the syrup samples were as follows:

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The recommendation of the Associate Referee was approved by the General Referee and Committee D and was adopted by the Association. See the General Referee and Committee reports, *J. Assoc. Off. Anal. Chem.* (1984) 67, March issue.
This report of the Associate Referee, M. F. Morselli, was presented at the 96th Annual International Meeting of the AOAC, Oct. 25–28, 1982, at Washington, DC.

1, Grade A—light amber with 20% cane sugar (added to sap before processing); 2, Grade A—light amber, pure; 3, Grade A—medium amber blended with 20% light corn syrup; 4, GradeA—medium amber, pure; 5, Grade A—medium amber blended with 20% dark corn syrup; 6, Grade A—dark amber, pure; 7, Grade A—medium amber, pure, identical to Sample 4. All maple syrups were certified pure by their producers. All samples were hot-packed (82°C) into 1 oz glass bottles. Collaborators were given instructions for correct storage conditions.

Sample Combustion and Determination of $\delta^{13}C$‰

Various methods of combustion of syrup to CO_2 are used before isotopic determination with mass spectrometers. Combustion method, instrument, and working standards used by the collaborators are given in Table 1. Working standards vary in each laboratory, but in all cases $\delta^{13}C$‰ values are reported relative to CO_2 generated from the reference standard Pee Dee belemnite (PDB).

The sample is completely combusted to CO_2 and H_2O. Combustion products are frozen in liquid nitrogen and distilled through a Dry Ice trap to remove water, and the purified CO_2 is collected in a second liquid nitrogen trap. The ratio of $^{13}C/^{12}C$ in the carbon dioxide is determined by mass spectrometry. The overall error is ±0.15‰.

Corn Syrup and Cane Sugar in Maple Syrup Carbon Ratio Mass Spectrometric Method First Action

Method Performance

Maple syrup pure (100%) av. $\delta^{13}C = -24.21$‰
Maple syrup 80%, cane syrup 20%: av. $\delta^{13}C = -21.12$‰
Maple syrup 80%, corn syrup 20%: av. $\delta^{13}C = -22.35$‰
(S_x = 0.63–1.52, S_o = 0.13–1.52)

Principle

Sample is burned completely to CO_2 and H_2O; CO_2 is purified, and $^{13}C/^{12}C$ ratio is measured in isotope ratio mass spectrometer. Differences in $^{13}C/^{12}C$ values for maple syrup (av. $\delta^{13}C = -24.21$‰), corn syrup (av. $\delta^{13}C = -11.29$‰) and cane sugar (av. $\delta^{13}C = -11.85$‰) provide measure of corn syrup and cane sugar added to maple syrup.

Apparatus

(a) *Combustion system.*—Vac.-tight glass manifold including quartz combustion furnace, liq. N trap, automatic Toepler pump, and high-vac. source. CuO may be used to aid combustion.

(b) *Purification system.*—Glass manifold interconnected with combustion system including trap, sample collection tube, and manometer [see *Geochim. Cosmochim. Acta* **3**, 54–55(1953)].

(c) *Mass spectrometer.*—Micromass 602, 602D, 903, or 602C (Kearns Group, 58 Buckingham Dr, Stamford, CT 06902), or equiv. instrument designed or modified for isotope ratio measurement and capable of accuracy of 0.01% of abundance at mass 45.

Preparation of Sample

Place 20–50 mg sample in Pt crucible, position sample in tube or furnace, and evacuate system. Admit to 600 mm Hg; tank O may be purified over CuO followed by liq. N trap. Heat sample to appropriate temp. (~1200° without CuO) in manifold in furnace, condensing CO_2 in liq. N trap. A 2- to 4-h combination time may be used; no recirculation is necessary. Isolate collection trap and purification system from combustion system and pump by valves, and pump off O. Cool purification trap with solid CO_2–acetone; cool sample tube with liq. N. Permit collection trap to warm, condensing impurities in solid CO_2 trap and CO_2 in sample tube.

Alternative Preparation of Sample

Place 5–10 mg sample (may be dried in vac.) in pyrex tube ⅜ in. diam. Add ~2 g ACS grade wire-form CuO. Evacuate tube to fore pump pressure and seal tube with oxy-methane flame. Let sealed tube react 2–4 h at 550°. Cool tube to room temp., crack tube on vac. line, and pump off noncondensable gas. Freeze contents in liq. N trap. Liq. N trap is replaced with dry ice–acetone slush to retain H_2O while transferring CO_2 to mass spectrometer.

Determination

Operate mass spectrometer according to manufacturer's instructions. Calibrate with ≥2 stds such as NBS SRM 20 Solenhofen limestone ($\delta^{13}C = -1.06$‰ against Pee Dee belemnite (PDB)), NBS SRM 21 graphite ($\delta^{13}C = -27.8$‰), or NBS SRM 22 crude oil ($\delta^{13}C = -29.5$‰). Correct values obtained for zero enrichment in inlet system, for mixing between sampling and std valves, for tailing of major on to minor peak signal, and for contribution of ^{17}O to mass 45 signal. Calcn:

$$\delta^{13}C(‰) = \left[\frac{^{13}C/^{12}C \text{ sample}}{^{13}C/^{12}C \text{ std}} - 1\right] \times 1000$$

Convert laboratory analyses, relative to std used, to PDB base by following this relationship:

$$\delta(X-\text{PDB}) = \delta(X-B) + \delta(B-\text{PDB}) + 10^{-3}\delta(X-B) \times \delta(B-\text{PDB})$$

where $\delta(X-B)$ and $\delta(X-\text{PDB})$ refer to analyses of sample (X) relative to std (B) and relative to PDB, and $\delta(B-\text{PDB})$ is analysis of std (B) relative to PDB, all δ values in parts per thousand. Sample with $\delta^{13}C$ value less negative than −23.49‰ relative to PDB suggests adulteration.

Results

Results of the collaborative study are compiled in Table 2. Because of high technical error, the means of the replicates

Table 1. Instruments and conditions used by collaborators

Coll.	Instrument	Combustion method	Reaction time	Working std	Reproducibility	Anal. error
1	Micromass 602	550°C w/CuO	4 h comb. time	NBS 22	± 0.15‰	± 0.05‰
2	VG Micromass 602D	550°C w/CuO	2 h comb. time	CO_2 from calcite w/phosphoric acid calib. to NBS 20	± 0.10‰	± 0.15‰
3	VG Micromass 602D	1200°C	none	marble	± 0.10‰	± 0.10‰
4	Micromass 602C	1200°C in pure O_2	none	int. carbonate std calib. w/PDB[a]	± 0.10‰	± 0.10‰
5	Micromass 903	1200°C in pure O_2	none	ultra pure tank – CO_2 calib. w/V-SMOW + NBS[b] stds	± 0.15/mL	± 0.10/mL
6	Micromass 602	1500°C w/CuO	none	oxalic acid and bender limestone	± 0.15‰	± 0.05‰

[a]PDB = Pee Dee belemnite:carbonate from the fossil skeleton of *Belemnitella americana* from the Pee Dee formation of South Carolina.
[b]NBS = National Bureau of Standards reference material.

Table 2. $\delta^{13}C$ (‰ vs. PDB) values for samples

Coll.	Samples 1	2	3	4	5	6	7
1	−20.17	−23.17	−22.19	−23.75	−21.64	−24.03	−23.53
	−20.31	−23.19	−21.98	−23.52	−21.80	−24.02	−23.50
2	−20.89	−23.79	−22.20	−23.76	−22.20	−24.37	−23.87
	−20.80	−23.63	−22.22	−23.66	−22.13	−23.37	−23.74
3	−21.43	−24.00	−22.51	−24.26	−22.61	−24.85	−24.03
				−24.21			
4	−22.45	−24.72	−22.55	−24.24	−22.74	−24.60	−25.50
	−22.24	−24.23	−22.64	−24.87		−24.85	−25.71
	−22.58	−24.30	−22.98	−24.51		−25.14	−25.96
						−25.45	
5	−21.60	−24.40	−22.90	−24.30	−22.70	−25.00	−24.30
	−21.40		−22.70		−23.00		−24.30
	−21.50						
6	−17.00	−23.90	−23.20	−23.40	−21.50	−24.10	−23.20
	−18.60	−23.90	−21.60	−24.30	−21.30	−27.50	−23.60
	−21.90	−22.70	−21.70	−26.30	−21.30	−23.90	−21.80
	−21.00	−25.30	−21.90			−21.70	−24.80
	−22.20		−20.80			−24.50	−24.00
	−20.90					−26.80	
	−20.50						
	−20.30						
Mean (of lab. means)	−21.12[d]	−23.94[b]	−22.36[c]	−24.18[ab]	−22.24[c]	−24.58[a]	−24.14[ab]

Means followed by the same letter are not significantly different at the 5% level of Duncan's multiple range test.

for each collaborator were used in all calculations. Analysis of variance and Duncan's multiple range test indicate that the $^{13}C/^{12}C$ ratios of adulterated syrup samples are statistically different ($P<0.05$) from the ratios of pure maple syrup samples (Table 3). Cane sugar-adulterated samples are also significantly different from corn syrup-adulterated maple syrups ($P<0.05$). The mean of the pure maple syrup δ‰ values is -24.21 ± 0.27. At the 95% confidence limit, we can assume that δ‰ values less negative than −23.49 indicate adulteration; at the 97.5% confidence limit that value improves to −23.38, at 99% to −23.26, and at 99.5% to −23.17.

We conclude that adulteration of maple syrup with 20% or more corn and cane products can be detected by $^{13}C/^{12}C$ isotopic analysis.

Recommendation

On the basis of the results reported here, carbon isotopic determination provides a method for detecting maple syrup adulteration with corn and cane sugars. Note that the limits were derived from a mean of 6 collaborators. Individuals may give higher or lower values than those reported here. Therefore, we recommend that at least one certified pure maple syrup sample be analyzed at the same time as the suspect syrup. The Associate Referee (Maria Franca Morselli) recommends that the $^{13}C/^{12}C$ mass spectrometric method be adopted official first action for detecting corn and cane products in maple syrup.

Table 3. Measures of precision in determination of isotopic ratio (x − 1)

Average[a]	Sample	Repeatability SD	Repeatability CV_o,%	Reproducibility SD	Reproducibility CV_x,%
20.94	1	1.27	6.06	1.38	6.57
23.94	2	0.71	2.97	0.71	2.97
22.27	3	0.59	2.66	0.63	2.81
24.24	4	0.81	3.36	0.81	3.36
22.08	5	0.13	0.60	0.65	2.95
24.64	6	1.52	6.18	1.52	6.18
24.12	7	0.75	3.10	1.11	4.60
23.72	7A[b]	0.84	3.54	0.84	3.54
24.18	4 + 7	0.81	3.36	0.93	3.87

Units are dimensionless × 1000.
[a]Of all individual values.
[b]Lab. omitted as Dixon outlier ($P = 0.05$).

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MYCOTOXINS

Analyst Performance with Aflatoxin Methods as Determined from AOCS Smalley Check Sample Program: Short-Term and Long-Term Views

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The International Smalley Aflatoxin Check Sample Program of the American Oil Chemists' Society has offered check sample series for aflatoxins in peanut meal, cottonseed meal, and corn meal since 1976, and an aflatoxin M in raw milk series since 1980. This paper provides the computed mean of all analysts' results and between-laboratory precision for each of the samples in each of the check sample series distributed in 1980–81 and 1981–82. In addition, a comparison is made of the relative measurement and analytical accuracy of those analysts who have participated in the peanut meal series for at least 4 years and in the cottonseed and corn meal series since their inception (6 years). For this comparison, each analyst's result for each sample was calculated as a percent of the mean for all analysts for that sample; these values were then averaged for each analyst over all the meal samples in all the series for each meal type in which the analyst had participated, to obtain an overall measure of analytical accuracy. A similar calculation was made using the reported results for the defined solution of aflatoxins included in each series, to obtain an overall measure of measurement accuracy. An evaluation of the meal series results for the past 2 seasons shows an overall within-laboratory precision in the range reported for the collaborative studies by which the methods were validated; the between-laboratory precision, although improved over past years, is still far from the collaborative study range. The precision data for the aflatoxin solution included in each series indicate this bias could be related, in large part, to the reference standards used. The extended period evaluation of analysts' performance shows no apparent correlation between measurement and analytical accuracy except for a general positive trend for those analysts using the BF method for aflatoxins in peanut meal. A comparison of the accuracy of the BF and CB methods for aflatoxins in peanut meal shows no significant difference between results by the 2 methods on the basis of the extended period evaluation, in contrast to the generally higher results for the CB method in the past 2 seasons' evaluation. The scatter in the average analysts' analytical accuracy is essentially the same, regardless of the method used.

The International Smalley Aflatoxin Check Sample Program of the American Oil Chemists' Society (AOCS) has offered check sample series for aflatoxins in peanut meal, cottonseed meal, and corn meal since 1976, and an aflatoxin M in raw milk series since 1980. Currently, each annual series consists of 7 individual samples provided singly to analysts at monthly intervals. Samples 1 and 3 of each series are specified as duplicates. Sample 2 of each meal series consists of an ampule of a benzene–acetonitrile (98 + 2) solution of the 4 aflatoxins B_1, B_2, G_1, and G_2, and in the milk series, a benzene–acetonitrile (90 + 10) solution of aflatoxin M_1, each solution to be used for direct spotting and quantitation. Comparison of each analyst's results with the true aflatoxin content of the solution provides information on standards quality and quantitation techniques, for correction and application to subsequent samples. Unknown to analysts, 2 of the remaining samples each season are duplicates. Results for these replicate samples provide insight into within-laboratory performance.

Analytical reports are edited to exclude from evaluation nonofficial or improper methods. Accepted results are processed to compute arithmetic mean, standard deviation, and coefficient of variation for each sample in a series with all results outside ±3 standard deviations excluded from the final evaluation This paper provides the computed statistics for each of the aflatoxin check sample series distributed in 1980–81 and 1981–82.

Because a considerable number of laboratories have participated in each of the aflatoxin check sample series for a number of years, it is now possible to study analyst performance over an extended period of time and numbers of samples. For this purpose, a statistic termed "C-value" has been used as a measure of analyst accuracy. The C-value for a sample result is calculated by dividing that analytical result by the mean of all accepted values for that sample and multiplying by 100 to obtain a value that expresses the analyst result as a percentage of the consensus value. For example: If a sample mean value for aflatoxin B_1 is 30 ng/g, and an analyst result is 15 ng/g, the C-value is 50 [(15/30) × 100]. A C-value of 100 represents a result equal to the mean of all accepted analyses for a sample.

While conventional measures of bias based on the variance associated with each set of analyses are influenced by changes in analysts' performance from one sample to another (1), the C-value is unaffected by between-laboratory precision factors; however, in the absence of a true value for the denominator, the unknown deviation of the consensus from the true value does introduce an unknown variable. Aggregate C-values may be treated by accepted statistical methods to obtain measures of precision for both repeatability (within-laboratory precision) and reproducibility (between-laboratory precision).

All analytical results for aflatoxin B_1 in meals and the solutions of aflatoxins (Sample 2) were transformed to C-values for each of the selected analysts. For C-values of the aflatoxin solution samples, the value used for the denominator was the true aflatoxin B_1 content of the sample solution rather than the mean of all analytical values, although in all cases the rounded values were equal. Mean C-values and resultant coefficients of variation for meals and working standard samples were calculated for each analyst. For comparative purposes, an additional transformation was made by subtracting 100 from the mean C-value of each analyst. The result of this transformation is to produce a value that directly expresses analyst average percent deviation from the mean, regardless of direction.

An analyst's C-value for each working standard sample, where only quantitation of a solution of pure aflatoxins is required, presents a best measure of that analyst's accuracy in measurement, while C-values for meal samples, where extraction, extract purification, and quantitation are required is a measure of that analyst's proficiency in analysis.

Results and Discussion

Evaluation of 1981–82 Analytical Results for Aflatoxins

The 1980–81 and 1981–82 sample series evaluations of aflatoxins in the 3 meal and the raw milk sample series are presented in Tables 1–4. All series continue to show high between-laboratory coefficients of variation similar to but

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Table 1. Evaluation of participant analyses for aflatoxins in peanut meal series

Sample	N[a]	Mean, ng/g				Std dev., ng/g				Coeff. of var., %			
		B_1	B_2	G_1	G_2	B_1	B_2	G_1	G_2	B_1	B_2	G_1	G_2
1980-1 Season													
1	61	29	5	6	2	17	3	6	3	58	55	96	130
2	53	(0.83	0.36	0.77	0.33)[b]	0.30	0.15	0.27	0.12	36	41	35	36
3	58	30	5	6	2	16	2	5	2	55	43	74	90
4	51	19	3	5	1	9	2	4	1	49	64	93	113
5[c]	52	23	4	5	1	14	2	4	2	59	60	74	122
6	46	25	4	3	2	13	2	3	2	52	53	70	130
7[c]	48	23	4	5	1	12	2	4	2	53	54	78	129
1981-2 Season													
1	58	116	22	18	3	65	14	12	2	56	64	64	58
2	52	(0.74	0.39	0.29	0.20)[b]	0.26	0.17	0.09	0.08	35	43	30	39
3	51	115	20	17	4	57	10	9	3	50	49	51	85
4	56	44	6	23	4	24	4	11	2	53	62	48	63
5[c]	48	37	6	17	3	20	5	12	2	54	76	66	82
6[c]	54	38	5	15	2	25	3	7	1	66	61	48	56
7	51	88	10	49	7	47	5	23	4	53	43	48	59

[a] Number of participant results

[b] Values for a working solution of the aflatoxin (μg/mL) for direct spotting

[c] Blind duplicate samples

Table 2. Evaluation of participant analyses for aflatoxins in cottonseed meal series

Sample	N[a]	Mean, ng/g				Std dev., ng/g				Coeff. of var., %			
		B_1	B_2	G_1	G_2	B_1	B_2	G_1	G_2	B_1	B_2	G_1	G_2
1980-1 Season													
1	29	33	7			17	5			51	69		
2	23	(0.95	0.38	0.77	0.35)[b]	0.37	0.15	0.30	0.19	39	39	39	56
3	26	33	8			11	3			34	42		
4	27	5	1			7	2			138	171		
5[c]	25	48	11			20	6			42	54		
6	26	111	27			41	11			37	43		
7[c]	24	47	10			19	5			40	53		
1981-2 Season													
1	29	16	3			7	3			48	69		
2	25	(0.72	0.35	0.29	0.18)[b]	0.32	0.18	0.12	0.10	44	51	43	55
3	24	14	3			6	2			46	62		
4[c]	24	52	12			27	7			52	59		
5	25	61	15			31	8			51	50		
6[c]	27	53	14			26	7			50	47		
7	26	88	10			47	5			53	43		

See Table 1 for footnote explanation

Table 3. Evaluation of participant analyses for aflatoxins in corn meal series

Sample	N[a]	Mean, ng/g				Std dev., ng/g				Coeff. of var., %			
		B_1	B_2	G_1	G_2	B_1	B_2	G_1	G_2	B_1	B_2	G_1	G_2
1980-1 Season													
1	37	33	7			24	5			73	68		
2	26	(0.86	0.36	0.79	0.31)[b]	0.36	0.14	0.23	0.11	42	39	29	35
3	29	31	6			14	3			45	53		
4	29	8	1			4	1			55	114		
5[c]	33	37	4			23	3			63	69		
6[c]	33	34	3			18	3			53	91		
7	28	40	5			20	5			50	95		
1981-2 Season													
1	37	5	1			4	1			64	189		
2	38	(0.80	0.41	0.33	0.22)[b]	0.23	0.16	0.11	0.10	29	38	33	44
3	35	5	1			2	1			45	162		
4[c]	37	16	2			9	1			55	65		
5[c]	33	15	1			9	1			58	99		
6	37	13	2			7	1			55	62		
7	35	10	2			5	1			44	83		

See Table 1 for footnote explanation

Table 4. Evaluation of participant analyses for aflatoxin M_1 in raw milk series

Sample	N[a]	Mean, μg/L	Std dev., μg/L	Coeff. of var., %
		M_1	M_1	M_1
1980-1 Season				
1	11	0.20	0.26	134
2	13	(0.42)[b]	0.28	67
3	12	0.14	0.21	149
4[c]	10	0.16	0.19	120
5[c]	10	0.17	0.28	165
6	9	0.05	0.07	153
7	11	0.20	0.26	132
1981-2 Season				
1	12	0.12	0.14	117
2	13	(0.98)[b]	0.32	33
3	9	0.06	0.06	91
4[c]	12	0.15	0.15	103
5[c]	10	0.15	0.12	78
6	11	0.13	0.09	66
7	8	0.09	0.10	119

See Table 1 for footnote explanation

generally lower than those reported in the evaluations of the previous 5 seasons of the Smalley Aflatoxin Series (2) and in the results of the International Aflatoxin Check Sample Program (3, 4) conducted by the International Agency for Research on Cancer. In both programs, analytical results for deoiled peanut and corn meal samples have between-laboratory coefficients of variation around 50% for aflatoxin B_1 at a level of approximately 40 ng/g.

The Smalley formula for rejection of results outside ±3 standard deviations of the mean value should theoretically accept 99.7% of analysts' results, assuming a normal distribution of values. For the past 2-season evaluation, 2.0, 0.3, and 2.5% of results from the peanut, cottonseed, and corn meal series, respectively, were rejected.

Although they have not been classified as outliers, the false negative, or no-detectable aflatoxin results from the past 2-season series were 0.1, 3.1, and 2.9% of all reported results for the peanut, cottonseed, and corn meals, respectively.

Evaluations of analyses for aflatoxin M_1 in milk (Table 4) reveal large but improved between-laboratory coefficients of variation for all samples. The pooled value for all milk samples was 143% for the 1980–81 series and 98% for the 1981–82 series. The improvement in milk sample analysis appears closely related to improvement in analysis of the aflatoxin M_1 solution (Sample 2). These results may not be of great significance as an indication of analyst performance since few analysts reported results by using an officially adopted method.

Table 5 is an evaluation of results from analysts using liquid chromatographic methods during the 1981–82 season; there were 9 analysts for the peanut, 6 for the cottonseed, and 5 for the corn series of samples. The mean of values for LC for peanut and cottonseed meal, respectively, were 15% and 11% greater for aflatoxin B_1 and 27% and 33% greater for aflatoxin B_2 than the mean values by all other methods. Means for aflatoxins G_1 and G_2 in peanut meal were essentially the same for values by LC and by all other methods. Means of values by LC in the corn meal series were essentially the same as the means of values by the other methods.

Results for aflatoxin B_1 in peanut meal by the BF and CB methods from analysts participating in the 1980–81 and 1981–82 series are compared in Table 6. The mean aflatoxin B_1 values for the 2 seasons were 34% higher for analysts using the CB method than for analysts using the BF method, while the between-laboratory coefficients of variation were approximately the same for results by either method. These comparisons agree closely with those from previous Smalley Check Sample Series for Aflatoxin Analysis (2) as well as those from the International Aflatoxin Check Sample Program (3, 4).

Results for the blind duplicates in each of the meal series for 1980–81 and 1981–82 are presented in Table 7. The pooled within-laboratory coefficient of variation (repeatability) of 27% compares favorably with the repeatability values found in collaborative studies of the methods used. This is in contrast to the large between-laboratory coefficients of variation (reproducibility) in relation to those found in the collaborative studies (about 50% vs about 30%), indicating a major between-laboratory bias component. The precision data for the aflatoxin solution (Sample 2) in each series indicate this bias could be related, in large part, to the reference standards used.

Table 6. Evaluation of participant analyses for aflatoxin B_1 (ng/g) in peanut meal by BF and CB methods

Sample	N[a]	BF method Mean	BF method Std dev.	BF method Coeff. of var., %	CB method Mean	CB method Std dev.	CB method Coeff. of var., %
			1980-1 Season				
1	26/15	23	12	51	37	18	49
3	20/16	22	9	42	42	21	50
4	19/11	12	6	45	19	9	47
5[b]	22/15	17	10	61	31	15	50
6	21/16	20	15	71	30	14	46
7[b]	24/14	18	10	54	33	12	36
			1981-2 Season				
1	25/13	109	64	59	127	63	50
3	21/11	98	39	40	114	62	55
4	26/15	38	14	37	63	20	32
5[b]	21/13	27	13	46	46	25	54
6[b]	22/14	28	14	49	41	25	60
7	23/13	79	35	44	114	61	54

[a] First number denotes number of participant analyses, BF method second number denotes number of participant analyses, CB method

[b] Duplicate samples, unknown to participants

Table 5. Evaluation of participant analyses for aflatoxins in 1981-2 series samples by LC methods

Sample	N[a]	Mean, ng/g B_1	B_2	G_1	G_2	Std dev., ng/g B_1	B_2	G_1	G_2	Coeff. of var., % B_1	B_2	G_1	G_2
						Peanut meal							
1	9	141	23	17	4	79	14	10	4	56	59	58	97
2	8	(0.81	0.44	0.30	0.22)[b]	0.27	0.17	0.08	0.09	33	39	28	40
3	9	105	21	18	4	65	12	10	2	62	58	53	52
4	9	54	7	27	4	24	3	9	2	45	48	33	43
5[c]	9	44	5	18	3	15	2	7	1	35	42	41	39
6[c]	9	39	6	17	2	21	4	13	1	53	70	79	43
7	9	124	14	54	8	35	4	17	3	28	30	33	40
						Cottonseed meal							
1	5	16	5			5	1			31	19		
2	6	(0.87	0.53	0.32	0.27)[b]	0.36	0.10	0.13	0.07	41	20	42	26
3	5	16	5			5	1			31	19		
4[c]	5	130	25			70	8			54	32		
5	6	69	15			31	6			45	42		
6[c]	5	44	11			21	6			46	57		
7	5	110	23			44	9			40	42		
						Corn meal							
1	4	6	0			1	0			19			
2	5	(0.70	0.49	0.32	0.29)[b]	0.30	0.11	0.10	0.62	48	24	36	21
3	5	6	0			2	1			37	224		
4[c]	5	21	2			5	1			23	50		
5[c]	4	14	1			5	1			32	48		
6	5	11	1			3	0			23	40		
7	5	11	1			1	0			12	40		

See Table 1 for footnote explanations

Table 7. Evaluation of laboratory performance with analyses of aflatoxin B_1 (ng/g) in unknown duplicate samples

	Peanut meal		Cottonseed meal		Corn meal	
	1980-1 Season	1981-2 Season	1980-1 Season	1981-2 Season	1980-1 Season	1981-2 Season
N[a]	43	42	24	22	25	27
Mean[b]	25	34	47	50	36	17
S_r	5.1	11.2	10.8	13.4	11.8	3.8
S_b	11.3	12.5	13.4	16.1	15.7	5.7
Coeff. of var. within-labs,%	21	33	23	27	33	23
Coeff. of var.[c] between-labs,%	50	49	36	42	55	41

[a] Number of participants who ran duplicate determinations

[b] Average of duplicate determinations

[c] Includes both S_r and S_b components

Extended Period Evaluation

The C-100 values were calculated for each analysis by laboratories that had participated in the peanut meal series for at least 4 years, in the cottonseed meal series for 6 years, and in the corn meal series for 6 years. These values were averaged over the total time frame for each laboratory in each series and the variability of the C-100 values for each laboratory in each series was calculated as a coefficient of variation. These data are presented in Table 8 for the peanut meal series, in Table 9 for the cottonseed meal series, and in Table 10 for the corn meal series.

On the assumption that C-100 values for the standard solutions (Sample 2) are a best criterion of analyst accuracy of measurement, tables of the means of analysts' C-100 values for each series report these values in descending order of analyst accuracy in measurement with equal weight given to deviations above or below the true standard sample concentration.

The average C-100 values for all analysts in each series were also determined as well as the averages for all analysts with C-100 values that were ≤ 10 for the standards solutions and again for all analysts with C-100 values that were ≤ 20.

The 23 analysts who used the BF method for peanut meal show a general correlation between C-100 values for the standards and for the meals, which is evident when the data are presented in a scatter plot (Figure 1). This correlation is not evident for those analysts who used the CB method for peanut meal (Figure 1) or for those analysts who participated in the cottonseed meal series (Figure 2) or the corn meal series (Figure 3). Neither is there any apparent correlation between the mean accuracy in measurement of analysts (C-100 for standards) and the scatter of each analysts' profi-

Table 8. Mean C-100 values for aflatoxin B_1 determined by participants in the peanut meal series 1977-82 and coefficients of variation of these values for each participant from 23 laboratories using the BF method and 12 laboratories using the CB method

BF method					CB method				
No. of analyses		Mean C-100		CV of C-100	No. of analyses		Mean C-100		CV of C-100
Stds	Samples	Stds (A)	Samples	Samples	Stds	Samples	Stds (A)	Samples	Samples
5	35	-1	39	38	5	35	2	3	47
5	35	1	5	61	5	35	-2	-16	35
4	24	-3	-3	25	5	35	2	21	24
5	35	4	-9	43	5	35	6	6	36
5	35	6	-21	48	5	35	8	9	46
4	24	7	-37	52	5	35	9	11	37
5	35	10	-4	54	5	35	15	5	23
5	35	12	2	43	5	35	16	31	26
5	35	-14	-21	46	5	35	17	-24	49
5	35	16	-18	53	5	35	-17	-41	101
5	35	17	24	27	5	35	18	72[a]	33
5	35	-19	-18	69	4	28	42	17	28
5	35	19	-7	47					
4	24	20	7	30					
4	24	20	22	35					
4	24	22	0	41					
5	35	25	4	25					
5	35	37	-1	37					
5	35	-40	-22	35					
4	24	44	-8	41					
5	35	49	-11	28					
5	35	51	13	36					
5	35	65	20	35					
All analysts		15	-2	41			9	2	41
A $\leq \pm$ 10[b]			-4					6	
A $\leq \pm$ 20[b]			-4					0.5	

[a] Classified as an outlier

[b] All analysts with mean C-100 for standards at or less than the designated value

Table 9. Mean C-100 values for aflatoxin B_1 determined by participants in the cottonseed meal series 1977-82 and the coefficients of variation of these values for each analyst of 21 laboratories using the Pons method

No. of analyses		Mean C-100		CV of C-100
Stds	Samples	Stds (A)	Samples	Samples
5	36	0	6	31
5	36	0	-28	46
5	36	0	20	54
5	36	3	-11	55
5	36	4	1	49
5	36	-5	1	44
5	36	7	13	31
5	36	-7	-8	60
5	36	8	26	29
5	36	-9	-9	43
5	36	10	6	18
5	36	10	-1	39
5	36	11	-23	60
5	36	-12	-8	49
5	36	15	15	29
5	36	17	19	50
5	36	18	11	31
5	36	19	6	31
5	36	-29	4	23
5	36	-29	-43	70
5	36	47	50	37
All analysts		4	2	44
$A \leq \pm 10$[a]			1	
$A \leq \pm 20$[a]			2	

[a] All analysts with mean C-100 values for standards at or less than the designated value

Table 10. Mean C-100 values for aflatoxin B_1 determined by analysts in the corn meal series 1977-82 and the coefficients of variation for each analyst of 24 laboratories using the AOAC and AACC method

No. of analyses		Mean C-100		CV of C-100
Stds	Samples	Stds (A)	Samples	Samples
5	36	2	4	39
5	36	-3	13	21
5	36	3	31	27
5	36	3	20	40
5	36	-6	1	33
5	36	8	-4	22
5	36	-8	19	25
5	36	8	4	33
5	36	8	13	37
5	36	-9	-19	47
5	36	9	32	49
5	36	10	-6	31
5	36	14	22	34
5	36	-17	-32	53
5	36	19	31	34
5	36	21	4	25
5	36	21	30	30
5	36	-21	23	30
5	36	27	5	49
5	36	-35	-36	46
5	36	35	-2	57
5	36	37	44[a]	66
5	36	40	72[a]	47
5	36	-41	-50	65
All analysts		5	6	41
$A \leq \pm 10$[b]			9	
$A \leq \pm 20$[b]			9	

[a] Classified as an outlier

[b] All analysts with mean C-100 values for standards at or less than the designated value

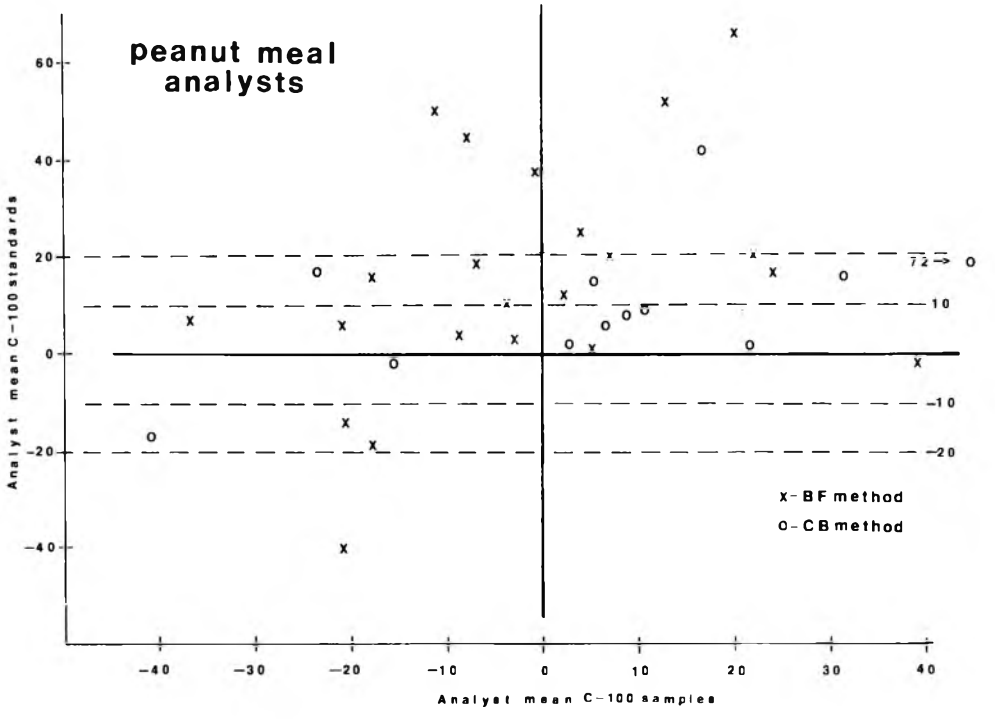

Figure 1. Scatter plot of peanut meal analysts' mean C-100 values for standards and meal samples, where analyst direction and magnitude of deviation from zero on vertical axis reflects accuracy in measurement, while direction and magnitude of deviation from zero on horizontal axis reflects accuracy of analysis.

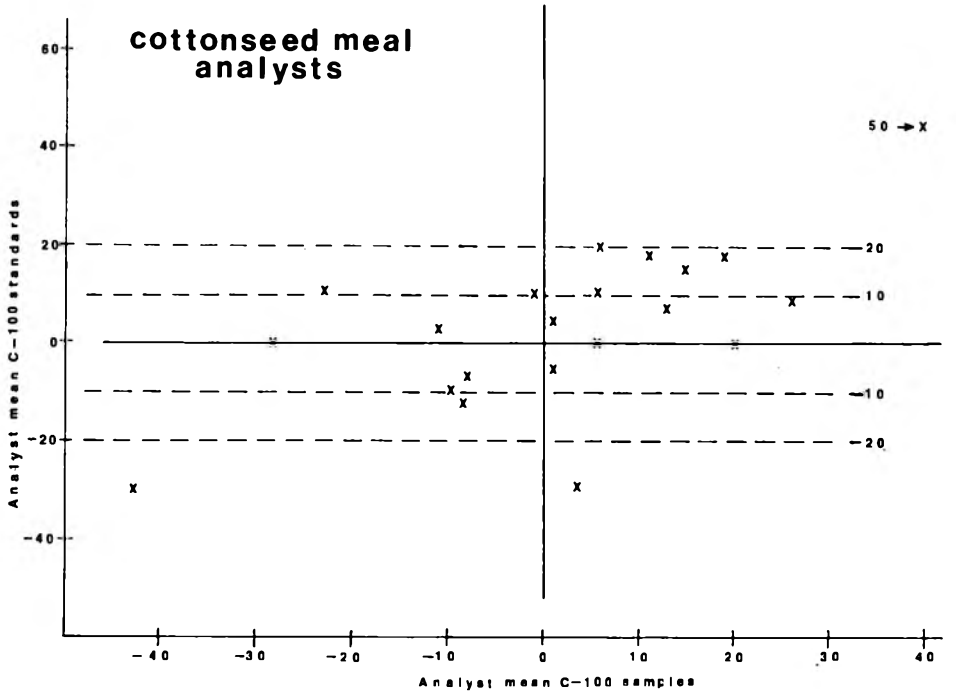

Figure 2. Scatter plot of cottonseed meal analysts' mean C-100 values for standards and meal samples, where analyst direction and magnitude of deviation from zero on vertical axis reflects accuracy in measurement, while direction and magnitude of deviation from zero on horizontal axis reflects accuracy of analysis.

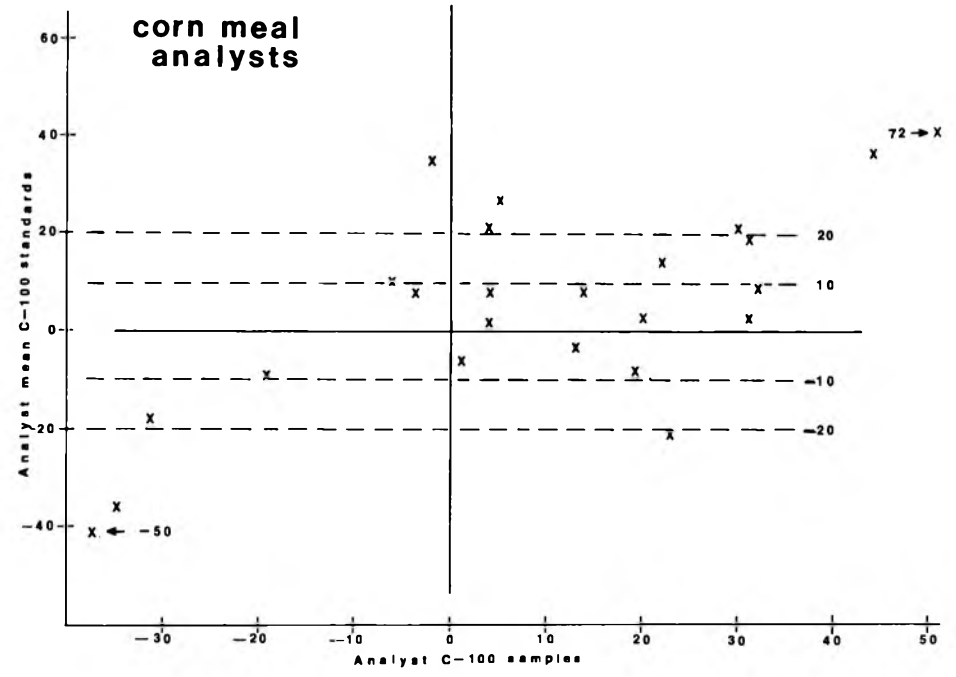

Figure 3. Scatter plot of corn meal analysts' mean C-100 values for standards and meal samples, where analyst direction and magnitude of deviation from zero on vertical axis reflects accuracy in measurement, while direction and magnitude of deviation from zero on horizontal axis reflects accuracy of analysis.

ciency with the total method (CV of C-100 for meals). Although the laboratories using the BF method for peanut meal averaged slightly lower in their aflatoxin B_1 results than did those laboratories using the CB method, the difference is not significant, considering the magnitude of the coefficients of variation for all analysts for these analyses; nor does the difference between laboratories using the 2 methods show any relation to the accuracy in measurement of the 2 groups (Figure 1).

An interesting coincidence is the scatter of the average analysts' proficiency with each of the total methods; these coefficients of variation for all analysts are in the tight range of 41–45%.

A summary is given in Table 11 of repeatability and reproducibility calculated from the blind duplicate sample results submitted for each of the 3 meal series for the 6 years of the series. The data in Table 7 for the 1980–81 and 1981–82 seasons compared with the equivalent data in Table 11 show

Table 11. Repeatability (within-laboratory coefficient of variation) and reproducibility (between-laboratory coefficient of variation, includes all error components) of analysts results of data accumulated over 6 years with blind duplicate samples in the 3 meal series

Meal series	Mean B_1 (ng/g)	B_1 range (ng/g)	Repeatability (R_o)	Reproducibility (R_x)	R_o/R_x
Peanut	36	17-38	41	70	0.59
Cottonseed	42	29-122	42	66	0.64
Corn	28	8-36	39	67	0.58

a marked improvement in both between- and within-laboratory precision. As previously noted, repeatability has improved to a level expected from collaborative studies of the methods used; improvement in reproducibility is more than the within-laboratory component, indicating improvement in the pure between-laboratory component also, but based on the collaborative studies of the methods, improvement in between-laboratory precision is possible.

Aflatoxin methods are sometimes deceptively simple, but their application may be difficult, as with other methods of analysis for trace organic compounds. Such analyses require constant, diligent effort to maintain quality of laboratory environment, purity and calibration of standards, purity of reagents, and control of each step of the methodology. Care must be devoted to all details of the analysis to minimize errors that can have escalating effects on final results of analyses for aflatoxins at the required ng/g levels.

Acknowledgments

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Preparation of Deoxynivalenol (Vomitoxin) from Field-Inoculated Corn

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A process was developed for production of gram quantities of deoxynivalenol (DON) from corn that had been inoculated in the field with *Fusarium graminearum* and was estimated to contain 400–500 mg DON/kg. Steps in the purification procedure included extraction with methanol–water (1 + 1), partition from an aqueous solution into ethyl acetate by using a hydrophilic matrix, defatting, Florisil column chromatography, methylene chloride–water partition, semipreparative liquid chromatography (LC), and crystallization from ethyl acetate. The average yield of crystalline DON produced by this method (or with minor variations) was 281 mg/kg.

Deoxynivalenol (DON), also known as vomitoxin, is a major emetic and refusal factor in swine exposed to *Fusarium*-infected corn (1, 2). There is now considerable evidence for the widespread natural occurrence of DON in grains in many parts of the world, including North America (1–4). To aid evaluation of the safety of DON-contaminated foods and feeds, it is necessary to isolate and purify gram quantities of DON for toxicological testing. The primary requirement is source material containing a high concentration of DON. Laboratory cultures of *Fusarium graminearum* grown on corn or rice have been estimated to contain up to 396 mg/kg but any yields on liquid media were low (5, 6). Several earlier reports describing isolation of DON included preparative thin layer chromatography (TLC) (6–9), but this technique does not readily lend itself to large scale purification. Recently, preparative or semipreparative liquid chromatography (LC) has been used (5, 10) but, again, quantities of DON processed were low and in one case (10) no indication was given that crystalline toxin was obtained. Supplies of highly DON-contaminated corn that had been inoculated in the field with *F. graminearum* were fortunately available to us. By including semipreparative LC in our method, we have obtained gram quantities of crystalline DON. The method developed for this purification is given below.

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Experimental

Extraction

Three hundred g ground corn was blended in a 3.8 L capacity Waring blender for 2 min (medium speed) with 1200 mL methanol–water (1 + 1). After filtration under reduced pressure through a bed of Hyflo-Super Cel (Fisher Scientific Co.), the filter cake was again blended for 2 min with 1200 mL methanol–water (1 + 1) and refiltered. The filter cake was rinsed with 150 mL methanol–water (1 + 1). Approximately 1 mL Antifoam A (Dow Corning) was added to the combined extracts, which were then evaporated to ca 200 mL on a rotary evaporator. The aqueous extract was added to a 300 mL capacity Chem Tube (Analytichem International, Harbor City, CA 90710) containing an inert hydrophilic matrix, and gentle suction was applied via a Buchner flask to absorb the

extract. The tube was eluted with suction by eight 100 mL portions of ethyl acetate. Alternatively, the aqueous extract could be extracted 4 times in a separatory funnel with 200 mL ethyl acetate; emulsions were centrifuged at 1000 rpm for 5 min. Combined ethyl acetate extracts from the separatory funnel were dried over 50 g anhydrous granular sodium sulfate and decanted, and the sodium sulfate was rinsed with 50 mL ethyl acetate. The ethyl acetate solution obtained by either procedure was evaporated to dryness under reduced pressure.

Column Chromatography

The extract residue was redissolved in 60 mL acetonitrile and defatted twice with 60 mL *n*-hexane, and the acetonitrile was evaporated under reduced pressure. The residue obtained from 1850–1875 g corn was dissolved in about 10 mL methylene chloride–methanol (95 + 5) and added to a 7.5 cm id column containing 200 g Florisil (60–100 U.S. mesh, Floridin Co., Berkeley Springs, WV 25411; Lot No. 079), slurry-packed in methylene chloride–methanol (97.5 + 2.5). The column was eluted with methylene chloride–methanol (97.5 + 2.5) and 20 mL fractions were collected using a fraction collector. Every 5th tube and subsequently all tubes at the beginning and end of the vomitoxin elution zone were analyzed for vomitoxin by TLC. (Generally vomitoxin was found between tubes 40 and 100.) TLC was carried out by spotting column fractions and standard (500 μg/mL in chloroform) on E. Merck silica gel 60 precoated 20 × 20 cm TLC sheets, then developing with chloroform–acetone (3 + 2). Vomitoxin was visualized by heating the TLC sheets on a hot plate after dipping in 15% sulfuric acid.

Additional Purification

Following evaporation of the methylene chloride–methanol (97.5 + 2.5) eluate, the residue (ca 3 g) was dissolved in ca 0.5 mL methylene chloride in a 50 mL round-bottom flask, transferred to a 4 dram Teflon-lined screw cap vial, and extracted 3 times with 10 mL distilled, deionized water by rolling the vial gently for 2 min. The combined water extracts were evaporated to dryness under reduced pressure in a 250 mL round-bottom flask; the resulting syrup was transferred in methanol to a 50 mL round-bottom flask and again evaporated. The residue was dissolved in 3–5 mL ethyl acetate and filtered using a 10 mL Luer tip glass syringe with a filter holder containing a LSWP 01300 Millipore filter. The syringe was rinsed with 0.5 mL ethyl acetate, the rinse was added to the filtrate, and the solution was evaporated under reduced pressure. The residue was dissolved in 5 mL water for semipreparative LC.

Semipreparative LC

The following apparatus and conditions were used: Altex Model 110A pump, Rheodyne Model 7125 injector with 1000 μL loop, Lobar pre-packed column (size B, 310 × 25 mm id) containing EM LiChroprep RP-8 (40–63 μm) (BDH Chemicals Ltd), Varian refractive index detector (range setting 128), and Perkin-Elmer recorder; the mobile phase was water–methanol (1 + 1), previously filtered and degassed under reduced pressure; flow rate was 4.5 mL/min at 90 psi. Crude DON (200–300 mg in 1 mL water) was injected onto the column and the eluant corresponding to the one observed peak (Figure 1) was collected in a 1 L round-bottom flask, evaporated to dryness under reduced pressure, transferred to a 50 mL round-bottom flask with methanol, and again evaporated. Between LC runs, the column was washed with

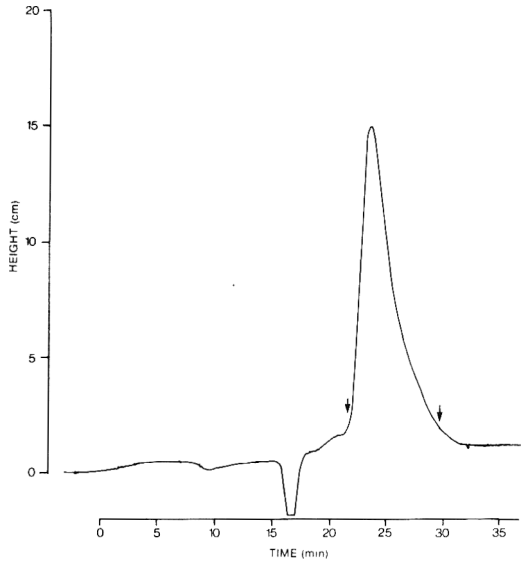

Figure 1. Semipreparative LC of DON. For conditions, see *Experimental*. Amount of crude DON injected was 230 mg. Arrows mark start and finish of collection.

300 mL prefiltered methanol at a flow rate of 3 mL/min, then re-equilibrated with 300 mL mobile phase.

Crystallization

After LC purification, the DON residue was dissolved in a minimum volume of ethyl acetate, seeded with a few DON crystals, and left overnight with a loose cover of Al foil over the flask. The resulting crystalline mass was broken up in 0.5–1 mL methylene chloride, drawn up in a Pasteur pipet cut at the constriction, and filtered with suction using a small, coarse porosity, sintered glass funnel. The crystals were redissolved in ethyl acetate and filtered to remove insoluble material, and the solution was evaporated. Crystals were obtained as before and dried overnight in a vacuum desiccator. Some samples were dried further at 100°C under reduced pressure to determine if previous drying was adequate.

Methylene chloride washings used to filter crystalline DON were saved in a separate 250 mL round-bottom flask and, when sufficient material had been collected, it was purified by LC and crystallization as above, starting with the ethyl acetate syringe filtration (under *Additional Purification*).

Analytical LC

Purity of crystalline DON was determined by LC using a Waters Associates Model 6000A pump, a Waters M 441 UV detector (229 nm filter), a 25 cm × 4.6 mm id stainless steel column packed with Spheri-5 RP-8 5 μm (Brownlee Laboratories, Inc., Santa Clara, CA 95050), and a 10 mV recorder. The mobile phase was filtered, degassed water–methanol (9 + 1) (10). Operating conditions were: flow rate 1.5 mL/min, pressure 3000 psi, chart speed 0.25 cm/min, and sensitivity 0.01 AUFS. Five μL of a 100 μg DON/mL solution in mobile phase was injected and peak heights were determined in duplicate.

Spectroscopy

The IR spectrum of DON was recorded as a Nujol mull, using a Perkin-Elmer 257 spectrophotometer, the UV spectrum in methanol on a Beckman Model 25 instrument, the mass spectrum at 70 eV with a VG Micromass ZAB-2F mass spectrometer, and the ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra in $CDCl_3$ (plus a drop of CD_3OD) on a Bruker WM-250 NMR spectrometer operating at 250 MHz.

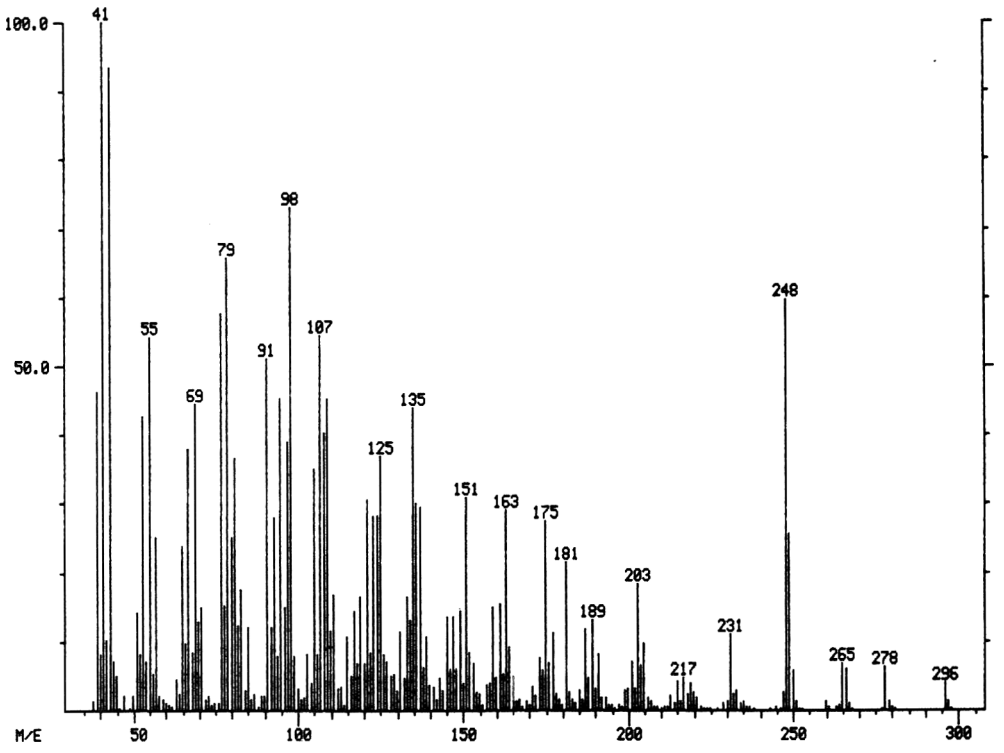

Figure 2. Electron impact mass spectrum of vomitoxin.

Results and Discussion

The method described above for preparation of DON was developed using field-inoculated corn obtained from Agriculture Canada and the University of Guelph. The latter corn was a ground sample of approximately 22 kg that we estimated to contain 473 mg/kg following TLC analysis of the defatted extracts (in acetonitrile) from 11 batches of this corn (1384–2225 g). This estimate was confirmed, albeit on only 50 g analytical sample, by gas chromatography with electron capture detection (3, 11) to be 454 mg/kg. Yields of crystalline DON obtained in 5 of 7 batches of the Guelph corn (1850–1875 g) processed by the method as described averaged 141 mg/kg (range 102–179 mg/kg); however, these do not include material purified from collected methylene chloride washings, which added to yields of crystals from the other 2 batches. The total yield of crystalline DON obtained from 22 kg corn, including material processed previously by slight variations in the method (no second crystallization) and from methylene chloride washings, was 6.15 g (281 mg/kg average). Loss in weight was negligible after heating the crystals at 100°C under reduced pressure.

The melting point of DON preparations ranged from 149–150.5°C to 153–154°C and for DON processed directly (without inclusion of material from methylene chloride washings) was at least 151–152.5°C.

The IR spectrum of DON showed bands at 3450, 3370, and 1680 cm^{-1} and the UV spectrum (0.032 mg/mL in methanol) had an absorption maximum at 216 nm (ϵ 6384). The electron impact mass spectrum (Figure 2) was closely comparable to previously published spectra (8, 12). The ^{1}H and ^{13}C NMR spectra were completely consistent with the DON structure; assignments were made by multiplicity sorting (2D-NMR).

Analytical LC was carried out on all crystalline DON preparations to determine purity. DON showed a single peak at a retention time of 10.8 min. Eleven samples prepared using the above method had purities averaging 95% based on the purest sample obtained (mp 153–154°C). There was, however, no general correlation between melting point and LC purity.

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Effects of Methanol Concentration and Solvent:Peanut Ratio on Extraction of Aflatoxin from Raw Peanuts

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Aflatoxin B_1 was extracted by a water slurry process using methanol concentrations of 55, 60, 65, and 70% in water and solvent:peanut ratios of 3, 4, 5, and 6 mL/g. Results failed to show that methanol concentration had an effect on amount of B_1 extracted; however, the amount of B_1 extracted increased with an increase in solvent:peanut ratio. Aflatoxin B_1 was also extracted by the official AOAC method II, using methanol concentrations of 55, 60, 65, and 70% in water and solvent:peanut ratios of 2, 3, 4, and 5 mL/g. Results showed that the amount of B_1 extracted increased with percent methanol at low solvent:peanut ratios but not at high ratios. Also, the amount of B_1 extracted increased with solvent:peanut ratios at all methanol concentrations.

A methanol–water solution is used to extract aflatoxin from peanuts according to the official AOAC Method II (BF) (1). The Agricultural Marketing Service (AMS) of the U.S. Department of Agriculture uses a modified version of the BF method (2). A water slurry (WS) method that was developed to reduce the amount of solvent required for aflatoxin analysis of peanuts also uses a methanol–water extraction solution (3). The percentage by volume of methanol in the methanol–water extraction solvent specified by the BF, AMS, and WS methods are 55, 55, and 71%, respectively. The ratios of solvent (methanol–water) volume to peanut weight specified by the BF, AMS, and WS methods are 5.0, 2.7, and 4.7 mL/g, respectively. Because efficient extraction of aflatoxin from the peanut sample is imperative for accurate analysis, more information is needed about effects of concentration of methanol and the solvent:peanut ratio on aflatoxin extraction efficiency.

Although it is desirable to increase aflatoxin extraction efficiency, extraction of other compounds that interfere with the analysis should be kept as low as possible. For example, the volume of residue remaining in the analytical portion of the extract after evaporation of chloroform causes error in the dilution factor used to compute the amount of aflatoxin in the portion (4). In addition, some extracted compounds may interfere with quantitation of aflatoxin on thin layer chromatographic (TLC) plates.

The objective of this study was to determine the effects of methanol concentration in the extraction solvent and the methanol–water solvent:peanut ratio on amount of aflatoxin extracted within the BF and WS methods. The effects of these treatments on the volume of other compounds extracted by the BF method were also determined.

Experimental

About 25 kg raw peanuts naturally contaminated with aflatoxin were comminuted in a mill similar to that used in AMS laboratories (5). Aflatoxin was extracted from samples of the comminuted peanuts by either the WS or BF method.

WS Method.—Eleven hundred g peanuts were blended with 1600 mL (1600 g) water for 3 min. The water blend (slurry) was divided into 12 samples weighing 175 g each. Each sample contained ca 101 mL (101 g) water and ca 74 g peanuts. Various volumes of methanol and water were added to each of the 12 samples of water slurry to achieve 12 different combinations of 4 concentrations of methanol in water (55, 60, 65, and 70% methanol) and 4 methanol–water solvent:peanut ratios (3, 4, 5, and 6 mL/g). Four other combinations (3 mL/g solvent:peanut ratio in combination with 60, 65, and 70% methanol concentration and 4 mL/g solvent:peanut ratio in combination with a 70% methanol concentration) were not possible because the samples of slurry contained too much water. Eighty mL hexane was added to each sample of slurry and blended 1 min.

The BF method was used for the remainder of the analytical procedure except that the methanol–water extract from the centrifuge bottle was filtered through coarse paper to remove peanut particles and oil. Approximately 75 mL methanol–water extract was placed on the filter, and the 50 mL analytical portion of the filtrate was removed before all of the extract passed through the filter. During filtration, most of the oil in the extract rose to the top of the extract retained by the filter and was discarded after the 50 mL analytical portion was filtered. To make more accurate comparisons of amounts of aflatoxin extracted by each treatment, extracts from all 12 samples were spotted on each of 4 TLC plates, and all spots were quantitated densitometrically (6, 7). The above test was replicated 5 times with five 1100 g samples.

BF Method.—Two thousand g peanuts were blended with 3000 mL (1986 g) hexane for 3 min. The hexane blend (slurry) was divided into sixteen 200 g samples. Each sample contained about 100 g peanuts and about 151 mL (100 g) hexane. Various volumes of methanol and water were added to each of the 16 samples of hexane slurry to achieve 16 different combinations of 4 concentrations of methanol in water (55, 60, 65, and 70% methanol) and 4 methanol–water solvent:peanut ratios (2, 3, 4, and 5 mL/g). Forty-nine mL hexane was then added to each sample and blended for 1 min.

The hexane blend was used in this procedure to reduce the variability in aflatoxin concentration among the sixteen 200 g samples. Waltking has used this procedure to reduce the subsampling error for chunk-style peanut butter (8). The 200 g hexane blend (100 g peanuts and 151 mL hexane) plus 500 mL of 55% methanol in water and 49 mL hexane is considered to be equivalent to the standard BF extraction method for raw peanuts, which uses 100 g raw peanuts, 500 mL of 55% methanol in water, and 200 mL hexane. The BF method was used for the remainder of the analytical procedure. Extracts from all 16 samples were quantitated on TLC plates as previously described for the WS method. The above test for the BF method was replicated 10 times.

The weight of oily residue remaining in the analytical portion of the extract after evaporation of the chloroform was determined by weighing each vial containing the residue and reweighing the vial after washing with chloroform. Weights were measured to the nearest 0.1 mg. The volume of the residue was computed by dividing the weight of residue by the density of the residue (0.938 g/mL) determined in a previous study (4). The volume of oily residue after evaporation of the chloroform was not determined for the WS method because previous studies (unpublished) have indicated that

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filtration of the methanol–water extract from the centrifuge bottle reduces the amount of oily residue for the WS method by approximately 81%.

Results and Discussion

Table 1 gives the average ng aflatoxin B_1/g peanut material extracted with each of the 12 treatments for the WS method. Each value in the table is the average of 20 observations (4 plates per replication × 5 replications). The data indicate that the amount of aflatoxin B_1 extracted is proportional to the solvent:peanut ratio. There appears to be no measurable effect of methanol concentration on the amount of B_1 extracted. An analysis of variance of the data shows that there was a strong solvent:peanut ratio effect ($F = 6.6$, probability $> F = 0.003$) and no detectable methanol concentration effect ($F = 1.7$, probability $> F = 0.200$) on the amount of aflatoxin B_1 extracted.

Table 2 gives the average ng aflatoxin B_1/g peanut material extracted by each of the 16 treatments for the BF method. Each value in the table is the average of 40 observations (4 plates per replication × 10 replications). The data indicate that the amount of aflatoxin B_1 extracted is proportional to the solvent:peanut ratio. For low solvent:peanut ratios, the amount of B_1 extracted generally increased as the methanol concentration increased, but the trend does not appear to exist for high solvent:peanut ratios. An analysis of variance of the data indicates a strong solvent:peanut ratio effect ($F = 24.4$, probability $> F = 0.0001$) and a weaker but significant methanol concentration effect ($F = 3.2$, probability $> F = 0.0278$) on amount of aflatoxin B_1 extracted. There was no measurable interaction between methanol concentrations and solvent:peanut ratios ($F = 0.9$, probability $> F = 0.565$).

The average volume of residue per 10 g peanuts extracted by the BF procedure for each of the 16 treatments is shown in Table 3. Each value in the table is the average of 10 replications. The data indicate that the amount of residue is directly proportional to the solvent:peanut ratio and to the methanol concentration of the extraction solvent. An analysis of variance shows that there is both a solvent:peanut ratio effect ($F = 24.4$, probability $> F = 0.0001$) and a methanol concentration effect ($F = 3.2$, probability $> F = 0.002$) on the volume of residue.

Results indicate that the methanol concentration used in the WS method can be reduced from 71 to 55% without reducing the amount of aflatoxin extracted. A solvent:peanut ratio higher than the presently specified ratios of 5:1 for the BF method and 4.7:1 for the WS method may be desirable. Further research is needed to determine the optimum ratio. Although a higher solvent:peanut ratio would increase the volume of residue extracted, previous studies (unpublished) indicate that filtration of the methanol–water extract after centrifugation removes most of the residue. We recommend this filtration step for the BF method and the WS method.

Table 1. Average ng aflatoxin B_1/g peanuts extracted with WS method for 12 combinations of methanol concentrations and solvent:peanut ratios

Solvent: peanuts, mL/g	% Methanol in methanol–water solvent			
	55%	60%	65%	70%
3	41.8	—	—	—
4	47.4	45.7	49.4	—
5	47.8	47.6	48.0	45.8
6	52.7	55.0	53.1	48.0

Table 2. Average ng aflatoxin B_1/g peanuts extracted with BF method for 16 combinations of methanol concentrations and solvent:peanut ratios

Solvent: peanuts, mL/g	% Methanol in methanol–water solvent				
	55%	60%	65%	70%	Av.
2	30.9	34.7	32.3	37.1	33.8
3	35.3	39.1	35.0	39.2	37.2
5	39.3[a]	40.8	41.0	40.8	40.5
6	42.1	42.7	43.1	42.0	42.5
Av.	36.9	39.0	37.9	39.8	

[a]Treatment which used 5 mL/g solvent:peanut ratio and 55% methanol–water solvent is equivalent to standard BF method.

Table 3. Average μL residue/10 g peanuts extracted with BF method for 16 combinations of methanol concentrations and solvent:peanut ratios

Solvent: peanuts, mL/g	% Methanol in methanol–water solvent				
	55%	60%	65%	70%	Av.
2	12.2	16.5	15.8	20.1	16.2
3	17.9	22.2	23.1	22.4	21.4
4	27.3	28.8	32.8	39.5	32.1
5	31.5	38.0	40.4	42.0	38.0
Av.	22.2	26.4	28.0	31.0	

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Fluorometric Screening Method for Citrinin in Corn, Barley, and Peanuts

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A fluorometric method is described for rapid screening of citrinin in corn, barley, and peanuts. The initial extraction uses an aqueous acidified acetonitrile solution, and the resulting extract is partially purified by several acid–base partition steps. Citrinin is determined by using a fluorometer. Mean recoveries of added citrinin from corn, barley, and peanuts were 56.4, 66.6, and 40.0%, respectively, at levels of 100–8000 ng/g.

Citrinin is a secondary metabolite of several *Penicillium* and *Aspergillus* species. Krogh (1) listed 14 *Penicillium* and 3 *Aspergillus* species capable of producing citrinin. Citrinin was first isolated from *P. citrinum* (2) and found to possess antibacterial activity (3). The toxic properties of citrinin precluded its use as a therapeutic drug, and it is now regarded as a potentially important mycotoxin that may be a contaminant of animal or human food (1).

Citrinin alone does not seem to be a carcinogen but causes kidney damage (4). A positive test was obtained when citrinin was tested for DNA-attacking ability in the rec assay using *Bacillus subtilis* (5). Synergistic effects of citrinin in combination with other nephrotoxic chemicals have been reported. In combination with *N*-nitrosodimethylamine (DMN), kidney tumors increased from 57% to about 90% when citrinin was administered after DMN to rats (4). *N*-(3,5-Dichlorophenyl) succinimide (NDPS) alone does not cause kidney tumors but treatment with citrinin after NDPS caused kidney tumors in 4 of 18 rats (4). Paired combinations of citrinin–ochratoxin A and citrinin–penicillic acid elicited synergistic lethal responses in test animals (6, 7). Citrinin and ochratoxin are frequently produced by *P. viridicatum* in moldy wheat and barley (1). The porcine nephropathy associated with moldy feed possibly results from synergistic combination of several nephrotoxic mycotoxins (1).

Citrinin, ranging from 0.07 to 80 mg/kg, has been found as a natural contaminant of wheat, rye, oats, barley (1, 8), and in trace amounts in apples (9). Several methods for determining citrinin in corn and barley have been published (8, 10–14). The method of Marti et al. (14) is a modification of Scott's 2A method (8) to improve the resolution of citrinin from interferences in liquid chromatography (LC) and thin layer chromatography (TLC).

Analysis for citrinin is difficult because it is not efficiently extracted and is thermally unstable. The modifications in our extraction method are the use of Na_2EDTA to help improve the recovery by taking advantage of the chelating properties of citrinin, and the use of a more concentrated KCl solution to avoid altering the extraction solution volume. This method was developed to provide a rapid screening nonchromatographic detection method.

METHOD

Apparatus and Reagents

(a) *Spectrofluorometer.*—Varian Model 330 with detector (Varian Associates, Houston, TX).

(b) *Shaker.*—Wrist-action (Burrell Corp., Pittsburgh, PA).

(c) *Solvents.*—Acetonitrile, ACS grade; 2,2,4-trimethylpentane, ACS grade; chloroform.

(d) *KCl solution.*—8% w/v.

(e) *Na_2EDTA solution.*—10% w/v.

(f) *$NaHCO_3$ solution.*—5% w/v.

(g) *Sulfuric acid.*—20% v/v.

(h) *Hydrochloric acid.*—12.1N.

Procedure

Weigh 25 g sample, ground to pass 20 mesh screen, into 500 mL glass-stopper Erlenmeyer flask. Add 180 mL acetonitrile, 10 mL 8% KCl solution, 10 mL 10% Na_2EDTA solution, and 2 mL 20% H_2SO_4. Secure stopper with masking tape and shake 15 min on wrist-action shaker. Filter through fluted paper and collect at least 100 mL filtrate.

Measure 100 mL filtrate into 250 mL separatory funnel, add 50 mL 2,2,4-trimethylpentane (isooctane), and shake vigorously ca 1 min. Let phases separate, and drain lower phase into clean 250 mL separatory funnel. Discard isooctane and rinse funnel with $CHCl_3$. Repeat with additional 50 mL isooctane, discard isooctane, and rinse funnel for use below. To extracts in separatory funnel, add 25 mL water and 50 mL $CHCl_3$. Shake gently ca 1 min and let phases separate. Drain lower $CHCl_3$ phase into rinsed funnel. Re-extract aqueous phase twice with 10 mL $CHCl_3$ and combine $CHCl_3$ extracts. Discard aqueous phase, rinse funnel with $CHCl_3$, and reserve for $NaHCO_3$ extraction. To combined $CHCl_3$ extracts, add 25 mL 5% $NaHCO_3$ solution. Shake gently ca 1 min and let phases separate. Drain lower $CHCl_3$ phase into rinsed funnel, and collect aqueous phase into 400 mL glass beaker. Re-extract $CHCl_3$ phase twice with 25 mL 5% $NaHCO_3$ solution and combine aqueous phases in 400 mL beaker. Discard $CHCl_3$ phase, rinse funnel with $CHCl_3$, and reserve for use below. Gradually acidify aqueous phase in 400 mL beaker to pH 1–2 with approximately 4 mL concentrated HCl. Transfer to rinsed 250 mL separatory funnel; rinse beaker with 50 mL $CHCl_3$, pour rinse into separatory funnel, and swirl funnel gently ca 30 s. Let phases separate and drain lower $CHCl_3$ phase into 125 mL Erlenmeyer flask. Repeat with 50 mL $CHCl_3$. Discard aqueous phase; combine $CHCl_3$ extracts into 125 mL Erlenmeyer flask and reserve for fluorometric analysis. For 25 g sample, 1 mL $CHCl_3$ extract represents 0.125 g of sample.

Transfer 3 mL $CHCl_3$ extract into fluorometer cuvet. Because citrinin deteriorates in light, samples should be read on fluorometer as soon as possible after extraction. Set fluorometer at excitation wavelength of 330 nm and emission wavelength of 500 nm. Take reading directly from fluorometer and calculate concentration of citrinin in sample. Fluorometer is always set at zero using sample of matrix containing no citrinin and extracted with each set of samples.

Calculate concentration of citrinin as follows:

$$\text{Citrinin, ng/g} = (Cs \times Mx)/(Ms \times W)$$

where Cs = concentration of standard, ng/mL; Mx = meter reading of sample; Ms = meter reading of standard; W = weight of sample represented in final extract, g/mL.

Results and Discussion

Citrinin was determined quantitatively in corn, barley, and peanut samples amended with 100–8000 ng citrinin/g (Table 1). Mean recoveries were 56.4% for corn, 66.6% for barley, and 40.0% for peanuts.

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Table 1. Recovery of citrinin from corn, barley, and peanuts

Added, ng/g	Found,[a] ng/g	CV, %	Rec., %
	Corn		
100	67.1 ± 5.5	8.2	67.1
200	121.0 ± 6.7	5.5	60.5
400	234.9 ± 10.7	4.6	58.7
800	486.0 ± 24.3	5.0	60.8
4000	1744.8 ± 125.2	7.2	43.6
8000	3803.1 ± 375.6	9.9	47.5
Mean		6.7	56.4
	Barley		
100	79.5 ± 7.5	9.4	79.5
200	162.5 ± 4.1	2.5	81.2
400	187.2 ± 17.6	9.4	46.8
800	545.0 ± 30.5	5.6	68.1
4000	2595.2 ± 389.3	15.0	64.9
8000	4470.6 ± 118.2	2.6	55.9
Mean		7.4	66.6
	Peanuts		
100	38.0 ± 10.0	26.3	38.0
200	76.9 ± 18.7	24.3	38.4
400	235.8 ± 4.6	2.0	58.9
800	288.0 ± 54.6	19.0	36.0
4000	1388.1 ± 178.1	12.8	34.7
8000	2735.6 ± 244.8	8.9	34.2
Mean		15.6	40.0

[a]Average of 3 consecutive analyses ± SD.

The Na_2EDTA competes with the citrinin as a chelating agent, perhaps improving the recovery. This procedure requires no heat at any point so the recovery process is not hampered by deterioration of citrinin due to heat. The initial standard stock solution was prepared in a foil-lined volumetric flask and placed in a freezer at −25°C. A standard curve was run using 10 points and was linear from 100 to 800 ng/mL. The development of this procedure took about 3 months. At the end of this time, another standard curve, run using the same 10 points, was also linear from 100 to 800 ng/mL. Because no deterioration of the citrinin in the original standard stock solution occurred, we concluded that a fresh standard stock solution did not have to be made daily. The time involved in a single determination is about 1 h including the separation phase and reading the sample on the fluorometer. Citrinin recoveries in corn were 71% by LC and 75% by TLC (6). This method is intended to provide an alternative detection method for laboratories not equipped to perform LC determinations and to eliminate some of the variability of TLC determinations.

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Simple Colorimetric Estimation of Cyclopiazonic Acid in Contaminated Food and Feeds

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A method has been developed for quantitative determination of cyclopiazonic acid, a mycotoxin produced by a common food contaminant, *Penicillium cyclopium*. The organism was grown successively in synthetic minimal medium, rice, corn, and wheat for 15 days. The toxin was extracted with chloroform followed by separation by thin layer chromatography. A colorimetric assay procedure has been successfully developed for the analysis of cyclopiazonic acid present in infected rice, corn, and wheat. The sensitivity of the method was tested by using recovery experiments.

The study of mycotoxicoses, especially aflatoxicoses (1, 2), confirms the existence of fungal metabolites in higher organisms. Many metabolites have been isolated from moldy commercial food and feed suspected of being involved in outbreaks of a number of diseases (3, 4). However, lack of quantitative assay methods made it difficult to determine suitability of the feed and to treat animals diseased by consumption of such contaminated feed because the amount of toxin could not be measured. It is therefore necessary to develop a rapid and sensitive analytical method for the detection and quantitative estimation of cyclopiazonic acid in agricultural commodities and consumer products.

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Chemically, cyclopiazonic acid is an indole tetramic acid which was isolated from culture of *Penicillium cyclopium* and found to be a mycotoxin (5). The intraperitoneal LD_{50} value was 2.3 mg/kg body weight in male rats (6). This compound is also produced by *Aspergillus versicolar* (7) and *A. flavus* Link (8). Reports of production of cyclopiazonic acid by several *A. flavus* isolates (9) which also produce aflatoxin have proved the possibility of a natural occurrence of the toxin in agricultural commodities in which aflatoxins are detectable. The observation lends further support for the necessity of an analytical method of estimation for cyclopiazonic acid in various agricultural commodities.

The present colorimetric determination of cyclopiazonic acid is based on the color reaction of Ehrlich's reagent with the indole group present in the acid structure (10, 11). The reagent *p*-dimethylaminobenzaldehyde reacts with α, β-unsaturated indoles, preferentially in the β-position (12), in presence of HCl to give colored cations. Since the β-position is saturated in cyclopiazonic acid (6), the condensation may

be taking place at the α-position to give a purple cation (Figure 1) which can be measured at 560 nm. The color produced obeys Beer's law.

Experimental

Reagents

(a) *Penicillium cyclopium.*—Isolated in our laboratory from warehouse-contaminated sample of rice and confirmed as *P. cyclopium* by U.S. Department of Agriculture Laboratory, Peoria, IL. Strain was grown in synthetic medium with the following composition (g/L): glucose, 50; tartaric acid, 5.0; ammonium tartrate, 5.0; $(NH_4)_2HPO_4$, 0.5; K_2CO_3, 0.5; $MgCO_3$, 0.5; $(NH_4)_2SO_4$, 0.25; $ZnSO_4$, 0.07; $FeSO_4 \cdot 7H_2O$, 0.07.

(b) *Chemicals.*—Chloroform, ethyl acetate, formic acid, *p*-dimethylaminobenzaldehyde (AnalaR grade obtained from BDH (India) Ltd).

(c) *p-Dimethylaminobenzaldehyde solution.*—Prepared by dissolving 500 mg in 100 mL absolute alcohol.

(d) *Standard cyclopiazonic acid solution.*—Prepare by dissolving 10 mg in 100 mL methanol.

TLC Separation of Cyclopiazonic Acid

Liquid culture was prepared by sterilizing 1 L minimal medium in a Hopkin's flask after adjusting the pH to 6.5. Other media were prepared by placing 500 g rice, wheat, or corn along with 100 mL water in separate flasks; flasks were autoclaved 15 min at 15 psi pressure, cooled, and then inoculated with 1.0 mL spore suspension of *P. cyclopium* (containing ca 10^6 spores) and kept at 20°C for 15 days. Toxin was extracted with chloroform by proper agitation and, in the case of pure culture, the mycelial toxins were also extracted. The extracts in each case were filtered through Whatman No. 41 paper and evaporated to dryness at 30°C. Each extract was redissolved in 2.0 mL chloroform, solvent was concentrated to 0.5 mL at 30°C, and the whole solution was spotted in the form of a thin line on thin layer chromatographic plates coated with silica gel G. Plates were developed with chloroform–ethyl acetate–formic acid–toluene (50 + 40 + 10 + 2) solvent system. After drying, a portion of the plate was sprayed with *p*-dimethylaminobenzaldehyde in *n*-butanol and exposed to HCl vapors. Corresponding areas containing the toxin in each case were scraped off separately and extracted with methanol. Extracts were diluted to 50 mL with methanol in separate standard flasks.

Recovery Experiments

Ten g rice, corn, or wheat to which known amounts of cyclopiazonic acid had been added was extracted twice with 25 mL methanol and filtered through Whatman No. 41 paper. Solutions were diluted to 50 mL in separate standard flasks and used for estimation.

A standard curve for cyclopiazonic acid was drawn (Figure 2) by adding 1 mL of 10, 20, 30, 40, and 50 μg/mL standard solutions in methanol to a series of test tubes containing 2.0 mL *p*-dimethylaminobenzaldehyde solution and mixing thoroughly. Finally, 10 mL 5N HCl was added and the color developed was read at 560 nm in a Klett-Summerson photoelectric colorimeter.

One mL portions of the extracted toxins were also placed in separate test tubes, mixed with *p*-dimethylaminobenzaldehyde and 5N HCl, and read at 560 nm. The amount of cyclopiazonic was determined from the standard curve.

Results and Discussion

The color developed when cyclopiazonic acid reacts with *p*-dimethylaminobenzaldehyde does not obey Beer's law

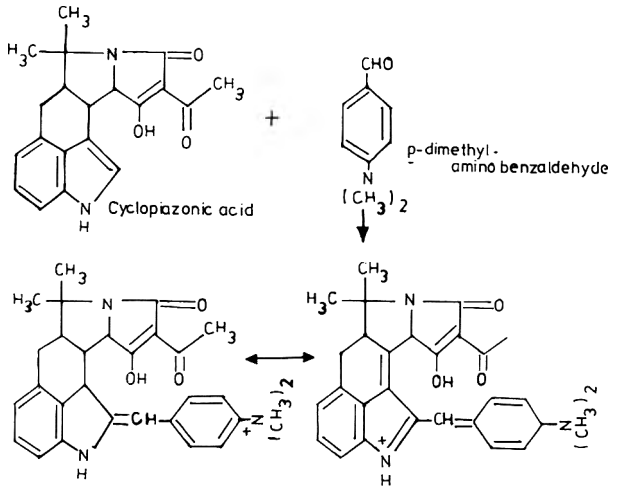

Figure 1. Color reaction of cyclopiazonic acid with *p*-dimethylaminobenzaldehyde.

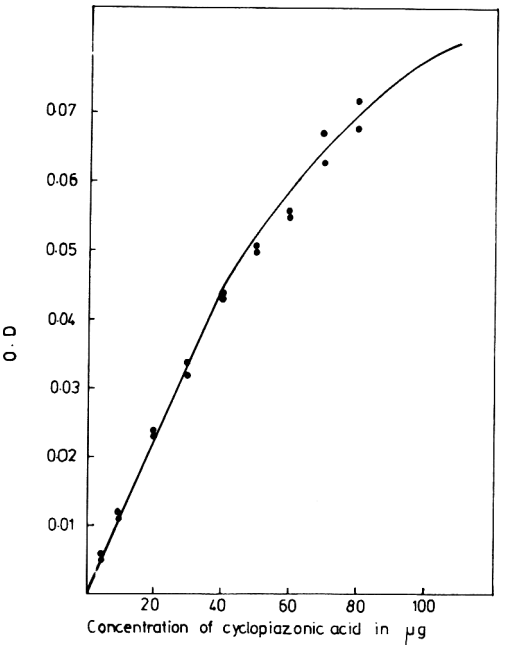

Figure 2. Standard curve for color reaction of cyclopiazonic acid.

beyond the range of 5–50 μg/mL. The optimum temperature for color development was 30°C (Klett reading 24, 25, 40, 37, 34, 0, at 0, 4, 30, 40, 60, and 100°C for 20 μg/mL) (Table 1) and the color was stable 30 min. Furthermore, paper chromatography of chloroform extract of the toxin, developed in *n*-butanol–acetic acid–water (40 + 10 + 50) solvent system and sprayed with ninhydrin reagent (Figure 3), clearly showed the complete absence of tryptophan in the crude extract, which eliminates possible interference of tryptophan in the color reaction.

Recovery experiments showed 63.4–95.1% recovery (Table 1). Methanol should be used directly for extraction in recovery experiments to avoid loss of toxin during double extraction.

Table 1. Recovery of cyclopiazonic acid added to various commodities

Commodity	Added, μg/100 g	Rec., %
Rice	205	85.7
	500	95.1
Wheat	250	63.4
	500	82.5
Corn	250	75.6
	500	88.8

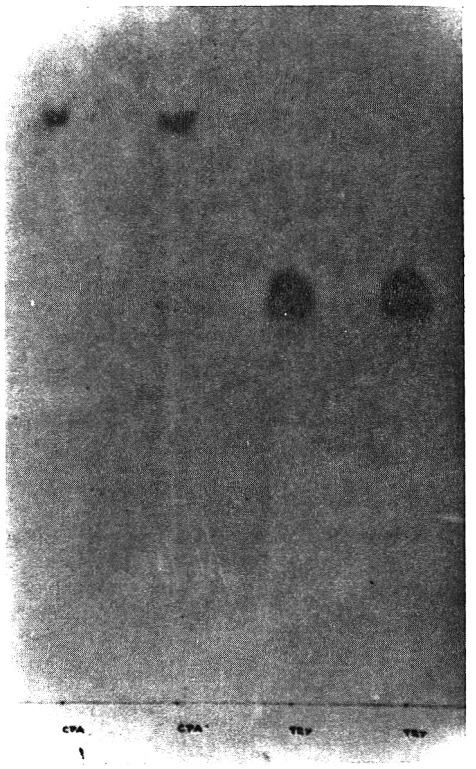

Figure 3. Paper chromatography of crude cyclopiazonic acid extract and tryptophan developed with *n*-butanol–acetic acid–water (50 + 40 + 10) solvent system.

The assay of cyclopiazonic acid produced on liquid minimal medium and contaminated rice, wheat, and corn showed maximum production of the toxin in the mycelia obtained in minimal medium (90, 18, 25, 30 mg/100 g, respectively). The toxin present in the culture filtrate can also be estimated by taking 0.5 mL culture filtrate directly for the assay. In this case, the color fades after 10 min because a number of components are present in the culture; hence, the reading must be taken immediately after color development.

The method is sensitive and can be used for screening stored grains and other consumer products before their delivery to the public.

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Thin Layer Chromatographic Determination of Deoxynivalenol in Wheat and Corn

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A thin layer chromatographic (TLC) method for determining deoxynivalenol (DON) in corn and wheat was developed. DON is extracted from the grain with acetonitrile–water (84 + 16) and filtered through a column of mixed alumina–charcoal–Celite (0.5 g + 0.7 g + 0.3 g). The solvent is evaporated on a steam bath. Ethyl acetate is added to the residue and heated to dissolve DON. After cooling, the residue is transferred to a vial with additional ethyl acetate and is dissolved in $CHCl_3$–acetonitrile (4 + 1) for TLC on an $AlCl_3$-impregnated silica gel plate with $CHCl_3$–acetone–isopropanol (8 + 1 + 1). The plate is heated in a 120°C oven for 7 min; a blue fluorescent spot is produced under longwave ultraviolet light. DON is quantitated visually and/or fluorodensitometrically by comparison with reference standards. The minimum detectable amount of DON is ca 20 ng/spot. The limit of DON determination is ca 40 ng/g for wheat and 100 ng/g for corn. Recoveries of DON added to wheat and corn at 100, 500, and 1000 ng/g levels were 85, 93, and 88% and 77, 80, and 80%, respectively.

Deoxynivalenol (DON) (3,7,15-trihydroxy-12,13-epoxytrichothec-9-en-8-one), also known as vomitoxin, is one of the toxic trichothecenes produced by various species of *Fusarium* and other mold genera. DON was first characterized in Japan (1) as a metabolite of *F. roseum (F. graminearum)* isolated from moldy barley, and was shown to be identical to the emetic factor (vomitoxin) isolated in the United States from corn that had been damaged by ear rot caused by *F. roseum* and which produced vomiting in swine (2). Since *F. roseum* is a common cause of scab or head blight of many small grains in addition to barley, the finding of DON in scabby wheat in Canada (3) and the United States (4) was not unexpected. Information on the toxic effects expected from exposure of animals or humans to DON is limited (5, 6); what little is known, plus the inference of possible deleterious effects by association with other trichothecenes that are potent toxins (5), suggests that more extensive information on DON occurrence might be desirable.

The only effective analytical method (7) currently in general use involves gas chromatographic/mass spectrometric (MS) instrumentation and is labor intensive. A much simpler and less time consuming method using thin layer chromatography (TLC) has been proposed (8), but in attempting to apply the method we encountered a number of problems. These have now been overcome, resulting in a method, presented here, that is practical and fills the need for a method that can be used in extensive surveys. Quantitation is improved by

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the use of densitometry, although visual estimation can be used if desired.

METHOD

Apparatus

(a) *Grinder.*—Centrifugal grinding mill equipped with 2 mm sieve (ZM-1, Brinkmann).

(b) *Chromatographic tube.*—Polypropylene (10 mm id × 50 mm) equipped with plastic filter disk and reservoir (QS-Q, California Scientific, 410 Martin Ave, Santa Clara, CA 95050).

(c) *TLC plates.*—Precoated 20 × 20 cm silica gel 60 plates (No. 5763, E. Merck). Dip plates in 15% $AlCl_3$ solution (see *Reagents* (d)) and let stand in vertical position 5 min to drain. Remove residual $AlCl_3$ from back of plate with wet paper towel. Air dry at room temperature 2 h and activate at 105°C 1 h. Store in dust-tight cabinet.

(d) *Viewing cabinet.*—Chromato-Vue C-6 (Ultra-Violet Products, Inc., San Gabriel, CA 91778), fitted with 15 W longwave ultraviolet (UV) lamp.

(e) *Fluorodensitometer.*—Schoeffel SD 3000 (excitation 365 nm, emission filter cutoff at 440 nm) with electronic integrator (SP4100, Spectra Physics).

(f) *Sample vial.*—2 dram, with foil- or Teflon-lined screw cap.

Reagents

(a) *Activated carbon.*—Darco G60 (J. T. Baker Chemical Co.).

(b) *Neutral alumina.*—Chromatographic grade (No. 9296, Matheson, Coleman & Bell).

(c) *Diatomaceous earth filter aid.*—Acid-washed Celite 545 (Johns-Manville).

(d) *$AlCl_3$ solution.*—Mix 15 g $AlCl_3.6H_2O$ with 15 mL water, and add 85 mL ethanol; then mix and warm on steam bath until dissolved.

(e) *DON standard.*—Myco Lab Co., PO Box 321, Chesterfield, MO 63017. *Stock solution.*—1 mg/mL ethyl acetate. Warm as required to dissolve. *Working solution.*—Dilute portion of stock solution to 20 ng/μL with ethyl acetate. Keep solutions tightly sealed at room temperature.

Sample Preparation

Grind 5–10 lb (2–4 kg) sample to pass U.S. 20 mesh sieve and blend.

Extraction

Weigh 50 g ground, blended wheat or corn into 500 mL glass-stopper Erlenmeyer flask. Add 200 mL acetonitrile–water (84 + 16); secure stopper with masking tape. Vigorously shake 30 min on shaker. Filter through Whatman No. 2 paper. Collect 20 mL filtrate in 25 mL graduated cylinder.

Wheat.—Proceed with column chromatography.

Corn.—Transfer 20 mL filtrate to 125 mL separatory funnel. Add 50 mL hexane and shake 1 min. Draw lower aqueous acetonitrile layer back into cylinder for transfer to column described below. Proceed with column chromatography.

Column Chromatography

Secure chromatographic tube on 125 mL filter flask. Place small ball of glass wool in bottom of tube and add ca 0.1 g Celite.

Weigh 0.7 g charcoal, 0.5 g alumina, and 0.3 g Celite. Place in 50 mL beaker and thoroughly mix with spatula. Add mixture to tube and lightly tap tube to settle packing. Apply suction and compress packing with tamping rod. (Note: Experimentally determine tamping pressure that will provide channel-free packing porous enough to allow 2–3 mL/min flow rate for extracting solvent with 20 cm Hg vacuum. Columns may be prepared ahead of time and stored in upright position in beaker covered with aluminum foil for use as needed.) Add ball of glass wool on top of adsorbent. Apply 20 mL extract to column. As solution reaches top of packed bed, rinse cylinder with 10 mL acetonitrile–water (84 + 16) and then add to column and continue aspiration until flow stops. Do *not* let column go dry between addition of extract and solvent. Add 4 boiling chips to filtrate in filter flask and evaporate to dryness on steam bath. It is essential that no water droplets remain in flask. Add 3 mL ethyl acetate to residue and heat to boiling on steam bath; then remove flask and gently swirl to dissolve DON. Cool to room temperature. Transfer extract solution to 2 dram vial and rinse with three 1.5 mL portions of ethyl acetate. Evaporate extract to dryness on steam bath under stream of nitrogen. Retain dry extract for TLC. Final extract represents 5 g sample.

Quantitation

Dissolve residue in vial in 100 μL $CHCl_3$–acetonitrile (4 + 1). Apply 5 and 10 μL aliquots of sample solution alongside 20, 40, 100, 200, and 400 ng reference standard spots on scored TLC plate (1 cm channels). Develop plate with $CHCl_3$–acetone–isopropanol (8 + 1 + 1) in unequilibrated tank (development time is ca 1 h). Remove plate from tank and let solvent evaporate from plate at room temperature in well ventilated hood ≥5 min. Residual solvent can result in fading of DON spots during subsequent operations. Before heating, briefly examine plate under longwave UV light for possible blue fluorescing interferences. Heat plate 7 min in upright position in 120°C convection oven. Place plate on cool surface in dark 1 min. Observe DON as blue fluorescent spot under longwave UV light at R_f ca 0.6. Spot should be well resolved from other background fluorescent spots. Quantitate DON by comparing fluorescence intensity of sample spot with those of standard DON spots visually or densitometrically in manner analogous to techniques used for aflatoxins (9). When using densitometer, scan spots from top to bottom, parallel to direction of development. Response must be linear relative to standard concentration, at least for 100, 200, and 400 ng spots. The 20 and 40 ng spots can be used for qualitative estimate of concentrations ≤100 ng/g. At these concentrations, sample must be rechromatographed using more sample extract. After scanning all channels of plate, repeat scan for first spot measured. Values for the 2 scans should agree within instrument replication capabilities as previously determined. Ozone produced by instruments equipped with xenon lamps may fade DON spots. Fading can be prevented by covering TLC plate with clean glass plate before and during densitometric scanning or by venting ozone.

Calculation

Calculate average response per 100 ng DON for 100, 200, and 400 ng spots. Use average response to calculate amounts of DON in sample spots that are in range of these standards.

$$\text{ng/g} = R_u \times (100/R_s) \times (V_d/V_s) \times (1/W)$$

where R_u = densitometric response for DON in unknown, counts; R_s = response for 100 ng DON standard, counts; V_d = dilution volume of final extract used for spotting, μL; V_s = volume of sample spotted, 10 μL; W = weight of sample equivalent in final extract, 5 g.

Results and Discussion

The method (8) on which this study was based uses extraction of the substrate with acetonitrile–water (84 + 16) for a short period in a high-speed blender, separation of the DON from interferences in the extract with an activated carbon–alumina column, further separation of the DON on silica gel TLC, and visualization of the developed DON spot on the TLC plate by charring with sulfuric acid. In applying the method to samples, the following difficulties were encountered: in some samples, the volume of eluting solvent was inadequate to obtain complete removal of the DON from the adsorbent column; the adsorbent column was packed in a manner that impeded solvent flow and allowed passage of some of the carbon into the eluate; a residue in the final extract interfered with application of extract to the TLC plates; the sulfuric acid charring of the DON was only sensitive to a level of 500–1000 ng/spot. The charred spots were difficult to quantitate and were of insufficient intensity to achieve the desired limit of determination.

Following the 20 mL extract with an additional 10 mL eluting solvent proved adequate for attaining the desired recovery from the adsorbent column. To improve column porosity and packing retention, a piece of glass wool and a layer of diatomaceous earth filter aid were placed on top of the plastic filter disk, the charcoal and alumina were mixed, and filter aid was added to the mixture.

The problem of the residue was resolved by substituting ethyl acetate for acetone–methanol as the transfer solvent. The residue is less soluble in this solvent than in acetone–methanol and is left behind. DON, however, is less soluble in ethyl acetate than in acetone–methanol and complete transfer therefore necessitated heating the extract with the ethyl acetate.

Several changes were made to optimize the TLC conditions. $CHCl_3$–acetonitrile was substituted for methanol-acetone as the sample application solvent to give more compact spots. For corn, partitioning the original acetonitrile-water extract against hexane removed materials that made spotting difficult. $AlCl_3$, which on heat activation produces an intense blue fluorescence with DON (10), was substituted for sulfuric acid charring as the detection reagent to lower the detection limit to about 20 ng/spot. The plates can be sprayed with $AlCl_3$ solution after development, but this must be done very thoroughly and evenly to obtain reproducible fluorescence. The plates also can be dipped in $AlCl_3$ solution and reactivated before development or $AlCl_3$ can be incorporated in the water used for the silica gel slurry when TLC plates are prepared. The latter 2 techniques afford the most uniform incorporation of the $AlCl_3$ and hence give reproducible fluorescence intensity for quantitation. For the commercial plates used in most of this study, the dipping technique was the sole option for uniform application of $AlCl_3$. The unheated plate should be examined under UV light for the presence of blue fluorescent spots that might interfere with the DON spot.

The fluorescence intensity of DON spots depended on the $AlCl_3$ concentration in the silica gel layer and heating time. For dipped plates there was no gain in fluorescence when the $AlCl_3$ concentration exceeded 15 g $AlCl_3.6H_2O$ in 100 mL ethanol–water (85 + 15); for laboratory spread plates, a 10% $AlCl_3$ aqueous solution was optimum for making the silica gel slurry. Tests with heating times of 3, 7, 10, and 15 min in a 120°C convection oven showed that 7 min was optimum for maximum fluorescence intensity and stability.

The final modification in the TLC procedure was the use of $CHCl_3$–acetone–isopropanol in place of ethyl ether–acetone as the mobile phase. This resulted in improved resolution and more compact, intense, and stable fluorescent spots.

The above refinements have resulted in a method suitable for densitometric quantitation of DON. Two other densitometric methods (11, 12) for DON and several other trichothecenes have been proposed; one of these produces a colored derivative with an absorption maximum of 610 nm (11). The minimum detection limit was not reported for DON; however, the minimum detection limit for the structurally similar nivalenol was 200 ng/spot.

The other method is a fluorodensitometric method (12) with a detection limit of 25 ng/spot for DON standard. The fluorescence is developed only after a complicated and tedious procedure. Neither method was validated by application to more than one sample with added DON.

The recovery results for DON added to wheat and corn are reported in Table 1. The recovery is about the same at all added levels from both wheat and corn. Precision of measurement is better at the high levels. The combination of sample size used for quantitation (0.5 g) and the resolution of DON from interferences provided an approximate limit of determination of 40 ng/g for wheat and 100 ng/g for corn. The higher detection limit in corn results from the interfering yellow fluorescent tailing. The method presented here is rapid, simple, and inexpensive in terms of reagents and equipment. It is accurate as demonstrated by the recoveries.

To evaluate the applicability of the method to naturally contaminated samples, 3 corn samples and 2 wheat samples were analyzed for DON. DON was detected in all samples: DON levels in corn were 36 000, 14 000, and 140 ng/g and in wheat were 800 and 150 ng/g. The identity of the DON from naturally contaminated samples was confirmed but not quantitated by using capillary gas chromatography–negative chemical ionization MS with methane as the reagent gas (13). In addition, the method has been applied to the analysis of more than 50 corn samples. Two samples were negative, 19 had DON levels below 200 ng/g, and the remaining had DON levels ranging up to 2100 ng/g (unpublished data).

Table 1. Recovery of DON added[a] to wheat and corn

Commodity	DON added, ng/g	Av. rec.,[b] % of added	SD	CV, %
Wheat[c]	100	85 (85, 99, 96, 68, 70)[d]	14.3	17
	500	93 (97, 88, 96, 85, 99)	6.1	7
	1000	88 (87, 88, 90, 85, 92)	2.7	3
Corn[c]	100	77 (69, 72, 72, 83, 90)	8.9	12
	500	80 (84, 76, 82, 73, 86)	5.5	7
	1000	80 (76, 86, 74, 83, 83)	5.1	6

[a]DON was added to ground sample and allowed to stand 2 h before extraction was carried out.
[b]Corrected for blank value.
[c]Blank corn contained ca 30 ng DON/g and this value was subtracted from DON found in spiked sample before recovery was calculated; none was detected in wheat.
[d]Individual values are in parentheses.

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Deoxynivalenol in Winter Wheat: Thin Layer Chromatographic Method and Survey

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A rapid method for the determination of deoxynivalenol (DON) in wheat was used to analyze 57 wheat samples collected from 4 midwestern states where the winter wheat crop was contaminated with Fusaria. The method involves sample extraction with acetonitrile–water (84 + 16), cleanup by charcoal–alumina column chromatography, and determination by thin layer chromatography (TLC), using an $AlCl_3$ solution spray and heat to form a fluorescent derivative. Recoveries of DON added to wheat at levels as low as 0.2 μg/g averaged >80%. DON was detected at an average level of 3.6 μg/g; the levels ranged from 0.2 to 9.0 μg/g in 54 of 57 of the wheat samples. The quantity of DON was, in general, proportional to the percentage of total damaged kernels (grade). The chemical identity of DON was confirmed by mass spectrometry after isolation with preparative TLC.

Deoxynivalenol (DON, vomitoxin) is one of a group of closely related secondary fungal metabolites called trichothecenes. The trichothecenes are associated predominantly, although not exclusively, with some species of *Fusarium*, which, under certain climatic conditions, invade grains in the field and in storage. DON is one of the 4 known naturally occurring trichothecenes (the others are T-2 toxin, diacetoxyscirpenol, and nivalenol); it has been detected in samples of corn, barley, and wheat (1–7). These toxins, when present in grains or feeds either singly or in combination, have been implicated as causing farm animal diseases known as fusariotoxicoses (2, 8, 9).

During spring 1982, unusual weather conditions in parts of the central United States were conducive to the infection of winter wheat by Fusaria. Strains of Fusaria are responsible for pink scab and tombstone kernels in wheat and pink ear rot in corn, and are associated with the occurrence of DON. To quickly assess the extent to which DON might be found in commercial wheat harvested in the affected areas, surveys were needed.

Although several methods for the analysis of grains and feeds for DON had been reported, including those based on the use of thin layer chromatography (TLC), gas chromatography (GC), and GC-mass spectrometry (MS) in the determinative step (10), none of these methods met our needs for speed, simplicity, accuracy, and limit of determination. The method described in this paper is based on a rapid, efficient extraction and cleanup procedure (11) combined with a TLC determinative procedure (12). This method was applied to the analysis of 57 wheat samples collected in selected areas of 4 midwestern states where unusual contamination of winter wheat with Fusaria was reported. The chemical identity of DON in 4 of the positive samples was confirmed, using MS.

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METHOD

Reagents

(a) *Adsorbents for cleanup column.*—(Note: Prepacked cleanup columns and reservoirs are available from Mycolab Co., PO Box 321, Chesterfield, MO 63017.) Activated charcoal, Darco G-60 (J. T. Baker Chemical Co., Phillipsburg, NJ 28865); neutral alumina, 80–200 mesh (No. A-950, Fisher Chemical Co., Pittsburgh, PA 15219). To prepare cleanup column, insert empty tube into apparatus (c) and apply vacuum (350–400 torr). Add activated charcoal to height of 3 cm (ca 4 mL charcoal, 0.7 g); then add 1 cm neutral alumina, and finally tamp small plug of pyrex wool lightly onto top of packing. Packed columns may be stored for indefinite time.

(b) *$AlCl_3$ spray solution.*—Dissolve 20 g $AlCl_3.6H_2O$ in 100 mL ethanol–water (1 + 1).

(c) *DON standard solutions.*—(DON is available from Mycolab Co.) *Stock solution.*—0.5 mg/mL. Weigh 5.0 mg DON into 10 mL glass-stopper volumetric flask, dilute to volume with acetone–methanol (2 + 1), and vigorously shake to dissolve. *TLC standard solution.*—20 μg/mL. Pipet 1.0 mL DON stock solution into 25 mL volumetric flask and dilute to volume with acetone–methanol (2 + 1). Refrigerate DON solutions when not in use.

Apparatus

(a) *Wrist-action shaker.*—Burrell Corp., Pittsburgh, PA 15219.

(b) *Vacuum pump or water aspirator.*—Capable of producing 400 torr vacuum.

(c) *Vacuum system assembly.*—125 mL filter flask, No. 4 rubber stopper with 7/16 in. (11 mm) diameter single hole, and vacuum regulator (optional) (No. 8-670-32, Fisher Scientific Co.).

(d) *Cleanup tube.*—Polypropylene (10 mm id × 50 mm) with plastic filter disk (No. 15-1570, Bio-Lab Products, 3031 Tisch Way, San Jose, CA 95128).

(e) *TLC apparatus.*—50 μL syringe, spotting template, developing tank with cover, spray bottle, and viewing cabinet with longwave (365 nm) ultraviolet (UV) light.

(f) *TLC plates.*—20 × 20 cm glass, Adsorbosil-Plus 2 Prekotes, Hard Layer (Applied Science Laboratories, State College, PA 16801).

(g) *Filter paper.*—32 cm fluted (No. 588, Schleicher & Schuell, Keene, NH 03431).

Extraction and Column Cleanup

Grind samples to pass 2 mm sieve. Weigh 50 g ground sample into 500 mL glass-stopper Erlenmeyer flask, add 200 mL acetonitrile–water (84 + 16), stopper, seal with masking tape, and shake 30 min, using wrist-action shaker set at fast rate. Filter sample through fluted paper into graduated cylinder. Collect 20 mL filtrate.

Attach charcoal–alumina cleanup column to 125 mL filter flask vacuum assembly. Transfer 10 mL acetonitrile–water extract to column and apply vacuum (400 torr). After first 10 mL extract has entered column, add second 10 mL extract. Continue to apply vacuum until all extract has entered column; then add 10 mL acetonitrile–water extraction solvent as column wash. After all solution has passed through column, disconnect flask, add a few boiling chips, and evaporate to dryness on steam bath. Add 1 mL acetone–methanol (2 + 1) to flask and dissolve residue, using ultrasonic bath or vortex mixer for 1 min. Transfer solution to 3 dram (11 mL) vial through tightly packed plug of Pyrex wool in short-stem funnel. In same manner, rinse flask with 3 additional 1 mL portions of acetone–methanol and combine solutions in vial. Evaporate this solution just to dryness under stream of nitrogen on steam bath.

Thin Layer Chromatography

Add 100 μL acetone–methanol (2 + 1) to vial and dissolve extract, using ultrasonic bath or vortex mixer (1 min). Spot 25 μL sample extract and 1, 2, 5, 10, and 20 μL DON standard solution (20 μg/mL) ca 1.5 cm apart on imaginary line ca 4 cm from bottom of plate, using warm air stream or heated plate to aid in evaporating solvent. Develop plate in closed, unequilibrated tank with ethyl acetate–ethyl ether (1 + 1) to height of ca 12 cm. Remove plate, air dry, and spray evenly with $AlCl_3$ solution. Heat plate in 100°C forced-draft oven 10 min. Remove plate and examine under longwave (365 nm) UV light. DON appears as blue fluorescent spot with R_f ca 0.6. Compare sample extract to standards, and, if DON is present, match intensity of sample spot with one of standard spots. Estimate dilution required for samples having spots with greater intensity than 20 μL standard spot, quantitatively dilute sample with acetone–methanol (2 + 1), and respot with same standard range. Additional confidence in the identity of DON may be obtained by developing second plate with chloroform–methanol (93 + 7).

Calculate amount of DON in sample, using the following formula:

$$\text{DON, ng/g sample (ppb)} = S \times (C/X) \times (V/W)$$

where S = μL standard equal to sample, C = concentration of standard solution (20 μg/mL), V = final volume of sample (μL), X = μL sample spot that had fluorescence intensity equal to standard spot, and W = amount of sample represented by final extract (5 g).

When dilutions are required, extract solution equivalent to 1.25 g sample is used for initial determination; therefore extract solution equivalent to 3.75 g sample is left in vial.

Results and Discussion

This method was designed for the rapid screening of wheat samples for DON, and, in practice, it has been found to be very useful. One analyst can analyze 12 samples in a day and, with assembly-line techniques, an even greater samples/analyst output can be achieved. Recoveries of DON added to "blank" wheat at 4 levels ranging from 0.2 to 5.0 μg/g averaged >80%. The limit of determination is estimated to be 0.2 μg/g, although an experienced analyst can detect DON at levels as low as 0.1 μg/g.

Six samples of wheat that were naturally contaminated with DON were exchanged with another laboratory, where they were analyzed by a longer method which uses GC in the determinative step (6). The results (μg DON/g) for the 6 samples were 1.7, 3.5, 4.6, 0.2, 1.4, and 1.0 for the GC method and 0.5, 4.0, 8.5, not detected, 1.4, and 3.0 for the TLC method, respectively.

To determine DON in winter wheat, a survey was carried out in cooperation with the Federal Grain Inspection Service (FGIS) of the U.S. Department of Agriculture (USDA). Through the FGIS, the independent grader stations in the affected areas supplied samples of scabby wheat which had been submitted for grade determination. Included with each sample was the grader determination of the percent of total damaged kernels (TDK) in that lot. The samples were arranged by the reported percent TDK in stepwise categories of 1% ranging from 0 to 20% TDK (i.e., 0 to 1% TDK, 1.1 to 2.0% TDK, 2.1 to 3.0% TDK, etc.). When there was not enough sample for analysis, the sample was combined with another sample from the same grader station which had the same percent TDK. A total of 57 samples were analyzed. A summary of the results is shown in Table 1.

This survey was designed to give an indication of the levels of DON which might be present in the specific areas where mold-damaged wheat was being harvested. Because samples having TDK were selectively collected, the survey does not represent a significant portion of the 1982 winter wheat crop from the affected areas. The results of this survey do indicate the levels of DON which may be present in wheat when there are significant percentages of TDK present, and, more important, that there is a correlation between the percent of TDK

Table 1. Analysis of wheat samples for DON

Total damaged kernels, %	No. of samples	Av. DON detected, μg/g	Range DON detected, μg/g
0– 1.0	9	0.4	ND[a]–0.8
1.1– 2.0	9	2.2	0.2–4.0
2.1– 4.0	9	2.1	0.5–6.0
4.1– 6.0	8	4.7	3.0–8.0
6.1– 8.0	8	5.3	2.0–9.0
8.1–12.0	7	5.7	4.0–8.5
12.1–20.0	7	6.0	4.0–8.5
	57	3.6	ND–9.0

[a]ND = no DON detected.

and the level of DON present. Thus, the percent of TDK in wheat as determined by the independent graders will give an excellent immediate indication of the possible presence and level of DON.

The procedure used for confirming the presence of DON in sample extracts involves isolation by TLC followed by MS analysis, and is based on a procedure developed for the confirmation of aflatoxins in various commodities (13). A portion of the sample extract, calculated to contain at least 100 ng DON, was applied as a single spot on a TLC plate and spots of standard DON were applied at both sides of the same plate. After development, the central portion over the sample was covered with a glass plate and the standards were sprayed with the $AlCl_3$ solution and heated to effect visualization. The area of the sample having the same R_f as the standards was eluted in situ using a Camag Eluchrom elution system with 2 mL chloroform–acetone–isopropanol (14 + 3 + 3) as the eluting solvent. Negative ion chemical ionization MS was used for confirmation of identity (14). In this study, samples were introduced by on-column capillary GC-MS and analyzed by resonance electron capture, using methane as the reagent gas. This procedure was used to confirm DON in 4 wheat samples in which TLC had shown DON to be present at levels ranging from 0.2 to 9.0 μg/g.

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Enzyme-Linked Immunosorbent Assay of Ochratoxin A in Wheat

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An enzyme-linked immunosorbent assay (ELISA) was developed for the quantitation of ochratoxin A amended to wheat. Ochratoxin A conjugated to horseradish peroxidase (HRP) was used as an enzyme marker in the assay. At toxin levels below 30 ppb, a cleanup treatment was necessary for ELISA. Among 3 cleanup methods tested (solvent partition, Sep-Pak cartridge treatment, solvent partition and cartridge treatment), reverse phase cartridge treatment was the most simple and effective. In the analysis, ochratoxin A was extracted from wheat with methanol. The methanolic extract was diluted with water to a final 10–15% methanol content, and then passed through a cartridge. Ochratoxin A was eluted from the cartridge with 85% methanol which was then concentrated. The final solution, in 0.1M, pH 7.5 sodium phosphate–Tween 20 buffer and 5% methanol, was then subjected to ELISA. ELISA allowed minimal detection of the toxin in wheat at the 1–2 ppb level after cleanup. Recoveries of toxin added to wheat samples in the 1.0–30 ppb range were 85–90% with standard deviations of 10–15%.

Ochratoxin A is one of the most toxic isocoumarin-containing mycotoxins produced by various species of *Aspergillus* and *Penicillium* (1–3). Because of its association with certain mycotoxicoses in animals and possibly in humans, it has received considerable attention in recent years (4, 5). To avoid the risk of consumption of the toxins by humans and animals, a rigorous program for monitoring ochratoxin A in agricultural commodities is essential. Although thin layer chromatography (6–8), spectrophotometry using carboxypeptidase A (9), and liquid chromatography (LC) (10–12) have been developed for determining ochratoxin A in agricultural commodities, most of these methods require extensive cleanup steps before analysis. In view of the recent development of immunoassay for mycotoxins including ochratoxin A (13–17), we attempted to develop a practical enzyme-linked immunosorbent assay (ELISA) for ochratoxin A.

Investigation in our laboratory has shown that the minimum detection level of ochratoxin A by direct ELISA was 25 pg per assay (16). Most recently, Morgan et al. (18) reported that as little as 10 pg ochratoxin A could be detected by indirect ELISA. However, application of such a method to determine toxin in agricultural commodities has not been tested. In the present study, ochratoxin A was added to wheat samples and recovery was determined by a direct ELISA. We found that a cleanup step is necessary for determining the toxin at levels below 50 ppb; thus, the efficiency of 3 cleanup methods for subsequent ELISA were compared. Details of the cleanup and ELISA protocols, and techniques for coating antibody to the solid phase, are described in this paper.

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Experimental

Materials

Ochratoxin A was supplied by Robert E. Peterson (USDA, Northern Regional Research Center, Peoria, IL). Tritiated ochratoxin A was prepared according to the method of Chang and Chu (19) by New England Nuclear Co. (Boston, MA). Bovine serum albumin (BSA), Tween-20, and horseradish peroxidase (HRP) were obtained from Sigma Chemical Co. (St. Louis, MO). Ethylenediamine (EDA)-modified BSA was prepared according to the method described by Chu et al. (20). Ochratoxin A protein conjugates were prepared by the water-soluble carbodiimide method described previously (13, 16), using 1-(3-dimethylamino)-propyl-3-ethylcarbodiimide·HCl (Aldrich Chemical Co., Milwaukee, WI) as the coupling reagent. The ratio of toxin conjugated to the proteins, determined by a spectrophotometric method (1), was 12:1, 20:1, and 2:1 for toxin-BSA, toxin-EDA-BSA, and toxin-HRP, respectively. All other chemicals were either reagent grade or chemically pure.

Preparation of Antiserum

Antiserum specific for ochratoxin A was prepared by immunization of toxin-BSA or toxin-EDA-BSA in rabbits similar to those previously described (13). The resulting antiserum was purified by the ammonium sulfate fractionation method of Herbert et al. (21).

Determination of Antibody Titer by Radioimmunoassay

Protocols for titration of antiserum radioimmunoassay (RIA) were similar to those described by Chu et al. (22). Briefly, 100 μL of different concentrations of antiserum was incubated with 100 μL ^{3}H-toxin (ca 10 000 dpm) (specific activity 0.4 Ci/mmole) at room temperature for 30 min and then in a refrigerator overnight. After incubation, the mixture was precipitated with 0.3 mL 67% saturated ammonium sulfate solution in 0.1M, pH 7.2 sodium phosphate buffer (NaPB), allowed to stand at room temperature for 1 h, and then centrifuged. The supernatant solution was decanted, and the precipitate was resuspended in 0.5 mL 40% saturated ammonium sulfate in NaPB, allowed to stand at room temperature for 30 min, and again centrifuged. The precipitate was then redissolved in 1 mL water. The radioactivity of the combined supernatant solution (F:free ^{3}H-toxin) and of the redissolved precipitate solution (B:bound ^{3}H-toxin) was then determined. The titration curve was established by plotting % bound vs log concentration of antiserum. Antibody titer by RIA was defined as the reciprocal of the amount of antiserum (mL) needed to give 50% binding of ^{3}H-toxin under stated conditions.

Determination of Antibody Titers by Enzyme-linked Immunosorbent Assay

For the titration of antibody titers of ochratoxin A by ELISA, both Falcon Microtest III tissue culture plates (No. 3070 plate; Catalog No. 4–3070–0 (k), Becton Dickinson & Co., Oxnard, CA) and Dynatech Immulon Substrate flat bottom plates (Catalog No. 011–010–3250, Dynatech Laboratories, Alexandria, VA) were tested for their effectiveness in coating antibody to plates. In each analysis, 50 μL portions of appropriate dilutions of antiserum in 0.1M, pH 7.5 phosphate buffer were coated on polystyrene ELISA plates either by air-drying (40°C) the antiserum directly (DD method) onto the plate (16) or by incubating antibody in 0.1M, pH 9.6 sodium carbonate buffer in the plate at 4°C overnight (carbonate method) (23). Excess antibody was washed with 0.1M phosphate buffer and pH 7.5–0.5M NaCl with 0.1% (v/v) Tween 20 (PBS–Tween), and then 50 μL ochratoxin A-peroxidase conjugate (100 ng/well) in PBS-Tween was added to each well. The plates were incubated at 37°C for 2 h and washed 4 times with PBS–Tween, and bound enzyme was determined by reading absorbance at 414 nm after incubation with 100 μL substrate containing 1.2mM hydrogen peroxide and 0.4mM 2,2′-azino-di-ethyl-benzthiazoline-6-sulfonate (ABTS) (16, 17) at 37°C for 20 min. Controls were run with preimmune sera in the same way. Antibody titer by ELISA was defined as the reciprocal of the amount of antisera (mL) needed to give 80% of maximum absorbance under stated conditions.

Competitive Ochratoxin A ELISA

Competitive assays were carried out on microtiter tissue culture plates coated with toxin antiserum (1:200–1:400 dilution) by the DD method. Antibody-treated plated were washed 4 times with PBS-Tween, and then 25 μL of a 1:100 dilution ochratoxin A-peroxidase solution (0.4 mg/mL) was added to each well. This was followed by addition of 25 μL standard ochratoxin A(1–50 ng/mL) or 25 μL sample extracts, all diluted in 0.1M PBS-Tween. The plates were incubated 2 h at 37°C. After washing, the residual enzyme was determined (16).

Preparation of Wheat Samples

The 50 g wheat samples were blended with 150 mL methanol in a Waring blender for 2 min and filtered. Residues were extracted with 2 additional 150 mL portions of methanol. Combined methanolic extracts were then subjected to 3 cleanup methods to test their effectiveness in preparing extracts for ELISA.

(a) *Solvent partition method.*—The methanolic extracts were concentrated and dissolved in 10 mL 1% sodium bicarbonate solution, and impurities were extracted with 20 mL chloroform. The sodium carbonate solution was then neutralized to pH 7.5 with HCl before ELISA.

(b) *Cartridge cleanup.*—Two mL of the methanolic extracts (or concentrated extracts) were mixed with 10 mL water and passed through a C_{18} reverse phase Sep-Pak cartridge (Part No. 51910, Waters Associates, Milford, MA). After cartridge was washed with 10 mL 15% aqueous methanol (washing discarded), samples were eluted with 10 mL 85% aqueous methanol. After cleanup, all samples were concentrated in vacuum to remove solvent, redissolved in a small amount of methanol, and diluted with 0.1M sodium phosphate buffer to a final methanol concentration of less than 5%.

(c) *Solvent partition plus cartridge cleanup.*—Extracts were concentrated and redissolved in 20 mL 1.0% sodium bicarbonate solution. Impurities were extracted once with chloroform (twice the sample volume), the aqueous layer was acidified to pH 1.8, and toxin was extracted with chloroform 3 times (twice the sample volume). The acidic chloroform extract was concentrated, redissolved in 10 mL methanol, and passed through a Sep-Pak C_{18} cartridge as described in procedure **(b)**.

Recovery Studies

Recovery studies were carried out by mixing appropriate amounts of unlabeled ochratoxin A with 85 000 dpm pure ^{3}H-toxin in methanol with 50 g wheat flour to final concentrations of 1.0, 5.8, and 30.8 ppb. The mixtures were dried at room temperature, and then carried through extraction and cleanup treatments. Recoveries of radioactivities of ochratoxin A from the samples were compared.

Interference Studies

To test the effect on the ELISA system of interfering substances in wheat extracts prepared by different cleanup procedures, 50 μL aliquots of solutions containing different amounts of extracts were incubated with toxin-HRP in the absence of free ochratoxin A. The percentage of inhibition of binding of the toxin-HRP was calculated as previously described (17):

$$\% = (1 - A \text{ sample ext}/A \text{ without sample ext}) \times 100$$

Results

Production of Antisera by Using Two Different Toxin-Protein Conjugates

The effect of 2 different protein conjugates on antibody production is shown in Table 1. Although the degree of the amount of ochratoxin A conjugated to 2 different carriers was different (12 vs 20 moles toxin/mole), the amount of antibodies elicited by the rabbits after immunization with both conjugates was similar. Present results indicate that both toxin-BSA and toxin-EDA-BSA were good immunogens for producing antibodies against ochratoxin A. The antibody dilution necessary for optimum ELISA analysis was 50–200 times higher than obtained from RIA. Since high specific radioactive ochratoxin A is not available at present, ELISA is a more sensitive system for titration of antibody titer.

Titration of Antiserum by ELISA

In the ELISA titration assays, 2 different brands of ELISA plates, i.e., Falcon and Dynatech, and 2 different antibody coating methods were compared. Ochratoxin-peroxidase concentration of 100 ng/well (50 μL) produced the most satisfactory results as determined in the optimization experiments, so this concentration of toxin-HRP was used in all subsequent titrations. As shown in Figure 1, coating antibody by the DD method on the Falcon plates (curve FD) gave a satisfactory titration curve within the maximal absorbance of 0.77 at an antiserum dilution of 1:160. Incubation of antibody in carbonate buffer in Dynatech plates also resulted in a good titration curve with the maximum absorbance of 0.39 at 1:320 dilution (curve DB). Controls run with preimmune sera indicated that toxin-HRP bound in a similar manner with only neligible levels of nonspecific binding. Coating antibody into Dynatech plates by the DD method (curve DD) generally was less satisfactory.

Table 1. Production of antibody against ochratoxin A by different protein-toxin conjugates[a]

Week of immunization	Toxin-BSA		Toxin-EDA-BSA	
	Rabbit			
	1	2	3	4
8 (1)[b]	55[c]	281	61	322[c]
9 (2)	400	299	74	125
10 (3)	175	322	106	196
16 (1)	87	115	172	263
18 (3)	200	285	476	256
22 (1)	59	61	52	114
23 (2)	50	50	32	111
24 (3)	64	91	41	167

[a]Titer values determined by RIA were defined as the reciprocal of the amount of antiserum (mL) needed to give 50% binding of ^{3}H-toxin under stated conditions. Antibody titer values were negligible before 6 weeks after initial immunization.
[b]Number in parentheses indicates number of weeks after booster.
[c]Antibody titers for rabbits 1 and 4 at the 8th week, as determined by ELISA, were 1.08×10^4 and 1.32^4, respectively.

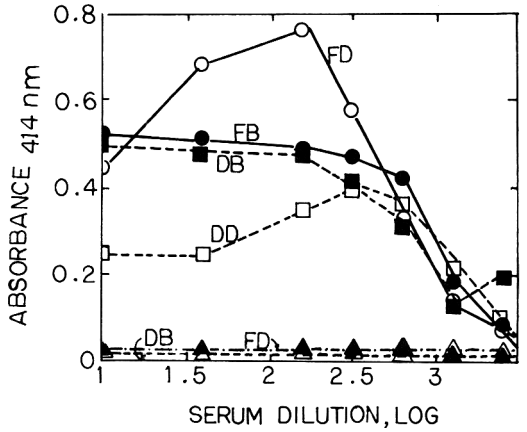

Figure 1. ELISA titration curves for ochratoxin A antiserum on Falcon (F, circles) and Dynatech (D, squares) plates coated by different methods. FD (-○-○-): drying antiserum directly on F; FB (-●-●-): coating antiserum on F by bicarbonate method; DD (—□—□—): drying antiserum on D; DB (—■—■—): coating antiserum on D by bicarbonate method. Preimmune serum coated to F by drying method, FD (—▲—▲—) and to D by bicarbonate method, DB (-△—△—) was used as control.

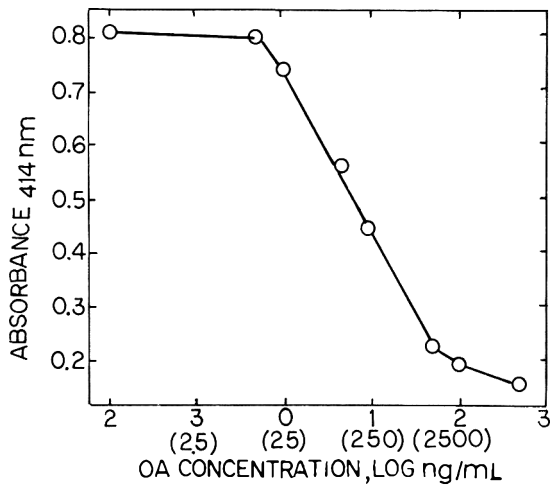

Figure 2. Standard curve for ELISA of ochratoxin A. Results are averages of quadruplicate analyses. Values in parentheses (X-axis) are in pg ochratoxin A/ assay well.

Competitive ELISA

Competitive assays were carried out either on Falcon plates (antibody coated by the DD method) or Dynatech plates (antibody coated by the bicarbonate technique). A typical competitive standard curve obtained on the Falcon plates is shown in Figure 2. The results indicated that the displacement of toxin-HRP from the antibodies by free ochratoxin A was most efficient between 25 and 1250 pg/assay. The minimal detectable level for ochratoxin A was about 25–50 pg/assay.

Efficiency of Extracting and Cleanup Procedures

To test the efficiency of the methods used in the present study, ^{3}H-labeled toxin (85 000 dpm) together with unlabeled toxin was added to the wheat samples to final concentrations of 1, 5.8, and 30.8 ppb, and the recovery of radioactivity in each step was determined. Results showed that 75–85% of toxin added to wheat was recovered after the first methanolic extraction, and 92.1–93.5% was recovered after all combined methanol extractions. Cartridge treatment of crude extracts did not result in significant loss of toxin, and 86.5–89.6% of added toxin was recovered. Solvent partition procedure and solvent partition plus cartridge cleanup appeared to lose more

toxin than use of cartridge alone. Recovery of toxin after these treatments was generally in the range 73.3–78.7%.

Effect of Wheat Extracts on ELISA

Results on the effect of wheat extracts obtained from different treatments on the binding of toxin-HRP to the antibody are shown in Table 2. When extracts equivalent to 1 g original wheat/mL (25 mg/well) prepared by the cartridge cleanup procedure were used, no detectable interfering effect on the ELISA system was observed. However, the assay system could not tolerate extracts containing more than 0.4 g wheat/mL (10 mg/well), which were prepared by the solvent partition method only. It is apparent that the cartridge cleanup step removed considerable amounts of interfering substances, while the solvent partition procedure had little effect on removing interfering substances.

Recovery Test by ELISA

To test the efficiency of ELISA of ochratoxin A in wheat flour, samples were spiked with standard toxin in the range 0.2–30 ppb and were subject to different treatments. Results (Table 3) showed that the best recovery of added toxin was obtained in samples that were treated by the solvent partition plus cartridge cleanup; at the 0.5 ppb level, an estimate of 89.6% ± 10.8% of added toxin was recovered. However, cartridge treatment only produced comparable, satisfactory results at the 1.0 ppb level, with recovery of 87.6% ± 10.8% of added toxin. After solvent partition, the minimal detectable level was 5 ppb in which 81.6 ± 2.8% of added toxin was recovered.

Discussion

Although several analytical methods have been developed for determining ochratoxin A in agricultural commodities, most of them are insensitive and time consuming (6–9). Some of the newer techniques such as LC (10–12, 24) have the ability to detect as low as 0.5–5 ppb toxin in bakery products and cereals; however, LC requires specially trained personnel and instrumentation. In addition, sample cleanup is still necessary to achieve high sensitivity and only one sample can be analyzed at a time. Results obtained from our study indicate that ELISA can be used as an alternative method for ochratoxin A analysis with an additional advantage of analyzing a large number of samples at one time.

Because the lower detection limit for ochratoxin A by ELISA is 1–2 ng/mL (25–50 pg/assay), and also because the assay system can tolerate about 1 g wheat/mL (25 mg/assay) after cartridge cleanup (Table 2), ELISA should allow ochratoxin A to be detected at 1.0–2 ppb. The recovery experiments confirm such observations (Table 3). Use of the combination of solvent partition and cartridge cleanup slightly improved cleanup efficiency. The assay system can tolerate up to 2 g extract/mL (50 mg/assay); thus with ELISA, ochratoxin A can be detected at 0.5–1.0 ppb. However, this procedure is time consuming and may not be suitable for routine assays. The solvent partition procedure alone was not effective in removing interfering substances for ELISA. The sensitivity for ELISA could be improved further by using more extensive cleanup steps. On the other hand, because extracts equivalent to 50 mg extract/mL (1.25 mg/assay) without any cleanup did not show any interfering effects, the cleanup step can be omitted when the contamination level is greater than 30 ppb.

It has been noted that selection of the solid phase and method of antibody coating on the solid phase is very important for the success of ELISA (25–27). In this study, the method of drying antibody directly onto the plates was suitable for Falcon plates and the method of incubating antibody in 0.1M, pH 9.6 sodium carbonate buffer overnight at 4°C was appropriate for Dynatech plates. These data are consistent with the result obtained by Pestka and Chu (27) and further reiterate the importance of selecting appropriate brands of plates and coating methods for ELISA.

Table 2. Effect of wheat extracts prepared by different cleanup procedures on binding of toxin-peroxidase to toxin-antibody

Ext equiv. to original sample, mg/well	Inhibition[a]			
	Methanol ext	Solv. partn	Sep-Pak	Solv. partn plus Sep-Pak
1.25	0	—	—	—
5.0	4.7	0	—	—
10.0	20.8	8.5	—	—
25	34.7	20.8	0	0
50	41.5	39.6	9.4	0
100	44.2	52.8	27.4	10.8
150	—	—	39.6	18.9
200	—	—	48.9	37.5

[a]See text for calculation.

Table 3. Effect of sample cleanup on recovery (%) of ochratoxin A from wheat by ELISA[a,b]

Toxin added, ppb	Solv. partn	Sep-Pak	Solv. partn plus Sep-Pak
0.2	—	322.0 ± 43.5	171.2 ± 35.0
0.5	703.0 ± 122.4	182.0 ± 32.0	89.6 ± 10.8
1.0	492.0 ± 96.0	87.6 ± 10.8	86.6 ± 9.2
2.5	166.4 ± 31.2	85.2 ± 14.4	77.6 ± 16.2
5.0	81.6 ± 12.8	88.3 ± 15.1	72.0 ± 6.5
30	80.0 ± 11.3	90.3 ± 10.1	75.3 ± 15.3

[a]Extractions performed on single set of 50 g samples.
[b]Data are means of 7–8 analyses ± standard deviation.

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Determination of Aflatoxin M_1 in Milk by Liquid Chromatography with Fluorescence Detection

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A liquid chromatographic (LC) method is proposed for the determination of aflatoxin M_1 in milk. The method was successfully applied to both liquid whole and skim milk and also whole and skim milk powder. The samples are initially extracted with acetonitrile–water followed by purification using a silica gel cartridge and a C_{18} cartridge. Final analysis by LC was achieved using a radial compression module equipped with a 5 μm C_{18} column and a fluorescence detector. The method was successfully applied to samples at levels of 10 to 0.08 ppb added aflatoxin M_1 with recoveries in the range of 70–98%.

The contamination of a large variety of nuts by aflatoxins produced by fungi such as *Aspergillus flavus* and *A. parasiticus* presents a need for screening and evaluation methods, because of the carcinogenic potential of the aflatoxins (1–5). The detection and evaluation in milk is of prime importance. Groundnuts and extracted groundnuts from oil refinery, mixed with milk, are commonly used to feed cattle. Aflatoxin B_1, the more potent carcinogen at the 1% level, is excreted as aflatoxin M_1 in the milk. This metabolite has the same toxicity as aflatoxin B_1 toward animals (6).

Published methods such as 2-dimensional thin layer chromatography (7), column chromatography, and liquid chromatography (8, 9) with an extended cleanup either are not sufficiently sensitive or are too tedious. In this paper, we describe a method using acetontrile–water as the extracting solvent. The sample is purified by using a commercial silica gel cartridge followed by a C_{18} reverse phase cartridge (10–12). The limit of detection was 0.08 ppb, and aflatoxin M_1 was confirmed by using the trifluoroacetic acid (TFA)-catalyzed water derivative according to Beebe (13).

Experimental

Apparatus

(a) *Wrist-action shaker.*—Burrell Corp., Pittsburgh, PA 15219.

(b) *Liquid chromatograph.*—Series 4, with No. 7125 Rheodyne loop injector (Perkin Elmer Canada Ltd).

(c) *Fluorescence detector.*—Model LS-4 fluorescence spectrometer with separate excitation and emission monochromators (Perkin Elmer Canada Ltd).

(d) *Liquid chromatographic column.*—10 cm × 5 mm Radial-Pak 5 μm C_{18} cartridge used in conjunction with radial compression separation system (Waters Associates Inc., Milford, MA 01757).

(e) *Laboratory data system.*—Model 3354 (Hewlett-Packard Co., Avondale, PA 19311).

(f) *Mixer.*—Thermolyne Maxi Mix.

(g) *Filter paper.*—7.0 cm, Whatman No. 41.

Reagents

(a) *Solvents.*—Distilled in glass (Caledon, Georgetown, Ontario, Canada). Chloroform had an ethyl alcohol content of 0.75%.

(b) *Adsorbents.*—Sep-Pak silica and Sep-Pak C_{18} cartridges (Waters Associates Inc.).

(c) *Mobile phases.*—Sep-Pak silica cartridge: chloroform and chloroform–methanol (95 + 5). Sep-Pak C_{18} cartridge: acetonitrile–water (35 + 65). Radial-Pak C_{18} column: 0–5 min, acetonitrile-water (2 + 8); 5–10 min, acetonitrile-water (3 + 7); 10–15 min, acetonitrile-water (4 + 6); 15–20 min, acetonitrile-water (4.5 + 5.5).

(d) *Trifluoroacetic acid (TFA).*—PCR Research Chemicals Inc., Gainesville, FL 32602.

(e) *Aflatoxin M_1.*—Gift from H. P. Van Egmond (National Institute of Public Health, PO Box 1, 37220BA, Bilthoven, The Netherlands).

(f) *Aflatoxin M_1 analytical standard.*—Dissolve aflatoxin M_1 in acetonitrile–water (2 + 8) to prepare 10 pg/μL standard.

Extraction

Transfer 50 mL milk or 50 g powdered milk to 250 mL glass-stopper Erlenmeyer flask, add 200 mL acetonitrile–water (80 + 20), stopper, and shake 1 h, using wrist-action shaker set at fast rate. Filter sample through Whatman No. 41 paper, and rinse paper twice with 50 mL portions of acetonitrile. Evaporate to ca 50 mL over 50°C water bath, using rotary evaporator. Add 100 mL $CHCl_3$, shake, and transfer to 250 mL separatory funnel. Drain lower organic layer through bed

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of Na_2SO_4 into 300 mL round-bottom flask. Partition aqueous layer with another 100 mL $CHCl_3$ and combine lower layer with first fraction in round-bottom flask. Evaporate to dryness over 50°C water bath, using rotary evaporator. Dissolve residue in 2–3 mL $CHCl_3$.

Cleanup

Attach Sep-Pak silica cartridge to 10 mL glass syringe. Transfer residue from round-bottom flask to syringe. Elute entire residue into cartridge. Wash round-bottom flask with two 3 mL portions of $CHCl_3$ and quantitatively transfer to syringe, again eluting washings from round-bottom flask into cartridge. Discard $CHCl_3$ washings from cartridge. Add 5 mL chloroform–methanol (95 + 5) to syringe, again eluting into cartridge. Collect entire $CHCl_3$–methanol fraction containing aflatoxin M_1 in 15 mL graduated centrifuge tube. Evaporate solvent to dryness over hot water bath, using gentle stream of nitrogen.

Attach Sep-Pak C_{18} cartridge to 10 mL glass syringe. Transfer residue from silica gel cartridge with 1 mL acetonitrile–water (35 + 65) and transfer to syringe eluting into C_{18} cartridge. Add another 3 mL acetonitrile–water (35 + 65) to centrifuge tube from previous step, again eluting into C_{18} cartridge. Collect entire acetonitrile–water fraction and retain for aflatoxin M_1 analysis by LC.

Preparation of Standard Curve

Set LC flow rate to 1.0 mL/min. Set fluorescence detector parameters as follows: excitation wavelength, 357 nm; emission wavelength, 425 nm; excitation slit, 15 nm; emission slit, 20 nm; set recorder scale to 75.

Use aflatoxin M_1 analytical standard (**f**) to prepare analytical calibration curve by plotting different amounts of M_1 (pg) vs observed peak area. Under above conditions, 10 pg aflatoxin M_1 could be easily detected, with retention time of 18.1 min.

Confirmation

Evaporate sample from C_{18} cartridge, which was previously analyzed by LC, to dryness under gentle stream nitrogen. Add 100 μL TFA and vortex-mix. Dilute sample with acetonitrile–water (2 + 8) to same volume as before evaporation. Inject into LC system under identical conditions as for aflatoxin M_1. Repeat above step with standard. Under above conditions, retention time of derivatized aflatoxin M_1 standard or sample is 16.1 min.

Table 1. Recovery of aflatoxin M_1 added to milk[a]

Sample	Added, ppb	Rec., %
Skim milk powder	10	91.0
SD		3.6
CV, %		3.9
Whole milk powder	1	97.3
SD		3.0
CV, %		3.0
Whole milk	1	97.6
CD		4.0
SV, %		4.0
Whole milk	0.5	90.0
SD		9.5
CV, %		10.5
Whole milk powder	0.2	82.5
SD		4.9
CV, %		5.9
Whole milk	0.08	70.0
SD		10.0
CV, %		9.0

[a]Results of triplicate analysis.

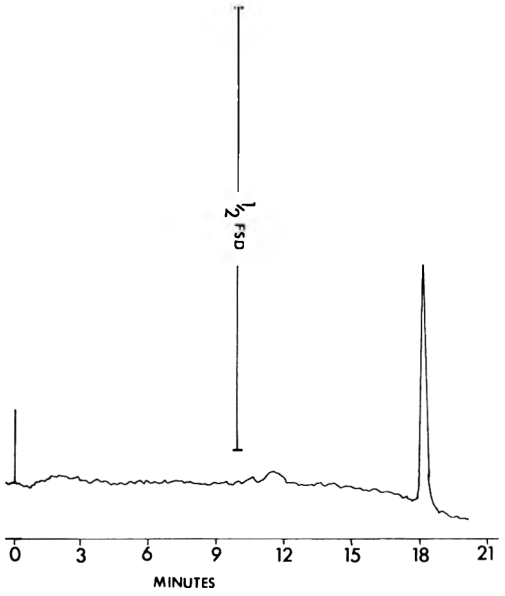

Figure 1. Chromatogram of 40 pg aflatoxin M_1. See text for LC conditions.

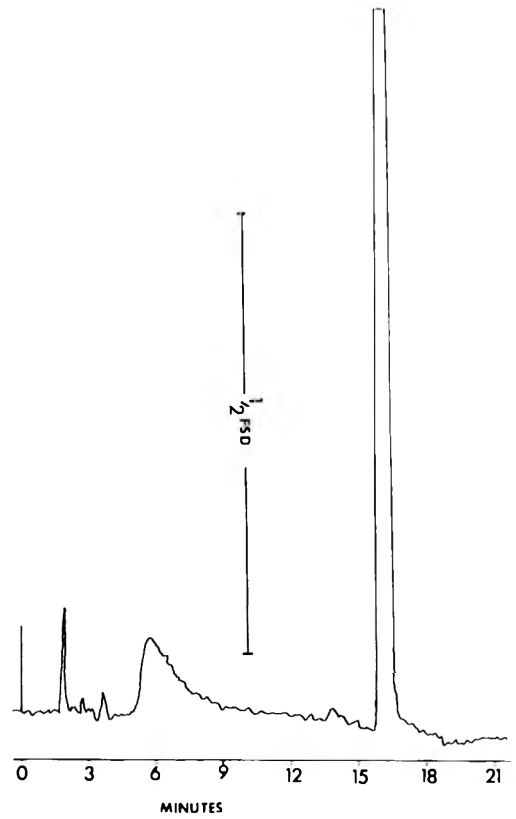

Figure 2. Chromatogram of 50 mL whole milk blank sample in 0.5 mL. Injection volume 2 μL. See text for LC conditions.

Results and Discussion

Use of the Radial-Pak 10 cm × 5 mm column reduced required operating pressure, and the larger diameter column also allowed an increase in sample size. The radial compression separation system allowed us to increase the organic content of the mobile phase while still achieving baseline separation. The linear solvent gradient program allowed elution of more polar material while retaining aflatoxin M_1. With the increase of the organic phase, aflatoxin M_1 eluted with a retention time of 18.1 min (Figure 1).

The Sep-Pak silica cartridge provided a quick and easy purification step before final purification by the C_{18} cartridge.

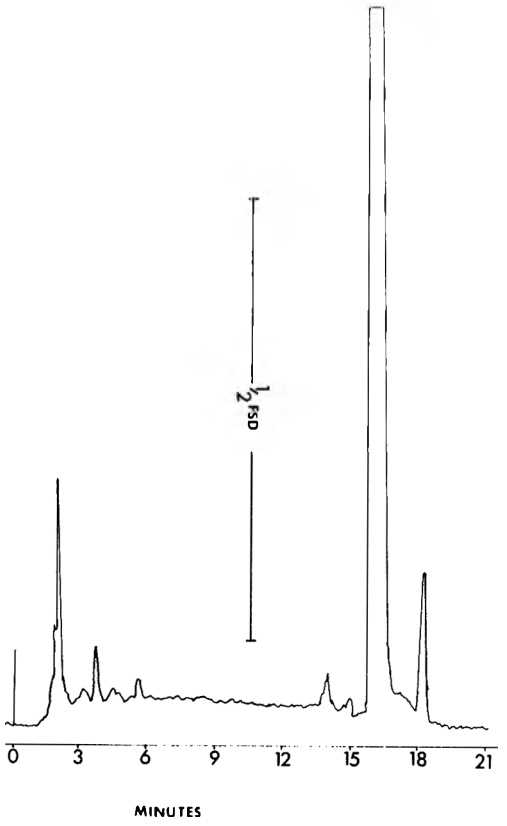

Figure 3. Chromatogram of 50 mL whole milk with 4 ng added aflatoxin M_1 in 0.5 mL. Injection volume 6 μL. See text for LC conditions.

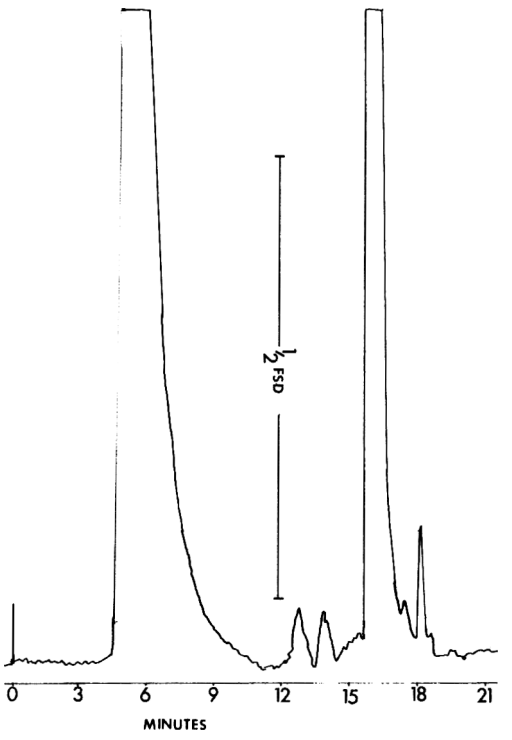

Figure 4. Chromatogram of 9.5 g whole milk powder in 1 mL showing presence of 0.4 ppb aflatoxin M_1. Injection volume 5 μL. See text for LC conditions.

Aflatoxin M_1 is totally eluted from the reverse phase cartridge with minimal solvent.

Aflatoxin M_1 was completely resolved from other contaminants in the milk by using the above purification system. Aflatoxin M_1 was confirmed using the TFA reaction. Most samples contained artifacts that were not resolved from the derivatized aflatoxin M_1. The disappearance of the parent aflatoxin M_1 was judged satisfactory for confirmation.

Aflatoxin M_1 gave a linear response from 10 to 200 pg.

Representative recoveries of aflatoxin M_1 added to different types of milk are shown in Table 1. All samples were analyzed in triplicate along with a separate blank. Recoveries ranged from 70 to 98%.

Figure 1 shows a typical chromatogram of 40 pg aflatoxin M_1. Figure 2 is a chromatogram of a blank whole milk sample, showing no interference. Figure 3 is a chromatogram of whole milk spiked at 0.08 ppb; Figure 4 is a chromatogram of whole milk powder showing the presence of 0.4 ppb aflatoxin M_1.

In conclusion, the method described allowed detection of aflatoxin M_1 in different types of milk at levels as low as 0.08 ppb. There were no discernible interferences in the final analysis by LC. The solvent gradient program permitted resolution of unknowns in the sample, which prevented interference in the analysis of aflatoxin M_1. Only 5min equilibration was needed before another injection could be made.

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Rapid Determination of Deoxynivalenol (Vomitoxin) by Liquid Chromatography Using Modified Romer Column Cleanup

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A modification of the Romer method for determining deoxynivalenol (DON) provides rapid sample cleanup and includes liquid chromatographic (LC) quantitation. The method was evaluated using wheat, wheat flour, and other wheat products. The sample is extracted with acetonitrile–water (84 + 16), and an aliquot of the extract is subjected to activated charcoal–alumina column chromatography. The extract is then evaporated and diluted to volume with mobile phase, and DON is quantitated using liquid chromatography. The relative standard deviation based on duplicate samples is 6.07%. The detection limit is 30 ppb based on 2 × signal/noise ratio. Results by this method compared with the results obtained by the Scott GC method showed a correlation coefficient of 0.992 with a mean vomitoxin content of 779 ppb by this method and 716 ppb by the Scott method for 14 samples.

The recent occurrence for deoxynivalenol (vomitoxin) is scab-damaged wheat in the central United States had prompted the U.S. Food and Drug Administration to establish certain "levels of concern" (1). Although it is still difficult at present to estimate the potential public health hazard posed by this toxin, monitoring deoxynivalenol is becoming a major concern in the grain and food industries. A routine rapid method of isolating and quantitating deoxynivalenol (DON) will be of great importance and convenience to the industries and regulatory agencies monitoring wheat and other samples.

Numerous methods of analysis of DON have been published since its discovery in 1972 in Japan (2, 3). An extensive review of methods dealing with trichothecenes in general was published by Scott in 1982 (4). Schweighardt et al. (5) and Bennett et al. (6) investigated the use of liquid chromatography (LC) for DON quantitation.

By modifying the Romer method of sample cleanup (7) to increase the efficiency of analyte recovery from the charcoal–alumina column and by incorporating a sensitive UV detector, using a cadmium lamp with a UV filter at 229 nm, a rapid and sensitive LC method is obtained. This method was investigated for possible use in routine assay of DON in wheat, wheat flour, and wheat products. A comparison of this method with the Scott GC method (8) has also been carried out.

METHOD

Apparatus

(a) *LC pump.*—Model 110A, constant flow (Beckman Instruments, Inc., 1780 Fourth St, Berkeley, CA); or equivalent.

(b) *LC injector.*—Model LC420 autosampler equipped with 20 μL loop (Perkin Elmer, Norwalk, CT), or equivalent.

(c) *LC detector.*—Model 160 UV detector with cadmium lamp and 229 nm filter (Beckman), or equivalent. Set sensitivity to 0.005 AUFS.

(d) *LC column.*—Reverse phase C_{18} 4.6 mm id × 25 cm Zorbax (Du Pont, Wilimington, DE), or equivalent.

(e) *Activated charcoal–alumina column and reservoir.*—Myco Lab Co., PO Box 321, Chesterfield, MO.

(f) *Vacuum pump or water aspirator.*—Capable of producing minimum vacuum of 15 in. Hg (Figure 1).

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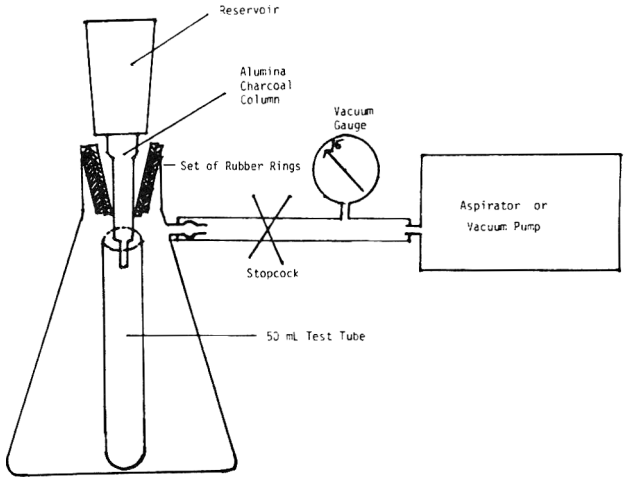

Figure 1. Sketch of cleanup column assembly.

Reagents

(a) *Extracting solvent.*—Combine 840 mL acetonitrile and 160 mL water and mix well.

(b) *Cupric carbonate basic.*—Mallinckrodt, or equivalent.

(c) *Deoxynivalenol standard.*—Obtained from Myco Lab Co. *(1) DON stock solution.*—20 μg/mL. Dissolve 2.0 mg DON in acetone and dilute to volume of 100 mL with acetone in volumetric flask. Store solution in freezer. *(2) LC working standard.*—2.0 μg/mL. Pipet 1.0 mL stock solution into 10 mL volumetric flask, evaporate to dryness under N_2. Redissolve in 10 mL mobile phase, using vortex mixer for minimum of 20 s.

(d) *LC mobile phase.*—Combine 230 mL methanol and 770 mL water; stir slowly and continually during use. Degas solution under vacuum if it is to be used same day as preparation. Flow rate 1.0 mL/min.

Calibration of Column Activity

Set up activated charcoal-alumina column as follows: Place 50 mL test tube or centrifuge tube into 1 L suction flask. Insert column fitted with reservoir on top of test tube, with hollow rubber ring surrounding reservoir/column to make system air-tight as shown in Figure 1. Pipet 1 mL stock solution (1) (20 μg/mL) into 10 mL volumetric flask. Evaporate to dryness under N_2. Redissolve in 10 mL extracting solvent, using vortex mixer. Transfer this solution into column, using 5 mL extracting solvent to rinse flask. Proceed as in determination and LC quantitation steps below. Determine by LC the quantity of DON recovered. Recovery should be >80%. If recovery is <80%, repeat recovery test, using two 10 mL portions of extracting solvent to elute toxin. Recovery should be >80%; otherwise, repeat test using three 10 mL portions of eluting solent. Record number of 20 mL aliquots necessary for >80% recovery.

Preparation, Extraction, and Determination

Grind sample to pass 30 mesh screen. Weigh 50 g ground sample into 40 oz Waring blender; add 10 g basic cuprice carbonate and 200 mL extracting solvent. Blend 3 min at high

speed. Filter through 24 cm 2V paper and collect portion of filtrate. Pipet 10 mL (represents 2.5 g sample) onto top of activated charcoal-alumina column with number of 10 mL portions of eluting solvent determined in calibration.

Start vacuum pump or aspirator. When solvent reaches top of packing, pipet 10 mL extracting solvent (NOT the extract) into column and continue suction until solvent reaches top of packing again. Repeat number of elutions determined during column calibration for recovery >80%. Note: Do not interrupt vacuum once siphoning is underway; otherwise, partial bubbling of eluant will cause loss of DON.

Quantitatively transfer eluted solvent extract in test tube to 200 mL round-bottom flask with aid of acetonitrile in squeeze bottle. Rinse tube with acetonitrile from squeeze bottle to complete transfer. Evaporate acetronitrile to dryness in rotary evaporator and quantitatively transfer extract to 1 dram vial with acetone. Evaporate acetone to dryness under gentle flow of N_2 with gentle heating. Immediately dissolve dried extract in 2.0 mL mobile phase for LC by vortex-mixing vial ca 20 s.

LC Quantitation

Set flow rate at 1.0 mL/min and let column and detector equilibrate 30–45 min. Repeat injection of standard solution until peak heights are constant (Figure 2). Inject sample extract interspersed with standards as frequently as necessary to assure accurate quantitation.

Calculations.—

$$\text{DON, ng/g or ppb} = (C \times PH \times V_1)/(PH' \times W)$$

where C = concentration of DON standard in ng/μL (2 ng/μL); PH and PH' = sample and standard peak heights, respectively; V_1 = μL injection solvent used to dissolve sample or test extract; W = weight of sample (2.5 g).

Results and Discussion

In our experience, this method is quite straightforward and requires considerably less time for sample extraction and cleanup compared with the Scott method. Assuming that LC and GC procedure require approximately the same amount of time, comparable analytical results can be attained with a considerable savings of time for sample preparation.

Fifteen samples of wheat and wheat products were ground and analyzed in duplicate by this method and also analyzed by the Scott method using GC quantitation. The results are shown in Table 1. Chromatograms of 2 different samples are shown in Figures 3 and 4. These samples were naturally contaminated hard red winter wheat and flour from similar wheat, obtained from various areas of the midwest United States in the summer of 1982. Based on duplicate analysis, the relative standard deviation of this method is 6.1%. Relative standard deviations of 10–10.2% have been reported for the GC method (5). For the 15 samples quantitated by the LC method and the GC method, the results were comparable. The mean value obtained by LC was 779 ppb and by GC, 716 ppb. The standard deviation of the difference between methods was 112 ppb with a correlation coefficient of 0.992; the hypothesis of no difference between methods is accepted at the 0.05 level of significance.

Recovery results for artificially contaminated samples spiked with various concentrations of standard DON in acetone and analyzed in duplicate were 86.6 and 91.2 ppm at 88 ppm spike; 175 and 174 ppm at 176 ppm spike; 348 and 366 ppm at 440 ppm spike, and 1050 and 1080 ppm at 1320 ppm spike. Clean wheat and corn were used that had been analyzed first to determine the absence or presence of DON before spiking. The average recovery was 90.6% (relative standard deviation 3.24% based on duplicates). It should be noted that the recovery is nearly 100% at the lower DON levels.

To confirm the purity of the DON peak, a sample extract containing 11.7 ppm DON was selected and the ratio of detector response of 229 to 254 nm (7444/1704 = 4.37) was compared with the ratio of response of standard DON (3552/814 = 4.36).

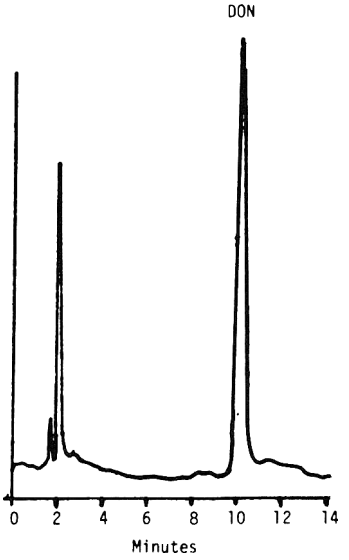

Figure 2. Chromatogram of DON standard, 40 ng/20 μL.

Table 1. Comparison of LC and GC methods for determining DON in wheat (ppb)

Sample	GC method	LC method	
		Dupl.	Av.
Flour	295	261 241	251
Flour	472	546 497	522
Flour	1030	1280 1320	1300
Flour	1190	1210 1250	1230
Macaroni	132	170 191	181
Wheat	—	1700 1670	1690
Wheat	3430	3520 3430	3480
Wheat	724	606 679	643
Wheat	769	912 1030	971
Wheat	723	979 1020	1000
Flour	267	256 231	244
Wheat	122	138 137	138
Wheat	150	157 170	164
Wheat	363	349 341	345
Wheat	359	440 429	435
Mean	716		779

Between-method standard deviation = 112 ppb;
Correlation coefficient = 0.992.

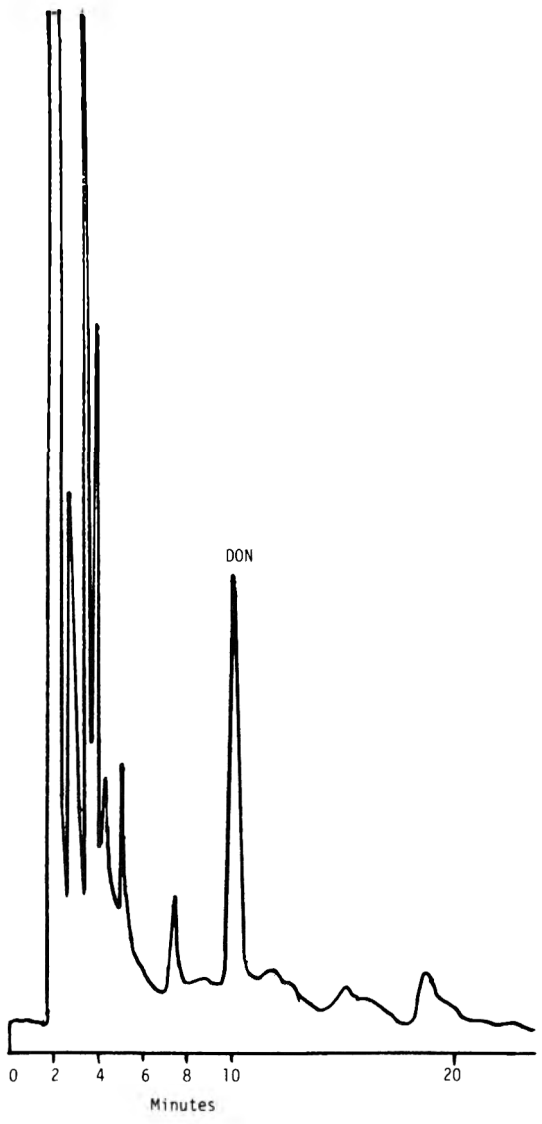

Figure 3. Chromatogram of sample of naturally contaminated wheat screenings. Peak represents 36.7 ng DON injected, equivalent to 11.7 ppm DON in sample. Final dilution of extract was 16.0 mL.

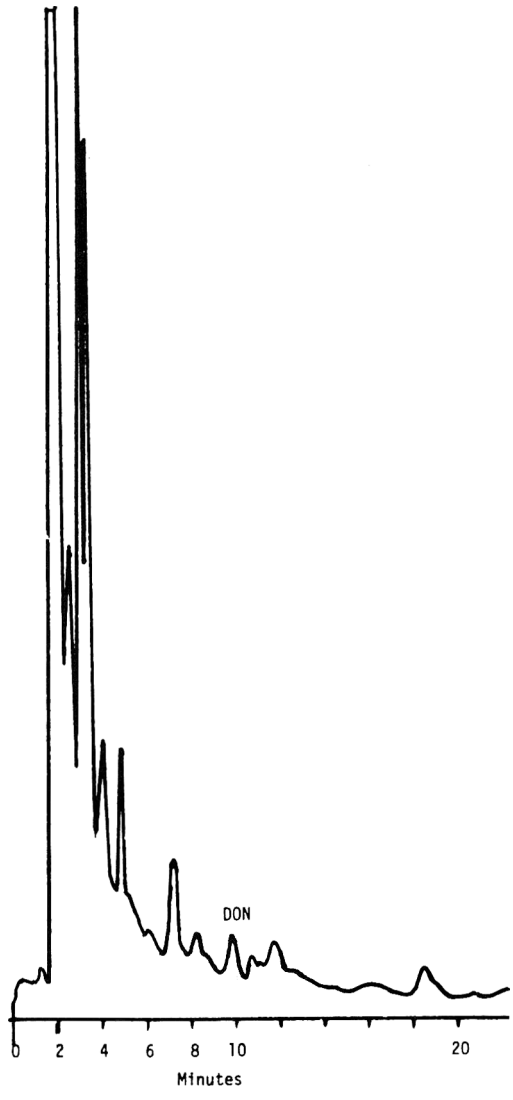

Figure 4. Chromatogram of sample of naturally contaminated wheat. Peak represents 2.89 ng DON injected, equivalent to 115 ppb DON in sample. Final dilution of extract was 2.0 mL.

In this study, we found that the recovery of the original Romer extraction and charcoal–alumina column cleanup can be improved by assuring that the quantity of extraction solvent used is sufficient to remove all DON from the column. For this reason, each new batch of columns should first be calibrated before use.

Acknowledgment

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COLOR ADDITIVES

Reverse Phase Liquid Chromatographic Determination of Intermediates and Subsidiary Colors in D&C Green No. 5

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A reverse phase liquid chromatographic method is presented for the simultaneous determination of 2-amino-5-methylbenzenesulfonic acid, 5-amino-2-methylbenzenesulfonic acid, External D&C Violet No. 2, and the lower sulfonated subsidiary color in D&C Green No. 5. The method uses octadecylsilanized silica gel as the stationary phase and gradient elution with a mobile phase of aqueous acetonitrile solutions containing ammonium acetate. A wide dynamic range is achieved in a single analysis with quantitation of the intermediates at relatively low levels, up to 0.2% (w/w) each, and subsidiary colors at relatively high levels, up to 3.0% (w/w) each. In addition, the method separates the 3 primary disulfonated isomers of D&C Green No. 5 as well as numerous unidentified components. The results of a recovery study and a survey of 16 commercial samples and the pharmacology sample are presented. Recoveries of the intermediates and subsidiary colors from samples of D&C Green No. 5 ranged from 96.8 to 103.8%. The calibration and recovery data are treated statistically to evaluate the performance of the method.

D&C Green No. 5 (Colour Index No. 61570) is one of several regulated color additives belonging to the anthraquinoid class of dyes. It is currently permitted for use in coloring ingested drugs, externally applied drugs and cosmetics, and nonabsorbable surgical sutures (1).

D&C Green No. 5 is manufactured by the sulfonation of 1,4-bis[(4-methylphenyl)amino]-9,10-anthracenedione (D&C Green No. 6) with fuming sulfuric acid (2–4). The principal product of the reaction is 2,2′-[(9,10-dihydro-9,10-dioxo-1,4-anthracenediyl)diimino]-bis(5-methylbenzenesulfonic acid) (*o,o*-isomer) (Figure 1), which is the structure generally shown for D&C Green No. 5. Consideration of the normal isomeric distribution observed during aromatic sulfonation reactions (5) suggests that significant levels of isomeric products are also formed.

Principal among the disulfonated products are 2-[[9,10-dihydro-9,10-dioxo-4-[(4-methyl-3-sulfophenyl)amino]-1-anthraceneyl]amino]-5-methylbenzenesulfonic acid (*o,m*-isomer) (Figure 2) and 5,5′-[(9,10-dihydro-9,10-dioxo-1,4-anthracenediyl)diimino]-bis(2-methylbenzenesulfonic acid) (*m,m*-isomer) (Figure 3).

Reverse phase liquid chromatography (LC) with the use of buffered eluants has been found to be applicable to the analysis of aromatic sulfonated compounds (6–8), including synthetic organic dyes (9). When this technique was used in the investigation of the chemical composition of D&C Green No. 5, it became apparent that, in addition to providing a powerful research tool, it would also be useful for the quantitation of intermediates and subsidiary colors as required during enforcement of the color additive specifications. Currently, the intermediates in D&C Green No. 5 are determined by elution chromatography and solvent extraction (10, 11), and the subsidiary colors are determined by thin layer chromatography (TLC) (12).

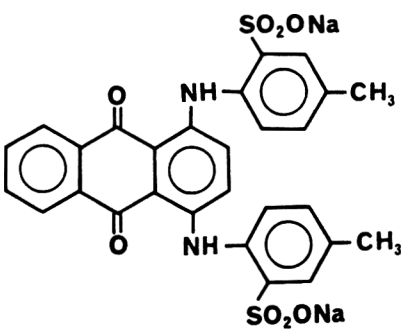

Figure 1. D&C Green No. 5 (*o,o*-isomer).

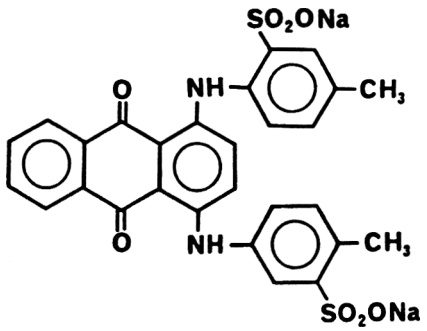

Figure 2. *o,m*-Isomer of D&C Green No. 5.

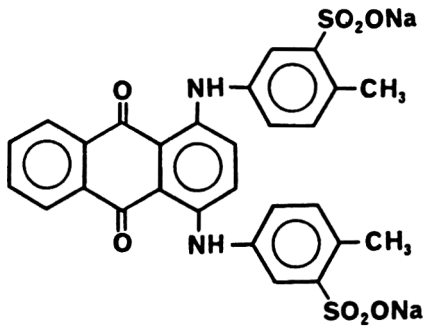

Figure 3. *m,m*-Isomer of D&C Green No. 5.

Because D&C Green No. 5 is manufactured by the sulfonation of D&C Green No. 6, the latter compound is, strictly speaking, the "intermediate" for the color additive. However, for the purpose of regulating the composition of the color additive, the intermediates are considered to consist of "sulfonated toluidines," and these are limited to a total of not more than 0.2% by weight of D&C Green No. 5 (1). The sulfonated toluidine constituents arise from the sulfonation of *p*-toluidine, which is a contaminant of D&C Green No. 6.

The principal monosulfonated *p*-toluidine is 2-amino-5-methylbenzenesulfonic acid, commonly known as *p*-toluidine-*m*-sulfonic acid (PTMS) (Figure 4), with smaller amounts of 5-amino-2-methylbenzenesulfonic acid, commonly known as *p*-toluidine-*o*-sulfonic acid (PTOS), also being formed (Figure 5).

The *o,m*- and *m,m*-isomers of D&C Green No. 5 are not considered subsidiary colors of the dye for regulatory pur-

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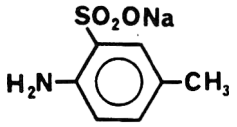

Figure 4. 2-Amino-5-methylbenzenesulfonic acid (PTMS).

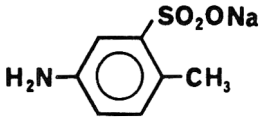

Figure 5. 5-Amino-2-methylbenzenesulfonic acid (PTOS).

poses. One of the subsidiary colors is 2-[[9,10-dihydro-9,10-dioxo-4-[(4-methylphenyl)amino]-1-anthraceneyl]amino]-5-methylbenzenesulfonic acid, a monosulfonated product from the sulfonation of D&C Green No. 6 that is referred to as the lower sulfonated subsidiary color. It should be noted that another isomeric form of the lower sulfonated subsidiary color is possible. The other principal subsidiary color of D&C Green No. 5 is 2-[[9,10-dihydro-9,10-dioxo-4-hydroxy-1-anthraceneyl]amino]-5-methylbenzenesulfonic acid (External D&C Violet No. 2) (Figure 6). This subsidiary color arises from the sulfonation of D&C Violet No. 2, which is also a contaminant of D&C Green No. 6. As with the lower sulfonated subsidiary color, another isomeric form of Ext. D&C Violet No. 2 is also possible. The amounts of lower sulfonated subsidiary color and Ext. D&C Violet No. 2 are limited by regulation to a total of not more than 3% by weight of D&C Green No. 5 (1).

The method presented here permits the determination of the sulfonated toluidine intermediates, the lower sulfonated subsidiary color, and Ext. D&C Violet No. 2 subsidiary color in a single analysis. In addition, it separates the *o,o*- and *o,m*-isomers of D&C Green No. 5 and, with modification, also the *m,m*-isomer.

Method

Apparatus and Reagents

(a) *Liquid chromatograph.*—Altex Model 312MP with 2 Model 110A pumps, Model 420 microprocessor-controller, and loop injector fitted with 50 μL loop (Altex Scientific, Inc., Berkeley, CA 94710).

(b) *Detector.*—Waters Model 440 dual wavelength UV detector operated at 254 nm (Waters Associates, Inc., Milford, MA 01757).

(c) *Liquid chromatography column.*—Bio-Rad Biosil-ODS 5S, 25 cm × 4 mm id, 5 μm particle size, Cat. No. 125-0080 (Bio-Rad Laboratories, Richmond, CA 94804).

(d) *Recording integrator*—Shimadzu Model C-R1A (Shimadzu Scientific Instruments, Inc., Columbia, MD 21045).

(e) *Acetonitrile.*—LC grade (Fisher Scientific Co., Pittsburgh, PA 15219).

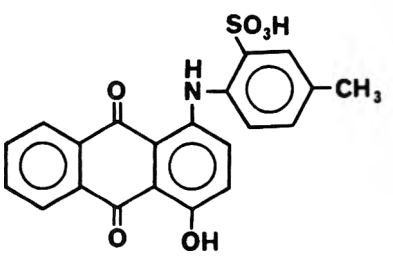

Figure 6. External D&C Violet No. 2 (*o*-isomer).

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

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

(h) *LC eluants.*—(*1*) *Solvent A.*—1.5 g ammonium acetate/100 mL 0.5% (v/v) acetonitrile in water. (*2*) *Solvent B.*—1.5 g ammonium acetate/100 mL 50% (v/v) acetonitrile in water. Prepare solvent B by placing appropriate amounts of ammonium acetate and acetonitrile in volumetric flask and diluting to volume with water.

(i) *Reference compounds.*—(*1*) *PTMS.*—Division of Color Technology (DCLT) reference compound. Purity and identity were established by elemental analysis, ^1H nuclear magnetic resonance spectrometry, and LC analysis. (*2*) *PTOS.*—Eastman Laboratory Chemicals, Rochester, NY 14650 (Cat. No. 566) and characterized in the same manner as PTMS. This compound is no longer supplied by Eastman. It is available from Pfaltz & Bauer, Inc., Stamford, CT 06902 (Cat. No. A29260). (*3*) *Lower sulfonated subsidiary color.*—DCLT reference compound used in the development of the TLC method for this compound (12). Strength and purity were checked by elemental analysis, TLC analysis, and LC analysis. It was found to be free of interfering contaminants. (The TLC procedure consisted of spotting the methanol solution of the lower sulfonated subsidiary color (10 μL solution containing 0.05 g/100 mL) on a reverse phase TLC plate (Whatman KC_{18}, Cat. No. 4803-600) and developing with methanol. This procedure allowed the detection of D&C Green No. 6 in the color as determined by comparison with an authentic sample.) (*4*) *Ext. D&C Violet No. 2.*—Certified sample AA9871. Strength and purity were established by elemental analysis and LC analysis. It was found to be free of interfering contaminants.

Analytical Parameters

(a) *LC gradient elution program.*—5 to 100% B in A (linear) in 25 min; hold at 100% B for 10 min; hold at 5% B in A for 10 min to allow column to equilibrate before injection. Flow rate is 1 mL/min.

(b) *Integrator settings for data collection and presentation.*—Width 65, slope 100, drift 55, min ar 100, t-dbl 1000, lock 3, stptm 35, attn 0, speed 5, method 241, spl wt 100, is. wt 1. These conditions provide a chart speed of 0.5 cm/min and inhibit integration for the first 3 min of the analysis. Time programmable parameter changes: time 0 min, min ar 10, attn 0; time 11 min, min ar 1000, attn 3; time 24.1 min, drift 15. An attenuation setting of 0 corresponds to 0.02 AUFS, whereas a setting of 3 corresponds to 0.16 AUFS.

Calibration

Select sample of D&C Green No. 5 which contains little background contribution for the 4 constituents. Run color blanks to determine background levels, which are subtracted from responses obtained. The dye concentrations of the color blanks are the same as that used in the analysis (0.250 g/100 mL). Prepare stock solutions for PTMS, PTOS, and Ext. D&C Violet No. 2 in water and calibrate these 3 components simultaneously by adding aliquot of each material to dye to span desired range in more or less equal steps (see Tables 1–4 for levels and range). Because of its low water solubility, prepare stock solution for the lower sulfonated subsidiary color in methanol and calibrate in absence of other components by adding appropriate aliquots to solution of dye. For each component in solution, calculate C, % by weight relative to the amount of D&C Green No. 5 in the solution:

$$C = V \times C' \times 0.4$$

Table 1. PTOS[a] calibration data

Run	t_r, min	Added, %	Area, i.c.[b]
1	4.97	0.0971	6203
2	4.95	0.194	12 422
3	4.95	0.0485	3266
4	4.93	0.243	15 348
5	4.93	0.146	9369
6	4.93	0	190
7	4.92	0	169
8	4.97	0	170
9	5.03	0	174
10	4.96	0.0728	4756
11	4.98	0.170	10 957
12	5.00	0.218	13 992
13	4.98	0.121	7842
14	4.96	0.0243	1693
15	4.96	0.0121	924

[a]*p*-Toluidine-*o*-sulfonic acid.
[b]Integrator counts.

Table 2. PTMS[a] calibration data

Run	t_r, min	Added, %	Area, i.c.[b]
1	8.78	0.160	11 415
2	8.76	0.0533	4241
3	8.75	0.213	15 145
4	8.74	0.107	7915
5	8.73	0.267	18 813
6	8.78	0	542
7	8.71	0	531
8	8.72	0	521
9	8.76	0	532
10	8.78	0.240	16 987
11	8.78	0.133	9660
12	8.79	0.187	13 326
13	8.77	0.0267	2381
14	8.78	0.080	6030
15	8.78	0.0133	1452
16[c]	8.86	0.0883	6041
17[c]	8.85	0.0442	3018

[a]*p*-Toluidine-*m*-sulfonic acid.
[b]Integrator counts.
[c]Added as calibration points during the recovery study (prepared without added dye).

Table 3. Ext. D&C Violet No. 2 subsidiary color calibration data

Run	t_r, min	Added, %	Area, i.c.[a]
1	24.41	1.48	463 911
2	24.40	1.11	336 444
3	24.38	2.78	881 158
4	24.39	2.22	697 725
5	24.41/24.70[b]	0.130	36 230
6	24.39	0.185	52 458
7	24.39	0.556	163 729
8	24.43	0.370	103 717
9	24.40	1.85	575 663
10	24.40	0.093	29 495
11	24.40	0.741	216 259
12	24.39	1.30	390 851
13	24.39	0.926	268 879
14	24.40/24.67[b]	0	3761
15	24.39/24.67[b]	0	3606
16[c]	24.40	1.123	336 297
17[c]	24.42	0.562	155 900

[a]Integrator counts.
[b]Detected as 2 responses. Area obtained by summing the responses.
[c]Added as calibration points during the recovery study (prepared without added dye).

where V = volume of aliquot of stock solution (mL) taken; C' = concentration of stock solution (mg/mL); and 0.4 = 100 (%)/250 (mg). Using integrator, measure peak areas on the chromatogram for PTMS, PTOS, Ext. D&C Violet No. 2, and the lower sulfonated subsidiary color. Obtain 11–15 calibration points for each component. Calculate regression line equation for each component according to the method reported by Bailey et al. (13).

Table 4. Lower sulfonated subsidiary color calibration data

Run	t_r, min	Added, %	Area, i.c.[a]
1	31.25	1.06	262 389
2	31.21	2.12	516 222
3	31.24	0.635	159 563
4	31.19	3.17	767 547
5	31.22	1.48	359 974
6	31.22	0.212	54 404
7	31.18	2.54	620 067
8	31.20	0.423	108 819
9	31.12	0.846	209 025
10	31.12	0.106	28 716
11	31.10	1.693	415 163
12	31.15	0	6275
13	31.13	0	6298
14	31.15	0	6304
15	31.24	0	6327
16[b]	30.93	1.97	493 376
17[b]	30.95	0.984	247 896

[a]Integrator counts.
[b]Added as calibration points during the recovery study (prepared without added dye).

Determination

Place 0.250 g D&C Green No. 5 sample in 100 mL volumetric flask and dilute to volume with water. Let solution stand, with occasional mixing, at least 1 h before analysis to ensure complete dissolution of the dye.

Inject 50 μL dye solution and chromatograph according to parameters described for LC gradient elution program. Calculate % by weight of each component in sample from corresponding regression line equation.

Recovery Study

Recovery solutions were prepared and submitted to the analyst as unknowns. Recovery samples were prepared so as to contain all 4 components in a single solution of the dye. Four levels of each component were prepared and these levels were repeated in random combinations 5 times. Five color blanks were included for the purpose of determining background levels. Also, 2 calibration solutions prepared without added dye were provided. The additional calibration points were incorporated with those previously obtained and the data were combined for calculation of the regression line equation.

Calculations

All calculations used in calibration and in the statistical analysis of the data were made according to the method reported by Bailey et al. (13), and the constituents observed in the recovery study and sample survey were quantitated by using the calibration equations.

Results and Discussion

LC Separation

A typical LC chromatogram for a spiked commercial sample of D&C Green No. 5 obtained under the conditions previously described is shown in Figure 7. PTOS and PTMS are well resolved from one another and produce sharp, symmetrical peaks, thus permitting ready detection and integration. These compounds were detected only at low levels so that the early part of the chromatogram (Figure 7) was recorded at high sensitivity (0.02 AUFS) and low peak rejection (10 integrator counts). At a gradient elution program time of 11 min (after the elution times for PTOS and PTMS—5.00 and 8.73 min, respectively), the recorder sensitivity was lowered (0.16 AUFS) and the peak rejection area was increased (1000 integrator counts) for the presentation and reporting of the remaining components of the dye.

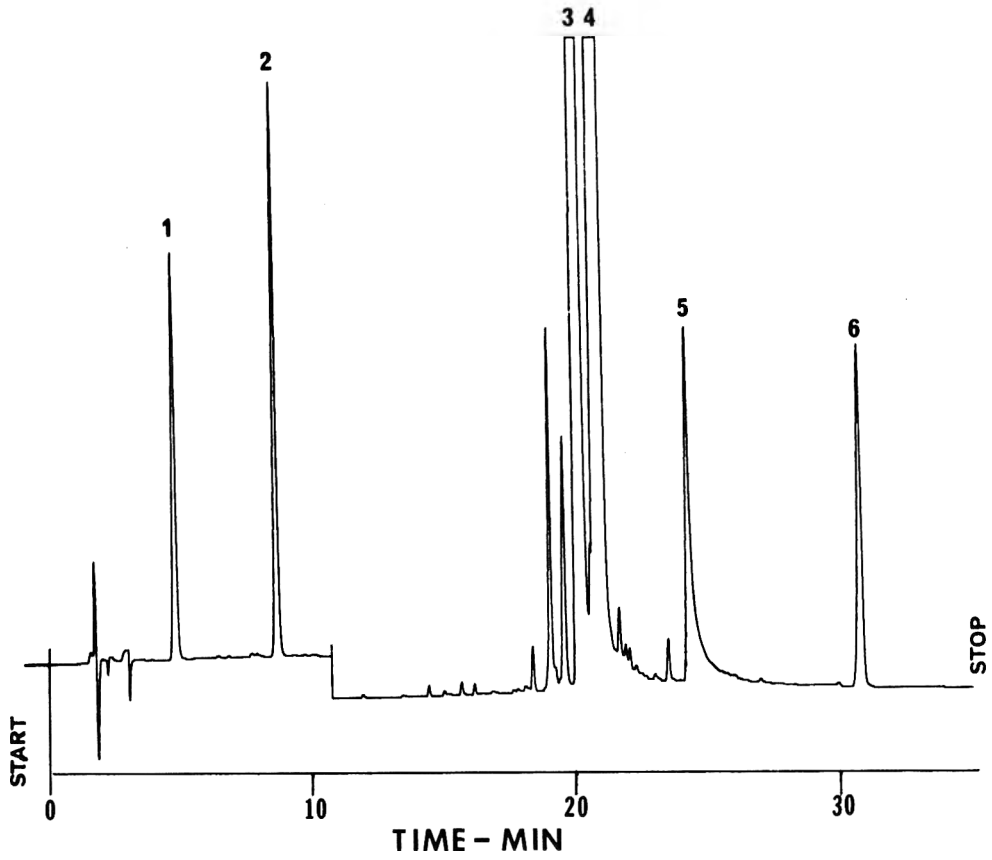

Figure 7. LC chromatogram of D&C Green No. 5 (0.250 g/100 mL). 1 = PTOS (5.00); 2 = PTMS (8.73); 3 = *o,m*-isomer (20.21); 4 = *o,o*-isomer (20.95); 5 = Ext. D&C Violet No. 2 (24.38); 6 = lower sulfonated subsidiary color (31.08). Numbers in parentheses are elution times (min).

In the case of the Ext. D&C Violet No. 2 subsidiary color, a tailing peak was obtained that suggests the possibility of a mixed retention mechanism (14). The tailing nature of this peak under the chromatographic conditions employed here presented a problem in selecting a suitable column to achieve separation and quantitation. Although columns supplied by several manufacturers were tested, only the Bio-Rad column produced peaks for Ext. D&C Violet No. 2 that were satisfactory for quantitation. Several different Bio-Rad columns were examined and all produced peaks for Ext. D&C Violet No. 2 that were of sufficient sharpness to permit detection and integration.

Attempts to quantitate Ext. D&C Violet No. 2 by using peak height measurements to generate the calibration curve were not successful because of the nonlinear relationship which was obtained. On the other hand, the use of peak area presented a problem because of peak tailing. The integrator tended to report low areas for samples of low concentration because of early sensing of the baseline. This problem was minimized by increasing the baseline sensitivity of the integrator at the elution time of the Ext. D&C Violet No. 2. This had the effect of causing the integrator to detect the tailing portion of the peak at a point closer to the original baseline and also to detect riding peaks separately.

An additional problem encountered in the detection and quantitation of Ext. D&C Violet No. 2 occurred because of the presence of the isomeric form of the subsidiary color in some dye samples. As mentioned earlier, Ext. D&C Violet No. 2 is principally the *o*-sulfonated isomer. However, the *m*-sulfonated isomer occurs to varying degrees, depending on the conditions of sulfonation; the method developed here can separate these isomers although the degree of separation depends on the absolute and relative amounts present. In some cases, the *m*-isomer is observed only as a slight shoulder on the Ext. D&C Violet No. 2 peak which the integrator does not detect as a separate peak. However, when the amount of the *m*-isomer relative to the amount of the *o*-isomer is large, the *m*-isomer may be detected by the integrator as a separate peak riding on the tail of the *o*-isomer peak. Also, when the absolute amount of these isomers is small, they may be detected as separate peaks. In those cases where the integrator detected 2 peaks, the areas were added together to produce a composite response which was then employed in the calculations. This procedure will not exert a significant effect on the results; the areas would be expected to be additive, and the response factors for the 2 isomers are similar because the absorption spectra and molar absorptivities for these 2 compounds are also similar (15).

The response obtained for the lower sulfonated subsidiary color was a single symmetrical peak, usually observed as the last response in the chromatogram. There was no sign of resolution of the 2 isomers of the lower sulfonated subsidiary color in the reference compound for this component or in any of the commercial D&C Green No. 5 samples analyzed. In the absence of reference compounds for each of the possible isomers of the lower sulfonated subsidiary color, it is assumed that they are not resolved by this method, and the single peak is used to quantitate both isomers. The reference compound would be expected to be a mixture of the isomers since it was produced by the sulfonation of D&C Green No. 6 (12).

Calibration

Calibration data for the 4 constituents of D&C Green No. 5 are presented in Tables 1–4 and the statistical treatment of these data is shown in Table 5. All calibration solutions were prepared in the presence of the dye except those provided as part of the recovery study. PTOS and PTMS were calibrated in the same solutions with Ext. D&C Violet No. 2; however,

Table 5. Statistical analysis of calibration data

Component	Points	Regression equation	Corr. coeff.	Y_{UB}[a]	X_{LD}[b]	Y_Q[c]	Prediction limits,[d] %
PTOS[e]	11	$Y' = 62\,980X + 2$	0.9999	226	0.007	443	0.2 ± 0.004
PTMS[f]	13	$Y' = 68\,395X + 20$	1.0000	124	0.003	225	0.2 ± 0.002
Lower sulfonated subsidiary color	13	$Y' = 241\,878X + 5630$	0.9998	18 659	0.11	32 236	3.0 ± 0.06
Ext. D&C Violet No.2	15	$Y' = 318\,582X - 16\,908$	0.9996	5609	0.15	27 693	3.0 ± 0.09

[a] Y_{UB} is the 99% upper prediction limit on the blank (area).
[b] X_{LD} is the limit of detection with 99% confidence (in units of % of dye).
[c] Y_Q is the peak area corresponding to X_{LD}.
[d] Prediction limits calculated at the specification level.
[e] *p*-Toluidine-*o*-sulfonic acid.
[f] *p*-Toluidine-*m*-sulfonic acid.

2 of the Ext. D&C Violet No. 2 solutions were prepared without any other added components. All of the lower sulfonated subsidiary color calibration solutions were prepared without addition of other components. The 2 calibration solutions provided as part of the recovery study are shown as the last 2 entries in Tables 2–4. In the case of PTOS, these additional points could not be utilized because of interference caused by the methanol solvent used to dissolve the lower sulfonated subsidiary color stock solution (see recovery study discussion below).

The results of the statistical analysis of the calibration data calculated as in reference (13) and shown in Table 5 demonstrate the excellent performance of the method. The correlation coefficients for all 4 calibration plots show a linear relationship between the points. The calculated lower limits of detection (X_{LD} in Table 5) suggest that the method performs to levels well below those proposed as specifications. The prediction intervals at the specification levels are quite narrow even at the higher concentrations required for the subsidiary color components.

Recovery Study

Data obtained from the recovery study are contained in Table 6. The recovery solutions were prepared in the presence of the dye. Four levels of each component were prepared and each level was repeated 5 times in random combinations with the other components. A total of 20 recovery solutions were analyzed.

The solutions containing the higher levels of the lower sulfonated subsidiary color produced distorted peaks for PTOS which interfered with the results for this component. This distortion was caused by the methanol solvent required to dissolve the subsidiary color. In those recovery solutions containing high levels of methanol (i.e., those with high levels of the lower sulfonated subsidiary color), the early eluting peaks were split as the result of a transient gradient imposed by the solvent. This effect was observed only for the PTOS peak and did not appear to interfere with the PTMS peak. In spite of this problem, 10 data points were successfully collected for the PTOS recovery study. The solutions which produced split peaks for PTOS are omitted from Table 6. The 10 data points collected are adequate to gauge the performance of the method for PTOS since 1 level (0.0419%) contained all 5 data points and 2 of the remaining 3 levels contained 2 data points.

The data in Table 6 demonstrate excellent accuracy and precision and suggest that the LC method would perform adequately in measuring the levels of these components in commercial batches of the color additive.

Table 6. Recovery of intermediates and subsidiary colors added to D&C Green No. 5

Added, %	Found, %	Rec., %	SD	CV, %
	PTOS[a]			
0.0105	0.0109(2)[b]	103.8	0.000 424	3.9
0.0419	0.0421(5)	100.7	0.000 200	0.5
0.0838	0.0833(1)	99.4	—	—
0.2094	0.2054(2)	98.1	0.001 414	0.7
	PTMS[c]			
0.0110	0.0112(5)	101.8	0.000 212	1.9
0.0442	0.0445(5)	100.7	0.000 200	0.4
0.0883	0.0881(5)	99.8	0.000 593	0.7
0.2208	0.2221(5)	100.6	0.000 675	0.3
	Ext. D&C Violet No. 2			
0.281	0.2867(5)	102.0	0.008 86	3.1
0.562	0.5438(5)	96.8	0.005 75	1.0
1.123	1.0970(5)	97.7	0.005 50	0.5
2.810	2.7738(5)	98.7	0.007 95	0.3
	Lower Sulfonated Subsidiary Color			
0.164	0.1679(5)	102.4	0.013 0	7.7
0.984	0.9922(5)	100.8	0.010 1	1.0
1.970	1.9941(5)	101.2	0.016 4	0.8
3.28	3.3188(5)	101.2	0.011 2	0.3

[a] *p*-Toluidine-*o*-sulfonic acid.
[b] Number of runs in parentheses.
[c] *p*-Toluidine-*m*-sulfonic acid.

Survey of Commercial Samples

Sixteen commercial samples of D&C Green No. 5 that had been certified and the pharmacology sample (AA4700) were analyzed by the LC method described here. The results are presented in Table 7 together with the results obtained for intermediates (elution chromatography) (10) and subsidiary colors (TLC) (12) during certification analysis. Generally, the results agree favorably with 1 or 2 exceptions. As indicated by the results in Table 7, the levels of PTOS and PTMS are quite low.

Examination of the LC chromatogram for D&C Green No. 5 (Figure 7) shows that the method described here also produces a nearly baseline separation for the *o,m*- and *o,o*-isomers of D&C Green No. 5, as determined by chromatographing authentic reference compounds (work in progress in Division of Color Technology). However, at a D&C Green No. 5 concentration of 0.250 g/100 mL, the response of the *m,m*-isomer is obscured by that of the *o,o*-isomer. After dilution (3:100), the response of the *m,m*-isomer emerges as a tailing peak on the response for the *o,o*-isomer (Figure 8). The separation of the 3 D&C Green No. 5 isomers can be optimized by using isocratic elution at 60% B in A (Figure 9). Thus this method, as described or with slight modification,

Table 7. Survey of commercial D&C Green No. 5 samples

Sample	PTOS,[a] %		PTMS,[b] %		Ext. D&C Violet No. 2, %		Lower sulfonated subsidiary color, %	
	LC	EC[c]	LC	EC	LC	TLC	LC	TLC
AA9989	<0.007	nil	<0.003	nil	0.22	0.51	1.2	1.1
Z4857	<0.007	nil	0.003	nil	0.28	<0.5	<0.11	<0.5
AA3004	<0.007	nil	<0.003	nil	0.62	0.5	<0.11	0.1
AA9728	<0.007	nil	0.005	nil	0.42	0.56	2.06	1.43
AA4700[d]	<0.007	nil	0.003	nil	0.72	0.5	0.25	0.3
AA6925	<0.007	nil	0.004	nil	0.51	0.69	0.22	0.4
AA8934	<0.007	nil	0.015	nil	0.34	NR[e]	<0.11	NR
AA9550	<0.007	nil	0.006	nil	0.61	0.65	0.13	<0.2
AA9080	<0.007	nil	<0.003	nil	0.52	0.52	<0.11	trace
ZB0573	<0.007	nil	<0.003	nil	0.60	0.4	<0.11	trace
ZB0669	<0.007	nil	<0.003	nil	<0.15	<0.1	<0.11	<0.1
AA9900	<0.007	nil	<0.003	nil	0.40	0.5	2.57	2.1
AA9548	<0.007	nil	0.006	nil	0.60	0.88	0.16	<0.2
AA9875	<0.007	nil	<0.003	nil	0.40	0.29	2.44	2.25
AA9178	0.008	nil	0.016	nil	<0.15	trace	<0.11	trace
ZB0565	<0.007	nil	<0.003	nil	<0.15	0.02	0.88	1.1
AA9946	<0.007	nil	<0.003	nil	0.43	<0.1	2.68	2.39

[a] *p*-Toluidine-o-sulfonic acid.
[b] *p*-Toluidine-*m*-sulfonic acid.
[c] Elution chromatography.
[d] D&C Green No. 5 pharmacology sample.
[e] Results not reported.

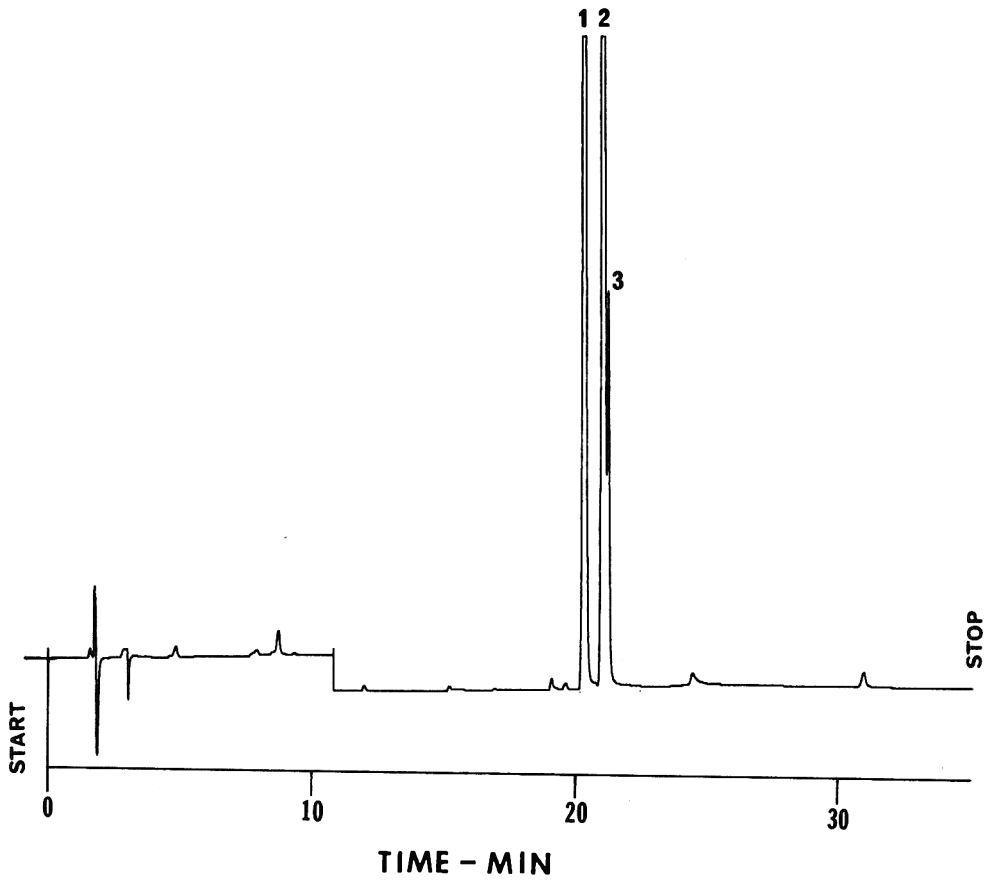

Figure 8. LC chromatogram of D&C Green No. 5 (0.0075 g/100 mL). 1 = *o,m*-isomer (20.31); 2 = *o,o*-isomer (21.03); 3 = *m,m*-isomer (21.21). Numbers in parentheses are elution times (min).

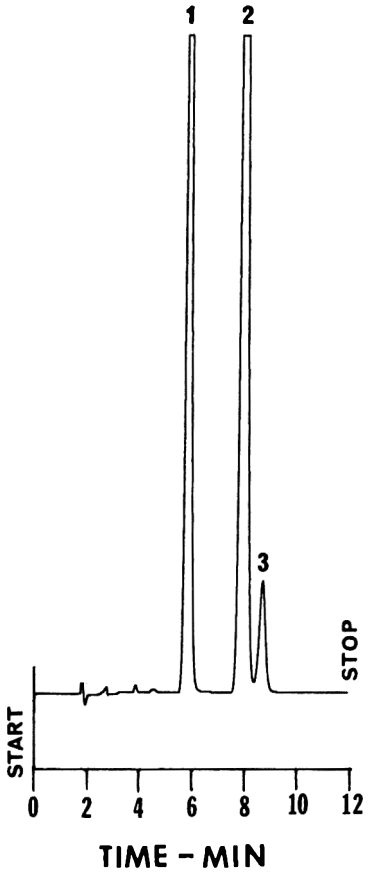

Figure 9. LC chromatogram of D&C Green No. 5 (0.0075 g/100 mL) obtained by using isocratic elution with 60% B in A. 1 = *o,m*-isomer (5.69); 2 = *o,o*-isomer (7.79); 3 = *m,m*-isomer (8.48). Numbers in parentheses are elution times (min).

can provide a useful tool for the study of the chemistry, composition, and manufacture of D&C Green No. 5.

Acknowledgment

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VITAMINS AND OTHER NUTRIENTS

Improved Reverse Phase Liquid Chromatographic Determination of Vitamins A and D in Fortified Milk

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A method for determination of vitamins A, D_2, and D_3 on the same 6 mL sample of fortified milk is described. A special indicator permits selection from the cleanup column of a fraction that contains all vitamin D and is free of cholesterol interference for final measurement by reverse phase liquid chromatography. Conservation of analyst's time and expensive solvents results from the relatively small sample processed. The average recoveries and standard deviations were, respectively, 94.1% and 4.8 for vitamin D_2, 91.1% and 5.2 for vitamin D_3, and 92.2% and 2.6 for vitamin A in nonfat skim milk; 86.5% and 9.4 for vitamin D_2, and 83.7% and 4.9 for vitamin D_3 in homogenized whole milk. Detectable minima are about 100 IU vitamin A/qt and 50 IU vitamin D_2 or D_3/qt.

The routine monitoring of vitamin fortification of milk has become practical as a result of the development and introduction of analytical techniques based on liquid chromatography (LC). Henderson and Wickroski (1) used a reverse phase system and an ODS-HC-Sil X-1 column to determine vitamin D in 100 mL samples of milk. For vitamin A in margarine, infant formula, and milk, Thompson and Maxwell (2) recommended a LiChrosorb reverse phase column. Henderson and McLean (3) refined and combined the methods of Henderson and Wickroski (1) and Thompson et al. (4) for the simultaneous determination of vitamins A and D in skim milk and chocolate milk, using a Vydac column. Muniz et al. (5) claimed that in their hands the procedure of Henderson and Wickroski (1) failed to adequately eliminate the interference from cholesterol and proposed the removal of cholesterol by precipitation with digitonin in a separate step. Separations of vitamins D_2 and D_3 were obtained for 100 mL samples with a Vydac column.

The method now reported incorporates an indicator for sample cleanup that permits selection of a fraction showing interference-free, well separated LC peaks for vitamins D_2 and D_3. This eliminates the need for a separate precipitation of cholesterol. Reduction of the sample size from 100 to 6 mL significantly decreases analysis time and amounts of expensive solvents. Vitamin A is determined on a saponified and extracted aliquot of the same sample.

Experimental

Reagents

(a) *Solvents.*—LC grade acetonitrile, methanol, hexane, benzene, and chloroform (containing 1% alcohol) (Burdick & Jackson Laboratories, Muskegon, MI 49442).

(b) *Potassium hydroxide solution.*—In volumetric flask, dissolve 400 g KOH in water, cool, and dilute to 500 mL.

(c) *Ethanolic pyrogallol solution.*—Dissolve 1 g pyrogallol in 100 mL ethanol.

(d) *Neutral aluminum oxide.*—Beckman Grade I (J. T. Baker Chemical Co., Phillipsburg, NJ 08865). Deactivate according to procedure outlined by Henderson and Wickroski (1).

(e) *Mobile phases.—(1)* Methanol–water (90 + 10); *(2)* acetonitrile–methanol (90 + 10).

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(f) *Vitamin D marker.*—Chlorophyll-a, No. C-5753 (Sigma Chemical Co., Box 14508, St. Louis, MO 63176). Dilute 1 mg chlorophyll-a to 20 mL with chloroform. Store in dark glass bottle and refrigerate.

(g) *Vitamin A standard solutions.—(1) Stock solution.*—Dissolve 300 mg crystalline *trans*-retinol (Sigma Chemical Co.) and dilute with methanol to 50 mL in volumetric flask. *(2) Intermediate standard solution.*—Dilute 5 mL stock solution to 50 mL with methanol in volumetric flask. *(3) Chromatographic standard solution (ca 6 μg/mL).*—Dilute 0.5 mL intermediate standard solution to 50 mL with methanol in volumetric flask. Determine exact concentration from absorption at 325 nm, using $E^{1\%}_{1cm}$ 1832.

(h) *Vitamin D standard solutions.—(1) Stock solutions.*—Dissolve 100 mg each of crystalline ergocalciferol (D_2) and crystalline cholecalciferol (D_3) (Sigma Chemical Co.) separately and dilute to 200 mL with methanol in volumetric flasks. *(2) Intermediate standard solution.*—Combine 1 mL of each stock solution in single 100 mL volumetric flask and dilute to volume with methanol. *(3) Chromatographic standard solution (0.5 μg/mL each vitamin).*—Dilute 5 mL intermediate standard solution to 50 mL in volumetric flask with methanol.

Apparatus

(a) *Chromatographic tubes.*—10 mm id × 300 mm glass with sealed-in fritted disc (Kontes Chromaflex K 420540, or equivalent).

(b) *Liquid chromatograph.*—Laboratory Data Control Constametric II G pump equipped with Rheodyne 7105 syringe injection valve with 175 μL sample loop, Spectromonitor *III* variable wavelength detector (LDC, Riviera Beach, FL 33404), and Chromatopak Model CRIA data processor (Shimadzu Corp., Kyoto, Japan). *Chromatographic column.*—Vydac reverse phase 10 μm, 3.2 mm id × 250 mm stainless steel, No. TP 201 (The Separation Group, 8738 Oakwood Ave, Hesperia, CA 92435).

Saponification and Extraction

Place small magnetic stirring bar in 50 mL low-actinic glass Erlenmeyer flask, add 6 mL milk sample. 10 mL 1% ethanolic pyrogallol solution, and 2.5 mL 80% KOH. Let mixture saponify overnight at room temperature (6) with slow constant stirring. Transfer saponified mixture to 125 mL separatory funnel. Rinse flask with two 8 mL portions of water, 5 mL ethanol, and 15 mL hexane, add rinses to separatory funnel, and shake. Drain lower layer into second separatory funnel and re-extract with 3 additional 15 mL portions of hexane, adding extracts to first hexane shake-out. Wash combined extracts with 15 mL KOH. Draw off KOH layer, re-extract with 15 mL hexane, and add extract to previous combined extracts. Wash combined hexane extracts with 15 mL portions of water until there is no reaction to phenolphthalein. There is no adverse effect if any emulsion that forms is dispersed with a few drops of ethanol. Dry stem of funnel and empty contents into clean, dry beaker that is used

to pour mixture into 125 mL 24/40 standard taper glass-stopper Erlenmeyer flask. Let water droplets remain in beaker. Rinse separatory funnel, its stem, and beaker with additional hexane. Place flask in water bath at 30°C, connect flask to rotary evaporator, evaporate hexane just to dryness, and disconnect flask. Immediately add exactly 6 mL hexane and swirl flask to dissolve residue. Remove 1 mL solution by pipet and place in 2 mL Kontes Microflex vial. Evaporate hexane just to dryness with stream of nitrogen, keeping vial warm in 30°C water bath. Add 100 μL LC grade methanol, swirl vial, and set aside for vitamin A analysis.

To remaining 5 mL hexane solution in 125 mL Erlenmeyer flask, add 0.1 or 0.2 mL marker solution and 1 g dry 8% deactivated neutral aluminum oxide. Connect flask to rotary evaporator, immerse in 30°C water bath, and evaporate solvent until alumina is dry and free-flowing. Use extreme caution to prevent loss of powdered sample as flask is disconnected from evaporator.

Aluminum Oxide Column Chromatography

Prepare 10 mm id glass chromatographic column (**b**) as described by Bell and Christie (7) with the following modifications: Pour 15 g portion of 8% deactivated alumina through funnel into column which is vibrated with portable engraving tool to facilitate packing. Prepare column just before use and use column only once.

Immediately pour dried alumina containing portion of sample for vitamin D analysis on top of prepared alumina column. Use vibrator to settle mixture, and place thin pad of glass wool on top. Rinse flask with 10 mL hexane and add rinse to column. When all hexane has entered column, rinse flask with 2 mL benzene and add benzene to column. When benzene has entered column, rinse flask with chloroform and add chloroform to column. Continue to add chloroform to column until marker, which is seen as compact green band (pink or red under longwave UV light), reaches 6 cm above bottom of column. If 0.1 mL marker is used, progress of the band is followed under ultraviolet light. If 0.2 mL marker solution is added, band can be followed without difficulty in subdued light. At this point, continue adding chloroform but collect next 6–8 mL eluate (or until marker reaches 2 cm from bottom of column) in 50 mL Erlenmeyer flask. Operate column under gravity only. Use of vacuum or pressure on column is unsuitable. Evaporate this eluate to ca 1 mL with rotary evaporator with flask immersed in 30°C water bath. Quantitatively transfer contents with chloroform to 3 mL tapered Kontes Microflex vial and evaporate just to dryness with stream of nitrogen, keeping vial in 30°C water bath. Immediately add 100 μL LC grade methanol, swirl vial, and proceed with LC analysis for vitamin D.

Liquid Chromatography

Vitamin A.—Use these operating conditions: ambient temperature; chart speed, 60 cm/h; sensitivity, 1.0 AUFS; flow rate, ca 0.75 mL/min; detector wavelength, 325 nm UV; recorder, 1 mV; injection volume, 10 μL.

Chromatograph 10 μL standard and then 10 μL sample in mobile phase 1 on Vydac reverse phase column. Identify vitamin A from retention times. Quantitate by electronic integration or calculate as follows from average peak heights of duplicate injections of standard and sample:

$$\text{Vit. A in sample (IU/qt)} = 946\,000\,HC/RH'$$

where H *and* H' = average peak heights of sample and standard, respectively; C = IU in injected standard; R = recovery percent.

To obtain R, with each group of milk samples, run sample of milk to which known amount of vitamin in normal range has been added.

Vitamin D.—Use these operating conditions: ambient temperature; chart speed, 60 cm/h; sensitivity, 0.1 AUFS; flow rate, ca 0.75 mL/min; detector wavelength, 265 nm UV; recorder, 1 mV; injection volume, 20 μL.

Chromatograph 20 μL standard and then 20 μL sample in mobile phase 2 on Vydac reverse phase column. Identify vitamins D_2 and D_3 from retention times. Quantitate by electronic integration or calculate as follows from average peak heights of duplicate injections of standard and sample:

$$\text{Vit. } D_2 \text{ or } D_3 \text{ (IU/qt)} = 189\,200\,HC/RH'$$

where H and H' = average peak heights of sample and standard, respectively; C = IU D_2 or D_3 in standard; R = recovery percent.

Results and Discussion

Reconstituted unfortified nonfat skim milk was fortified with retinol acetate and vitamins D_2 and D_3 at levels corresponding to those normally found in fortified market milks, 2000 IU/qt of vitamin A and 400 IU of each D vitamin, to determine recoveries after extraction and saponification. Results are shown in Table 1.

Known amounts (400 IU/qt) of vitamins D_2 and D_3 were added to homogenized whole milk that had not been previously fortified and for which the absence of the vitamin was verified by analysis. Standards were added to the milk before saponification. Recovery results are shown in Table 2.

Figure 1 is a chromatogram of vitamin A standard. Figure 2 is a chromatogram of an unfortified sample of reconstituted skim milk showing the absence of interfering peaks for vitamin A. Figure 3 is a chromatogram of skim milk fortified with 2000 IU vitamin A/qt. Figures 4, 5, and 6 are chromatograms of, respectively, a standard containing a mixture of vitamins D_2 and D_3, an unfortified homogenized milk sample, and a

Table 1. Recovery of vitamins added to reconstituted nonfat skim milk

	Vitamin A (200 IU/qt addec)		Vitamin D_2 (400 IU/qt added)		Vitamin D_3 (400 IU/qt added)	
	IU	%	IU	%	IU	%
	1808	90.4	369	92.3	338	84.5
	1792	89.6	369	92.3	349	87.3
	1934	96.7	381	95.3	355	88.8
	1824	91.2	380	95.0	358	89.5
	1864	93.2	411	102.8	387	96.8
	1906	95.3	387	96.8	369	92.3
	1769	88.5	348	87.0	342	85.5
	1847	92.4	353	88.3	385	96.3
	1956	92.8	388	97.0	394	98.5
Av.	1856	92.2	376	94.1	364	91.1
SD	65	2.6	19	4.8	21	5.2

Table 2. Recovery of vitamin D added to homogenized whole milk

	Vitamin D_2 (400 IU/qt added)		Vitamin D_3 (400 IU/qt added)	
	IU	%	IU	%
	357	89.3	354	88.5
	336	84.0	349	87.3
	341	85.3	333	83.3
	304	76.0	312	78.0
	348	87.0	303	75.8
	315	78.9	348	87.0
	420	105.0	343	85.8
Av.	346	86.5	335	83.7
SD	37	9.4	20	4.9

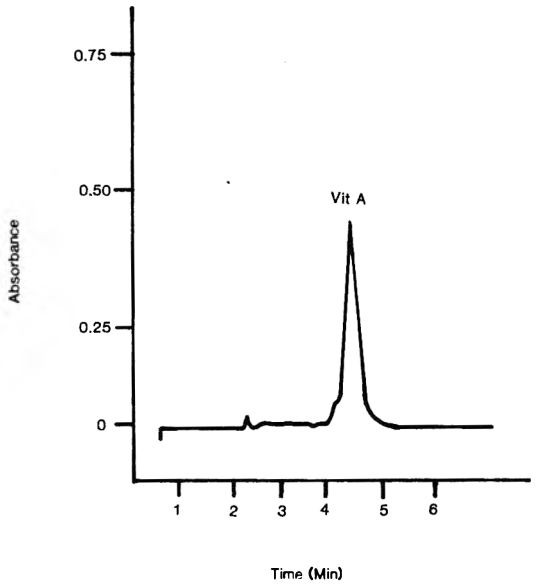

Figure 1. Chromatogram of vitamin A standard on Vydac reverse phase column. Mobile phase, methanol–water (90 + 10), flow rate ca 0.75 mL/min, wavelength, 325 nm, sensitivity, 1.0 AUFS. Vitamin A = 60 ng.

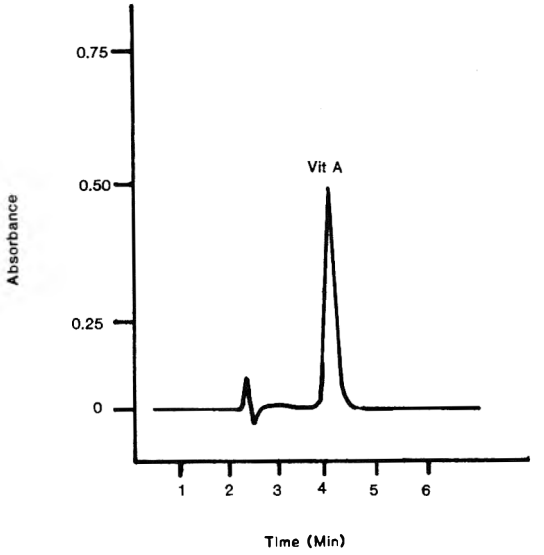

Figure 3. Chromatogram of skim milk fortified with 2000 IU vitamin A/qt on Vydac reverse phase column. Operating conditions same as in Figure 1.

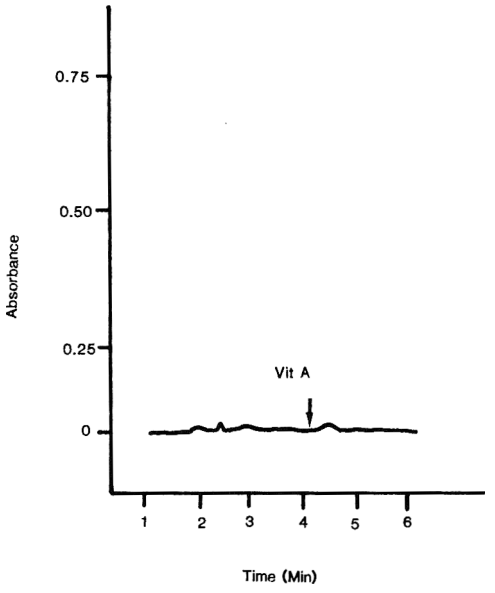

Figure 2. Chromatogram of skim milk without added vitamin A on Vydac reverse phase column. Arrow indicates position of vitamin A. Operating conditions same as in Figure 1.

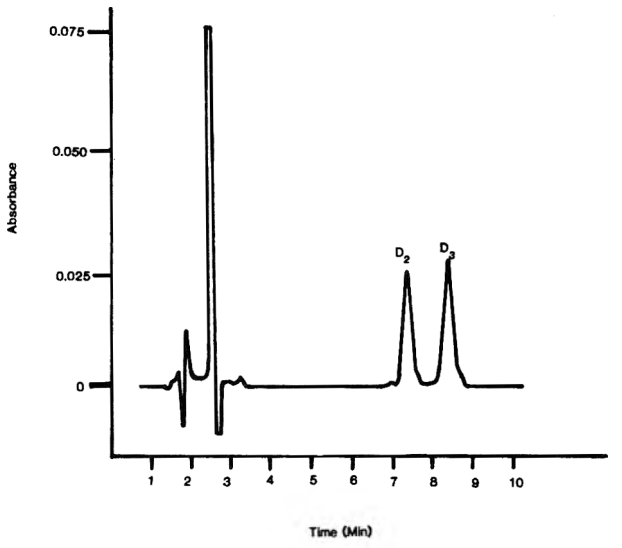

Figure 4. Chromatogram of vitamin D_2 and D_3 standards on Vydac reverse phase column. Mobile phase, acetonitrile–methanol (90 + 10), flow rate, ca 0.75 mL/min; wavelength, 265 nm; sensitivity, 0.1 AUFS. Vitamin D_2 = 10 ng, vitamin D_3 = 10 ng.

sample of homogenized milk fortified with 400 IU vitamin D_3/qt.

The indicator used in the aluminum oxide column cleanup step acts not only as a visible tracer for locating and collecting the portion of the sample containing the vitamin D regardless of variations in the column makeup, but is also sufficiently specific in indicating the position of the vitamin that cholesterol contamination of the LC fraction is eliminated.

The marker used to follow the progress of the vitamin D band through the cleanup column has not been conclusively identified chemically, but it appears to be associated with the chlorophyll in plants. It can be extracted from green leafy material including grass. A convenient source is commercially available chlorophyll-a in which it is present as a minor ingredient. The material was found in a somewhat crude sample of carotene that was available in our laboratory but not in more highly purified carotene samples. It is present in trace amounts in some milk fat and in vegetable oils. When 100 mL samples of whole milk were treated as outlined in a previous method (1), the progress of the indicator that was usually naturally present could be followed reasonably well as a yellow fluorescent band under longwave ultraviolet light. However, when the procedure was used for low-fat milk or when the sample size was reduced to 6 mL, there was not enough marker present to give a visible band. When the marker is used as directed in this procedure, a distinct, compact green band visible under ordinary light (pink or red under longwave ultraviolet light) is produced on the column and the band proceeds through the column in consistent relationship to the vitamin band.

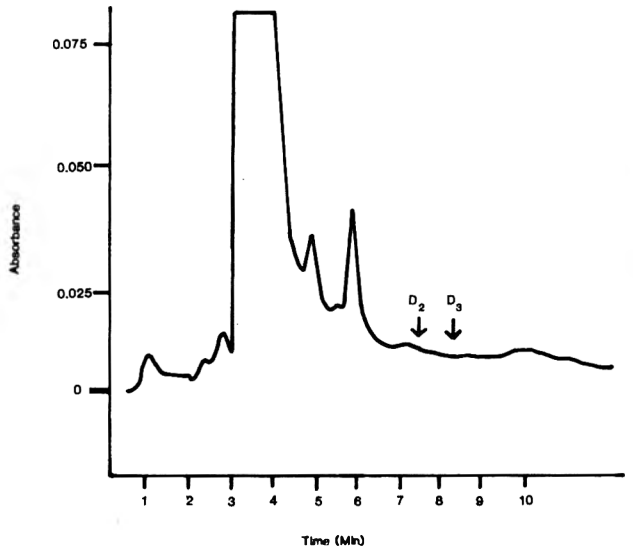

Figure 5. Chromatogram of homogenized milk (3.8% fat) without added vitamin D on Vydac reverse phase column. Arrows indicate position of D_2 and D_3. Operating conditions same as in Figure 4.

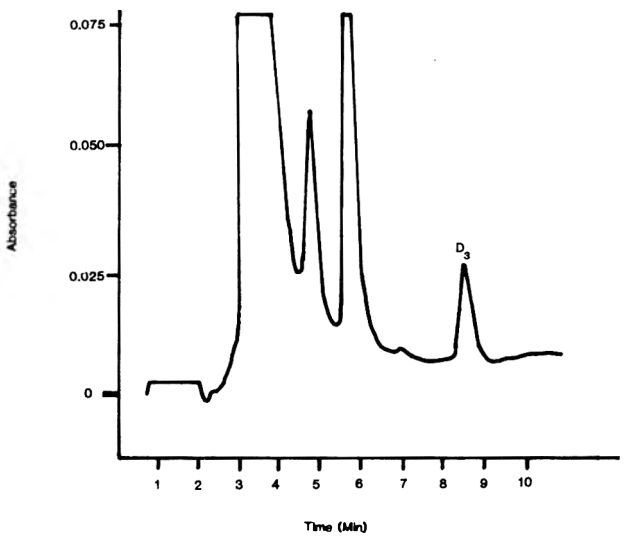

Figure 6. Chromatogram of homogenized milk (3.8% fat) fortified with 400 IU vitamin D_3/qt on Vydac reverse phase column. Operating conditions same as Figure 4.

Although cholesterol normally does not produce a measurable interfering peak at 265 nm, if it is not eliminated it appears as a late-eluting, ill-defined peak in subsequent chromatograms that can totally obscure the vitamin peaks. The alternative is a time-consuming column cleanup procedure after each 2 or 3 injections.

The reduction of sample size to 6 mL for this method compared with the 100 mL sample used by Henderson and Wickroski (1) and by Muniz et al. (5) results in more rapid analyses and savings in the amounts of solvents and reagents needed. We have used this method to analyze more than 200 samples of commercially fortified skim, low-fat, and homogenized milks, as well as the vitamin concentrates used by the dairies and have found it to be an efficient, rapid, and reliable method for routine laboratory use.

The minimum detectable amounts were about 100 IU vitamin A and 50 IU vitamin D_2 or D_3 per quart. The estimates are based on the choice of the lowest concentration with a reasonably detectable response for a range of small amounts of vitamins added to unfortified milk.

Acknowledgment

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Protein Efficiency Ratio: AACC/ASTM Collaborative Study

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Eight laboratories (7 of the laboratories conducted animal experiments) participated in a collaborative study to standardize some of the methodology associated with animal bioassays for determining protein efficiency ratios and to suggest improvements which would reduce the variation among laboratories. One-, 2-, 3-, and 4-week protein efficiency ratios (PER) with 0-, 2-, or 4-day adaptation periods were obtained from each laboratory, respectively, for 6 protein sources: casein, lean beef, lactalbumin, textured vegetable protein, peanut flour, and wheat flour. Analyses were computed for PER and adjusted PER (APER). From the analysis of variance for PER and APER, significant ($P < 0.05$) effects were observed due to laboratories, adaptation length, protein sources, and/or interactions among these variables. In general, APER values show much less variation among laboratories than PER values. The reproducibility and repeatability variances were significantly ($P < 0.05$) greater for an assay length of 2 weeks than they were for 3- or 4-week assays. Two protein sources, casein and textured vegetable protein, were fed at both high (10%) and low (6%) levels of protein. Analysis of variance of PER values shows a significant ($P < 0.05$) laboratory by protein level by assay length interaction.

A collaborative study on the protein efficiency ratio (PER) was initiated with an ultimate objective to standardize the PER procedure. This research was stimulated by the renewed interest in protein quality that was created with the promulgation of nutritional labeling regulations (1). The official method for determination of protein quality in the United States is the PER procedure outlined by AOAC (2). Several investigators have reviewed the PER procedure, as well as other protein quality bioassays (3–9). None of the animal bioassays are totally satisfactory for predicting protein quality for humans because we consume a variety of protein sources each day in differing quantities. The PER assay has been the most widely used procedure and the most severely criticized; however, it is still a useful tool for evaluation of protein quality.

Two major criticisms of the PER procedure are that it does not show the value of a protein for maintenance and it does not rank proteins on a scale of 0 to 1. However, the latter could be accomplished by calculating a relative PER based on a casein-plus-methionine control diet. Background information on the AACC/ASTM collaborative study has been published (10).

The main overall objective was to better define the specifications of the procedure for determination of PER with the ultimate goal of making some useful changes in the assay. Also of interest was the measurement of variation of the data between laboratories. With the preceding guidelines, the following variables were selected: length of adjustment period, length of PER assay, protein analysis by each investigator compared with an analysis performed by Laboratory 7, and level of protein in the diet for low protein-containing foods. In the development of the preceding variables, several other parameters were considered (10).

Experimental

Seven laboratories participated in the feeding of rats for determination of PER (10). The statistical analysis of the data was conducted by an eighth laboratory which participated in the study. Six of the 7 laboratories used rats from the same supplier (ARS Sprague Dawley, Mogul Corp., Madison, WI), while one laboratory (Laboratory 2) obtained Sprague Dawley-derived rats from Harlan Industries, Cumberland, IN. The weanling rats were 21 days old when shipped. They arrived within 24–48 h at the laboratories.

Experimental Design

The experimental design for the PER study is shown in Table 1. It was our hypothesis that the amount of time allowed for rats to adjust to their new environment and to recover from shipping stresses was an important consideration in the PER assay.

Protein Sources

Five protein sources (ANRC casein, lactalbumin, lean beef, textured vegetable protein, and peanut flour) were used in the main portion of the study to evaluate the effect of length of adjustment period on PER, while ANRC casein, textured vegetable protein (TVP), and white wheat flour were used in the low protein portion of the study which had only a 2-day adjustment period.

ANRC reference casein and lactalbumin used in this study were donated by Humko Sheffield Chemical, Memphis, TN. Defatted peanut flour was obtained from Goldkrist, Inc., Atlanta, GA. The sample of wheat flour was contributed by General Mills, Inc., Minneapolis, MN. Textured vegetable protein was a sample of unflavored Tex-Pro contributed by Archer Daniels Midland Co., Decatur, IL. The sample of beef was prepared by the eighth participating laboratory. Seven rounds of beef (440 lb) were trimmed to remove the fat between muscles and fat and connective tissue on the outside of the muscles, ground through a ½ in. plate, well mixed in a large vacuum mixer (Buffalo), reground through a 3/16 in. plate in a Butcher Boy meat grinder, and again well mixed. The ground meat was frozen in stainless steel trays (2.0 kg of meat per tray in even layer) and freeze-dried in a Stokes shelf dryer with a vacuum of 1 mm Hg and a platten temperature of 40°C to about 1% moisture in 24 h. The lyophilized beef was ground in a Wiley mill to pass a 2 mm screen, using Dry Ice to prevent the beef and mill from heating during grinding. The ground sample was held in a refrigerator at 3–5°C until the CO_2 had evaporated, mixed thoroughly in a

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Table 1. Experimental design for protein efficiency ratio (PER) study

Variable	10% Protein diets[a]	6% Protein diets[b]
Adaptation periods, days	0, 2, 4	2
Adaptation diet	10% casein	10% casein
Animals	10 rats/trial	10 rats/trial
Protein sources for test diets	ANRC casein freeze-dried lean beef lactalbumin tex. veg. protein peanut flour	ANRC casin wheat flour text veg. protein
Test period, days	28	28
Data collection Rats and food consumption	rat wt on arrival at lab. rat wt on 0 day of adaptation rat wt on 0 day of PER test rat wt gain and food consumption every 7th day	rat wt on arrival at lab. rat wt on 0 day of adaptation rat wt on 0 day of PER test rat wt gain and food consumption every 7th day

[a]The diets fed ranged from 9.5 to 10.5% protein.
[b]The diets fed ranged from 5.5 to 6.5% protein.

vacuum mixer (no change in weight of the ground lyophilized meat), sampled for analysis, and stored in tightly closed plastic food storage bags (2 per sample) at 3°C until weighed for the rat diets. About 52 lb meat was produced for the PER collaborative study diets.

The protein sources (except beef), dextrose, cornstarch, cellulose, vitamin mix, and mineral mixture were analyzed by Laboratory 7 for protein (N × 6.25), ether extractable material, crude fiber, minerals, and water (Table 2). The proximate analysis of the beef sample was performed by the eighth laboratory. The analytical values obtained by both laboratories were used in computing the composition of the diets.

Composition of the Diets

The composition of the diets is shown in Table 3. Except for corn oil and water, each ingredient was weighed and packaged individually for a specific diet and shipped to each laboratory. One laboratory was unable to mix the large quantities, 15 thousand to 18 thousand g; consequently, this collaborator received 3 batches of ingredients for a diet, 5000 g × 3 to 6000 g × 3. The dry ingredients were thoroughly mixed; then the corn oil and water were added with additional mixing. Aliquots were then removed from each diet for chemical analysis by each collaborator and Laboratory 7.

Rat Feeding Procedure

The rats were allotted to groups of 10 rats based on body weight, and groups were randomly assigned to the experimental diets (10). During the study, rats were housed in cages that met or exceeded requirements for care and handling of rats (11). Other environmental conditions were as follows: room temperature, 73 ± 2°F; relative humidity, 50 ± 2%; light, 12 h and dark, 12 h. Feed and water were given ad libitum. Food consumption, taking spillage into account, was recorded at weekly intervals. In addition, any contaminated food was removed, weighed, and replaced with fresh food as necessary. Any dirty or contaminated water bottles or tubes were replaced as needed and all water bottles and watering tubes were cleaned (or changed) at least once per week. Rats were weighed at weekly intervals at the same time by each collaborator.

Calculation of Results

All collaborators reported data on the weight gain and the amount of food consumed by each animal from the beginning feeding for each weekly period. Values of protein efficiency ratio (PER) and adjusted protein efficiency ratio (APER) for the 4 weekly periods were then calculated for each animal in the study according to the following equations:

$$\text{PER} = \text{gain of test animal, g/wt of protein consumed, g}$$

$$\text{APER (12)} = \text{PER of test protein} \times 2.50/\text{av. PER of casein (for same week and adaptation)}$$

Statistical Analysis

Values of PER and APER were analyzed by analysis of variance to test the significance of effects due to laboratories, protein source, adaptation period, duration of assay, and their interactions. In addition, because the laboratories should be considered a random factor, a mixed model was used in the analysis of variance. Estimates of reproducibility and repeat-

Table 2. Proximate composition of dietary ingredients, [a] %

	Protein (N × 6.25)	Ether extract	Crude fiber	Ash	Water
ANRC[b] casein	92.6	0.0	0.42	1.06	4.36
Lactalbumin	82.3	2.19	0.48	1.03	3.99
Textured veg. protein	52.4	0.70	3.90	6.40	6.23
Peanut flour	61.8	0.06	4.43	4.72	4.90
Wheat flour	10.8	1.12	0.85	0.50	8.00
Beef	79.9	13.02	NA	4.14	2.94
Vitamins	16.7	0.40	0.14	0.16	0.61
Corn starch	0.30	0.00	0.51	0.10	7.33
Dextrose	0.0	0.45	0.05	0.05	0.06
Cellulose	0.03	0.33	95.06	0.19	4.39
Corn oil	NA	NA	NA	NA	NA
Salt mix (BT)[c]	0.0	0.03	0.46	89.3	1.02

[a]NA = not analyzed.
[b]Animal Nutrition Research Council.
[c]BT = Bernhart-Tomarelli modified mineral mixture.

Table 3. Composition of experimental test diets, g

Component	10% Protein diets					6% Protein diets		
	ANRC casein[a]	Lactalbumin	Beef	TVP	Peanut flour	ANRC casein	TVP	Wheat flour
ANRC casein	1727.55	—	—	—	—	307.5	—	—
Lactalbumin	—	1943.70	—	—	—	—	—	—
Beef	—	—	2185.2	—	—	—	—	—
Textured veg. protein	—	—	—	2776.5	—	—	543.8	—
Peanut flour	—	—	—	—	2353.5	—	—	—
Wheat flour	—	—	—	—	—	—	—	2638.5
Dextrose[b]	6666.00	6494.40	7326.0	5257.5	5526.0	2224.5	2068.0	141.0
Cornstarch[b]	3300.00	3300.00	3600.0	3000.0	3000.0	1000.0	1000.0	1000.0
Vitamins[c]	165.00	165.00	180.0	150.0	150.0	50.0	50.0	50.0
Cellulose	833.25	836.55	921.6	654.0	658.5	254.5	233.5	233.5
Minerals[d]	897.60	895.95	900.0	636.0	709.5	274.0	239.0	264.0
Corn oil[e,f]	1620.30	1577.40	1461.6	1458.0	1474.5	489.5	486.5	469.5
Water, dist.[f]	1290.30	1287.00	1425.6	1068.0	1128.0	400.0	379.5	203.5
Total wt of diet	16 500	16 500	18 000	15 000	15 000	5 000	5 000	5 000

[a]Adaptation diet (8000 g) was the same percentage composition as this ANRC casein diet.
[b]Dextrose, anhydrous, CPC International, Industrial Division, Yonkers, NY.
[c]Vitamin mix, Catalog No. 40055, Teklad Test Diets, Madison, WI.
[d]Salt mixture, Bernhart-Tomarelli Modified, Catalog No. 902843, Nutritional Biochemicals Division, ICN Life Sciences Group, Cleveland, OH.
[e]Corn oil, Mazola, CPC International, Industrial Division, Yonkers, NY.
[f]Added to preweighed-diet ingredients by collaborator.

ability (13, p. 69) variances were obtained by calculating the appropriate components of variance and computing [13, p. 81(b)].

$$\text{Repeatability} = \text{mean square (error)} = \sigma_0^2$$

$$\text{Reproduciblity} = \text{mean square (error)} + \text{laboratory component of variance} = \sigma_0 + \sigma_L^2$$

Collaborative Results and Discussion

The protein content of each diet as determined by each collaborator and by Laboratory 7 is shown in Table 4. Eleven of the 56 values fall outside of the desired ranges (9.5–10.5% and 5.5–6.5%) as analyzed by the individual laboratories; however, all but one of the 56 values were within these ranges as analyzed by Laboratory 7. Statistical comparisons showed no consistent differences in determination of protein content among laboratories.

Cochran's test for homogeneity of variance (14) showed that Laboratories 2 and 6 had significantly ($P < 0.05$) large variances of PER values in the test groups of 10 animals in comparison with the other laboratories. Laboratory 2 purchased animals from a different source than the other collaborators. Laboratory 2 also semirestricted the quantity of the diets of the 0-day, 2-day, and 4-day adaptation trials for 6, 4, and 2 days, respectively, because they believed there was a shortage of diets. Laboratory 6 was unable to mix all 3 adaptation diets as one large lot. These were mixed individually for each adaptation period.

All subsequent statistical analyses were performed with and without Laboratories 2 and 6. The elimination of these laboratories did not affect the conclusions concerning the effects and interactions of the various factors under consideration.

Analysis of Data from 10% Protein Diets

The PER and APER values are summarized in Table 5 (ANRC reference casein = 2.50) (12) for the 7 laboratories and in Table 6 for 5 laboratories after removing 2 outlier laboratories. For protein sources at 9.5–10.5% crude protein of the diet, these values were analyzed by analysis of variance to detect the effects and interactions of laboratory, adaptation period, protein source, and assay length. For both PER and APER values, there is evidence of significant ($P < 0.01$) 3-factor interactions (Table 7). These interactions preclude any simple statements about the effect of a single factor because their presence indicates that the effect of a factor depends on the levels of the other factors involved in the interaction.

The analysis of PER values yields significant ($P < 0.01$) laboratory × adaptation period × assay length, laboratory

Table 4. Protein content (N × 6.25) of diets (%)[a] analyzed by each collaborator and by Laboratory 7

Lab.	ANRC casein	Lactalbumin	Beef	Textured veg. protein	Peanut flour	ANRC casein	Textured veg. protein	White wheat flour	Non-protein
1	09.4	09.8	09.7	09.3	09.1	5.9	5.8	5.6	0.04
7	10.2	10.0	10.1	10.0	09.9	5.9	5.9	5.8	0.26
2	10.6	10.0	10.2	10.4	09.7	6.6	6.4	5.7	0.09
7	10.2	09.8	10.3	10.3	09.6	5.9	6.1	5.8	0.28
3	10.0	10.0	10.0	10.0	10.0	5.6	6.0	5.9	—
7	10.2	10.0	10.1	10.6	09.5	6.0	5.8	5.8	0.22
4	10.1	10.1	10.3[b]	10.7	10.1	6.2	6.4	6.1	0.13
7	10.0	10.2	10.1[b]	10.0	09.6	6.0	6.0	5.7	0.23
5	10.6	10.3	10.6	10.4	10.1	6.6	6.1	6.1	0.60
7	10.1	09.9	10.2	10.3	09.9	5.9	6.3	6.0	0.33
6	10.0	10.3	10.4	10.2	10.3	6.9	6.6	6.3	NA
7	10.1	10.0	10.2	10.0	10.1	6.3	6.1	5.6	0.40
7	09.8	09.9	10.0	09.9	09.6	6.0	6.1	5.9	0.30
7	09.9	10.0	10.0	09.9	09.7	5.9	6.1	5.9	0.25

[a]NA = not analyzed.
[b]Last week of study used another mix which analyzed to be 9.9% protein by Lab. 4 and 9.9% protein by Lab. 7.

Table 5. Unadjusted (PER) and adjusted (APER) protein efficiency ratio average values and standard deviations from 7 laboratories[a]

Protein source	Adap., days	PER: week 1		2		3		4		APER: week 1		2		3		4	
							10% Protein										
	0	4.11	1.35	3.48	0.57	3.22	0.36	3.09	0.33	2.50	1.08	2.50	0.30	2.50	0.17	2.50	0.14
Casein	2	3.75	0.80	3.26	0.45	3.06	0.39	2.97	0.30	2.50	0.38	2.50	0.24	2.50	0.19	2.50	0.15
	4	3.55	0.86	3.03	0.43	2.99	0.32	2.86	0.28	2.50	0.45	2.50	0.21	2.50	0.15	2.50	0.14
	0	4.27	1.71	3.89	0.47	3.53	0.30	3.28	0.25	2.60	1.28	2.80	0.29	2.75	0.19	2.66	0.16
Beef	2	4.08	0.84	3.66	0.41	3.31	0.34	3.15	0.27	2.73	0.41	2.82	0.32	2.71	0.23	2.65	0.18
	4	3.88	0.91	3.43	0.39	3.27	0.28	3.11	0.21	2.73	0.48	2.84	0.29	2.75	0.19	2.62	0.19
	0	4.08	1.42	3.99	0.52	3.78	0.36	3.51	0.31	2.54	0.80	2.87	0.27	2.94	0.24	2.85	0.19
Lactalbumin	2	3.70	1.06	3.73	0.45	3.48	0.45	3.31	0.33	2.44	0.49	2.87	0.27	2.84	0.25	2.78	0.18
	4	3.79	1.00	3.54	0.53	3.39	0.35	3.30	0.29	2.66	0.54	2.92	0.32	2.84	0.19	2.85	0.21
	0	1.76	1.02	1.82	0.35	1.68	0.26	1.54	0.26	1.11	0.64	1.30	0.19	1.31	0.16	1.24	0.16
Peanut flour	2	1.32	0.77	1.51	0.38	1.41	0.32	1.35	0.28	0.87	0.47	1.15	0.22	1.14	0.18	1.13	0.18
	4	1.22	0.73	1.38	0.32	1.39	0.29	1.31	0.30	0.83	0.50	1.14	0.23	1.17	0.21	1.14	0.20
	0	3.08	1.02	3.01	0.38	2.73	0.28	2.56	0.26	1.90	0.93	2.17	0.21	2.12	0.16	2.08	0.15
Textured	2	2.62	0.84	2.68	0.43	2.47	0.37	2.39	0.31	1.74	0.49	2.06	0.43	2.02	0.37	2.01	0.17
veg. protein	4	2.67	0.90	2.47	0.43	2.41	0.31	2.31	0.28	1.87	0.58	2.04	0.31	2.03	0.22	2.03	0.20
							6% Protein										
Casein	2	2.35	1.36	2.51	0.76	2.33	0.55	2.25	0.43	2.50	2.15	2.50	0.64	2.50	0.42	2.50	0.33
Textured veg. protein	2	1.51	1.42	2.09	0.51	2.02	0.36	1.90	0.29	0.96	2.95	2.13	0.55	2.21	0.45	2.14	0.35
Wheat flour	2	−0.42	1.03	−0.15	0.42	0.09	0.27	0.04	0.25	−1.38	3.06	−0.16	0.44	−0.05	0.28	0.05	0.27

[a]Average is for 70 animals, SD = $\sum_{i=1}^{70} (X_i - \bar{X})^2/69$, where X_i = PER of individual animal and $\bar{X} = \sum_{i=1}^{70} X_i/70$.

Table 6. PER and APER protein efficiency ratio average values and standard deviations from 5 laboratories[a]

Protein source	Adap., days	PER: week 1		2		3		4		APER: week 1		2		3		4	
							10% Protein										
	0	4.50	0.69	3.58	0.52	3.29	0.35	3.19	0.32	2.50	0.34	2.50	0.22	2.50	0.15	2.50	0.13
Casein	2	4.02	0.65	3.36	0.46	3.20	0.34	3.08	0.24	2.50	0.26	2.50	0.23	2.50	0.14	2.50	0.10
	4	3.88	0.62	3.17	0.39	3.09	0.29	3.00	0.23	2.50	0.31	2.50	0.17	2.50	0.13	2.50	0.10
	0	4.88	0.69	4.07	0.38	3.60	0.29	3.35	0.23	2.72	0.38	2.86	0.26	2.75	0.21	2.63	0.15
Beef	2	4.34	0.81	3.76	0.43	3.41	0.36	3.21	0.27	2.7[illegible]	0.41	2.82	0.37	2.67	0.25	2.61	0.19
	4	4.36	0.44	3.56	0.35	3.36	0.24	3.18	0.18	2.83	0.35	2.82	0.30	2.72	0.18	2.71	0.19
	0	4.52	0.80	4.14	0.47	3.87	0.32	3.60	0.30	2.51	0.41	2.90	0.24	2.95	0.23	2.83	0.18
Lactalbumin	2	4.11	0.87	3.92	0.33	3.67	0.33	3.43	0.27	2.55	0.38	2.94	0.24	2.88	0.23	2.78	0.15
	4	4.25	0.60	3.73	0.44	3.51	0.29	3.39	0.25	2.75	0.37	2.94	0.30	2.84	0.16	2.89	0.21
	0	1.96	0.92	1.88	0.36	1.71	0.24	1.57	0.27	1.08	0.50	1.31	0.16	1.30	0.15	1.23	0.15
Peanut flour	2	1.40	0.85	1.59	0.38	1.49	0.33	1.40	0.31	0.86	0.50	1.17	0.21	1.15	0.19	1.13	0.19
	4	1.51	0.42	1.41	0.34	1.40	0.32	1.36	0.33	0.97	0.26	1.11	0.22	1.13	0.21	1.14	0.22
	0	3.38	0.50	3.10	0.36	2.77	0.28	2.61	0.27	1.88	0.23	2.17	0.17	2.11	0.17	2.05	0.13
Textured	2	2.76	0.83	2.78	0.37	2.58	0.34	2.46	0.32	1.69	0.39	2.07	0.25	2.01	0.19	1.99	0.16
veg. protein	4	3.04	0.55	2.58	0.37	2.46	0.31	2.39	0.28	1.96	0.33	2.04	0.27	1.99	0.21	2.03	0.20
							6% Protein										
Casein	2	2.42	1.52	2.64	0.70	2.47	0.49	2.39	0.37	2.50	2.49	2.50	0.53	2.50	0.37	2.50	0.30
Textured veg. protein	2	1.37	1.62	2.10	0.56	2.05	0.35	1.95	0.27	3.34	0.46	2.00	0.45	2.09	0.36	2.05	0.28
Wheat flour	2	−0.66	1.09	−0.21	0.45	−0.07	0.29	0.02	0.27	−2.01	3.41	−0.23	0.45	−0.08	0.29	0.01	0.29

[a]Average is for 50 animals, SD = $\sum_{i=1}^{50} (X_i - \bar{X})^2/49$, where X_i = PER of individual animal and $\bar{X} = \sum_{i=1}^{50} X_i/50$.

× adaptation period × protein source, and laboratory × protein source × assay length interactions. Figures 1–5 graphically illustrate these interactions. These 3 interactions are evidenced by the changing effect due to assay length among laboratories, adaptation periods, and protein sources. There is little change in the PER values for a particular diet after an assay length of 2 weeks within a given laboratory; however, there is noticeable variation among laboratories, especially for peanut flour and TVP diets.

Analysis of APER measurements exhibits significant ($P < 0.01$) laboratory × adaptation period × assay length, and laboratory × adaptation × protein source interactions (Table 7). These are shown in Figures 6–9. The relationship of assay length changes with adaptation period among laboratories for each of the given protein sources. In general, the APER values show much less variation among laboratories than the PER values. This is especially true for assay lengths of 3 or 4 weeks.

Analysis of Data from 6% Protein Diets

Data from the 6% protein diets (Table 5) were analyzed by analysis of variance (Table 8) to investigate the effects of laboratory, protein source, and assay length on PER and APER. Both PER and APER analyses resulted in a significant ($P < 0.01$) laboratory × protein source × assay length interaction. This interaction is a result of a change in relationship between assay length and laboratories for different protein sources as illustrated in Figures 10 and 11.

Analysis of Data from Diets at 6% and 10% Protein Levels

Two protein sources, casein and TVP, were present at both high (10%) and low (6%) levels of protein. An analysis of variance on PER values was performed to investigate the effect of laboratory, protein level, protein, source, assay length, and their interactions (Table 9). This analysis shows a significant ($P < 0.01$) laboratory × protein level × assay length interaction. This interaction is illustrated in Figures 1

Table 7. Analysis of variance (mixed model)[a] for PER and APER values from 7 laboratories, 3 adaptation periods, and 4 periods of assay length (weeks)

Source		Degrees of freedom	Sum of squares	Mean square used as error term	*F*-value	Prob. > *F*
			PER Values, Five 10% Protein Diets			
L =	Lab	6	458.93	Residual	406.03	0.0001
A =	Adapt	2	94.95	L×A	24.76	0.0001
D =	Diet	4	2726.30	L×D	219.80	0.0001
W =	Week	3	161.48	L×W	6.89	0.0028
	L×A	12	23.00	Residual	10.18	0.0001
	L×D	24	74.42	Residual	16.46	0.0001
	L×W	18	140.62	Residual	41.47	0.0001
	A×D	8	1.79	L×A×D	0.70	0.6903
	A×W	6	9.49	L×A×W	1.15	0.3530
	D×W	12	69.62	L×D×W	10.09	0.0001
	L×A×D	48	15.36	Residual	1.70	0.0020
	L×A×W	36	49.40	Residual	7.28	0.0001
	L×D×W	72	41.39	Residual	3.05	0.0001
	A×D×W	24	3.64	L×A×D×W	0.86	0.6510
	L×A×D×W	144	25.34	Residual	0.93	0.7006
	Residual	3780	712.08	—		
	Total	4199	4607.81			
			APER Values, Four 10% Protein Diets (excluding reference protein casein)			
L =	Lab	6	20.59	Residual	27.18	0.0001
A =	Adapt	2	3.50	L×A	4.14	0.0428
D =	Diet	3	1520.48	L×D	284.98	0.0001
W =	Week	3	30.61	L×W	9.71	0.0005
	L×A	12	5.07	Residual	3.35	0.0001
	L×D	18	32.01	Residual	14.08	0.0001
	L×W	18	18.92	Residual	8.32	0.0001
	A×D	6	5.38	L×A×D	3.63	0.0065
	A×W	6	0.81	L×A×W	0.29	0.9376
	D×W	9	6.72	L×D×W	5.29	0.0001
	L×A×D	36	8.91	Residual	1.96	0.0006
	L×A×W	36	16.64	Residual	3.66	0.0001
	A×D×W	54	7.63	Residual	1.12	0.2580
	A×D×W	18	2.52	L×A×D×W	1.35	0.1717
	L×A×D×W	108	11.20	Residual	0.82	0.9093
	Residual	3024	381.90	—		
	Total	3359	2072.90			

[a]Using laboratories as a random factor.

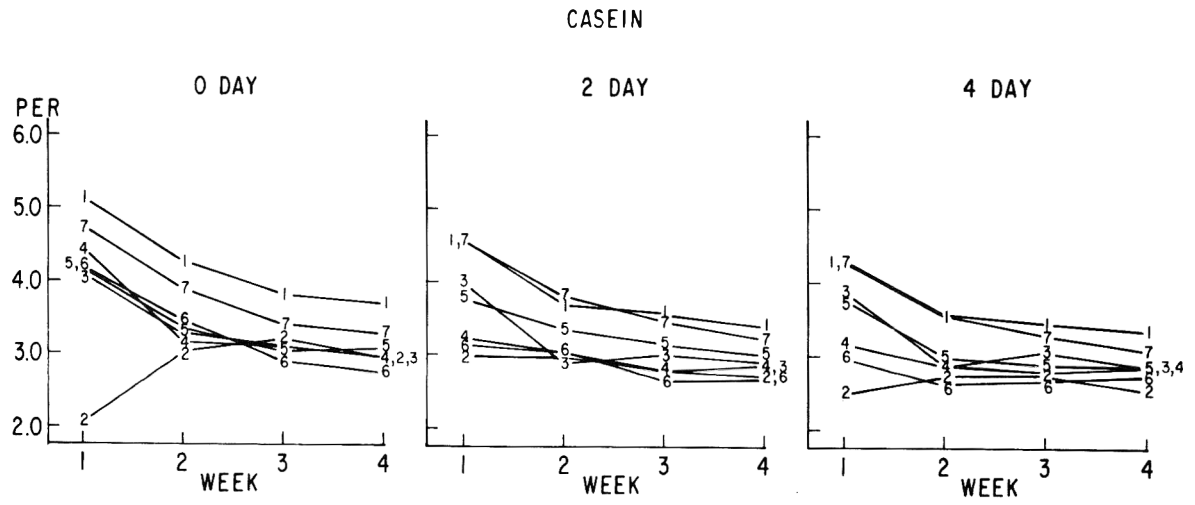

Figure 1. PER of ANRC casein (means for 10 rats fed 10% ANRC casein reference protein diet) following 0-, 2-, or 4-day adaptation period by each of 7 laboratories. Laboratory is denoted by numbers on connected line segments.

(2-day), 5 (2-day), and 10 and is explained by the change in relationship between assay length and laboratory at different protein levels. This change occurs for both protein sources.

Because the APER for casein is assumed to be 2.50, only the adjusted values for TVP were compared at the high and low protein levels. As Figures 9 (2-day) and 11 show, the effect of assay length on this measurement changes among laboratories and this change depends on which protein level is being used. This appears in the analysis of variance as a significant ($P < 0.01$) laboratory × protein level × assay length interaction (Table 9).

Reproducibility and Repeatability Variation

One of the main goals of this collaborative study was to estimate the reproducibility (i.e., among laboratories) variation and repeatability (i.e., within-laboratory) variation of PER and APER for each of the various combinations of factors. Tables 10 and 11 list the variances due to repeatability

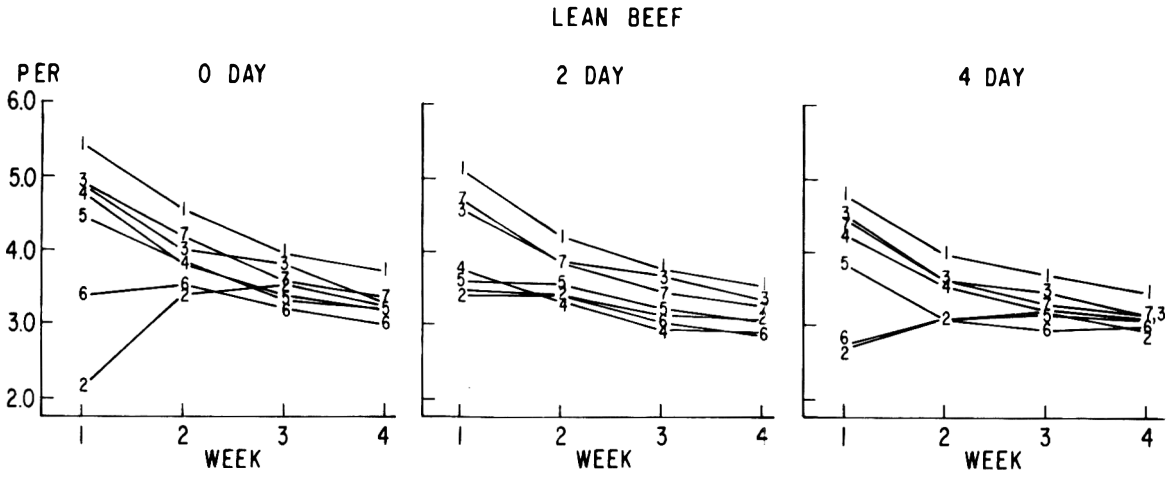

Figure 2. PER of beef (means for 10 rats fed 10% beef protein diet) following 0-, 2-, or 4-day adaptation period by each of 7 laboratories. Laboratory is denoted by numbers on connected line segments.

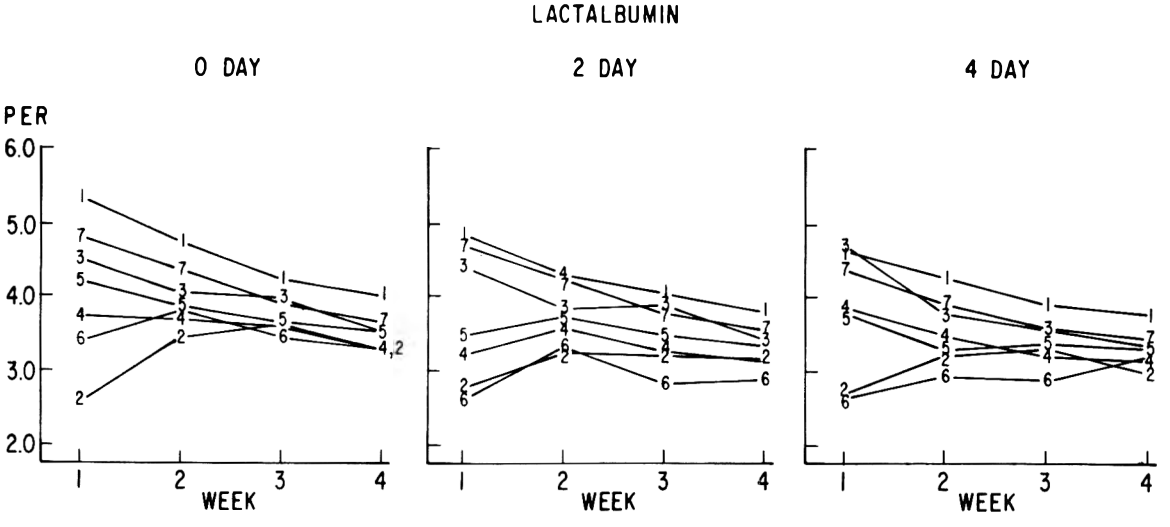

Figure 3. PER of lactalbumin (means for 10 rats fed 10% lactalbumin protein diet) following 0-, 2-, or 4-day adaptation period by each of 7 laboratories. Laboratory is denoted by numbers on connected line segments.

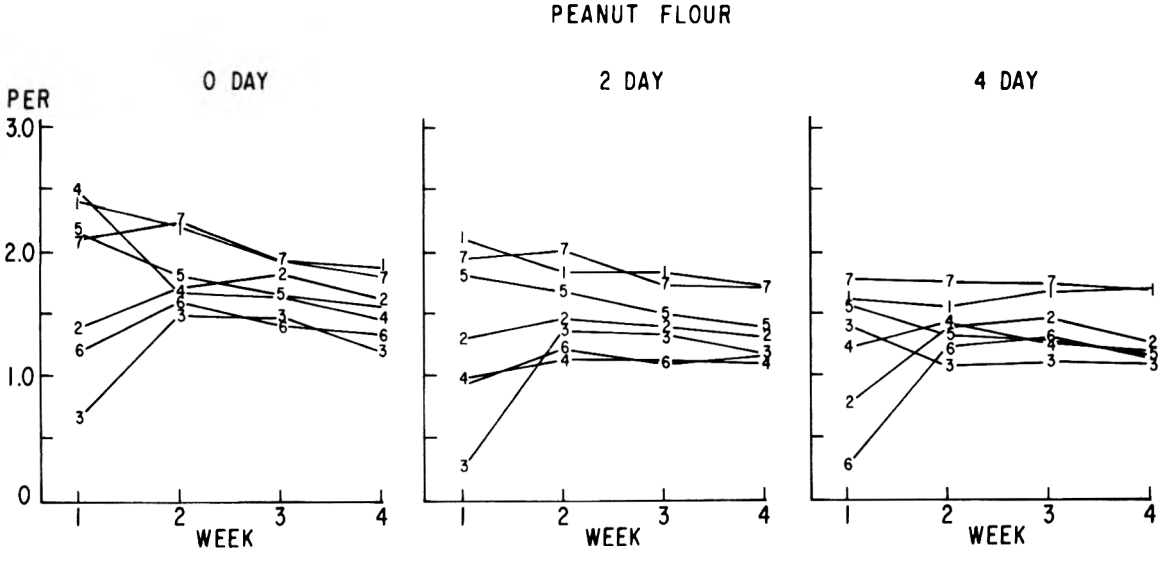

Figure 4. PER of peanut flour (means for 10 rats fed 10% peanut flour protein diet) following 0-, 2-, or 4-day adaptation period by each of 7 laboratories. Laboratory is denoted by numbers on connected line segments.

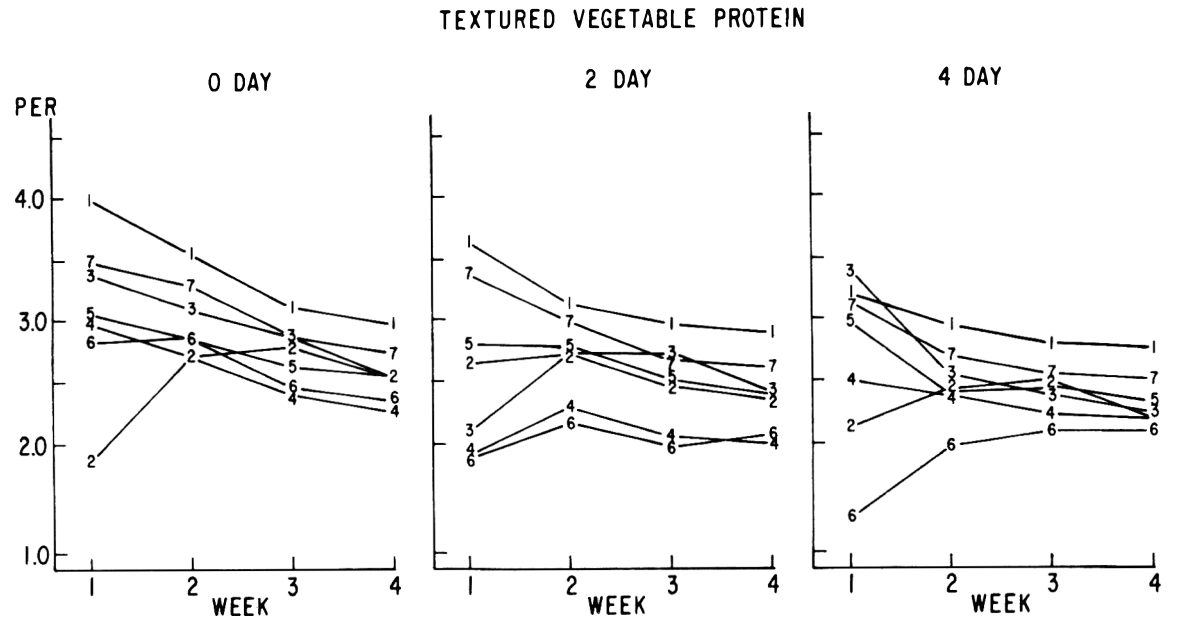

Figure 5. PER of textured vegetable protein (means for 10 rats fed 10% textured vegetable protein diet) following 0-, 2-, or 4-day adaptation period by each of 7 laboratories. Laboratory is denoted by numbers on connected line segments.

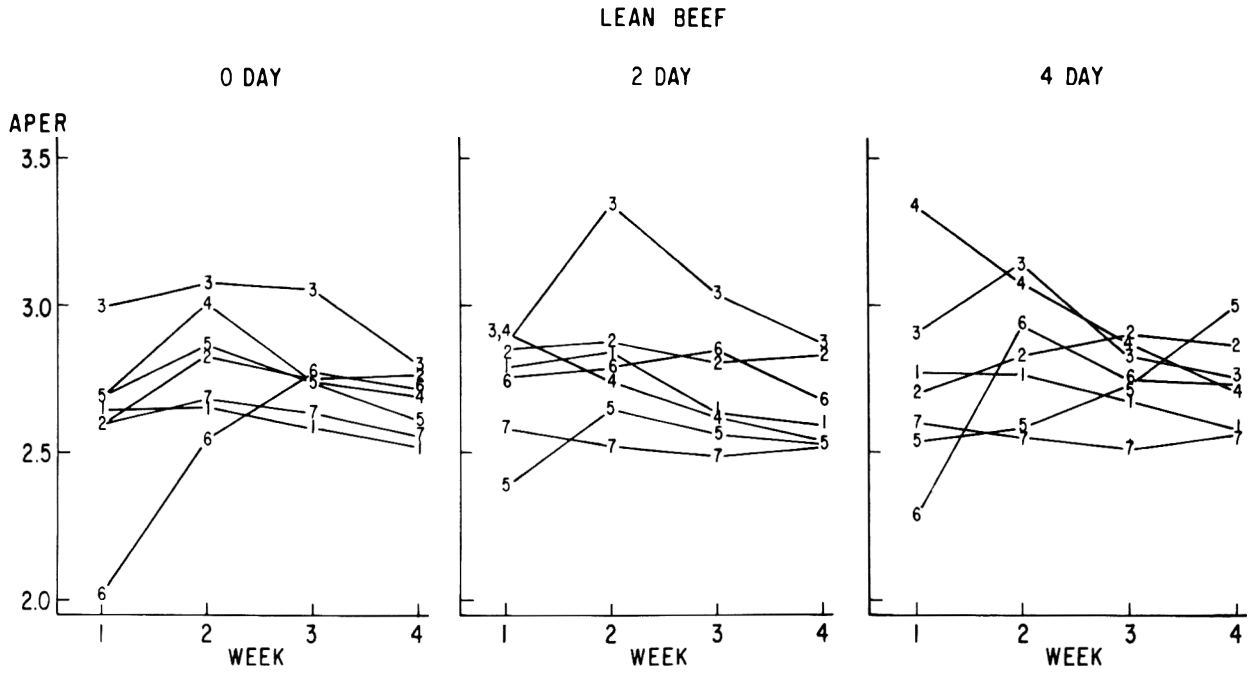

Figure 6. APER of beef (means for 10 rats fed 10% beef protein diet) following 0-, 2-, or 4-day adaptation period. Adjusted to the assumed PER of ANRC casein, 2.5. Data are for 7 laboratories. Laboratory is denoted by numbers on connected line segments.

and reproducibility of the PER and APER measurements for each protein source for each combination of adaptation period and length of assay. These variances were calculated using the values from all 7 laboratories (13).

Bartlett's test (15) has been applied to these variances to determine the minimal assay length needed to give repeatability and reproducibility variances which do not differ significantly from those of assays of a longer length (up to and including 4 weeks). These tests were performed for each of 18 diets in the study. The reproducibility comparisons showed that the variance due to reproducibility was significantly ($P < 0.05$) greater in the 2-week assay than in longer assays for every test diet except the three that involved peanut flour (Tables 10 and 11). These 3 diets showed no significant ($P < 0.05$) reduction in reproducibility variance when the assay was lengthened from 2 weeks; however, lengthening the assay to 2 weeks for the peanut flour diets did significantly ($P < 0.05$) reduce the reproducibility variation from that of one week. This was true for both PER and APER values.

Examination of the repeatability variances showed the same results except that even the peanut flour diet 2-week assays showed significantly ($P < 0.05$) larger repeatability variances in both PER and APER values than 3- or 4-week assays (Tables 10 and 11).

The comparison of standard deviations for the weekly repeatability and reproducibility of PER and APER values over all factors in this collaborative study—5 protein sources fed at 10% of the diet, 3 adaptation periods, 7 laboratories, and 10 animals/trial per laboratory (Table 12)—shows that lower standard deviations are obtained by adjusting PER

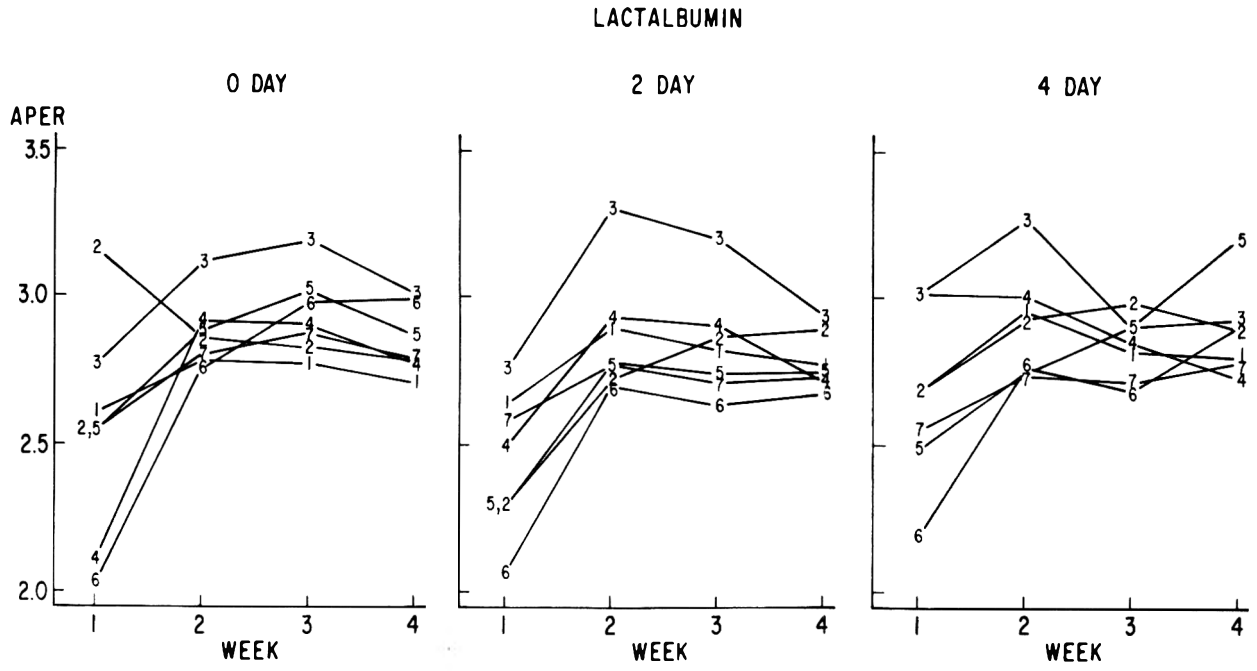

Figure 7. APER of lactalbumin (means for 10 rats fed 10% lactalbumin protein diet) following 0-, 2-, or 4-day adaptation period. Adjusted to the assumed PER of ANRC casein, 2.5. Data are for 7 laboratories. Laboratory is denoted by numbers on connected line segments.

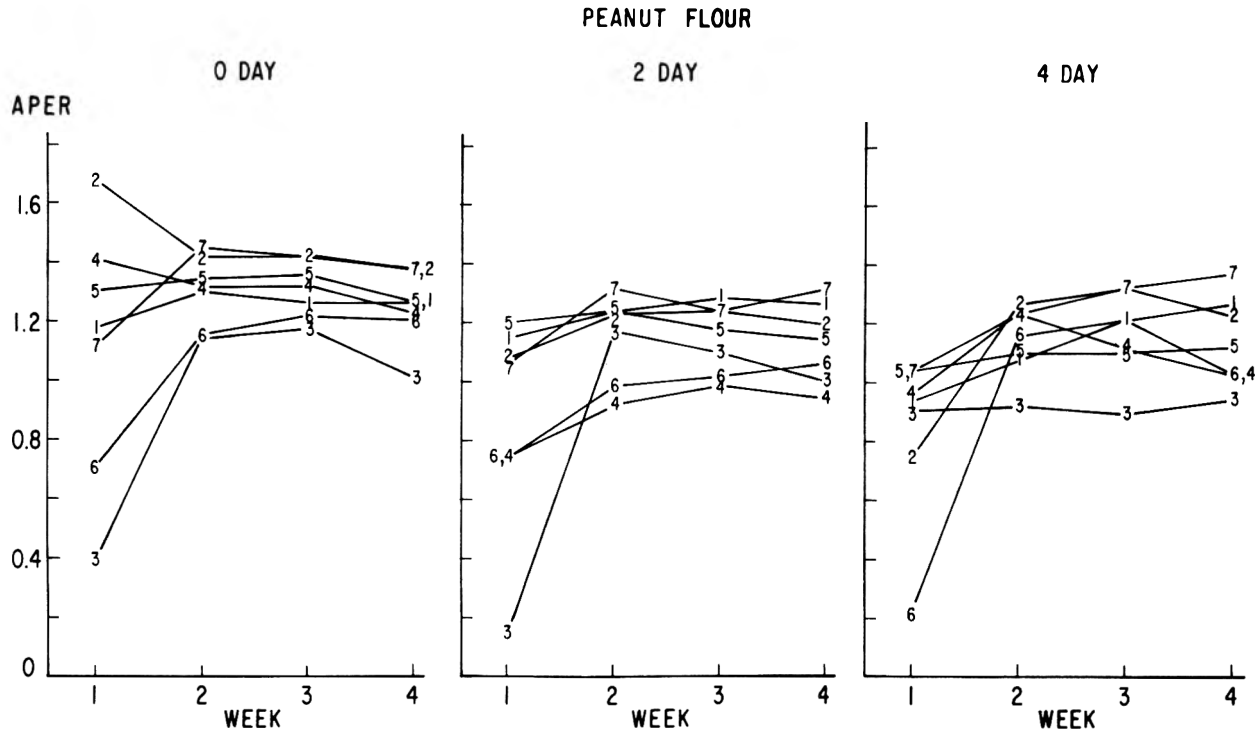

Figure 8. APER of peanut flour protein (means for 10 rats fed 10% peanut flour protein) following 0-, 2-, or 4-day adaptation period. Adjusted to the assumed PER of ANRC casein, 2.5. Data are for 7 laboratories. Laboratory is denoted by numbers on connected line segments.

values to that of the reference protein casein at 2.5. For both repeatability and reproducibility, standard deviations for the 3- and 4-week APER values are similar and at least 25% lower than standard deviations for the 2-week APER values.

The repeatability values of APER (Table 12) indicate that two 3-week results, each the average of data from 10 animals by the same analyst, should be considered suspect if the absolute value of their differences is greater than 0.15 APER. The value of 0.15 was established at the 95% level of confidence from data providing 756 DF.

The reproducibility values of APER (Table 12) indicate that two 3-week results, each the average of data from 10 animals, obtained by analysts in different laboratories should be considered suspect if the absolute value of their differences is greater than 0.30 APER, established at the 95% level of confidence from data providing over 756 DF.

The results from this collaborative study indicate that an assay period of 3 weeks has a reproducibility that is not statistically different from that of a 4-week assay. However, the reproducibility and repeatability variance is significantly ($P < 0.05$) greater for an assay length of 2 weeks than it is for 3- or 4-week assays.

Reasons for the decrease in the reproducibility variance in this collaborative study are probably related to the use of a

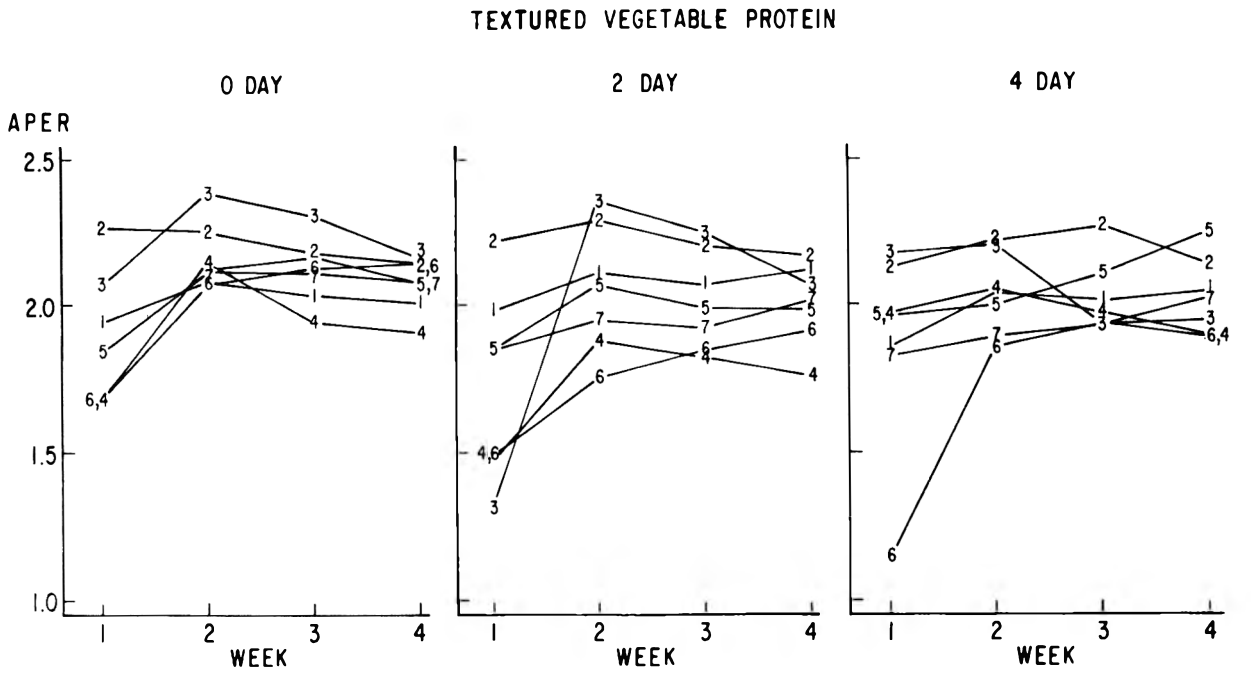

Figure 9. APER of textured vegetable protein (means for 10 rats fed 10% textured vegetable protein diet) following 0-, 2-, or 4-day adaptation period. Adjusted to the assumed PER of ANRC casein, 2.5. Data are for 7 laboratories. Laboratory is denoted by numbers on connected line segments.

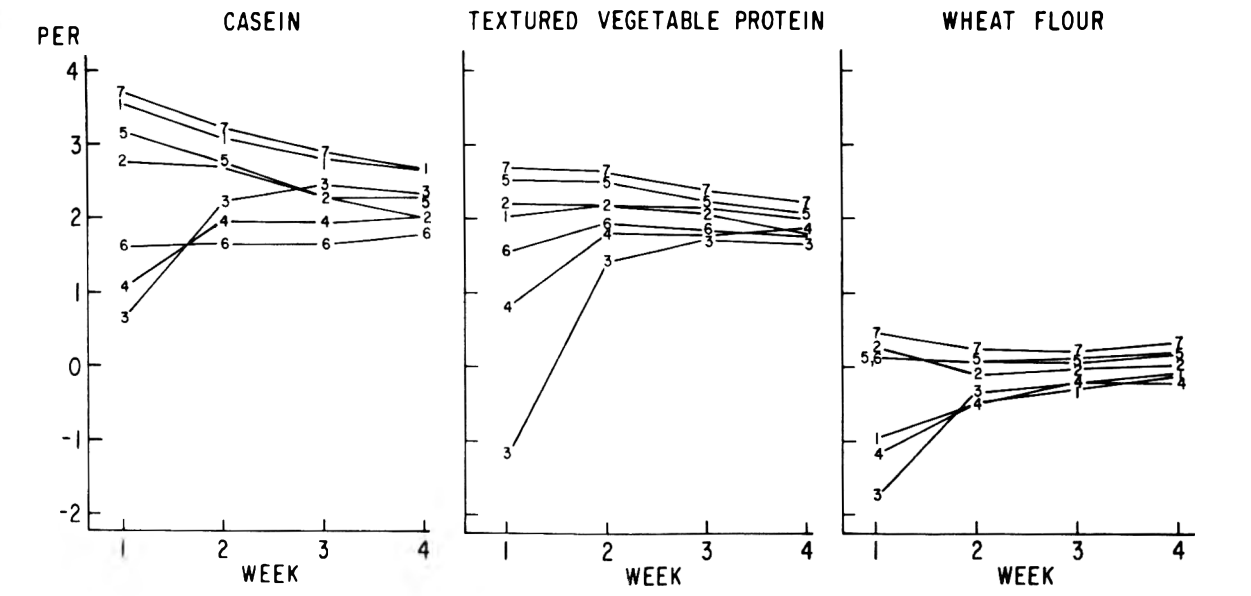

Figure 10. PER of ANRC casein, textured vegetable protein, and wheat flour (means for 10 rats fed 6% protein diets) following 2-day adaptation period by each of 7 laboratories. Laboratory is denoted by numbers on connected line segments.

single source of rats (with the exception of one laboratory that was identified as an outlier), the preparation and weighing of dietary ingredients by a single laboratory, and strict adherence to a detailed protocol. The analysis, preparation, and weighing of dietary ingredients and mixing of the diets followed by analysis of the diets probably represents an important source of variation of data between laboratories. Probably the largest single uncontrollable source of variation in a bioassay such as PER is the variation among the test animals.

PER Adjusted to Reference Protein Casein

Our PER values showed a significant laboratory effect, which is in agreement with other collaborative studies (16–19). However, adjusting the PER data to a casein standard diet equal to 2.50 reduced the laboratory effect. This is an important confirmation of earlier research (12) and suggests that the use of a casein standard is warranted.

Results of this study and of data from other researchers and workers indicate that PER of ANRC casein is higher than 2.5, the value suggested by Chapman et al. (8, 12). Hegarty questioned the wisdom of accepting a PER value of 2.5 for a 10% reference standard casein diet because of higher values obtained in recent literature and suggested this matter should be re-evaluated (8). Results of this collaborative study add considerable strength to his suggestion. Perhaps a better standard reference diet would be one using casein plus methionine (19). Since methionine (or total sulfur amino acids) is first limiting in casein for the rat, and some protein sources will produce PER values above that found for ANRC casein, the use of ANRC casein plus methionine as the standard refer-

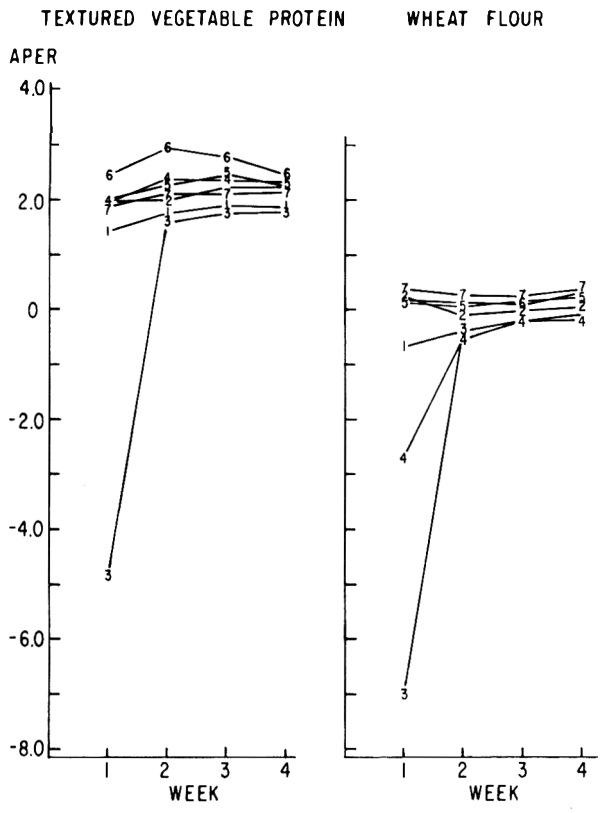

Figure 11. APER of textured vegetable protein and of wheat flour (means for 10 rats fed 6% protein diet) following 2-day adaptation period. Adjusted to the assumed PER of ANRC casein. Data are for 7 laboratories. Laboratory is denoted by numbers on connected line segments.

ence protein would permit investigators to rank protein sources with a greater degree of confidence.

The effect of the length of time allowed for the rats to adapt to their new environment and to get over shipping stresses was also reduced by calculating an APER. Unpublished data on the length of adaptation from one of the collaborators (L. R. Hackler) suggest that this factor is important in the determination of APER. One possible explanation for this discrepancy may be related to the basal diet. In the AACC/ASTM collaborative study, the basal diet was ANRC casein at the 10% crude protein level, whereas in the aforementioned studies, the basal diet contained 25% casein or 22.5% crude protein. This 25% casein diet was not only higher in protein, but higher in minerals, vitamins, and fat, and contained a mixture of procaine penicillin and streptomycin sulfate to eliminate low-grade infections associated with shipping stresses. All of these factors may be involved with the ability of rats to overcome shipping stresses and to adapt to their new environment.

Protein level of the test diet was also examined in this AACC/ASTM study. The results show that the level of protein in the test diet has a significant effect on both APER (Figures 9 and 11) and PER (Figures 1, 5, and 10) determinations (Table 9). Again, the APER values reduce the effect of protein level.

As expected, the wide variety of protein sources produced a large range of PER values. The determination of APER significantly reduces the differences due to protein sources (Tables 10, 11).

Collaborative Recommendations

The data from this study indicate that several changes should be made in the AOAC official method for protein efficiency ratio (secs. **43.212–43.216,** *Official Methods of Analysis* (1980) 13th Ed., p. 774). Therefore, it is suggested that:

1. In sec. **43.213,** laboratory rats should be weaned at 21 days old and be no more than 23 days old on arrival at the laboratory.
2. In sec. **43.213,** when animals are transported from breeding colony to test laboratory, an acclimation period of 2 days should precede the test.
3. The PER method should be changed from a 28- to 21-day feeding test, immediately following the 2-day acclimation period.
4. Each laboratory should maintain strict adherence to the AOAC official method for the test.
5. The test should be standardized to a 10% protein (1.6% N) ANRC casein diet with a range no greater than 9.5–10.5%.

Table 8. Analysis of variance (mixed model)[a] for PER and APER values from 7 laboratories and 4 periods of assay length (weeks)

Source	Degrees of freedom	Sum of squares	Mean square used as error term	*F*-value	Prob. > *F*
PER Values, Three 6% Protein Diets					
L = Lab	6	136.96	Residual	111.47	0.0001
D = Diet	2	988.43	L×D	118.32	0.0001
W = Week	3	13.84	L×W	0.75	0.5388
L×D	12	50.12	Residua	20.40	0.0001
L×W	18	111.36	Residua	30.21	0.0001
D×W	6	10.76	L×D×W	4.49	0.0017
L×D×W	36	14.37	Residua	1.95	0.0008
Residual	756	154.81	—		
Total	839	1480.65			
APER Values, Two 6% Protein Diets (excluding reference protein casein)					
L = Lab	6	85.87	Residual	83.10	0.0001
D = Diet	1	573.36	L×D	233.05	0.0001
W = Week	3	19.10	L×W	1.62	0.2203
L×D	6	14.76	Residual	14.28	0.0001
L×W	18	70.84	Residual	22.85	0.0001
D×W	3	2.95	L×D×W	2.09	0.1377
L×D×W	18	8.47	Residual	2.73	0.0002
Residual	504	86.80	—		
Total	559	862.15			

[a]Using laboratories as a random factor.

Table 9. Analysis of variance (mixed model)[a] for PER and APER values for diets at 2 protein levels from 7 laboratories and 4 periods of assay length (weeks)

Source		Degrees of freedom	Sum of squares	Mean square used as error term	*F*-value	Prob. > *F*
PER Values, Casein and TVP at 6% and 10% Protein Levels						
L =	Lab	6	180.01	Residual	176.71	0.0001
D =	Diet	1	100.84	L × D	59.69	0.0002
	Level	1	170.68	L × Level	35.91	0.0010
W =	Week	3	10.23	L × W	0.84	0.4875
	L × D	6	10.14	Residual	9.95	0.0001
	L × Level	6	28..52	Residual	28.00	0.0001
	L × W	18	72.67	Residual	23.78	0.0001
	D × Level	1	4.08	L × D × W	1.68	0.2431
	D × W	3	14.03	L × D × Level	11.82	0.0002
	Level × W	3	20.89	L × Level × W	3.16	0.0498
	L × D × Level	6	14.63	Residual	14.36	0.0001
	L × D × W	18	7.12	Residual	2.33	0.0013
	L × Level × W	18	39.62	Residual	12.96	0.0001
	D × Level × W	3	0.20	L × D × Level × W	0.49	0.6943
	L × D × Level × W	18	2.47	Residual	0.81	0.6926
	Residual	1008	171.14	—		
	Total	1119	847.26			
APER Values, TVP at 6% and 10% Protein Levels						
L =	Lab	6	77.13	Residual	26.87	0.0001
	Level	1	1.26	L × Level	0.08	0.7893
W =	Week	3	58.38	L × W	2.23	0.1198
	L × Level	6	96.78	Residual	33.71	0.0001
	L × W	18	157.10	Residual	18.24	0.0001
	Level × W	3	22.09	L × Level × W	1.33	0.2957
	L × Level × W	18	99.62	Residual	11.57	0.0001
	Residual	504	241.15	—		
	Total	559	753.51			

[a]Using laboratories as a random factor.

Table 10. PER and APER variances for 10% protein diets[a]

Statistic	Adap., days	Casein, week 1	2	3	4	Lactalbumin, week 1	2	3	4	Textured vegetable protein, week 1	2	3	4
PER:													
Reproducibility	0	1.92	0.35	0.14	0.12	2.11	0.29	0.14	0.11	1.08	0.16	0.08	0.08
Repeatability		1.07	0.17	0.05	0.03	1.40	0.11	0.07	0.04	0.71	0.07	0.02	0.02
Reproducibility	2	0.68	0.22	0.17	0.10	1.23	0.23	0.23	0.12	0.77	0.20	0.16	0.11
Repeatability		0.28	0.10	0.05	0.03	0.41	0.06	0.05	0.04	0.31	0.08	0.03	0.02
Reproducibility	4	0.79	0.20	0.11	0.08	1.09	0.30	0.14	0.09	0.88	0.20	0.10	0.08
Repeatability		0.35	0.06	0.03	0.02	0.39	0.10	0.04	0.03	0.41	0.12	0.06	0.03
APER:													
Reproducibility	0	1.27	0.10	0.03	0.02	0.64	0.07	0.06	0.04	0.90	0.05	0.03	0.02
Repeatability		1.27	0.10	0.03	0.02	0.55	0.06	0.05	0.03	0.90	0.04	0.02	0.02
Reproducibility	2	0.16	0.06	0.04	0.02	0.25	0.08	0.06	0.03	0.25	0.10	0.05	0.03
Repeatability		0.16	0.06	0.04	0.02	0.21	0.04	0.04	0.03	0.16	0.06	0.02	0.02
Reproducibility	4	0.23	0.05	0.03	0.02	0.30	0.11	0.04	0.05	0.35	0.10	0.05	0.04
Repeatability		0.23	0.05	0.03	0.02	0.24	0.08	0.03	0.02	0.26	0.09	0.04	0.03

Table 10 (continued)

Statistic	Adap., days	Beef, week 1	2	3	4	Peanut flour, week 1	2	3	4
PER:									
Reproducibility	0	3.05	0.24	0.10	0.07	1.10	0.13	0.07	0.08
Repeatability		1.96	0.10	0.03	0.02	0.67	0.05	0.03	0.02
Reproducibility	2	0.76	0.18	0.13	0.08	0.65	0.16	0.11	0.09
Repeatability		0.32	0.08	0.03	0.02	0.23	0.05	0.04	0.02
Reproducibility	4	0.92	0.17	0.08	0.05	0.56	0.11	0.09	0.10
Repeatability		0.22	0.06	0.03	0.02	0.30	0.06	0.04	0.03
APER:									
Reproducibility	0	1.70	0.09	0.04	0.03	0.43	0.04	0.03	0.03
Repeatability		1.70	0.06	0.02	0.02	0.26	0.02	0.02	0.01
Reproducibility	2	0.17	0.11	0.06	0.04	0.24	0.05	0.03	0.03
Repeatability		0.15	0.05	0.02	0.02	0.11	0.03	0.02	0.02
Reproducibility	4	0.24	0.09	0.04	0.04	0.26	0.05	0.05	0.04
Repeatability		0.14	0.04	0.02	0.02	0.19	0.04	0.03	0.02

[a]Reproducibility and repeatability are over 70 animals (7 labs and 10 animals per lab). Take square root to convert to SD.

Table 11. PER variances for 6% protein diets[a] (2-day adaptation period)

Protein source	Statistic	Week 1	Week 2	Week 3	Week 4
	PER:				
Casein	reprod.	2.05	0.62	0.32	0.19
	repeat.	0.54	0.31	0.14	0.09
Textured vegetable protein	reprod.	2.26	0.28	0.14	0.09
	repeat.	0.47	0.12	0.08	0.06
Wheat flour	reprod.	1.14	0.19	0.08	0.06
	repeat.	0.45	0.12	0.05	0.03
	APER:				
Casein	reprod.	5.05	0.44	0.19	0.12
	repeat.	5.05	0.44	0.19	0.12
Textured vegetable protein	reprod.	9.50	0.32	0.22	0.13
	repeat.	3.23	0.15	0.11	0.08
White wheat flour	reprod.	10.27	0.21	0.08	0.08
	repeat.	3.22	0.13	0.05	0.04

[a]Reproducibility and repeatability are over 70 animals (7 labs and 10 animals per lab). Take square root to convert to SD.

Table 12. Standard deviations for repeatability and reproducibility of PER and APER values at each week of assay

Week	PER[a] Repeat.	PER[a] Reprod.	APER[b] Repeat.	APER[b] Reprod.
1	0.78	1.08	0.64	0.68
2	0.29	0.45	0.23	0.28
3	0.20	0.35	0.16	0.21
4	0.16	0.30	0.14	0.19

[a]Values are over 7 laboratories, 10 animals per laboratory per trial, five 10% protein diets (casein, beef, lactalbumin, peanut flour, and textured vegetable protein), and 3 adaptation periods.
[b]As in a, except casein diet is excluded.

6. For protein sources that contain less than 12% crude protein, the highest percentage crude protein diet possible should be used and PER should be determined on a reference standard ANRC casein diet at the same crude protein level and other ingredients at the same level as in the test protein diet.

Acknowledgment

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Liquid Chromatographic Determination of Ascorbic Acid in Foods

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Ascorbic acid has been determined quantitatively in a variety of fresh and frozen fruits and vegetables, fresh and fortified canned juices, and powdered drinks by liquid chromatography (LC). The method consists of blending the sample in a solution of 0.05% EDTA in 0.2N H_2SO_4, centrifuging or filtering the mixture, then injecting a portion into the LC system. An Aminex HPX-87 LC column was used with 0.009N H_2SO_4 as solvent with a flow rate of 0.5 mL/min. Detection was at 245 nm. The method is simple and sensitive, and yields a high recovery. It compares favorably with the AOAC method, and has the advantage of being more accurate for samples with interfering pigments.

Ascorbic acid is the nutrient most affected by processing fruits and vegetables, and its retention is often used as an indication of retention of other nutrients (1, 2). Present methods for quantitative determination of ascorbic acid in foods and food products include the AOAC visual titration and fluorometric methods (3), a polarographic method (4), a gas chromatographic (GC) method (5), and ion-pairing LC methods (6, 7). Fluorometric and polarographic methods yield values higher than the actual ascorbic acid content, and the GC method is rather time consuming (7). The original ion-pairing LC method failed to completely separate ascorbic acid from interfering compounds in potato extracts, and was modified to analyze ascorbic acid in only potatoes and potato products (7).

The most commonly used of these methods on foods and food products is the AOAC visual titration method because it is simple and sensitive. It is based on titration of sample extract with 2,6-dichloroindophenol to a rose-pink that lasts for at least 5 s. The method, however, has 2 main disadvantages: First, it is difficult to visually determine the end point in the presence of interfering pigments (7), and, second, reducing compounds, including reducing ions such as ferrous, stannous, and cuprous ions, interfere (3).

The object of the present study was to develop a sensitive and specific LC method for determining ascorbic acid in various foods and food products, which has the inherent advantages of LC.

Experimental

Apparatus

(a) *Liquid chromatograph.*—Waters Associates, equipped with Model 6000A pump, Model U6K injector, and data module (Waters Associates, Milford, MA 01757).

(b) *Detector.*—Gilson Model 222 (Gilson Medical Electronics, Middleton, WI 53562), set at 245 nm, and a sensitivity unit of 0.05.

(c) *LC column.*—300 × 7.8 mm Aminex HPX-87 with Micro-Guard ion exclusion cartridge (Bio-Rad Laboratories, Richmond, CA 94804).

(d) *Centrifuge.*—Sorvall Model RC-5 with rotor No. SS-34 (DuPont Co., Newton, CT 06470).

(e) *Blender*—Waring.

Reagents

(a) *0.2N H_2SO_4 solution.*—Prepare in LC water.

(b) *Disodium ethylenediaminetetraacetate (EDTA) solution.*—0.05%. Dissolve 500 mg EDTA (ACS, Fisher Scientific Co.) in 1 L of solution (a).

(c) *Ascorbic acid standard solution.*—20 μg/mL. Accurately weigh 20 mg L-ascorbic acid (anhydrous, Sigma Chemical Co., St. Louis, MO 63178) into 100 mL volumetric flask, dissolve and dilute to volume with solution (b). Dilute 10 mL aliquots of this solution to 100 mL with solution (b).

(d) *LC mobile phase.*—0.009N H_2SO_4. Prepare in LC water.

(e) *LC water.*—Pass distilled, deionized water through 0.45 μm filter membrane.

Sample Preparation

(a) *Liquids.*—Dilute appropriate volume of liquid samples (2 mL orange, lemon, grapefruit, and fortified pineapple juices) to final volume of 50 mL with solution (b). Filter through Whatman No. 1 paper and refrigerate until LC analysis. Final sample extract contains 10–20 μg ascorbic acid/mL.

(b) *Solids and semi-solids.*—Blend appropriate weight (tomatoes, peas, and potatoes, 20 g; strawberries, 10 g; green peppers, 2 g; orange concentrate, 1 g) with proper volume of solution (b) for 3 min, centrifuge at 15 000 rpm for 10 min, quantitatively transfer supernatant to 100 mL volumetric flask, and dilute to volume with solution (b). Refrigerate until LC analysis. Final sample extract contains 10–20 μg ascorbic acid/mL.

(c) *Powders.*—Dissolve appropriate weight (0.2 g brand A; 0.1 g brand B) in solution (b) to final volume of 50 mL. Filter through Whatman No. 1 paper and refrigerate until LC analysis. Final sample extract contains 10–20 μg ascorbic acid/mL.

LC Analysis

Set flow rate at 0.5 mL/min and inject 10 μL ascorbic acid standard solution into liquid chromatograph. Establish retention time and peak area, and calibrate data module accordingly (external standard method). Inject 10 μL sample extract and obtain amount of injected ascorbic acid directly from calibrated data module. Calculate ascorbic acid content in mg/100 mL for liquid samples and in mg/100 g for solid and semi-solid samples. Re-inject 10 μL ascorbic acid standard solution and recheck data module calibration at regular intervals.

Recovery Study

Add proper volume of ascorbic acid standard solution to sample so that ascorbic acid content of spiked sample approximately doubles. Determine ascorbic acid content of spiked sample as described previously and calculate percent recovery.

Comparison with AOAC Method

The ascorbic acid content of selected samples was also determined by the AOAC official method (3) and results were compared with those for the LC method.

Results and Discussion

The 0.2N H_2SO_4 solution used to extract ascorbic acid from various food samples had a pH of about 1.0 and contained 0.05% EDTA. Addition of EDTA to ascorbic acid standard solutions increased the area of the ascorbic acid peak more than 3 times (Table 1), and also increased the stability of

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Table 1. Detector response to ascorbic acid standards with and without 0.05% EDTA

Ascorbic acid, ng	Detector response (area units × 10^{-6})[a]	
	Without EDTA	With EDTA
100	1.2 ± 0.1	3.9 ± 0.1
200	2.7 ± 0.2	8.3 ± 0.2
300	3.7 ± 0.1	11.6 ± 0.3

[a]Average of 3 runs ± SD.

ascorbic acid in the standard solutions and sample extracts. When ascorbic acid standard solutions with 0.05% EDTA were kept at room temperature and analyzed every 24 h, no significant decrease in ascorbic acid content was detected after 48 h. EDTA is a chelating agent which reacts with the metal ions in the system and therefore prevents the oxidation of ascorbic acid. The presence of EDTA in the extraction solution was essential for increasing sensitivity and reproducibility. EDTA is not retained by the column and is eluted at a shorter retention time (7.9 min) than ascorbic acid (12.9 min). Therefore, it does not hinder the quantitative determination of ascorbic acid (Figure 1).

Detector response to ascorbic acid was studied at different wavelengths ranging from 210 to 270 nm. Under the LC conditions used, response was maximum at 245 nm. Linear response was obtained over the range 50–500 ng ascorbic acid in the presence of EDTA. Results also indicated that the LC method is sensitive since ascorbic acid could be detected at nanogram levels (Figure 1).

The ascorbic acid content of the food samples analyzed by the LC method is presented in Table 2; selected sample chromatograms are shown in Figure 2. In all samples, the ascorbic acid peak was well resolved with no interference. In samples spiked with standard ascorbic acid, only the peak identified as ascorbic acid increased in size.

The recovery of ascorbic acid from selected spiked samples by the LC method is presented in Table 3. Results ranged from 97.5 ± 3.7 to 103.6 ± 2.8% with coefficients of variation ranging from 2.7 to 4.8%. These results indicate that the LC method is accurate and precise.

The ascorbic acid content of selected food samples determined by the LC and AOAC methods is shown in Table 4. Results obtained for ascorbic acid in fresh lemon juice, fresh grapefruit juice, frozen peas, and brand A powdered drink by the LC method agree well with results by the AOAC method. However, in the case of tomatoes and strawberries, the AOAC method yielded higher values than the LC method. This was because in the AOAC method it was difficult to determine the rose-pink end point in the red-colored sample extracts. In all determinations, the tendency was always to

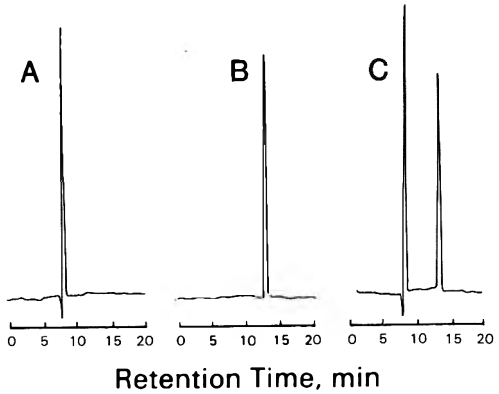

Figure 1. Chromatograms of EDTA (A), ascorbic acid (B), and ascorbic acid in EDTA (C).

Table 2. Ascorbic acid in food samples determined by LC method

Sample	Ascorbic acid,[a] mg/100 g	CV, %
Fresh orange juice	51.6 ± 2.2[b]	4.3
Fresh lemon juice	32.7 ± 1.5[b]	4.6
Fresh grapefruit juice	27.4 ± 1.5[b]	5.5
Tomatoes	12.8 ± 0.6	4.7
Green peppers	86.1 ± 3.6	4.2
Potatoes	12.5 ± 0.7	5.6
Frozen orange concentrate	165.0 ± 7.5	4.5
Frozen peas	15.2 ± 0.7	4.6
Frozen strawberries	26.9 ± 0.8	3.0
Canned fortified pineapple juice	44.1 ± 1.5[b]	3.4
Brand A powdered drink	411.3 ± 13.0	3.2
Brand B powdered drink	749.2 ± 9.9	1.3

[a]Average of 6 determinations ± SD.
[b]mg/100 mL.

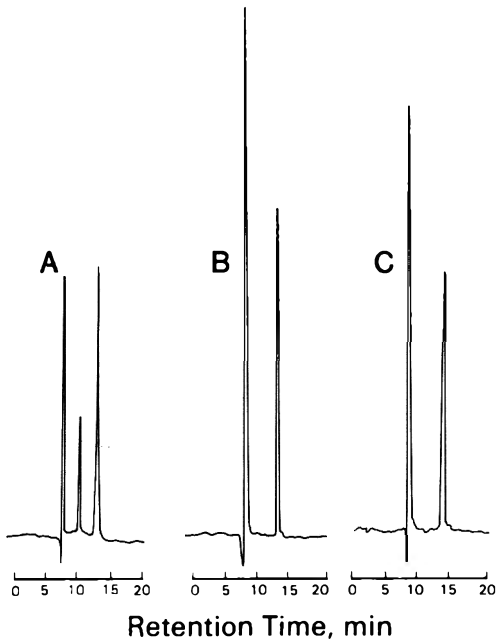

Figure 2. Chromatograms of fresh lemon juice (A), green peppers (B), and potatoes (C). Ascorbic acid peak (t_r 12.9 min) represents 140 ng (A), 178 ng (B), 137 ng (C).

Table 3. Recovery of ascorbic acid from spiked samples by LC method

Sample	Rec.,[a] %	CV, %
Fresh lemon juice	103.6 ± 2.8	2.7
Fresh grapefruit juice	101.3 ± 3.2	3.2
Green peppers	100.5 ± 4.8	4.8
Brand A powdered drink	97.5 ± 3.7	3.8

[a]Average of 6 determinations ± SD.

Table 4. Determination of ascorbic acid in foods by LC and AOAC methods

Sample	Ascorbic acid, mg/100g		CV, %	
	LC[a]	AOAC[b]	LC	AOAC
Fresh lemon juice	32.7 ± 1.5	31.4 ± 0.4	4.4	1.1
Fresh grapefruit juice	27.7 ± 1.5	26.3 ± 1.4	5.4	5.3
Tomatoes	12.8 ± 0.6	15.8 ± 1.0	4.7	6.3
Frozen peas	15.2 ± 0.7	16.5 ± 0.5	4.6	3.0
Frozen strawberries	26.9 ± 0.8	31.5 ± 1.5	3.1	4.8
Brand A powdered drink	411.2 ± 13.0	401.2 ± 3.9	4.4	1.0

[a]Average of 6 determinations ± SD.
[b]Average of 3 determinations ± SD.

over-titrate to obtain a visible change in color, which resulted in higher values. No pigment interference was encountered in the LC method.

The data obtained from this study indicated that the developed LC method is simple, accurate, and precise. It suffers no interference and is specific to ascorbic acid, and, moreover, it is more versatile than the AOAC method and other reported LC methods.

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METHOD PERFORMANCE

Performance of Methods of Analysis Used for Regulatory Purposes. I. Drug Dosage Forms. A. Chromatographic Separation/Spectrophotometric Measurement

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The precision attributes of one of the most widely used methods for the assay of drug dosage forms, partition chromatographic separation on diatomaceous silica and spectrophotometric measurement (ultraviolet, visible, or fluorometric), were recalculated on a consistent statistical basis, using a computer program FDACHEMIST. Eighteen collaborative studies of methods approved by AOAC during the past 10 years, of 24 compounds involving 69 dosage forms (here designated as materials), conducted by an average of 10 laboratories each, were reviewed.

The original data with no outliers removed were used for the calculation of mean, median, and standard deviations and coefficients of variation, both within- (CV_o) and between-laboratory (CV_x), for each material. Outliers in each data set were flagged by the Dixon and Grubbs tests for extreme values, the Cochran test for excessive variance within a laboratory, and the Youden rank sum test for consistent systematic deviations by specific laboratories.

The change in CVs if outliers are removed is tabulated and evaluated, generally using a 20% or greater outlier removal rate as excessive. That CV_x is chosen for each material which, with a minimum rejection of outliers (frequently zero), is acceptable in the light of the purpose of the assay and historical values. The method is approximately characterized by a CV_x of 2.25% and CV_o of 1.25% (CV_o/CV_x ratio of 0.56), with an average outlier rejection rate of 2.5% of the reported values. The precision parameters from the analyses of 80% of the materials studied could be accepted as is (without flagged outliers removed), although data from analyses of 48% of the materials included contained outliers at the 95% or higher confidence level (single tail test).

The CVs appear to be independent of the chemical nature of the analyte (phenylalkanolamines, thiazides, steroids, alkaloids, and miscellaneous) or the nature of the matrix (tablets, capsules, solutions). The CV_x to some degree depends on concentration with most of the values for CV_x falling within the following crude prediction limits (95% confidence level) for each indicated analyte level: 100% concentration, 0–2.5%; 10%, 0.5–3.0; 1%, 1.0–3.5; 0.1%, 1.5–4.0; and 0.01%, 2.0–4.25. Systematic deviations were not significant for this method, since means from the analyses of synthetic formulations were usually within 98–102% of the target value.

The performance of analytical methods for the determination of 57 active ingredients in pharmaceutical dosage forms in 36 collaborative studies published in the *Journal* of the AOAC during the period 1972 to early 1977 was reviewed by Horwitz (1). The methods were classified roughly according to their predominant operational feature—a liquid chromatographic separation step or a gas chromatographic, spectrophotometric, or automated determinative step. The average overall coefficient of variation (CV) between-laboratories* (CV_x) was 2.6% and the average CV within-laboratories (CV_o) was 1.5%, over the concentration range of active ingredient of 0.1–100%. The rounded down CV value of 2% from this study was used to establish the 100% concentration point of the general CV_x-concentration curve constructed by Horwitz et al. (2), which empirically appears to be independent of analyte, matrix, and method of analysis.

Other important conclusions from the initial review of the performance of drug methods were as follows: *(1)* Systematic error was not an important component of the analytical error of these AOAC-approved drug methods; *(2)* the ratio of within-laboratory to between-laboratory CVs was ordinarily 0.50 to 0.67; and *(3)* the performance of automated methods was approximately the same as manual methods, although automated methods produced fewer outliers.

In conjunction with a program to more fully and systematically assess the performance of analytical methods used in regulatory work (3), the collaborative studies published in the *Journal* of the AOAC subsequent to the previous paper (1976) through 1982 have been examined. During this time, we have also become aware of the importance and significance of the tendency of a method and its operation to produce outliers as a performance characteristic of methods and operators (3). Consequently, the previous set of drug collaborative studies have been re-examined in greater detail with respect to outliers and statistical values have been recalculated on the same basis as the subsequent studies.

The purpose of this paper is to examine the performance characteristics of the methods of analysis for drug dosage forms which have been adopted by AOAC during approximately the past 10 years. The results of this examination may lead to criteria and guidelines for the acceptance of methods of analysis for regulatory work, a process previously handled on a case by case basis. The principles and procedures used with this commodity and concentration range will be extended to other commodities and concentration ranges. A review of the performance characteristics of the methods for sulfonamides in animal feeds and tissues (4) and a preliminary review of methods for aflatoxins in foods and feeds (5) have been published. These studies may need to be re-examined on the basis of present outlier criteria.

Procedures

Nomenclature.—Statistical terms used in this paper are ordinarily applied as used by Youden and Steiner (6), where the reproducibility coefficient of variation, CV_x, includes both within- and between-laboratory variability, and repeatability, CV_o, is used for only the within-laboratory variability. An important exception is the substitution of the term "material" for "sample." The word "sample" can have many different meanings, and it is not always clear from the context in what sense the word is being used. This term is used in different ways even in the *Statistical Manual.* In this paper, the word "material" is defined as *the homogeneous substance assembled by the Associate Referee for subdividing into identical laboratory samples* which are the one or more (replicate) portions sent to the participants in the collaborative study. Several materials are usually submitted in a single collabo-

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*The nomenclature used in this paper does not correspond completely to that subsequently recommended by the Committee on Statistics and the Committee on Interlaboratory Studies. However, the symbols, and particularly the subscripts, employed here are those used by Steiner (6), and are identical with those recommended by the Committees. To correspond with the currently recommended nomenclature, the only change that needs to be made is to replace the phrase "between-laboratory(ies)" with "reproducibility (including within-laboratory (ies)" as used in conjunction with the terms "variance," "standard deviation," and "coefficient of variation."

rative study. The materials analyzed in a collaborative study are serving as surrogates for true samples (portions representing a lot) ordinarily analyzed in actual practice by the same method.

Statistical calculations.—A computer program in the APL language designated as FDACHEMIST has been developed for the statistical analysis of collaborative study data. The data are introduced into the computer material by material for each study and the data from each material are assigned a string name so that the data may be recalled for later manipulation, if desired. The current routine is to obtain the following output:

(1) The actual input data arranged line by line by laboratory number (numbers must be used to identify the laboratories in this program) for a check of input accuracy.
(2) The data rearranged in column format in ascending order of concentration accompanied by the laboratory number (useful for tentatively identifying outliers at the ends of the distribution).
(3) Also available from a subsidiary computer program are the results from a Youden rank sum test (6) and a statement of which laboratories exceed the minimum and maximum allowable sum of ranks (test is applied over all the materials in a study but is not ordinarily applied when fewer than 5 materials are involved).
(4) The average and median concentrations (the relationship between the 2 values give an indication of skewness of the data); the total number of readings and the number of laboratories supplying the readings (indicates the experimental design and provides an editorial check for completeness of input).
(5) The standard deviations and the corresponding CVs calculated for within-laboratories (σ_o), "pure" between-laboratories (σ_L), and between-laboratories ($\sigma_x = \sqrt{\sigma_o^2 + \sigma_L^2}$).
(6) The repeatability ($2\sqrt{2}\sigma_o$) and reproducibility ($2\sqrt{2}\sigma_x$) intervals as defined by the International Organization for Standardization (ISO) Standard ISO 5725-1981 (7) (for quality control purposes).
(7) The results of the Dixon outlier test (6) as applied to individual values and as applied to laboratory averages (when replicates are present) to determine the presence of extreme values (at the 95, 97, 98, and 99% one-tail confidence levels).
(8) The results of the Grubbs outlier test (8) as applied to individual values and as applied to laboratory averages, also to determine the presence of extreme values (at the 95, 97.5, and 99% one-tail confidence levels).
(9) The results of the Cochran outlier test (9) at the 95% and 99% confidence levels to test for an extreme variance within any one laboratory (applied only when replicates are present). Intermediate confidence levels are assigned by interpolation to the nearest whole number.
(10) Finally, a graph, compatible with typewriter output, is drawn with laboratory number on the X-axis and the corresponding concentration (limits can be arbitrarily assigned) on the Y-axis, giving a visual presentation of the data and the laboratory averages in ascending order (for visual examination of the means, ranges, and laboratory and individual outliers).

The program will not remove outliers automatically, but requires a deliberate decision on the part of the operator to edit the data to remove selected outliers. If outliers are to be removed, the entire program must be recycled.

The program will handle unbalanced designs, but such a design complicates interpretation. On those few occasions where one or two multiple results were provided in a series of single values, the laboratory average was often substituted for the replicates to avoid complex interpretations.

This program was applied to all collaborative studies of drugs which resulted in approved AOAC methods so that the data would be calculated and interpreted on a consistent basis.

Concentration estimation.—In most studies the concentration of active ingredient was given or enough information was present to permit a direct calculation. In some published collaborative studies, the concentrations were expressed only on a unit dosage basis (per tablet or capsule), on an absolute weight basis, or as percent recovery. In most such cases, the original dosage weight could be obtained by contacting the original author or laboratory in order to place all data on a common concentration basis, usually percent. When the data were not available from this source, the weight of a unit dose was then obtained in one of the following 3 ways, listed in order of preference: *(1)* weighing a sample of current production of the presumably equivalent product or by consultation with the manufacturer; *(2)* estimating from information in the original report, usually based on the concentration of the simulated standard in the study, if present; and *(3)* if no longer manufactured, estimating by comparison of the picture in the Identification Guide of the appropriate edition of the PDR (10) with a tablet or capsule whose weight is known. Because of the importance of concentration in analytical work, collaborative reports should provide these values or should permit their calculation from the information given (e.g., synthetic formulations). In those few cases where it was necessary to estimate dosage weights, it is believed that the weights are in error by not more than about 10%.

Outlier treatment.—The most important single factor affecting precision statements of a method is the treatment of outliers. The importance of this factor was not fully realized during the compilation of the previous paper (1). Therefore, the statistics for the previous studies were recalculated according to the present procedure, on a material by material basis, so that the number and type of outliers and their effect on precision may be highlighted for examination.

Considerable experience in the review of collaborative studies has led to the following current procedure for handling outliers:

(1) Laboratories which are reported not to have followed the method are rejected as procedural deviates and are usually excluded from the statistical analysis, unless the procedural deviation is minor and no significant change in parameters results from inclusion or exclusion. Authors should not include in their tables procedural deviate data which are not used in the calculations. The inclusion of such data can lead to input data errors.

(2) Laboratories rejected by the rank sum test can be considered for elimination, unless their removal leaves fewer than 5 laboratories, in which case the study is deemed inadequate. For example, if a collaborative study includes 6 laboratories and 2 are shown to be rank sum outliers, only 4 laboratories would be left. Obviously, a rejection rate of 33% of the participants is excessive. A reasonable rejection limit imposed by the discontinuous nature of the number of laboratories constituting a collaborative study meeting the Youden minimum rule of (laboratories × materials) = 30 is one laboratory of 6 (17%). Viewed as a percentage, this is still a fairly large number, but to reject the study because of the

single failure would impose a large economic penalty on the other 5 participants. The penalty to be exacted for proceeding in the face of a large outlier rate is to include another method-characterizing parameter, namely "% outliers." The precision would then be expressed as a "CV of X% accompanied by Y% outliers." This gives fair warning to future users of the method that there may be a potential for the production of outliers. Such a stigma can be removed only by the absence of or a significantly lower level of outliers in additional collaborative studies of the method. If a method must be used for analyses before additional collaborative studies are performed, such analyses must be accompanied by sufficient replication to verify the absence of the potential gross errors. This verification is easily obtained for drugs by the preparation and concurrent analysis of a synthetic material or may be obtained also by the use of another validated method on the same material which is sometimes available for drugs in the official compendia.

When replication is present in a study, laboratory means are used in the rank sum test. With studies containing fewer than 5 materials, the rank test is not ordinarily used. In actual practice, it is often found that when rank sum laboratory outliers are present, many of the same values are outliers by extreme value tests, such as the Dixon and Grubbs tests. Because of allegations that the Youden rank sum test unjustifiably removes laboratories which are not true outliers (H. C. Hamaker, personal communication), special attention was given in this study to the results of the application of this test.

(3) Individual extreme values are examined for outliers by the Dixon test. The Dixon test is used by Youden (6), Steiner (6), and ISO (7) because of its simplicity and because it was designed for small numbers of values such as are usually present with each material examined in a collaborative study. When replicates are present, there are 2 ways of applying the Dixon tests—to individual values and to laboratory means. Both are used in this program. When a laboratory average is shown to be a Dixon outlier, all values from that laboratory for that material are rejected; when only an individual value is exposed, only that value is omitted. When applied to individual values, the Dixon test requires a single normal population, which in turn requires that the laboratory means be effectively identical. This assumption is frequently violated in actual practice, but in such cases, if a value is really an outlier, it is also typically caught by one of the other outlier tests.

Here, as in the case of the rank sum test, the possible distortion in the statistical parameters produced by retaining an outlier must be balanced against attempting to make a reasonable estimate of the merits of the method. First the between-laboratory CV is calculated with no outliers removed. *If this CV is acceptable as judged against the CVs for comparable methods and analytes, the parameter is accepted regardless of whether or not outliers are indicated by statistical tests.* If the CV is relatively high and an outlier is present at a conventional confidence level (CL), e.g., 95% or above, the CV is recalculated with the outlier removed. This procedure is repeated until no further outliers are indicated. Then the CVs and % outliers are tabulated and evaluated, using the generalization that an outlier rate greater than 20% is excessive. *That value of CV is chosen which is acceptable in the light of historical CV values as developed in this and the previous paper with (1) the minimum rejection of outliers.* Several examples of the practical use of this evaluation procedure will be discussed in conjunction with the review of the tabulated statistics. This procedure obviously entails some prejudgment, but it is based on experience which indicates that the judgment leads to consistent, practical answers.

(4) Extreme values are also examined by the Grubbs test. This test has only recently been added to compensate for the lack of sensitivity of the Dixon test when 2 extreme values appear close to each other (the masking effect). The Grubbs test is based on the difference between the extreme value and the average value (not the nearest value).

(5) Replicates are examined for excessive variance by the Cochran test. This test examines the variance contributed from each laboratory and compares the maximum laboratory variance with the sum of variances from all the laboratories for the same material. This test sometimes gives an apparent "false" indication of an outlying replicate variance when most of the other ranges are very small. Youden did not use this test because he suggested that known replicates are usually not independent and therefore are likely to have an artificially small range. However, the test is very useful for examining blind replicates.

Systematic error.—For pharmaceuticals, the systematic error, as judged by the recovery (usually 98–102%), is so small for most of the methods reviewed that it was not considered necessary to include this parameter in the tabulations. In general, the bias of these methods is negligible compared to the specification ranges given in compendia and regulations.

Results

The data for the new and re-examined collaborative studies will be presented in a series of papers organized primarily by the predominating analytical feature—separation or measurement technique. Where enough data exist, these groups are further subdivided into logical classes. The data for the chromatographic separation technique specifying the diatomaceous silica column are subdivided into 5 chemical classes: sympathomimetic amines, thiazides, alkaloids, steroids, and other.

As an example of the presentation, Table 1:01 is examined in detail. This table contains data about the on-column oxidation method of Clark (11), who performed 3 studies of the same method, one for the determination of phenylpropanolamine, and one each for liquid and solid dosage forms of ephedrine. The first 2 columns of the table give the compound studied, the dosage form, the AOAC *Journal* citation for the collaborative study, and the official method number, AOAC(1980). (In some cases, the method reference is given in the table title or in footnotes.) Column 3 contains the concentrations of the active ingredients obtained directly from the original report or, when necessary, by calculation, or as a last resort, by estimation. The total number of determinations and number of laboratories involved in the analysis of each material, material by material, are given in the next 2 columns. These numbers also give the design of the study: When only single determinations have been performed, both numbers are the same; when the number of determinations is an integral factor of the number of laboratories, this integer is most likely the number of replicates per laboratory; when the factor is not a whole number, one or more laboratories have provided a number of determinations different from the others, i.e., an unbalanced design. When outliers are present and the data have been recycled, a new line entry for the same material (dosage form) is given which omits mention of the dosage form, but which shows the new number of determinations and laboratories involved in the iterated calculation.

The first entry under the columns "Outliers/%/Kind" for each dosage form is always "0/0," indicating that the calculations have been made with no outliers removed. If no statistical outliers are present, the next line of the table continues with another material or another compound or study (with a new citation). But if outliers have been detected, the number removed can be determined by the difference in the number of determinations and the number of laboratories from the first line of that dosage form. When the data have been recalculated with outliers removed, columns 6 and 7 give the % outliers removed and their kind (DI = Dixon individual value; D = Dixon laboratory average; G = Grubbs; C = Cochran; R = Youden rank sum). Rank sum outliers are tested only at the 95% confidence level. The number associated with the D, G, and C tests indicates its one-tail confidence level (95, 97.5, 98, and 99%); if placed to the left, the number refers to a low-lying outlier, and to the right, a high-lying outlier. Numbers on both sides of a letter indicate 2 outliers of the same kind were flagged, one high and one low. A series of 99s indicates $n/2$ outliers at the 99% CL, where n = number of 9s. The confidence levels (CL) used were those for which tables were conveniently available. A "P" in the outlier column indicates a procedural deviate which should have been omitted from the tabulation from the outset. When more than one outlier response is signaled in a computer run, a "/" is used to indicate that the same analytical value responded to different statistical outlier tests but an "&" is used to indicate that different analytical values are flagged as outliers in the same statistical analysis. Whether the outliers removed are sequential or alternatives is determined by noting the "No. of" columns. If sequential, the numbers on the next line are lower; if alternative, the numbers are the same or higher.

The last columns give the within-laboratory (CV_o) and between-laboratory (CV_x) coefficients of variation. The statistical results are presented under each CV heading in 2 columns. The values on the left in each column include only those that were not used in the final interpretations. The values on the right were the accepted CVs aligned with the associated percent outliers (often 0). Within-laboratory values calculated from Youden pairs are indicated by a "Y." Other common abbreviations used in the tables are: Com = commercial formulation; Syn = synthetic (i.e., of known composition by formulation); tab = tablet; cap = capsule; injn = injection.

Table 1:01 Phenylalkanolamines. Collaborative study conditions and derived coefficients of variation for the determination by method AOAC (1980) 38.206-38.2.0[a]. Values with no dosage form indicated and nonzero values under "Outliers" column are recalculations of the previous dosage form with the indicated outliers omitted. The CVs displaced to the right in the last column are those accepted with the associated per cent outliers (often zero).

Analyte JAOAC Reference	Dosage Form	Concn %	No. of Detns	No. of Labs	Outliers %	Outliers Kind	Coefficients of Variation Within-lab	Coefficients of Variation Between-lab
Phenylpropanolamine 56, 100(1973)	Com elixir	0.25	12	12	0	0		1.27
	Com elixir	0.096	12	12	0	0		2.58
	Syn elixir	0.1	12	12	0	0		2.71
	Syn elixir	0.25	12	12	0	0	1.86Y	2.10
Ephedrine, Liquids 58, 852(1975)	Com sirup	0.40	14	14	0	0		6.95
		0.41	12	12	14	99D99		1.66
	Com sirup	0.23	14	14	0	0		6.73
		0.24	13	13	7	99D		2.25
	Syn sirup	0.25	14	14	0	0		3.29
		0.25	13	13	7	D95		2.41
	Syn sirup	0.50	14	14	0	0		4.80
		0.50	13	13	7	98D		3.35
Ephedrine, solids 63, 672(1980)	24 mg tab	4.75	9	7	0	0		4.73
		4.80	8	6	11	95D/R		2.96
	Blind dupl	4.82	8	7	0	0		4.16
		4.66	7	6	13	R		4.13
		4.76	7	6	13	D98		2.14
		4.59	6	5	25	99D&R	2.20Y	1.49
	25 mg cap	14.2	9	7	0	0		9.79
		14.6	8	6	11	97.5G/R		5.53
		14.8	7	5	22	99D		1.66
	Syn mix	8.04	9	7	0	0		1.41
		8.06	8	6	11	R		1.37
	Syn mix	9.45	8	7	0	0		1.80
		9.47	7	6	13	R		1.88
	Syn mix	6.73	8	7	0	0		7.04
		6.89	7	6	13	99D/R		1.88

[a] Elution from weakly basic column, retention on weakly acid column, elution with base onto periodate column for on-column oxidation to benzaldehyde, determined by ultraviolet spectrophotometry.

Table 1:06 summarizes the previous tables (Tables 1:01–1:05). Under the heading "No. of" are the totals of the number of independent materials (mat.) analyzed for each class; the number under "detns" is the total number of values reported with no outliers omitted. Under "Average no. of labs" is the average number of laboratories participating in the studies of each chemical class. Under "% outliers" is the average percent outliers associated with the accepted CVs, calculated on a number of determinations basis. The final entries are the average accepted CVs and their associated standard deviations calculated from the individual accepted CVs for all the materials in that class. The standard deviation is given only as a rough indication of the spread of the accepted CVs, and not as a characteristic of the method.

Finally, computer-generated graphs are presented, Figures 1:01–1:05, labeled with a figure number corresponding to the equivalent table number, of the accepted CV_x (vertical linear scale) plotted against the concentration (horizontal on a log scale but labeled as a linear scale). The graphs are combined in Figure 1:06.

Table 1:02 Thiazides. Collaborative study conditions and derived coefficients of variation for the determination by the AOAC(1980) methods[a]. See Table 1 for explanation of row format.

Analyte JAOAC Reference	Dosage Form	Concn %	No. of Detns	No. of Labs	Outliers %	Outliers Kind	Coefficients of Variation Within-lab	Coefficients of Variation Between-lab
Benzthiazide (2)[a]	Syn 50 mg tab	24.9	30	10	0	0	0.63	1.59
55, 161(1972)	Com 50 mg tab	22.9	30	10	0	0	1.11	1.97
		23.0	29	10	3	95DI 0.90	1.52	
		23.0	27	9	10	95D 0.92	1.26	
		23.0	26	9	13	DAC96 0.48	1.03	
Hydrochlorthiazide (2)[a]	Syn 50 mg tab	24.7	30	10	0	0	1.09	1.46
	Com 50 mg tab	22.9	30	10	0	0	0.80	1.63
		22.9	29	10	3	99DI 0.44	1.09	
		22.9	27	9	10	99D 0.44	0.75	
Chlorothiazide (2)[a]	Syn 250 mg tab	67.6	24	10	0	0	0.48	0.70
56, 637(1973)	Com 250 mg tab	67.6	24	10	0	0	0.78	0.92
		67.6	22	9	8	C97 0.53	0.83	
Methyclothiazide (2)[a]	Com 2.5 mg tab	1.27	24	10	0	0	0.86	2.65
	Syn 2.5 mg tab	1.27	24	10	0	0	0.80	1.91
Polythiazide (3)[a]	Syn 13 mg tab	13.1	27	9	0	0	0.97	2.36
57, 716(1974)		13.1	24	8	11	C95 0.73	1.99	
		13.2	21	7	22	CC98 0.50	1.84	
	Com 1 mg tab	4.8	27	9	0	0	1.55	2.61
Bendroflumethiazide	Com 2.5 mg tab	0.77	24	8	0	0	0.59	0.80
59, 90(1976) (1)[a]	Syn 2.25 mg tab	0.73	24	8	0	0	0.62	0.97
		0.69	21	7	13	D95 0.65	0.31	
Cyclothiazide (1)[a]	Syn 0.62%	0.626	24	8	0	0 0.70	3.64	
		0.619	21	7	-	P	0.60	2.20
	Com 2 mg tab	0.615	24	8	0	0 1.08	3.07	
		0.611	21	7	-	P	0.69	2.07
		0.615	18	6	14	95D/ C99 0.61	1.13	

[a] (1) 37.168-37.174; (2) 37.189-37.194; (3) 37.195-37.199. Because of differences in their weakly acidic properties and solubilities, different extraction solvents and columns of different pHs must be used for the individual thiazides. A special step is incorporated in method (3) to remove vanillin used in polythiazide tablets. In all cases, final measurement is by ultraviolet spectrophotometry.

Table 1:03 Steriods. Collaborative stucy conditions and derived coefficients of variation for the determination by AOAC(1980) methods[a]. See Table 1:01 for explanation of row format.

Analyte JAOAC Reference	Dosage Form	Concn %	No. of Detns	No. of Labs	Outliers %	Outliers Kind	Coefficients of Variation Within-lab	Coefficients of Variation Between-lab
Estradiol valerate (1)[a]	Com oil injn	0.86	7	7	0	0		2.67
56, 86(1973)	Com oil injn	0.41	7	7	0	0		3.55
	Syn oil injn	0.355	7	7	0	0		3.51
	Com oil injn	0.95	7	7	0	0		3.24
	Syn oil injn	0.97	7	7	0	0		3.08
	Com oil injn	1.98	7	7	0	0		3.31
	Syn oil injn	1.84	7	7	0	0		2.48
Dienestrol (2)[a]	Com 0.1 mg tab	0.051	10	10	0	0		3.61
56, 674(1973)	Syn 0.1 mg tab	0.063	10	10	0	0	2.63Y	2.80
	Com 0.5 mg tab	0.15	10	10	0	0		3.09
	Com 0.5 mg tab	0.29	10	10	0	0	1.71Y	3.47
		0.29	9	9	10	97D	2.00	
Ethinyl estradiol (3)[a]	Com 50 ug tab	0.0470	26	13	0	0	1.81	2.81
57, 747(1974)		0.0472	24	12	8	99D 1.87	2.20	
		0.0469	24	12	8	C96 1.23	2.67	
		0.0472	22	11	15	99DAC 1.26	1.99	
		0.0473	21	11	19	99D 1.10		1.52
	Com 50 ug tab	0.0493	26	13	0	0	2.52	2.52
	Com 100 ug tab	0.099	26	13	0	0	1.89	2.90
Mestranol (4)[a]	Syn 150 ug tab	0.146	36	20	0	0 2.57		7.38
58, 75(1975)		0.148	34	19	6	99D/C98 1.61		4.86
		0.150	32	18	11	99D	1.51	2.92
	Com 150 ug tab	0.149	37	20	0	0 2.40		5.47
		0.151	35	19	5	99D	1.84	2.76
		0.151	33	18	11	98D 1.68		2.11
		0.151	31	17	16	C95 0.64		1.86
Norethindrone (5)[a]	Com 350 ug tab	0.359	28	14	0	0	0.75	1.78
60, 922(1977)		0.359	24	12	14	99D95 0.54		0.81
Norgestrel (5)[a]	Com 500 ug tab	0.491	28	14	0	0	0.58	1.58
		0.492	26	13	7	95D 0.60		1.30
		0.493	25	13	11	98DI/97.5G 0.53		1.06
Norethynodrel (5)[a]	Com 250 ug tab	1.63	28	14	0	0	2.36	2.91
		1.62	26	13	7	D199 1.88		2.20

[a] (1) 39.008-39.013; (2) 39.018-39.022; (3) 39.025-39.028; (4) 39.033-39.036; (5) 39.041-39.045. All methods utilize a partition chromatographic separation and reaction to a colored complex measured by visible spectrophotometry, except (1) which uses fluorometry and (2), UV.

Table 1:04 Alkaloids. Collaborative study conditions and derived coefficients of variation for the determination of alkaloids by AOAC methods[a]. See Table 1:01 for explanation of row format.

Analyte / JAOAC Reference / AOAC(1980) Reference	Dosage Form	Concn %	No. of Detns	No. of Labs	Outliers %	Outliers Kind	CV Within-lab	CV Between-lab
Codeine in APC tabs	Syn 16 mg tab	2.97	9	9	0	0		1.72
55, 142(1972)		2.97	8	8	11	R	1.83	
(1)[a]		2.98	7	7	22	99DAG/R	0.93	
	Com 14 mg tab	2.66	9	9	0	0		3.05
	Com 30 mg tab	5.52	9	9	0	0		1.50
		5.50	8	8	11	R	1.07	
	Com 59 mg tab	10.66	9	9	0	0		1.38
		10.62	8	8	11	R	1.03	
	Com 8 mg tab	1.52	9	9	0	0		1.51
		1.52	8	8	11	R	1.47	
	Syn 1 mg tab	0.18	9	9	0	0		3.84
		0.18	8	8	11	R	3.17	
	Com 1 mg tab	0.19	9	9	0	0		3.14
		0.19	8	8	11	R	2.83	
Neostigmine bromide	Syn 16 mg tab	7.6	11	11	0	0		1.81
57, 725(1974)	Com 15 mg tab	7.2	11	11	0	0	1.99Y	2.20
(2)[a]	Ophthmc soln	6.0	11	11	0	0		1.51
	Std soln (ppm)	5.51	12	12	0	0	1.95	
		5.54	11	11	9	99D	0.79	
Procaine	Syn injn soln	1.9	14	14	0	0		1.67
58, 93(1975)	Syn injn soln	1.9	14	14	0	0		1.60
(3)[a]			13	13	7	97D	1.08	
	Syn injn soln	1.9	14	14	0	0		1.50
Proporycaine	Syn injn soln	0.40	14	14	0	0		1.92
Tetracaine	Syn injn soln	0.147	14	14	0	0	8.92	
(3)[a]		0.145	12	12	14	95D99/G99	3.57	
		0.143	12	12	14	D9999	2.26	
		0.144	11	11	21	99D9999	1.51	
Reserpine-reacin-	NF Reference	0.17	11	11	0	0		2.91
namine alkaloids	Com 100 mg tab	0.037	11	11	0	0		2.32
60, 1018(1977)	Com 50 mg tab	0.028	11	11	0	0	14.0	
(4)[a]		0.027	10	10	9	D99		4.62
	Syn 45 mg tab	0.021	11	11	0	0	12.8	
		0.020	10	10	9	D99	4.35	
		0.020	9	9	18	98D59		2.25

[a] (1) 38.012-38.013; (2) 38.168-38.171; (3) 38.218-38.220; (4) 38.091-38.098: by elution of extraneous compounds from the partition column followed by elution of the analyte, which is extracted into acid solution for measurement by ultraviolet spectrophotometry or by fluorometry in the case of the reserpine-reacinnamine alkaloids.

[b] Method not adopted for this compound

Table 1:05 Other compounds. Collaborative study conditions and derived coefficients of variation for the determination of compounds not falling into the previous chemical classes, but assayed by partition chromatography and ultraviolet spectrophotometry. See TABLE 1:01 for explanation of row format.

Analyte / JAOAC Reference / AOAC(1980) Reference	Dosage Form	Concn %	No. of Detns	No. of Labs	Outliers %	Outliers Kind	CV Within-lab	CV Between-lab
Sodium butabarbital	Com 15 mg tab	12.4	26	13	0	0	0.51	1.48
55, 152(1972)	Syn tab	15.7	26	13	0	0	0.77	1.56
37.085-37.088		15.5	24	12	8	99C/D99	0.37	1.13
		15.7	22	11	15	99DC	0.38	0.81
		15.7	20	10	23	99DDC	0.39	0.47
		15.7	25	13	4	DI99	0.37	1.17
		15.7	24	12	8	99D	0.80	1.34
		15.7	23	12	12	99DDI99	0.38	0.86
Sodium diphenyl-	Syn powder	40.7	30	15	0	0	0.84	1.65
hydantoin	Com cap	40.9	30	15	0	0	1.07	1.68
55, 170(1972)		40.9	28	14	6	C99	0.56	1.58
37.089-37.092								

Abbreviations for all tables:

Com = Commercial; Cap = Capsule; Ophthmc = Ophthalmic; Syn = Synthetic; Mix = Mixture; Soln = Solution; Tab = Tablet; Injn = Injection

D = Dixon applied to laboratory averages; DI = Dixon applied to individual values; Y = From Youden pair; / = Same values; G = Grubbs; R = Rank sum; & = Different values

Table 1:06 Summary of Tables 1.01-1.05 Chromatographic separation on diatomaceous silica with spectrophotometric determination.

Chemical Class	No. of mat.	No. of detns	Average No. of labs	Outliers %	CV Within-lab	CV Between-lab
Sympathomimetic amines	14	155	10.4	6.7		2.16±0.60
Thiazides	14	366	9.3	0.0	0.83±0.28	1.70±0.66
Steroids	19	324	11.1	0.8	1.76±0.68	2.89±0.55
Alkaloids	18	196	10.9	1.5		2.25±0.92
Other	4	112	14.0	0.0	0.80±0.23	1.59±0.09
Average or (total)	(69)	(1153)	10.7	2.0[a]	1.30[a]±0.55	2.25[a]±0.52

[a] Weighted for materials

Discussion

Because of the addition of new information regarding the design of the collaborative study and because of the more detailed treatment of outliers, it is necessary to include some tabular material from the previous paper.

The original classification of methods according to their predominating operational feature, either type of separation or measurement technique, has been retained. One new classification has been added—liquid chromatography (LC). The present paper deals only with the partition chromatographic separation and spectrophotometric determination (visible, ultraviolet, or fluorometric). It covers 18 separate published collaborative studies of 24 compounds analyzed in 69 materials by an average of 10 laboratories per study. Subsequent papers will review other categories of methods of analysis for drugs.

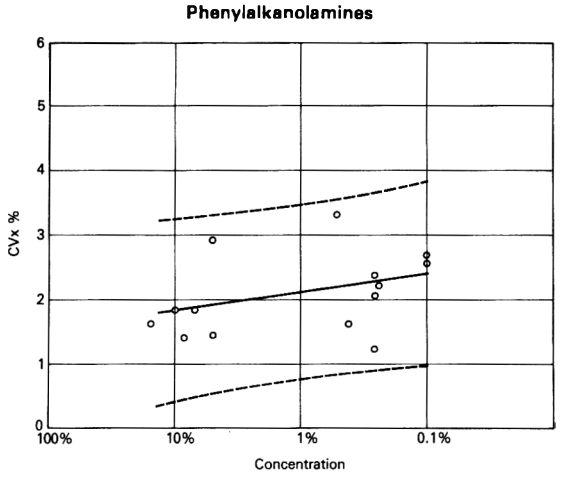

Figure 1:01. The overall coefficient of variation between-laboratories (CV_x) as a function of concentration (spaced logarithmically but labeled in percent), for the accepted CV_x data from the collaborative studies for phenylalkanolamines (Table 1:01). The center line is the line of best fit which is bracketed by the 95% prediction limits.

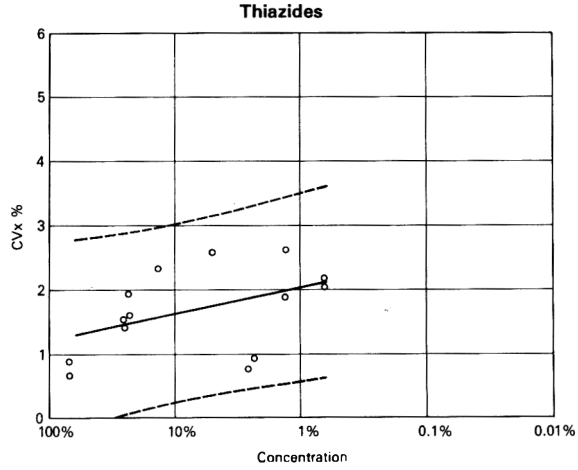

Figure 1:02. The accepted CV_x data for thiazides (Table 1:02) plotted as in Figure 1:01.

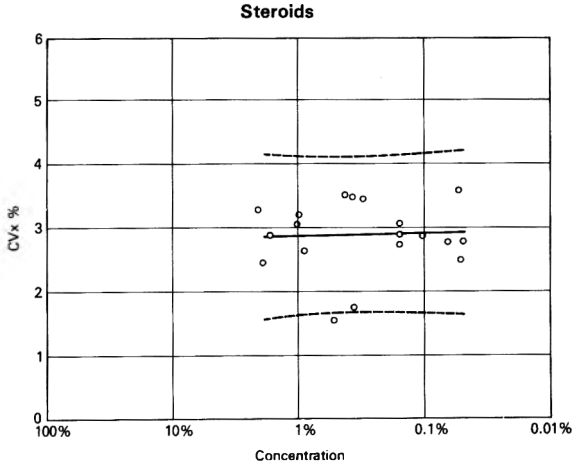

Figure 1:03. The accepted CV_x data for steroids (Table 1:03) plotted as in Figure 1:01.

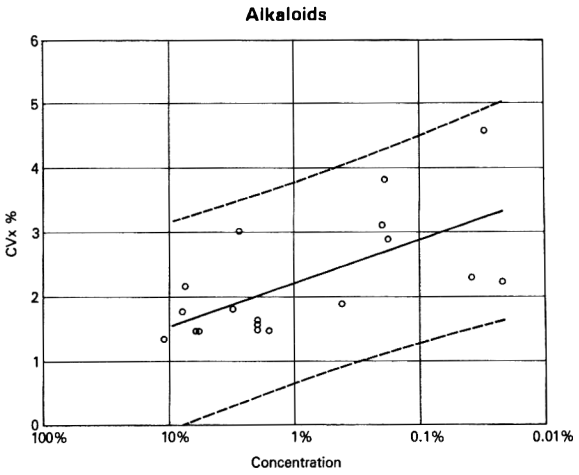

Figure 1:04. The accepted CV_x data for alkaloids (Table 1:04) plotted as in Figure 1:01.

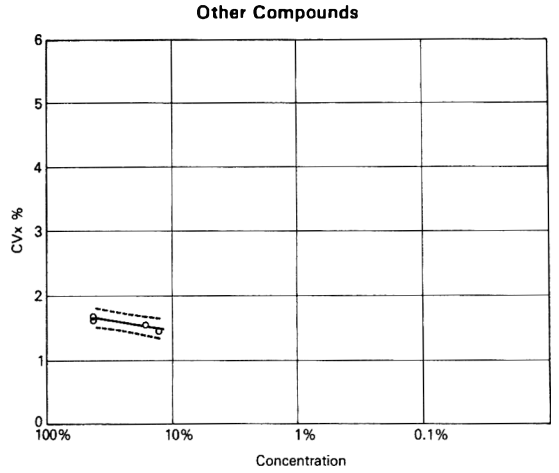

Figure 1:05. The accepted CV_x data for miscellaneous compounds (Table 1:05) plotted as in Figure 1:01.

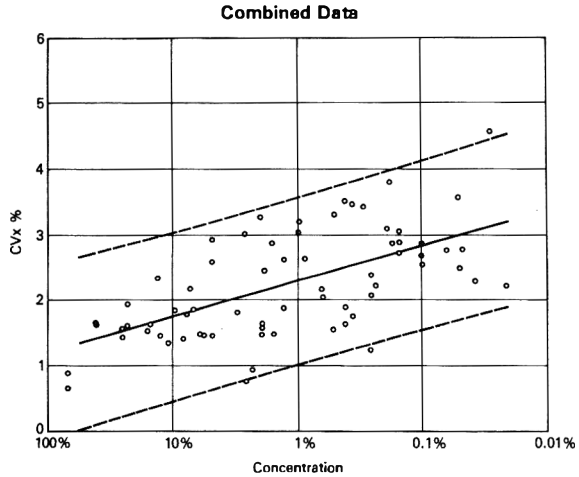

Figure 1:06. Figures 1:01–1:05 combined.

The most common analytical method category for pharmaceuticals used by regulatory agencies during the period examined is partition chromatographic separation using diatomaceous silica as an inert support for an immobile phase, usually an aqueous acid, base, or buffer. The isolated drug is generally measured spectrophotometrically. The wide proliferation of methods of analysis of commercial dosage forms of drugs, both alone and in combination with other drugs, by this technique, followed the work of Banes (12) and of Levine (13). In several cases, a number of the same or related compounds have been studied in separate collaborative studies, permitting a comparison of the statistical parameters from independent studies.

Tables 1:01–1:06 summarize the 18 studies of methods of analysis approved during the past 10 years that utilized the partition chromatographic separation and spectrophotometric determination. The most striking fact is that only 2 of these studies (11%) contained no statistical outliers, as defined here, although several studies contained only one outlier in 40–100 determinations. The 2 studies with no outliers were on radically different drugs, dosage forms, and final measurement methods: estradiol valerate (Table 1:03) in oil by a fluorometric measurement, and phenylpropanolamine (Table 1:01) by a relatively complex on-column oxidation to benzaldehyde which in turn was determined by UV spectrophotometry. This observed lack of outliers is not characteristic of at least the on-column oxidation procedure, since studies of the same method applied to ephedrine do contain outliers.

Sympathomimetic Amines (Table 1:01).—A method for ephedrine was subjected to 2 separate collaborative studies. The method was applied to liquid dosage forms in one study and to solid dosage forms in the other. The study with liquids had 9% Dixon laboratory average outliers (extreme values), whereas the study with solid dosage forms had one laboratory as a rank sum outlier (consistently low over all 6 materials) and several Dixon outliers, for an overall rate of 11%. The phenylpropanolamine method study also showed a rank sum outlier, according to the author, but we do not apply a rank sum test to studies with 4 or fewer materials. The author chose to ignore the indication of outliers given by this rank sum test because "no one value was an outlier, indicating that the results were not exceptionally high." The author permitted common sense to overrule the indication of the statistical outlier test, in that an overall CV of 2.17% (range 1.27–2.71% for 4 materials) is acceptable performance, notwithstanding a consistent bias on the part of one laboratory of 12.

A collaborative study of the previous phenylpropanolamine method applied to liquid ephedrine preparations showed a different outlier pattern, with all the outliers being of the Dixon variety. Fourteen laboratories submitted acceptable data. A fifteenth was omitted as a procedural deviate for failure to follow the method. The results from each of the 4 materials contained a Dixon outlier, with the data from one of the materials containing a Dixon outlier at both ends. All of the outliers were contributed by 2 laboratories. When these 5 outliers were omitted (9% outlier rate), the CV_x (reproducibility) was reduced from 5.4 to 2.4%, consistent with the CV_x for the same method as applied to phenylpropanolamine. The outliers are shown visually in Figure 2 where the data are plotted in ascending order of concentration, on the same absolute scale of percent for all 4 materials. Note that the high Dixon outlier of Synthetic Material 1 and Commercial Material 1 attained this outlier status because the non-outlying points are very close together.

The collaborative study of the phenylpropanolamine method applied to solid dosage forms of ephedrine showed one laboratory as a rank sum outlier as applied to 6 materials. In addition, results for 2 of the materials showed Dixon outliers after removal of the rank sum outlier. The progression of CV_x values with outlier removal is shown in Table 1:01, but can be roughly summarized by averaging the 6 materials as follows (the numbers in parentheses are the number of individual determinations used in each calculation):

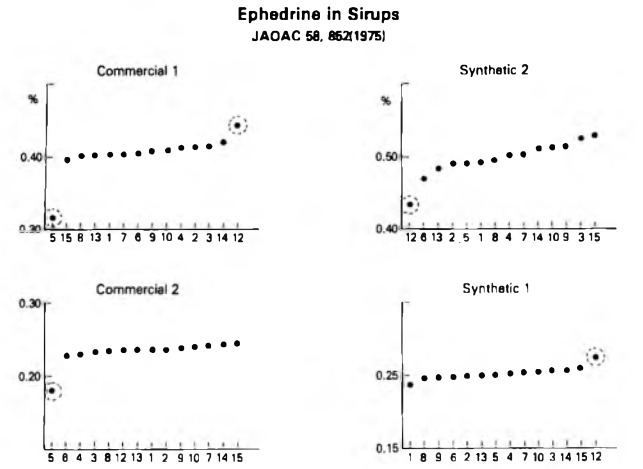

Figure 2. The data from the collaborative study of the determination of ephedrine in syrups; concentration in percent vs laboratory number, in ascending order. The circled points are Dixon outliers.

Statistical Procedure	*% Outliers*	$CV_x \pm SD$, %
No outliers removed (51)	0	4.82 ± 3.19
Rank sum outliers removed (45)	11	2.96 ± 1.60
Rank sum and subsequent Dixon outliers removed (43)	15	1.87 ± 0.57
Only Dixon and Grubbs outliers removed (46)	10	1.98 ± 0.54

This table still does not reflect the actual state of affairs. Because 2 of the 7 laboratories supplied 2 values each, and the other 5 laboratories supplied only one value each, the percentage of outliers was calculated on the basis of total number of values. The percentage of outliers would be slightly higher if calculated on the basis of the number of laboratories. This study also contained a set of blind duplicates. The removal of the rank outlying laboratory significantly affects the CV in the case of one of the blind duplicates but not in the other. The unaffected sample contained an additional Dixon outlier.

Note also that in this case the use of the rank sum test for outlying laboratories was redundant. The laboratory exhibiting a consistent systematic error also showed extreme values for 3 of the 6 materials, a fact that was also detected by the extreme value tests: the Dixon at 95% and higher CLs and the Grubbs at the 97.5% CL. In the other 3 cases where the rank sum test alone would have "unjustly" eliminated non-outlying values, the corresponding CVs were hardly affected by the removal of those values. In the case of the blind duplicate material, when the rank sum outlying laboratory was removed first, a 99% CL Dixon outlier was exposed; if the rank sum outlier was not removed, the same value was a Dixon outlier at the 98% CL. On the basis of this particular case, the accusation that non-outlying values are removed by the rank sum test, although true, is really immaterial, since their removal has practically no effect on the precision indices. In fact, the most efficient use of outlier tests in this case is the application of the Dixon test (the Grubbs test did not add any new information) to provide a CV_x of 2.0% at an outlier rate of 10%.

There is no pattern that emerges from these 3 studies of the same analytical method, assuming a choice of representative laboratories. The data can be accepted with no outlier removal, giving a pooled CV between-laboratories of 4.4%, or at the other extreme by removing all outliers shown by the Dixon test at not less than the 95% CL, giving a CV of 2.0% and a 10% outlier rate. A balanced estimate of the between-laboratory precision for the method is that the CV is about 3% (range 2–4%) with a possible outlier rate of about 10%, over the concentration range of 0.1–15%, based on examination of the data from 14 independent materials containing 2 compounds, and examined by 7–14 laboratories.

Thiazides (Table 1:02).—Twelve dosage forms of 6 different thiazide compounds were analyzed in triplicate by all laboratories in 4 separate studies. Seven of the materials showed no outliers at the 95% CL, but the other 5 showed Dixon individual value, Dixon laboratory average value, and Cochran (unduly large range of the triplicates) outliers. The outliers are spread through all 4 studies. The between-laboratory CVs as a function of outlier removal are as follows:

Statistical Procedure	*% Outliers*	$CV_x \pm SD$, %
No outliers removed (366)	0	1.88 ± 0.90
All Dixon and Cochran outliers removed (345)	6	1.44 ± 0.70

Here, little improvement in CV is seen by removal of 6% outliers, so the thiazide method may be characterized with a CV_x of 2% (range 0.7–2.7%, or rounded to 1–3%) with no outliers over the concentration range of 1–70%, based on the examination of 14 individual materials of 6 compounds by 8–10 laboratories.

The thiazide method was not approved as applicable to cyclothiazide. This action was apparently taken because cyclothiazide was analyzed in the same study with bendroflumethiazide, the analysis for which happened to show an unusually low CV_x of 0.9%. Including the results from one laboratory which reported color being extracted from the sample, an observation which should have immediately indicated that something was wrong, the CV_x for the 2 samples of cyclothiazide was about 3.4%; without the deviate laboratory, the CV_x was 2.1%, which is in line with the values for the other thiazides. If a Dixon laboratory outlier is also removed from one of the materials, the CV_x for cyclothiazide becomes 1.7% with a 7% outlier rate. It appears that the method actually is just as applicable to cyclothiazide as it is to the other thiazides studied in this series.

As pointed out by Anscombe (14), the purpose of outlier rejection rules is to guard against the adverse effects of outliers. He also pointed out that the application of outlier rejection rules is much like the purpose of a householder's fire insurance policy. In choosing a policy, 3 questions are considered: *(1)* the premium (equivalent to the increase in variance due to falsely retaining a true outlier—a larger CV is obtained and used than is really the case); *(2)* the protection in the event of fire (the reduction in variance when spurious readings are present and correctly excluded from the calculation); and *(3)* the danger of a fire (proneness to outliers). In this thiazide case, the tendency to the production of outliers is determined from the 12 materials of 6 compounds. The premium to be paid is the increase in the estimation of the CV_x by using the rejection rule which results in retaining observations which are not part of the normal population. The protection given is the reduction in CV_x when spurious observations are present and actually rejected. If we cover all hazards and reject no values as outliers, we will be operating with a CV_x of 1.7% (0.7–2.7% range); if we are willing to accept a probability of a fire (outlier rate of 6%, range 0–22%), we can accept a better precision of 1.4%. Since a 1.7% CV_x is an acceptable precision for drug methods, accepting a still better precision with a deductible (stigma) of 6% outliers saves a small premium at the expense of a chance of 1 in 16 exposure to the hazard of outliers. We can eliminate the damage that might be caused by the hazard (6% outliers) by paying the premium of increasing the CV_x from 1.4% to 1.7%, which is equivalent to a no-outlier situation.

The situation was reversed with the on-column oxidation method, where accepting an outlier rate (hazard) of 15% reduced the CV_x from 5.1 to 1.9%. It is sometimes profitable to accept a reasonable outlier rate (if less than 20%) since additional and greater insurance protection can later be bought in the form of analyzing additional independent samples when the analytical result on an actual sample from a lot is near a specification limit. (The small probability that acceptance of a true outlier will result in passing a defective lot is not covered by this option.)

Steroids (Table 1:03).—Five collaborative studies involving 7 compounds and 19 materials have been performed on steroids. Steroids are dispensed at relatively low concentrations (for pharmaceuticals), typically 0.1–1.0%. The CVs are near the 3% level, and the outlier rate is typically low. If no outliers are removed, the CV_x is 3.27% ± 1.28 (± SD) with 2 high values coming from one compound, mestranol. The reasonable outlier rejection review resulted in the acceptance of an average CV_x of 2.89% ± 0.55 with an average of 0.8% outlier rejection rate. If all indicated outliers at the 95% CL were to be removed, the average CV_x is 2.55% ± 0.84 with a 5.2% outlier rejection rate. The reasonable outlier rejection rule is obviously the "best buy."

The first commercial 50 μg ethinyl estradiol tablet exhibits an interesting outlier progression pattern. The original set of 26 determinations from 13 laboratories (in duplicate) show an acceptable CV_x of 2.81%, but also show the lowest value as an extreme Dixon outlier at the 99% CL and a pair of values from a different laboratory as a Cochran outlier. Therefore, there is a choice of 3 options for outlier removal: *(1)* Remove the Dixon laboratory pair outlier and reduce the CV_x of 2.81 to 2.20% with an outlier rate of 8%; *(2)* remove the Cochran outlier and reduce the CV_o from 1.81 to 1.23% with an outlier rate of 8% with little reduction in CV_x; and *(3)* remove both simultaneously to a CV_x of 1.99% and a CV_o of 1.26% with 15% outliers. However, the removal of the latter pair of outliers exposes another Dixon outlier at 99% CL. Removal of this outlier brought the final CV_x and CV_o to 1.52 and 1.10%, respectively, almost half the original values, but at the expense of a 19% outlier rejection rate. But in this case it was not necessary to remove the apparent outliers, since the original values with outliers included were typical for steroids. This example, however, shows that outlier removal can be noncommutative.

The mestranol study is a case where outliers must be removed to obtain acceptable CVs. Without outliers removed, the data from 20 laboratories result in a CV_x of 7 and 5% for 2 materials. One laboratory for the first material is both a Dixon laboratory average and a Cochran outlier. Its removal still results in a high CV_x but a masked Dixon 99% CL outlier is exposed, whose removal gives an acceptable CV_x of 2.9% at an outlier rate of 11%. Only one iteration (5% outliers) has to be performed on the second material in this study to obtain an acceptable CV_x value, although a Dixon outlier at the 98% CL and a Cochran outlier still contaminate the data.

Alkaloids (Table 1:04).—There were 4 studies of methods for alkaloids. In the study of the method for codeine in APC (aspirin, phenacetin, and caffeine) tablets, 7 different tablet materials, covering the concentration range of 0.2–11%, were examined by 9 laboratories. One laboratory is a rank sum outlier and one laboratory with one material is a Dixon outlier. The progression of CVs with outlier removal is as follows:

Statistical Procedure	*% Outliers*	$CV_x \pm SD$
All values (63)	0	2.31 ± 1.01
Rank sum outliers removed (56)	11	1.96 ± 0.84
Rank sum and 1 Dixon outlier removed (55)	13	1.83 ± 0.93

The precision with no outliers removed is acceptable in this case. Removal of outliers does not substantially affect the CVs. Since the concentration range in this study covers almost 2 orders of magnitude, it is not unexpected that the 2 lowest concentrations are beginning to exhibit the upward sweep of the CV vs log concentration curve which delineates the limit of reliable measurement, as shown in Figure 3.

The procaine method as applied to tetracaine was not adopted by AOAC because difficulties became apparent. The outlier progression pattern with this application of the method is also interesting. Two high outliers were obvious on inspection. Application of the Dixon test showed that the highest value was an outlier at the 99% CL and the lowest value was also a Dixon outlier at the 95% CL. Removal of these 2 outliers (14%) reduced the CV_x from an original excessive 8.92% to a considerably lower 3.57%. But removal of only the highest outlier (7%) produced almost the same CV, 3.94%. The second highest value was also a Dixon outlier at the 99% CL. If the 2 highest Dixon outliers were removed simultaneously, rather than the highest and lowest, the resulting CV_x is 2.26%, at the same outlier rate of 14%. When only the highest Dixon outlier was removed, the lowest value became a 99% CL Dixon outlier rather than the 95% CL initially indicated. Removal of all 3 outliers reduced the CV_x to 1.51%, but with an accompanying outlier rate of 21%. Therefore, it appears not only that the CV depends on which outlier is chosen for removal first but also that the CL at which an outlier appears depends to a certain extent on the past history of outlier removal in the statistical analysis. The outlier progression for this case is shown in detail in Table 2.

A method for reserpine-rescinnamine alkaloids (15) specifying fluorometric measurement was originally rejected by the reviewing subcommittee because of a CV_x of almost 6% with 8% outliers. With the substitution of a new reagent for the development of the fluorophore, the CV_x was reduced to 3.0 ± 1.09% with 7% outliers. The concentration range for 3

Table 2. Progression of coefficients of variation with outlier removal for collaborative study of tetracaine (JAOAC (1975) 58, 93)

Operation	Outliers removed		CV, %	Remaining outliers indicated
	No.	%		
No outliers removed	0	0	8.92	n = 1(95D) n = 14(D99/G97.5)
Highest outlier (n = 14) removed	1	7	3.94	n = 1(95D) n = 13(D99/G99)
Lowest (n = 1) and highest (n = 14) removed	2	14	3.57	n = 13(D99)
Two highest outliers (n = 13, 14) removed	2	14	2.26	n = 1(99D/97.5G)
All 3 indicated outliers (n = 1, 13, 14) removed	3	21	1.51	

of the materials, 0.02–0.04%, was among the lowest concentration ranges reported in this series.

Two solid and one liquid dosage forms of neostigmine at a concentration level of about 7% showed a CV_x of 1.84 ± 0.35% with no outliers. A 5.5 ppm standard solution submitted simultaneously showed a CV_x of 1.95%; with one Dixon outlier removed (9%), the CV_x was 0.8%. In this study, the CVs are more or less the same over a concentration range of almost 10^4, i.e., from 11×10^{-2} to 6×10^{-6}, with no outliers removed. This observation suggests the reason for the relative constancy of the CVs in this series of studies—the analyte is diluted to more or less the same concentration for the spectrophotometric measurement for all compounds.

Other compounds.—The 2 remaining studies in this series, one of a method for sodium butabarbital and the other for sodium diphenylhydantoin, show similar acceptable CV_x values of 1.5 and 1.7%, respectively, with no outliers removed. However, data for one of the 2 materials in each of the studies showed statistical outliers. One set of duplicates was a Cochran outlier in the diphenylhydantoin method study but removing this pair (6% of the data) merely changed the CV_x from 1.68 to 1.58%. As expected, the removal of this outlier had considerable influence on CV_o, reducing it from 1.07 to 0.56%.

A new aspect of outlier decisions is shown by the collaborative study of the method for sodium butabarbital. The original data (13 duplicates for a total of 26 values, 13 laboratories) are shown in Figure 4, all on a comparable absolute percent basis, arranged in ascending order of laboratory means. The material represented by the data on the left was a composite of commercial tablets. Statistical analysis of the data

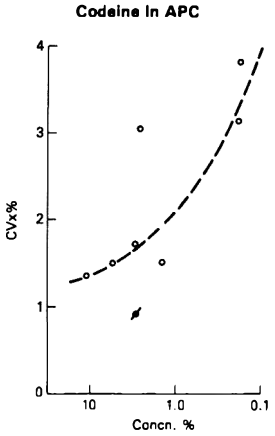

Figure 3. The coefficient of variation of the determination of codeine in APC tablets as a function of concentration (log scale), showing the increase in CV with decreasing concentration. The point ø has had 2 indicated statistical outliers removed. The other points have had no outliers removed.

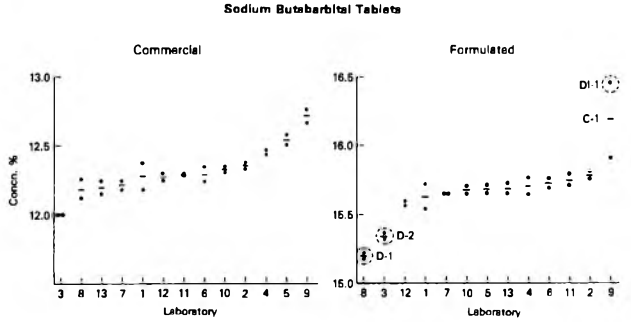

Figure 4. The original data from the collaborative study of the determination of butabarbital in tablets; concentration in percent vs laboratory number, in ascending order. Although CV_x values are approximately the same for the 2 materials, the commercial material shows no statistical outliers, but the formulated material shows 5 outlying points from 3 laboratories. See Figure 5 for the progression of CV_x with outlier removal from the formulated material.

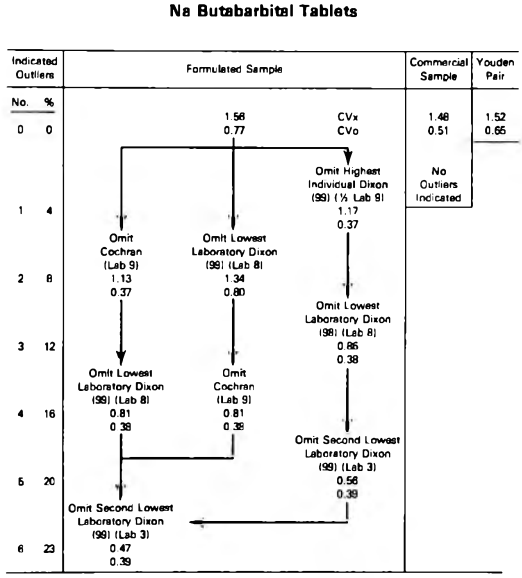

Figure 5. Actual overall CV between-laboratories (upper value of the pairs, CV_x) and within-laboratories (lower value of the pairs, CV_o) generated by the repeated application of the Dixon and Cochran outlier tests applied to the collaborative study data for commercial and formulated butabarbital tablet materials. Values in parentheses are indicated confidence levels. Cochran test in this case was used at the 95% level. Each material was examined by 13 laboratories in duplicate. Note comparability of the original data for formulated material with no outliers removed and data for presumably equivalent commercial material where no outliers are indicated by statistical tests. Note also that once Laboratory 9 is removed as Cochran (or individual Dixon) outlier, there is no further reduction in CV_o with additional removal of laboratory average outliers.

for this material shows no outliers by either the Dixon or Cochran test and they have a CV_x of 1.48%. The data from statistical analysis of the formulated material are shown on the right; they have approximately the same CV_x, 1.56%, as the data from the other material. On the first iteration, the highest value from Laboratory 9 and the lowest mean from Laboratory 8 are indicated as Dixon outliers at the 99% CL. The laboratory average of Laboratory 9 is not a Dixon outlier (95% CL) despite the large gap between the highest average and its nearest neighbor. This anomaly results from the structure of the Dixon test which for 11 to 13 *(n)* measurements examines the gap between the extreme value and its second nearest neighbor ($n - 2$) in the numerator and the extreme point minus the second extreme point from the opposite end in the denominator. When potential outliers are present at both ends, the various combinations can provide apparently strange outlier decisions.

The situation is even more incongruous when several outlier tests are used. Not only does the removal of outliers determine a new set of CVs, but the order of application of the outlier tests makes a difference, as diagrammed in Figure 5. If the Cochran test is applied first, the CV between laboratories decreases from 1.56 to 1.13% at an outlier rate of 8%. If the Dixon individual value test is applied first, the CV_x decreases to about the same value, 1.17, but at an outlier rate of only 4%. Note also that once the Cochran outlier is removed, CV_o remains constant at about 0.38%, regardless of the progression of between-laboratory outliers. If all outliers are removed as indicated by the 2 iterations (the DI-1, D-1, and C outliers are all indicated on the first iteration; see Figure 4), the ultimate CV_x of 0.47% and CV_o of 0.39% are obtained, at an outlier rate of 23%. But this has to be reconciled with the data from the analysis of equivalent commercial material which, because they showed no outliers, have a CV_x approximately 3 times as large. The reconciliation in this particular case is easy: Accept the CVs as found with no outlier removal, for a CV_x of 1.5% and a CV_o of 0.65%. Both the premium and

the hazard are very low. Acceptance of a CV_x of 0.5% at a 23% outlier rate is unrealistic, would be very hazardous, and provides little protection from outliers.

This discussion of the relation between CVs and outlier rates serves to emphasize that measurements of precision are inherently variable and are at the mercy of the appearance of unaccountable outliers. However, these relatively variable CVs are usually well within the acceptable range for the performance of their intended analytical function.

Summary of the Chromatographic Separation/Spectrophotometric Measurement Studies.—A collection of the analytical data averaged for each of 5 chemical classes from a total of 1153 determinations for 69 dosage forms of 24 compounds from 18 collaborative studies involving an average of 11 laboratories per study is given in Table 1:06. Included are the weighted (for the number of materials in each chemical class) averages for the 3 precision parameters, CV_o, CV_x, and the associated percent outliers. The diatomaceous silica separation/spectrophotometric measurement method may be roughly characterized by an average CV_x of 2.25%, increasing slightly with decreasing analyte concentration, and an average CV_o of 1.25% (CV_o/CV_x ratio of 0.58) with an average 2.0% outlier rate. The distribution of the CVs of the materials within those 4 chemical classes which contain substantial data appears to be the same for each class (homogeneous).

The data are also summarized in the form of Figures 1:01–1:06, labeled to correspond to the tables from which the data were taken. The center line of each graph represents the line of best fit for CV vs log concentration, bracketed by its prediction limits (the region where 95% of future such regression lines and similarly constructed confidence bands would be expected to encompass the long term value). The Y-axis of these figures is CV_x on a linear scale and the X-axis is concentration on a $\log_{10}$ scale beginning at log C = 2 (100%) and extending to log C = −2 (0.01%). The change in the CV(%) per change in log concentration (%), estimated from the figures, ranges from a low of 0.0 (steroids) to a high of 0.7 (alkaloids), with 0.3 and 0.4 for phenylalkanolamines and thiazides, respectively, and with an overall slope for all classes of compounds of 0.55. These slopes correspond to the change in CV for a 10-fold decrease in concentration. The various chemical classes of drugs are fairly evenly distributed on both sides of the best-fit line for the diatomaceous silica separation.

An examination of the composite figure shows that there is no segregation according to chemical type, except insofar as the potency places the active ingredient in a certain concentration range. Thus the CVs are independent of the chemical classification and the excipients of the various dosage forms. The overall CV_x as taken off the line of best fit for CV_x vs log concentration in Figure 1:06 extends from approximately 1.25% at the 100% concentration point to 3.5% at the 0.01% point, a concentration range of 4 orders of magnitude.

Other interesting aspects of the precision statistics for these collaborative studies are as follows: (*1*) Of the 18 published collaborative studies, only 2 (11%) studies (of methods for phenylpropanolamine and estradiol valerate) did not include at least one dosage form for which the method data contained an outlier flagged by the statistical tests used; (*2*) of the 24 compounds whose methods were studied, only 2 compounds (8%) did not have at least one dosage form (materials, assays) for which the method data contained an outlier flagged by the statistical tests used; (*3*) of the 69 individual materials (assays, dosage forms) the method data of 33 (48%), or approximately half the materials, had no outliers flagged by the statistical tests used; (*4*) despite the appearance of outliers throughout the studies, the precision results from method studies for 55 (80%) of the materials could be accepted either directly (no outliers indicated) or regardless of the presence of flagged outliers, because the CV_x with the outliers included was acceptable for the intended use of the methods for determining compliance with specifications. The distribution, by type of statistical test, of outliers that had to be omitted is given in Table 3. All significant outliers were indicated by the Dixon test; the Grubbs and rank sum tests did not add any information in these cases. More than half of these outliers were indicated at the 99% CL. It appears that, although the appearance of outliers was a frequent occurrence in these collaborative studies, they were often of no practical significance. Those outliers which had to be removed had a profound influence on the precision parameters.

Table 3. Numbers of materials by chemical class for which the original analytical data had to be reiterated to obtain "acceptable" between-laboratory CVs because of the presence of the indicated type of outliers

Outlier type	No. of materials examined					
	Phenylalkanolamine	Thiazides	Sterols	Alkaloids	Other	Total
None	6	12	17	16	4	55
Dixon, 99% CL	4		2	2		8
Dixon, 98% CL	2					2
Dixon, 95% CL	2					2
Procedural		2				2
Total	14	14	19	18	4	69

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PESTICIDE AND INDUSTRIAL CHEMICAL RESIDUES

Evaluation of Capillary Gas Chromatography for Pesticide and Industrial Chemical Residue Analysis. I. Comparison of Retention Ratios Obtained on Methyl Silicone-Coated Capillary Columns with Published Values for Packed Columns

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Many residue laboratories use the retention ratio data compiled by the Food and Drug Administration in its *Pesticide Analytical Manual* (PAM) for the tentative identification of pesticide and industrial chemical residues detected in food samples. These data were compiled using packed gas chromatographic columns. A study was undertaken to determine if these retention ratios could be used when applying capillary gas chromatography to residue analysis. Retention ratios of a selected group of 108 of the materials listed in the PAM tables were determined isothermally with split injection on 4 WCOT capillary columns obtained from 3 commercial suppliers and coated with 4 different methyl silicone coatings. Of the tested reference materials, 90 displayed retention ratios that matched the PAM values on all columns, 10 differed on at least one of the 4 columns, and only omethoate and monocrotophos gave retention ratios differing on all 4 columns.

Applications of capillary gas chromatography to the quantitation of a wide variety of pesticide and industrial chemical residues in environmental samples have been reported (1–4) in recent years.

Tables of gas chromatographic peak retention ratios relative to chlorpyrifos for numerous pesticide and industrial chemicals have been compiled by the Food and Drug Administration over the years. These tables, which are contained as an addendum entitled "U.S. FDA Listing of Chromatographic Properties" (PESTDATA) in the FDA *Pesticide Analytical Manual* (PAM) (5), are useful in tentatively identifying residues encountered during sample analysis. The retention ratios listed in the PESTDATA tables were determined isothermally at 200°C on packed columns containing supports coated with several different liquid phases including the methyl silicone DC-200 or its equivalent, OV-101. Krijgsman and Van De Kamp (1, 3) have shown that retention ratios determined under isothermal conditions differ from those determined with temperature programming. To make a valid comparison between capillary retention ratios and the PESTDATA values, the capillary values must also be determined isothermally. Splitless and on-column injection techniques require programming the column temperature. Split injections, although less sensitive, are made at isothermal column temperatures. The sensitivity criteria specified by PAM Vol. I, sections 143.21 and 311.412, for screening samples for pesticide and industrial chemical residues can easily be attained using the instrument parameters described under Apparatus.

Capillary gas chromatographic columns coated with OV-101 and other methyl silicones (SP-2100, OV-1, SE-30, etc.) are currently available from several sources. A study was undertaken to determine if the PESTDATA retention ratios obtained on OV-101 coated packed columns can be applied when using capillary columns coated with methyl silicones, for residue analysis.

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Experimental

Apparatus

(a) *Gas chromatograph.*—Hewlett Packard Model 5880A equipped with Model 7672A automatic sampler, split-splitless injector operated in the split mode (40:1), alpha-numeric keyboard, BASIC option, cartridge tape, and ^{63}Ni constant current electron capture detector. Carrier gas: hydrogen at 35 ± 1 cm/s linear velocity flow. Detector make-up gas: 5% methane in argon sufficient to give 30 mL/min flow at detector exit when added to column flow. Temperatures: injector 220°C, detector 350°C, column oven determined as described in *Procedure*. Sensitivity: attenuation 2 ↑ 6 (64×10^{-12} Hz/cm); this sensitivity produced a peak of approximately ½ full scale deflection (½ FSD) for 0.4 ng chlorpyrifos injected with a 40:1 split (approximately 10 pg to detector).

(b) *Capillary columns.*—(*1*) WCOT, Carbowax® 20M-deactivated, fused silica columns, 0.2 mm id × 50, 25 and 12.5 m lengths, coated with SP-2100, film thickness not given, Cat. No. 19091-60050 (Hewlett Packard, Avondale Division, Route 41, Avondale, PA 19311). (*2*) WCOT, fused silica column, 0.25 mm id × 25 m length, coated with OV-101, film thickness 0.2 μm, Cat. No. SLP-OV-101-25M (Quadrex Corp., PO Box 3881 Amity Station, New Haven, CT 06525). (*3*) WCOT, fused silica column, 0.31 mm id × 50 m length, coated with crosslinked methyl silicone (OV-1), film thickness not given, Cat. No. 19091-61350 (Hewlett Packard). (*4*) WCOT, fused silica column, 0.252 mm id × 60 m length, coated with DB1 (crosslinked methyl silicone equivalent to SE-30), film thickness 0.25 μm, Cat. No. 122-1062 (J&W Scientific, Inc., 3871 Security Park Dr, Rancho Cordova, CA 95670).

Procedure

The initial step of the study was to determine the column temperature for each column at which the retention ratios relative to chlorpyrifos most closely matched those given in the PESTDATA tables. A solution containing a mixture of lindane, heptachlor, aldrin, heptachlor epoxide, dieldrin, endrin, and *p,p'*-DDT in isooctane was used for this procedure.

A simple BASIC program was written for the gas chromatograph which instructed the instrument to inject the standard solution over a range of column temperatures starting at 190°C. At the end of each chromatographic run, a printout of retention ratios was generated, the column temperature was increased 1 or 2°C, and, after a 2 min hold for temperature stabilization, the standard solution was again chromatographed. This cycle was repeated to a column temperature of 210°C.

After the optimum operating temperatures were determined, a group of 100 additional pesticides and industrial chemicals was selected from the laboratory inventory for evaluation. These consisted of both essentially pure individ-

ual compounds and technical grade mixtures which were obtained as FDA/EPA reference materials.

Any of the materials that did not easily dissolve in isooctane were first solubilized in 2–3 mL acetone and then diluted to the appropriate concentration with isooctane. A few materials such as acephate and allethrin required acetone as the final solvent. The "ng ½ FSD" values from the PESTDATA table were used to estimate the relative quantity of each material required to give peaks between 40 and 100% FSD at the GC sensitivity specified under *Apparatus*.

The retention ratios for these materials were determined on the 4 capillary columns listed under *Apparatus* with the autosampler programmed to inject 2.5 μL of each solution of reference material at 50 min intervals. When these determinations were performed, the solution containing the 7 chlorinated reference materials previously prepared for the optimal temperature determination was placed in every tenth position in the autosampler rack to check the thermal stability of the system during the entire run. A second solution containing β-BHC, alachlor, chlorpyrifos, octachlor epoxide, endosulfan I, endosulfan II, *cis*-nonachlor, and endosulfan sulfate was injected following each injection of the chlorinated reference material mixture. Retention ratios relative to chlorpyrifos were calculated by the same formula used in obtaining PESTDATA values:

$$RRT_c = (\text{elution time of compd} - \text{elution time of solv.})/(\text{elution time of chlorpyrifos} - \text{elution time of solv.})$$

The gas chromatografic data system was programmed to perform this calculation after each injection.

Results and Discussion

The relationship between column temperature and relative retention ratios for the 7 selected chlorinated materials is shown in Table 1. The sum of individual deviations from the PESTDATA values at each temperature was obtained by calculating the absolute numerical difference between the determined value and the PESTDATA value and summing these differences. The standard deviations were calculated using the ratios of the determined values to the PESTDATA values. A combination of these 2 values was used to determine the optimum temperature for each column. These values indicate optimum temperatures of either 200 or 201°C for the Quadrex OV-101 column, 200°C for the H.P. OV-1 and J&W DB-1 columns, and 202°C for the H.P. SP-2100 column. For convenience sake, all columns were tested at 200°C except the H.P. SP-2100 column. All three lengths of the SP-2100 coated columns showed 202°C to be the optimum column temperature. Each of these columns was purchased individually over a period of 13 months. Therefore, the 3 SP-2100 columns were assumed to represent different manufacturing runs. Only the values obtained with the 50 m column are included in Table 1. The optimum temperatures for the 12.5 and 25 m H.P. SP-2100, the Quadrex OV-101, and the J&W DB-1 columns were also determined using a Varian 3740 gas chromatograph. Both Hewlett Packard columns again showed 202°C as the optimum temperature and the Quadrex and J&W columns required 200°C. These optimum temperatures were subsequently used in determining the relative retention ratios of the additional 100 materials studied on these columns.

The 4 columns were tested as received from the manufacturers. The lengths of these columns are not conducive to rapid analysis, with the possible exception of the 25 m Quadrex column. Column length can be adjusted to give a more rapid analysis with no effect on retention ratios, if all other parameters are kept constant. This is evidenced by the similar retention ratios obtained on the 12.5, 25, and 50 m H.P. SP-2100 columns. Compared with the 50 m column, the resolution is reduced for the shorter columns but is significantly better than for a 6 ft packed column. A normal GC run for a typical multiresidue analysis of a sample by PAM methodology (5) requires about the same amount of time (20–25 min) on the 50 m H.P. SP-2100 column as on the 6 ft packed column specified in the PAM. This time is reduced to ca 6–7 min with 12.5 m H.P. SP-2100 column and 10–12 min with the 25 m Quadrex column under isothermal conditions. The same analysis on the 60 m J&W DB-1 column would require ca 30–35 min. Longer columns give better resolution with some peak broadening, but length does not appear to affect peak symmetry.

The thermal stability of the Hewlett Packard chromatographic system over a period of 72 h of unattended operation is demonstrated by the retention data shown in Table 2. All these retention ratios are based on the retention times of aldrin (7.88 min) and isooctane (2.58 min) which were determined just before the automated run was started.

A list of compounds which were each found to display only one significant peak with a retention ratio matching the respective PESTDATA value within ±5% on the 4 different columns tested is shown in Table 3.

The compounds listed in Table 4 were found to display peaks differing from the PESTDATA values by more than ±5% on at least one of these columns. The following absolute retention times in minutes for chlorpyrifos and the solvent, isooctane, will allow the calculation of retention time variability for the compounds listed in Tables 4 and 5: H.P. SP-2100 (7.64 and 2.58), Quadrex OV-101 (3.98 and 1.25), H.P. OV-1 (6.99 and 2.50), and J&W DB-1 (11.27 and 2.72). In addition, as can be seen from the listed retention values, numerous peaks were observed in some cases. This was

Table 1. Change in retention ratio relative to chlorpyrifos with change in column temperature

Compound	PESTDATA value at 200°C	Column and temp., °C: 50 m H.P. SP-2100				25 m Quadrex OV-101				50 m H.P. OV-1 crosslinked				60 m J&W DB-1 crosslinked			
		198	200	202	204	199	200	201	202	199	200	201	202	199	200	201	202
Lindane	0.48	0.485	0.486	0.488	0.492	0.479	0.480	0.480	0.482	0.482	0.485	0.485	0.486	0.485	0.488	0.490	0.492
Heptachlor	0.83	0.829	0.829	0.830	0.833	0.830	0.830	0.832	0.833	0.834	0.834	0.834	0.835	0.829	0.830	0.833	0.835
Aldrin	1.05	1.047	1.047	1.047	1.047	1.052	1.052	1.052	1.052	1.054	1.054	1.054	1.054	1.054	1.054	1.054	1.054
Hept. epox.	1.29	1.305	1.301	1.298	1.296	1.300	1.300	1.298	1.297	1.305	1.303	1.300	1.298	1.303	1.300	1.299	1.298
Dieldrin	1.91	1.935	1.926	1.918	1.908	1.930	1.920	1.918	1.913	1.933	1.928	1.920	1.915	1.932	1.928	1.924	1.917
Endrin	2.15	2.173	2.162	2.152	2.139	2.164	2.157	2.152	2.146	2.170	2.164	2.156	2.148	2.173	2.164	2.160	2.151
p,p'-DDT	3.18	3.257	3.222	3.178	3.136	3.213	3.181	3.171	3.149	3.201	3.180	3.155	3.133	3.210	3.181	3.169	3.140
Sum[a]		0.149	0.091	0.031	0.081	0.080	0.030	0.032	0.052	0.089	0.058	0.064	0.077	0.098	0.055	0.061	0.077
Mean[b]		1.0094	1.0063	1.0035	1.0015	1.0050	1.0026	1.0018	1.0007	1.0075	1.0064	1.0038	1.0022	1.0078	1.0064	1.0062	1.0047
SD		0.0092	0.0063	0.0066	0.0120	0.0051	0.0030	0.0029	0.0052	0.0035	0.0038	0.0058	0.0085	0.0047	0.0058	0.0074	0.0111

[a]Sum of individual deviations from PESTDATA value.
[b]Mean of ratio of determined value vs PESTDATA value.

Table 2. Chromatographic system stability using Hewlett Packard SP-2100, 50 m capillary column[a]

Compound	Time lapse from start of run, h										Av.	SD	CV, %
	0	3	12	21	30	39	48	56	65	72			
Lindane	0.468	0.468	0.468	0.468	0.468	0.468	0.467	0.468	0.467	0.467	0.4677	0.00048	0.103
Heptachlor	0.794	0.794	0.794	0.794	0.793	0.794	0.792	0.793	0.793	0.792	0.7933	0.00082	0.103
Aldrin	1.000	1.001	1.001	1.000	1.000	1.000	0.999	1.000	1.000	0.999	1.0000	0.00067	0.067
Hept. epox.	1.240	1.241	1.241	1.240	1.240	1.240	1.238	1.240	1.239	1.238	1.2397	0.00106	0.086
Dieldrin	1.829	1.832	1.831	1.829	1.829	1.830	1.827	1.829	1.827	1.827	1.8290	0.00170	0.093
Endrin	2.053	2.056	2.056	2.054	2.053	2.054	2.051	2.053	2.052	2.051	2.0533	0.00177	0.086
p,p'-DDT	3.044	3.048	3.047	3.043	3.042	3.042	3.038	3.040	3.037	3.036	3.0417	0.00403	0.132

[a]Single injection of solution containing 7 chlorinated insecticides was made at each time period. Values shown are retention ratios relative to aldrin which eluted in 7.88 min at time 0. Isooctane (solvent) eluted in 2.58 min.

Table 3. Compounds displaying only one significant peak with retention ratio matching respective PESTDATA value within ±5% on all 4 columns tested

Alachlor	*p,p'*-Dichlorobenzophenone	Octachlor epoxide
Aldrin	Dichlorvos	Ovex
Atrazine	Dichloran	Parathion
Benfluralin	Dieldrin	Parathion-methyl
α-BHC	Endosulfan I	Pentachloroaniline
β-BHC	Endosulfan II	Pentachlorobenzene
δ-BHC	Endosulfan sulfate	*cis*-Permethrin
Captan	Endrin	*trans*-Permethrin
Captafol	EPN	*p,p'*-Perthane olefin
Carbophenothion	Ethion	Phorate
Carbophenothion sulfone	Fenitrothion	Phosalone
Carbophenothion sulfoxide	Fenthion	Phthalate, butyl benzyl
cis-Chlordane	Fonofos	Phthalate, di-2-ethyl hexyl
trans-Chlordane	Heptachlor	Pirimiphos methyl
Chlordecone	Heptachlor epoxide	Quintozene (PCNB)
Chlorpyrifos	Hexachlorobenzene	Ronnel
Coumaphos	Isodrin	Sulfallate
Crotoxyphos	Lindane (γ-BHC)	4-(2,4,5-TB) methyl ester
DCPA	Malathion	*o,p'*-TDE
o,p'-DDE	Malathion oxygen analog	*p,p'*-TDE
p,p'-DDE	*p,p'*-Methoxychlor	Tecnazene (TCNB)
o,p'-DDT	Methyl trithion	Terbufos
p,p'-DDT	Mevinphos	Thionazin
DEF	Mirex	1,2,4-Trichlorobenzene
Diazinon	Nitrofen	Trifluralin
Dibromochloropropane	*cis*-Nonachlor	Zytron
o,p'-Dichlorobenzophenone	*trans*-Nonachlor	

Table 4. Compounds that vary by more than ±5% from PESTDATA values on one or more of 4 columns tested

Compound	PESTDATA value	H.P. SP-2100	Quadrex OV-101	H.P. OV-1	J&W DB-1
Trichlorfon	0.07	0.072	0.159	0.071	0.074
Acephate	0.25	0.140	0.128	0.129	0.248
Omethoate	0.31	0.203	0.240	0.241	0.242
Monocrotophos	0.41	0.347	0.295	0.295	0.295
Dimethoate	0.42	0.424	0.388	0.388	0.389
Terbacil	0.55	0.550	0.487	0.498	0.501
Parathion ox. anal.	0.80	0.786	0.747	0.746	0.747
Linuron	0.87	0.937	0.881	0.882	0.884
Folpet	1.27	1.350	1.275	1.283	1.284
p,p'-Perthane	2.23	2.214	1.529[a]	1.523[a]	1.527[a]
o,p'-Dicofol	0.84[b]	0.859[b,c]	0.836[b]	0.836[b]	0.840[b]
		1.052	0.917		
		1.204			
		1.439			
		1.787			
		2.792	2.735		
	4.2		4.264[c]		
p,p'-Dicofol	1.02[b]	1.043[b,c]	1.009[b]	1.009[b]	1.013[b]
		1.119			
		1.583			
		1.647			
		1.676			
		1.915			
		3.422	3.263		
	4.5		4.600[c]		

[a]Matches retention ratio of *p,p'*-perthane olefin.
[b]Matches retention ratio of the respective dichlorobenzophenone breakdown product.
[c]Indicates major peak. Multiple peaks indicate degradation of compound either in injector or in column.

assumed to be the result of compound degradation, either in the column or the injector.

Table 5 lists several compounds which were injected but are not listed in the PESTDATA table. The retention ratios of these compounds are generally in very close agreement on all 4 capillary columns. The 1,1,1-trichloroethane peak was unresolved from the solvent front and the retention time could not be measured on the Quadrex OV-101 and the Hewlett Packard OV-1 crosslinked columns.

At the time that the Hewlett Packard 50 m, SP-2100 column was removed and the Quadrex column installed, the glass insert in the injection port was changed because some evidence of peak tailing was observed. The new insert was the same design as the old one and was installed as received from the manufacturer, i.e., with a small quantity of 10% OV-1 on 80–100 mesh Chromosorb W-HP column packing sand-

Table 5. Retention ratios relative to chlorpyrifos for compounds not listed in PESTDATA table

Compound	H.P. SP-2100	Quadrex OV-101	H.P. OV-1	J&W DB-1
1,1,1-Trichloroethane	0.029	w/solv.	w/solv.	0.030
Hexachloroethane	0.044	0.044	0.043	0.045
Hexachlorobutadiene	0.075	0.074	0.074	0.075
Hexachlorocyclopentadiene	0.108	0.121	0.120	0.122
Hexachloronorbornadiene	0.203	0.201	0.202	0.204
Heptachloronorbornene	0.346	0.342	0.344	0.346
Pentachlorophenol acetate	0.597	0.593	0.594	0.597

wiched between 2 small plugs of silanized glass wool. Several compounds showed severe degradation as evidenced by multiple peaks when injected into this insert, especially folpet, captafol, linuron, phostex, *p,p'*-DDT, omethoate, carbophenothion sulfone, and carbophenothion sulfoxide. No peaks were observed for acephate, captan, and pentachlorophenol when this insert was used.

The new insert was then removed and replaced by one that had been previously used, but from which the column packing and glass wool had been removed. This insert had also been cleaned with chromic acid solution and thoroughly rinsed with dionized water, ethanol, acetone, and dichloromethane. It had then been soaked for several days in a 5% solution of dichlorodimethylsilane in toluene. The insert was preconditioned in the chromatograph by disconnecting the column and heating the injection port at 230°C for 1 h with a flow of hydrogen carrier gas vented to a fume hood. The column was reconnected, injector temperature was decreased to 220°C, and several of the compounds which had shown degradation on the packed insert were again injected. Significant improvement was seen for all of the compounds injected, although folpet, captan, captafol, and acephate still showed considerable degradation. Small parent peaks for *o,p'*-dicofol and *p,p'*-dicofol now appeared with retention ratios of 4.2 and 4.5, respectively. As can be seen in Table 4, these parent peaks had not been obtained on the Hewlett Packard SP-2100 column even when a thoroughly conditioned, packed insert was used. It was therefore assumed that the packed insert injection system was responsible for much of the observed compound degradation and that, in some cases, the compounds were absorbed by this insert to such an extent that no detectable amount entered the column.

In an attempt to further improve the injection system before evaluating the Quadrex column, the column was again disconnected and the effluent from the injector containing the unpacked and cleaned insert was vented to a fume hood. Three 10 μL injections of Silyl 8 (available from Pierce Chemical Co., Box 117, Rockford, IL 61105) were made over a period of 10–15 min at an injector temperature of 220°C. The column was reconnected after approximately 1 h, and those compounds showing degradation with the previous systems were again injected. *o,p'*- and *p,p'*-Dicofol still showed some degradation; however, the parent peaks were now the major peaks and the second largest peak in each case was the corresponding dichlorodibenzophenone. Captan, linuron, acephate, and omethoate each displayed a single peak. Carbophenothion sulfone and sulfoxide each displayed one large peak that matched the respective PESTDATA value in addition to several very small peaks.

Several injections of the mixed standard solution of 7 chlorinated pesticides were made during the experimentation with the clean, silane-treated insert. It was observed that following the treatment with Silyl 8, approximately twice the peak area response was obtained and the precision of the responses of replicate injections improved significantly for each of these compounds. A summary of integrated area responses and the precision of these responses for the 7 test compounds, using the 3 different insert conditions over specified time periods, is shown in Table 6. Removing the packing and treating the insert did not affect the retention times or retention ratios of the 7 chlorinated pesticides in Table 6. However, tailing was reduced for several other compounds, especially acephate, monocrotophos, and trifluralin.

In addition to the individual compounds shown in Tables 3 and 4, solutions of technical grade reference materials of chlordane, phostex, toxaphene, BHC, and Aroclors 1221, 1016, 1242, 1248, 1254, 1260, and 1262 were chromatographed on the 4 columns. All of these materials displayed peaks with retention ratios matching the PESTDATA values. Due to greater resolution on the capillary column, each material also displayed numerous additional peaks, ranging from 4 for phostex to 80–85 for toxaphene.

The PESTDATA retention ratios for technical reference material pyrethrins are listed as 2.62 and 3.7. A solution of this material was injected on each of the 4 columns. Each column produced a similar chromatogram which displayed 6 significant peaks. The retention ratios of the respective peaks varied by no more than 2% from column to column. The average retention ratios were 2.163, 2.842, 3.078, 6.604, 8.666, and 9.408 for the 6 peaks. The major peaks on each of the columns corresponded to the 2.163 and 3.078 average retention ratios.

The capillary columns gave chromatograms for the Aroclors which exhibit much greater detail and peak resolution than can be obtained using a packed column. Tuinstra et al. (2) published a procedure for the quantitation of PCBs in milkfat, using glass capillary gas chromatography, splitless injection, and temperature programming. The PCB residue was quantitated by comparison with individual chlorinated biphenyl reference materials by using a chromatograph equipped for automated sample injection and computerized data reduction (6). Identification of the PCBs was based on relative retention time compared with the retention times of 2 internal standards, pentachloroaniline and mirex. Since these authors have shown that PCBs can be identified using the internal standard procedure, the identification of PCBs should also be feasible when using an external standard of chlorpyrifos and a chromatographic system with the thermal stability of the one used for this study. Quantitation would require comparison with individual chlorinated biphenyl compounds, especially in determining environmentally weathered residues. Likewise, the comparison to the reten-

Table 6. Effect of different methods used for conditioning capillary column injector inserts on chromatographic response and precision

	Packed insert[a]			Unpacked insert with DCMS treatment[b]			Unpacked insert with Silyl-8 treatment[c]		
Compound	Area count range	Av. $n=10$	CV, %	Area count range	Av. $n=8$	CV, %	Area count range	Av. $n=4$	CV, %
Lindane	285–330	303	5.56	156–277	185	16.45	359–370	364	1.28
Heptachlor	681–762	720	3.47	299–378	331	6.38	647–667	659	1.27
Aldrin	1136–1224	1172	2.35	498–615	544	5.66	1035–1068	1050	1.45
Hept. epox.	1650–1800	1718	2.64	685–847	755	5.87	1400–1451	1433	1.62
Dieldrin	1846–1972	1919	2.10	755–949	851	6.36	1547–1623	1589	2.02
Endrin	1641–1700	1700	2.65	643–881	753	7.60	1414–1483	1460	2.15
p,p'-DDT	1186–1718	1478	10.74	804–1032	928	8.36	1683–1805	1745	3.06

[a]Injections on H.P. SP-2100 column over 72 h.
[b]Injections on Quadrex OV-101 column over 30 h.
[c]Injections on Quadrex OV-101 column over 33 h.

tion of p,p'-DDE = 100 as reported by Sawyer (7) and by Webb and McCall (8) should be feasible.

Conclusion

The majority of the data listed in the PESTDATA Tables can be used for the tentative identification of pesticide and industrial chemical residues when using capillary columns coated with the 4 methyl silicone coatings tested, and probably with any methyl silicone coating under isothermal operating conditions. Crosslinking of the coating in the column appears to have no effect on the retention of the tested compounds.

Several differences from the PESTDATA values have been tabulated in this report and others may exist. When using any published data developed by another laboratory, the chromatographer must not rely on the data in blind faith. Great care must be taken to ensure that the chromatographic equipment is functioning properly. This is especially true when using capillary columns. The quality of the chromatogram and the final result greatly depend on the chromatographic parameters, injection technique, condition of the columns, cleanliness of the injection port liner, and cleanliness of the sample.

Acknowledgments

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Method for Determination of Organohalogen Pesticide Residues in Vegetable Oil Refinery By-Products

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A method using gel permeation and Florisil column chromatographic cleanup techniques is described for determination of residues of nonpolar organohalogen pesticides and pesticide alteration products in vegetable oils and their refinery by-products. Supplemental Florisil separation and alkali cleanup techniques are used to facilitate determinations. Residues are determined with a ^{63}Ni electron capture gas chromatographic detection system used in conjunction with 3 different gas chromatographic columns. Residue identities are confirmed by gas chromatography-mass spectrometry. Recoveries of 7 organohalogen pesticides, ranging from 90 to 103%, were determined by the supplemental Florisil separation technique to augment previously reported recovery data determined for initial GPC and Florisil cleanup steps. Soybean, peanut, and cottonseed deodorizer distillates and crude and refined oil, as well as additional refinery by-products, were analyzed. Nine to 13 organohalogen residues ranging from 0.5 to 6.3 ppm were determined in the 2 soybean deodorizer distillate samples used to develop and test the method. Identities of residues present at ≥0.3 ppm were confirmed by gas chromatography-mass spectrometry. An intralaboratory trial of the method provided additional recovery and residue determination data as follows: Recoveries ranging from 102 to 116% were obtained for 4 pesticides added to peanut oil deodorizer distillate. Residues determined in 1 soybean deodorizer distillate sample supported previously obtained data for this sample.

The vegetable oil refining process removes pesticide residues and other undesirable components from fats and oils (1). These components may become concentrated in some of the resulting refinery by-products such as the deodorizer distillate (distillate material resulting from steam distillation of the partially refined oil) (1). These vegetable oil refinery by-products and their mixtures have potential for use as animal feed ingredients.

Methods traditionally used to determine nonpolar organohalogen pesticide residues in fatty foods (2, 3) were found to be inadequate for analysis of many refinery by-products and associated mixtures. Emulsions were frequently encountered in the liquid/liquid extraction step and/or deactivation of the Florisil chromatographic column used in sample cleanup. An alumina column chromatographic cleanup procedure described by Lane et al. (4) for determination of dieldrin in fatty foods was found to be satisfactory for analysis of many feed-grade fats for dieldrin and other nonpolar organohalogen pesticide residues. However, inadequate cleanup of some samples by the alumina column often prevented proper Florisil separation of pesticides present. Also, when a series of refinery by-product mixtures of unspecified composition was cleaned up through an alumina column, as much as 40% of the original sample material eluted with the pesticides.

A procedure described by ABC Laboratories (5) specifying gel permeation chromatography (GPC) cleanup consistently removed more than 85% of the sample material while providing for greater than 90% recovery of most pesticides studied. This technique produced sample solutions which did not alter Florisil elution patterns of organohalogen pesticides. Results of analyses using GPC followed by Florisil column chromatography have been previously reported (1). Residues were determined by ^{3}H electron capture (EC) detector in conjunction with 2 gas chromatographic (GC) columns. This technique was satisfactory for the analysis of vegetable oils and many associated by-products. However, deodorizer distillates frequently contained numerous pesticides as well as unidentified halogenated materials and hydrocarbon interferences. GC separation of pesticide residues and interfering materials was poor, and residue determination was often impossible. In addition, attempts to confirm identity of residues by gas chromatography-mass spectrometry (GC-MS)

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were unsuccessful because of inadequate residue separation and sample cleanup.

To improve separation of pesticide residues and remove interfering materials, a second (supplemental) Florisil column technique with 5 ethyl ether/petroleum ether eluants was developed. Additional GC columns were tested and 3 were selected which, when used with a ^{63}Ni detector, improved peak resolution, and facilitated determinations. Hydrocarbons which interfered with dieldrin determination were removed by treatment with alkali. Samples analyzed by the appropriate additional cleanup and separation technique were amenable to GC-MS analysis. Identities of residues present at ≥0.3 ppm were confirmed by electron impact (EI) and chemical ionization (CI) detection modes.

METHOD

Reagents

(a) *Florisil.*—60–100 mesh PR grade (Floridin Co., Berkeley Springs, WV 25411). Treat as described in **29.002(i)** (2). Standardize with lauric acid (6). Test elution characteristics of Florisil column by preparing pesticide standard solution containing hexachlorobenzene (HCB) (0.4 μg/mL), α-BHC (0.2 μg/mL), heptachlor epoxide (0.4 μg/mL), aldrin (0.2 μg/mL), *p,p'*-DDT (1.2 μg/mL), and dieldrin (0.8 μg/mL) in hexane. Place 1 mL standard on column prepared as in *Cleanup* and elute with eluants 1 and 2 as in *Cleanup*. Concentrate eluates to 5 mL. Transfer each eluate to a second Florisil column and elute as described in *Supplemental Separation and Cleanup*. Concentrate eluates to 10 mL and determine recovery of each compound as in *Determination*. Only Florisil that quantitatively separates compounds into the eluates specified in Table 1 is satisfactory.

(b) *Florisil eluants.*—Let all eluant mixtures reach room temperature before adjusting to final volume. (1) *Eluant 1.*—20% methylene chloride–hexane (v/v). (2) *Eluant 2.*—50% methylene chloride–0.35% acetonitrile–49.65% hexane (v/v/v). (3) *Eluant 3.*—50% methylene chloride–1.5% acetonitrile–48.5% hexane (v/v/v). (4) *Eluant 1A.*—Petroleum ether. (5) *Eluant 1B.*—3% ethyl ether-petroleum ether (v/v). (6) *Eluant 1C.*—15% ethyl ether–petroleum ether (v/v). (7) *Eluant 2A.*—6% ethyl ether–petroleum ether (v/v). (8) *Eluant 2B.*—Same as 1C.

(c) *Sodium sulfate.*—Anhydrous granular, reagent grade. Heat at 600°C overnight, cool in desiccator, and store in bottles with foil-lined screw-top lids.

(d) *Potassium hydroxide.*—Anhydrous pellets, reagent grade.

(e) *Solvents.*—Acetonitrile, ethanol, ethyl acetate, ethyl ether, hexane, isooctane, methanol, methylene chloride, petroleum ether, and toluene. Suitable for pesticide analysis (Burdick and Jackson Laboratories, Inc., 1953 S Harvey St, Muskegon, MI 49442, or equivalent).

(f) *GPC gel.*—Bio-Beads SX-3, 200–400 mesh (No. 152-2750 (Bio-Rad Laboratories, 2200 Wright Ave., Richmond, CA 94804).

(g) *GPC eluant.*—Toluene–ethyl acetate (1 + 3).

(h) *Pesticide reference standards.*—Aldrin, α-BHC, *cis*-chlordane, dieldrin, endrin, heptachlor, heptachlor epoxide, HCB, lindane, *p,p'*-DDE, *p,p'*-DDT, *p,p'*-TDE, *trans*-chlordane, and others as required (EPA Reference Standards, Environmental Protection Agency, Reference Standards Repository (MD-8), Research Triangle Park, NC 27711). Dissolve individual standards in hexane, and dilute to exact concentration as needed for residue identification, quantitation, and sample fortifications for recovery studies.

Apparatus

(a) *Gas chromatograph with electron capture detector*—Model 5710A equipped with constant current ^{63}Ni EC detector (Hewlett-Packard Co., Route 41, Avondale, PA 19311, or equivalent). Use 1.8 m × 4 mm id glass columns with the following packings: (1) 5% OV-101 on 80–100 mesh Chromosorb W HP, (2) 3% OV-225 on 100–120 mesh Chromosorb W HP, (3) 1% SP-1240 DA on 100–120 mesh Supelcoport (Supelco Inc., Supelco Park, Bellefonte, PA 16823); column and injection port 200°C (1240 DA column 170°C), detector 350°C; 5% methane in argon carrier gas, 60 mL/min; detector signal attenuated to give about ½ full scale recorder deflection (FSD) for 0.2 ng heptachlor epoxide.

(b) *Gas chromatograph with halogen selective detector.*—Model 560 with Hall 700A electrolytic conductivity detector (Tracor Inc., 6500 Tracor Lane, Austin, TX 78721, or equivalent). Use 1.8 m × 2 mm id glass GC column packed with 5% OV-101 on 80–100 mesh Chromosorb W HP. Parameters: *n*-propanol solvent, 0.35 mL/min; helium carrier gas, 20–60 mL/min; hydrogen reactor tube gas, 60 mL/min; reactor tube, 900°C; column, 200°C; injection port, 210°C; electrometer signal attenuated to give about ½ FSD for 1.0 ng heptachlor epoxide.

(c) *Gas chromatograph with flame ionization detector.*—Model 5710A (Hewlett-Packard Co., or equivalent). Use 1.8 m × 4 mm id glass column packed with 3% OV-101 on 80–100 mesh Chromosorb W HP. Parameters: column, 200°C; injection port, 250°C; nitrogen carrier gas, 60 mL/min; hydrogen and air combustion gases 60 and 120 mL/min, respectively; electrometer signal attenuated to give about ½ FSD for 1000 ng heptachlor epoxide.

(d) *GPC columns and column fittings.*—Glass column 2.5 cm id × 450 mm, Glenco series 3500 system designed for use with organic solvents, equipped with extra 16 cm adjustable plunger (No. 3500-A-25) and lower end plate, with specified Teflon supports, ferrules, and tubing connectors, and with ⅛ in. od × 0.065 in. id high pressure Teflon tubing (No. 3114-2-065) connected at both ends. Column extender (No. 3400-R-025) for connecting GPC columns in series (Glenco Scientific, Inc., 2802 White Oak Dr, Houston, TX 77007).

(e) *GPC eluant delivery system.*—Laboratory pump (No. RP-G20-1CSC) capable of delivering solvent at about 5 mL/min, with pulse dampener (No. PD-60-LF) and pressure gauge (No. 110345), and fitting kit (No. R479) (Fluid Metering Inc., 48 Summit St, Oyster Bay, NY 11771).

(f) *GPC sample introduction valve.*—Altex Tefzel sample injection valve (No. 201-56) equipped with calibrated 5.0 mL Teflon loading loop (No. 201-20) and Teflon female Luer adaptor (No. 200-18) sample introduction port (Rainin Instrument Co., Inc., Mack Rd, Woburn. MA 01801).

(g) *Valve, 3-port.*—Teflon 3-port "tee-bore" valve (No. 3001-2 × 2 × 2) specially bored to accept Teflon ferrules, with Teflon ferrules to replace Viton "O" rings, in-line connection immediately preceding lower end plate of GPC column, as a column by-pass device (Glenco Scientific, Inc.).

(h) *Tubing and tubing connectors.*—High pressure Teflon tubing, ⅛ in. od × 0.065 in. id (No. 3114-2-065), with PP nuts (No. 30221-1) for in-line GPC system connections. High pressure Teflon tubing 1/16 in. od × 0.031 in. id (No. 3114-1-031), with PP nuts (No. 3022-2) for loading loops and sample introduction port assembly. Flare tube couplers (No. FT-148) for tubing-tubing connections (Glenco Scientific, Inc.).

(i) *Sample filtration apparatus.*—Thirteen mm; 5 μm porosity Mitex filter pads (No. LSWP-013-000) contained in Swinny filter holder (No. XX30-012-00) for sample filtration before GPC cleanup (Millipore Corp., Bedford, MA 01730).

(j) *Evaporative concentrators.*—A 500 mL Kuderna-Danish (KD) flask (No. K-570001) fitted with 3-ball Snyder column (No. K-503000, size 121) via 24/40 ⅀ joints, and 10 mL graduated concentrator tube (No. K-570050, size 1025) or 15 mL lower tube (No. K-570002), connected via 19/22 ⅀ joints. A micro evaporative concentrator column (No. K-569251) fitted with 10 mL graduated concentrator tube via 19/22 ⅀ joints, is used for concentrations to less than 5.0 mL (Kontes Glass Co., Spruce St, Vineland, NJ 08360).

(k) *Chromatographic column.*—Chromaflex column, 22 mm id × 300 mm (No. K-420540) with Teflon stopcock and coarse porosity fritted disc (Kontes Glass Co.).

(l) *GC-MS system with pulsed positive ion-negative ion option.*—Model 4023 (Finnigan Instruments, San Jose, CA 95134). Use methyl silicone fused silica capillary column 37.5 m × 0.22 mm id (Hewlett-Packard Co.), and helium carrier gas with Hydro-purge gas filter (Varian Associates, 4701 Lydell Dr, Cheverly, MD 20781) and 99.99% minimum purity methane, chemical ionization reagent gas (Matheson Gas Products, East Rutherford, NJ 07073).

(m) *Syringe for GC-MS.*—Hamilton 2.0 μL syringe (No. 7102) with 7.5 cm needle, point style No. 2 (Hamilton Company, PO Box 10030, Reno, NV 89150).

Sample Preparation

(a) *Liquid samples.*—Shake vigorously 3 s or longer until a uniform sample mixture is obtained. Immediately weigh 5.0 g sample into 50.0 mL volumetric flask. Dilute to volume with toluene–ethyl acetate (1 + 3) to give final sample concentration of 100 mg/mL.

(b) *Viscous or solid samples.*—Heat sample gently to liquefy before mixing. Proceed as for liquid samples.

(c) *Samples containing particulate material.*—Mix as in (a) or (b) as appropriate. Immediately weigh 5.0 g sample into 50 mL Erlenmeyer flask and dilute with ethyl acetate. Transfer sample solution to Buchner funnel fitted with sharkskin filter paper and attached to 500 mL suction flask. Filter with gentle suction. Rinse flask, funnel, and filter paper with four 20 mL portions of ethyl acetate. Quantitatively transfer filtrate to 500 mL KD flask fitted with 10 mL graduated concentrator tube. Add 3–4 boiling chips. Immediately attach Snyder column and begin concentrating filtrate on steam bath. Concentrate to about 6 mL; then transfer to 50.0 mL volumetric flask, rinsing with toluene–ethyl acetate (1 + 3). Dilute to volume with toluene–ethyl acetate to give final sample concentration of 100 mg/mL.

(d) *Aqueous-oil sample mixtures such as soapstocks.*—Shake vigorously 30 s or longer until uniform sample mixture is obtained. Immediately weigh 250 g sample into 1 L beaker. Transfer to 1 L separatory funnel, rinsing with small portions of water. Test sample with pH paper and neutralize as necessary with 10% sulfuric acid or 10% potassium hydroxide. Add 500 mL water and 100 mL hexane. Shake vigorously 1 min, let layers separate, and discard lower layer. Wash hexane layer twice with 100 mL water; discard washes. Dry hexane layer by passing through 10 cm sodium sulfate in 22 mm id × 300 mm glass chromatographic column. Collect eluate in 500 mL KD concentrator fitted with 15 mL lower tube. Add 3–4 boiling chips, immediately attach Snyder column, and begin concentrating on steam bath. Concentrate to about 10 mL; then transfer solution to tared 100 mL beaker, rinsing with hexane. Let air-dry in hood until hexane vapors are no longer detected. Place beaker containing isolated oil in oven 1 h at 60°C. Remove and let cool in desiccator. Determine weight of oil extracted. Weigh 1.6 g maximum, or quantitatively transfer oil if less than 1.6 g, into 25 mL graduated cylinder and dilute to 16.0 mL with toluene–ethyl acetate (1 + 3) to give final sample concentration of 100 mg/mL or less. (Note: Less than 1.6 g oil may be obtained; soapstock is likely to contain less than 0.4% oil.)

Gel Permeation Chromatographic Column

(a) *Preparation.*—Weigh 45 g 200–400 mesh Bio-Beads SX-3 gel into 100 mL beaker. Slowly transfer gel in about 10 g portions to glass-stopper 2 L graduated cylinder containing 260 mL methylene chloride. Stir or mix cautiously 30 s after each addition of gel. Allow gel to swell 4 h. Add enough toluene (ca 250 mL) to produce ca 70 mL supernate (upper layer). Mix gently, let settle 30 min, and decant supernate. Repeat addition of toluene (ca 50–100 mL) to produce 70 mL supernate and subsequent decantation 3 times. Examine decanted solution for presence of suspended fines by direct observation or by weighing particulate material remaining after removal of solvent. Continue addition of toluene and subsequent decantation until fines are no longer present in supernate. Add enough methylene chloride to gel to produce balanced density slurry. (If too much methylene chloride is added, clear layer forms at bottom of cylinder. Correct by adding additional toluene until density of gel slurry is balanced. If excess toluene is added, resulting supernate should be decanted before addition of methylene chloride.) Let slurry equilibrate overnight. Transfer gel mixture to GPC column as follows: Connect two 2.5 cm id × 450 mm glass GPC columns in series, using GPC column extender, and attach and secure lower end plate to bottom column. Slowly add balanced density slurry to top of column. Rinse graduate with toluene and add rinse to column. As solvent solution drains out lower column end plate, keep top of gel bed covered with toluene. When height of gel bed has settled below 450 mm, remove upper GPC column. Attach adjustable plunger to top of lower column and firmly depress to compress gel. Secure plunger, attach GPC column to 3-port valve, and complete assembly of GPC system (Figure 1). Begin eluting column with toluene–ethyl acetate (1 + 3) at flow rate of 2–3 mL/min. If pressure approaches limit for column seals (ca 50 psi for Glenco columns), reduce flow rate. If pressure is still excessive, increase gel bed height. Elute column for 3–4 h, disconnect pump, loosen plunger, and compress gel bed. Final gel column length should be ca 270 mm.

(b) *Calibration.*—Prepare 25 mL toluene–ethyl acetate (1 + 3) fortification solution containing 10–100 μg/mL each of lindane, heptachlor, aldrin, *p,p'*-DDT, and *p,p'*-TDE. Prepare isooctane GC quantitation standard containing 0.1–1 μg/mL each of the above pesticides. Weigh 1 g filtered butterfat into 10 mL volumetric flask, add 1 mL fortification solution, and dilute to volume with toluene–ethyl acetate (1 + 3). Weigh 1 g vegetable oil by-product into separate 10 mL vol-

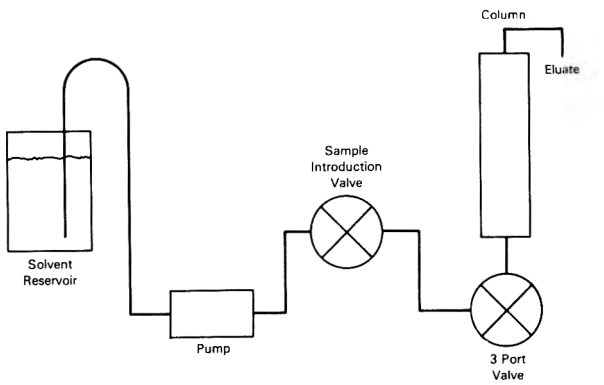

Figure 1. GPC cleanup assembly as described in Method.

umetric flask. Fortify and dilute as for butterfat. Load 5 mL butterfat solution into GPC column loading loop as described in *Cleanup*. Set sample introduction valve to load position and collect first 200 mL eluate from GPC column in consecutive 10 mL portions (portions 1–20). Collect next 50 mL eluted as 1 portion (portion 21). Subject each portion to azeotropic distillation with methanol, then hexane, as described in *Cleanup*. Dilute to 50 mL with hexane each of the 8 portions which represent the 80–160 mL volume (portions 9–16) of the total GPC eluate and determine pesticide levels in each. Determine pesticide levels in additional portions until pesticides are no longer detected. Determine total amount (μg) recovered (see note below) for each pesticide. Determine % pesticide recoveries for each portion ((μg pesticide in portion/total μg pesticide reovered) × 100). Concentrate each of the first 12 portions (first 120 mL GPC eluate) to less than 10 mL as necessary. Quantitatively transfer each portion to 100 mL tared beaker and let air-dry in hood until solvent vapors are no longer detected. Place beakers in oven 1 h at 60°C. Remove beakers, let cool in desiccator, and then weigh. Concentrate and weigh fat in additional portions until fat weighed has decreased to less than 1% of the amount placed on column. Determine total amount (mg) of fat recovered. (Note: Quantities of pesticides and sample weights often total only 75–85% of amount added for this calibration.) Determine % fat recovered in each portion ((mg fat in portion/total mg fat recovered) × 100).

Using above fat and pesticide recovery data, determine volumes of 2 GPC fractions which allow for best separation of high molecular weight sample materials (fraction 1) from organohalogen pesticides (fraction 2). Use recovery data for *p,p'*-TDE, to estimate cutoff volume between the 2 fractions. Fraction 2 should contain 90% or more recovered *p,p'*-TDE, as well as 95% or more recovered *p,p'*-DDT, lindane, heptachlor, and aldrin, and 10% or less of the recovered fat. Repeat above GPC cleanup of fortified butterfat, collecting one or two 5 mL portions of eluate before and after estimated cutoff volume. Determine optimum cutoff volume between fractions 1 and 2. (See Figure 2 for GPC separation obtained in our laboratory of 0.5 g butterfat from 3.63 μg *p,p'*-TDE and 0.805 μg aldrin.)

Load 5 mL fortified vegetable oil by-product solution onto GPC column as described in *Cleanup*. Collect and save volumes determined for fractions 1 and 2 above. Determine % sample material recovered in fraction 1 ((mg recovered/mg added) × 100). Determine % pesticide recoveries in fraction 2 ((μg recovered/μg added) × 100) as described above. About 85% or more oil sample should be contained in fraction 1. More than 85% of *p,p'*-TDE added and more than 90% of other pesticides added should be present in fraction 2. Volume adjustments may be made as long as no more than 15% of sample material is present in fraction 2. A GPC column prepared and calibrated as described above should be useful for the analysis of 100 or more vegetable oil by-product samples.

Cleanup

Prepare 1 L toluene–ethyl acetate (1 + 3) eluant and place in pump reservoir of GPC system. Adjust 3-port valve preceding column to allow eluant to by-pass GPC column. Start pump and allow solvent to flow for a few minutes. Discard solvent used. Set GPC sample introduction valve to by-pass position. Adjust 3-port valve preceding column to allow eluant to enter GPC column. Pump eluant through column at 4–5 mL/min for 15 min, or until any bubbles in column are removed. Fit 10 mL Luer-Lok syringe with 13 mm Swinny filter holder

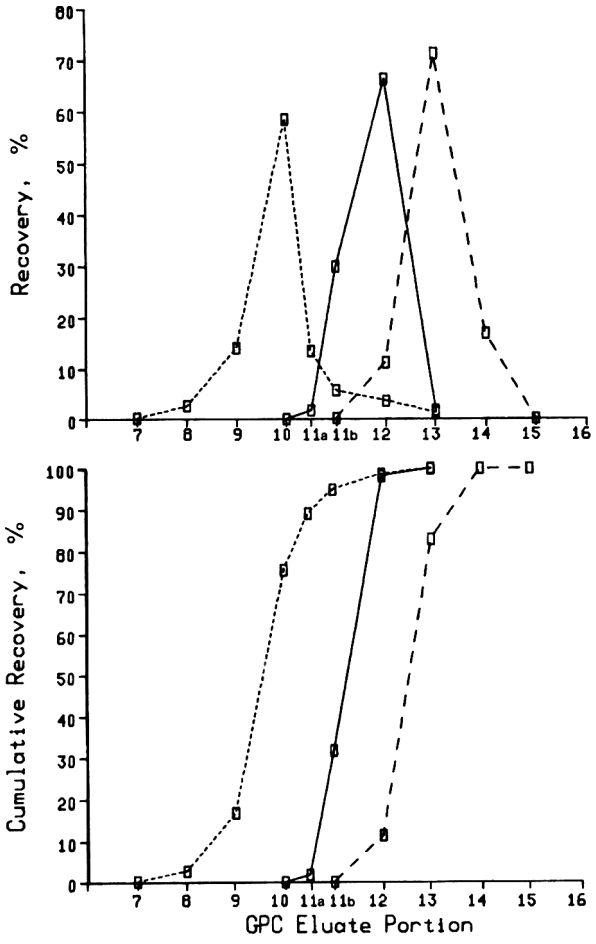

Figure 2. Separation of butterfat from 2 pesticides with column described in *Gel Permeation Chromatographic Column*.

The % of total amount recovered (upper) and cumulative % of total amount recovered (lower) are shown for portions of GPC eluate. See *Results and Discussion* for explanation of eluate portions. Butterfat, dotted line: *p,p'*-TDE, solid line; aldrin, dashed line.

containing 5 μm Mitex filter and attach in upright position to GPC sample introduction port. Prepare 100 mg/mL sample solution as described in *Sample Preparation*. Shake well. Immediately pour ca 8 mL sample solution into Luer-Lok syringe barrel. Insert plunger and slowly depress until most of sample solution has been forced from syringe onto 5 mL loop. Collect and discard excess sample solution as it drains from loop. Let ca 0.5 mL sample solution remain in syringe. Set sample introduction valve to load position. Collect first and second fractions in graduated cylinders, using volumes determined under *Gel Permeation Chromatographic Column*. Discard first fraction. Set GPC sample introduction valve to by-pass position. Remove Luer-Lok syringe and filter holder. Place new filter into holder and attach to introduction port. Pour second 8 mL portion of sample solution into barrel. Load sample loop and proceed with GPC cleanup above. Combine the 2 GPC fraction 2 eluates in 500 mL KD flask fitted with 15 mL lower tube. Rinse each graduated cylinder with 2 portions (5 mL each) of methanol. Add 200 mL methanol to KD concentrator. Add 3–4 boiling chips. Immediately attach Snyder column and begin concentrating on steam bath. Concentrate until solvent vapors are no longer observed at top of Snyder column. (Note: About 20 mL solution should be present in KD concentrator.) Remove KD concentrator from steam bath and add 100 mL methanol. Add 3–4 boiling chips and immediately begin concentrating solution. Concentrate until solvent vapors are no longer observed at top of Snyder column. Remove concentrator from steam

bath. Add 100 mL hexane and repeat concentration. If more than 5 mL solvent is present in lower tube, replace Snyder column with a modified micro evaporative concentrator column, add 3–4 boiling chips, and concentrate to about 2 mL. Remove lower tube and dilute residue to about 5 mL with hexane. Stopper and mix well.

Prepare Florisil eluants 1, 2, and 3 as described in *Reagents*. Add weight of Florisil determined from lauric acid test (*Reagents* (a)) to 22 mm id chromatographic column. Settle Florisil by gently tapping column. Top column with ca 1–2 in. anhydrous Na_2SO_4. Prewet column with 50–60 mL hexane. When solvent level has just reached top of Na_2SO_4, place 500 mL KD concentrator with volumetric or graduated collection vessel under column. Transfer GPC sample solution to column. Rinse container with 2 (ca 5 mL) portions of hexane and transfer rinsings to column. Let sample reach top of Na_2SO_4. Elute column at ca 5 mL/min with 200 mL eluant 1. Change receiver and elute with 200 mL eluant 2. Change receiver and elute with 200 mL eluant 3. Add 3–4 boiling chips to each KD concentrator. Immediately attach Snyder columns and begin concentrating on steam bath. Concentrate to less than 5 mL. Dilute each concentrated Florisil eluate to appropriate volume with hexane to obtain 100 mg equivalent of sample/mL.

Determination

Inject 6.0 μL of each Florisil eluate (equivalent to 0.6 mg sample) into gas chromatograph equipped with ^{63}Ni detector and 5% OV-101 GC column operated as described in *Apparatus*. Evaluate chromatograms and tentatively identify pesticide residues by relative retention time, using Appendix I of the FDA *Pesticide Analytical Manual* (PAM), Vol. I (3), and corresponding pesticide GC standard solutions described in *Reagents*. Prepare and inject additional reference standard solutions as required. Use ^{63}Ni EC detector with additional columns and other GC detector systems described in *Apparatus* to support tentative residue identification. If residues are detected in Florisil eluates 1 or 2, proceed to *Supplemental Separation and Cleanup* below. (See Table 1 for elution characteristics of residues found in soybean deodorizer distillate samples.)

Quantitate residues, using GC columns described in *Apparatus*. Store eluates in dark and refrigerate when possible until GC analysis has been completed. If confirmation of residue identity is required, concentrate Florisil eluate(s) to 20 μL or less and use GC-MS procedure described in *Confirmation of Identity*. (Generally, residue levels of ≥0.3 ppm can be confirmed by GC-MS.)

Supplemental Separation and Cleanup

(a) *Florisil chromatography.*—Prepare Florisil eluants 1A, 1B, 1C, 2A, and 2B as described in *Reagents*. Prepare Florisil column as described in *Cleanup*. Prewash column as described in *Cleanup*, using petroleum ether. Pipet equivalent of 500 mg oil sample from Florisil eluate 1 (obtained as described in *Cleanup*) onto column, and elute as described in *Cleanup*, using 200 mL each of eluants 1A, 1B, and 1C. Concentrate each eluate to less than 5 mL as described in *Cleanup*. Dilute each Florisil eluate to 5.0 mL with hexane. Prepare and prewash a second Florisil column as described above. Pipet equivalent of 500 mg oil sample from Florisil eluate 2 (obtained as described in *Cleanup*) onto column and proceed as described above, eluting with 200 mL each of eluants 2A and 2B. Concentrate and dilute to final volume as described above. Inject eluate 2B into gas chromatograph equipped with flame detector as described under *Apparatus*. If hydrocarbon co-extractives with retention times similar to pesticides are detected, proceed to alkali saponification described below. Quantitate residues in Florisil eluates as described in *Determination*.

Table 1. Elution behavior of chlorinated hydrocarbons with Florisil separation described in *Supplemental Separation and Cleanup*

Eluate 1 chemicals[a]		Eluate 2 chemicals	
Eluate 1A	Eluate 1B	Eluate 2A	Eluate 2B
Aldrin	BHC, α-	heptachlor epoxide	dichloro-benzophenone
DDE, *p,p'*-	chlordane, *cis*-		
Heptachlor	chlordane, *trans*-		dieldrin
Hexachlorobenzene	DDT, *p,p'*-		endrin
Mirex	TDE, *o,p'*-		
Polychlorinated biphenyls	TDE, *p,p'*-		

[a]No chemicals eluted in eluate 1C.

(b) *Alkali saponification.*—Place Florisil eluate 2B into 10 mL collection tube. Add 2.0 mL 2% potassium hydroxide-methanol (w/v) and proceed as described by Young and Burke (7), using 5.0 mL hexane for final volume. Transfer hexane layer into second collection tube with a disposable pipet. Quantitate residues as described in *Determination*.

Confirmation of Identity

A variety of techniques are useful for confirming the identity of individual organohalogen pesticide residues (3). However, because of the large number of pesticide residues and unidentified materials present in many deodorizer distillate samples, the following GC-MS procedure was used in this work.

Remove glass-lined steel capillary tubing that connects GC oven to MS interface. Push one end of 37.5 m fused silica capillary column through GC-MS transfer line and interface until tip of column is at entrance to MS ion source block. Thread other end of column into capillary injector until it is 0.5–1.0 cm below tip of a syringe inserted through injection port septum.

Place hydro-purge filter in helium supply line to remove oxygen from gas. Adjust helium carrier gas head pressure to 20 psi with septum purge flow of 100 mL/min. Linear velocity of gas through column is about 27 cm/s.

Adjust MS manifold temperature to 90°C. Maintain source temperature at 250°C for electron impact (EI) analyses and 140°C for chemical ionization (CI) analyses; injection port temperature at 250°C and transfer line region at 270°C. Inject up to 2.0 μL sample solution containing appropriate equivalent weight of sample, using splitless (Grob) mode with septum purge turned off. (Note: Injection volume containing ca 100 mg equivalent of sample is required for analysis at 1 ppm.) Restart septum purge 1 min after injection. Hold column temperature 1 min at 70°C after injection and then program to 170°C at 20°C/min followed by 8°C/min to 270°C. Hold the column temperature 10 min at 270°C. Acquire data as required for residue confirmations based on behavior of pesticide standards analyzed under these conditions.

Analyze for dichlorobenzophenone, α-BHC, and hexachlorobenzene, using methane CI. Introduce methane gas into ion source from makeup gas inlet. Analyze for all other pesticide residues in EI mode.

Results and Discussion

Samples Analyzed

Three different sets of vegetable oil and associated refinery by-product samples (2 sets of soybean oil samples received

in 1976 and 1980 and 1 set of peanut oil samples received in 1980) were analyzed by existing methodology (1) which uses techniques described in *Cleanup*. A crude oil, a completely refined oil, and a deodorizer distillate refinery by-product were included in each set. A soapstock (aqueous alkaline material resulting from base wash of crude oil) and a clabber stock (grease-like material obtained as scrapings from the refinery apparatus) refinery by-product were included in the 1976 soybean set, and a shell drain oil (also material resulting from steam distillation of partially refined oil) refinery by-product was included in the 1980 soybean and peanut oil sets. A cottonseed deodorizer distillate received in 1980 was also analyzed.

Results from analyses of the above samples are summarized in Table 2. Results from analysis of the 1976 soybean set agreed with those previously reported (1). The 1980 soybean crude oil sample contained 0.04 ppm dieldrin and 0.03 ppm α-BHC. No pesticide residues were found in the 1980 soybean completely refined oil and shell drain oil, or in the 1980 peanut oil samples. The limit of detection for these analyses was estimated to be 0.02 ppm heptachlor epoxide, based on injection of 1 mg equivalent of sample and EC-GC conditions where 0.2 ng heptachlor epoxide caused ½ FSD. A ^{63}Ni EC detector with 5% OV-101 and 1 + 1 5% OV-101/7.5% OV-210 GC columns was used to make these determinations. A residue in Florisil eluate 2 from the cottonseed deodorizer distillate analysis was determined to be 8.2 ppm *p,p'*-dichlorobenzophenone, as confirmed by GC-MS analysis. This residue is known to be the major alteration product of dicofol (Kelthane), which has been registered for use on cotton by EPA. Dicofol would completely elute in Florisil eluate 1 if present, but was not found.

The 1976 and 1980 soybean deodorizer distillate samples were used to develop and test the described method, because analysis of these samples by existing methodology (1) had presented unique residue separation and cleanup difficulties. The presence of numerous pesticide residues, as well as several unidentified halogenated residues and various hydrocarbon co-extractives, indicated that additional separation techniques were needed before identification and quantitation.

Table 2. Summary of pesticide residues found (ppm) in vegetable oils and associated refinery by-products[a]

1. Peanut oils and associated by-products	
crude oil	NF
deodorizer distillate	NF
shell drain oil	NF
refined oil	NF
2. Soybean oils and associated by-products (1976)	
crude oil	
dieldrin	0.05
endrin	0.01
soapstock (aqueous alkaline)	NF
deodorizer distillate	—[b]
clabberstock	—[c]
refined oil	NF
3. Soybean oils and associated by-products (1980)	
crude oil	
dieldrin	0.04
BHC, α-	0.03
deodorizer distillate	—[b]
shell drain oil	NF
refined oil	NF
4. Cottonseed deodorizer distillate	
dichlorobenzophenone, *p,p'*-	8.2

[a]NF, organohalogen pesticide residues not found.
[b]Multi-organohalogen residues determined, see Table 4.
[c]Multi-organohalogen residues determined presented previously; see reference 5.

The OV-101 and OV-101/OV-210 GC columns produced similar GC elution patterns that were insufficient for residue determination. Hydrocarbon co-extractives present in Florisil eluate 2 frequently prevented concentration to the low volumes required for GC-MS confirmation of identity. The following discussion describes techniques developed to eliminate these difficulties, as well as comments on general application of the method and results of an intralaboratory trial.

Chromatographic Cleanup and Residue Separation

The GPC column used in previously reported analyses (1) was purchased from ABC Laboratories (PO Box 1097, Columbia, MO 65201), already packed and calibrated. A second GPC column was prepared and calibrated as described in *Gel Permeation Chromatographic Column*, and used for analyses and method development reported here. The 2 columns displayed similar elution and separation characteristics for butterfat, vegetable oil by-products, and organohalogen pesticides. However, actual GPC eluant volumes required to elute high molecular weight materials and pesticides were different for the 2 columns. Figure 2 shows elution and separation characteristics for butterfat (0.5 μg), aldrin (0.805 μg),and *p,p'*-TDE (3.63 μg) obtained with the second GPC column. The portions correspond to consecutive 10 mL eluate volumes, except for portion 11, which was collected in 2 portions of 5 mL each to further characterize the best cut-off between fat and pesticide fractions. The % total fat and pesticides recovered (determined as described in *Gel Permeation Chromatographic Column)* and cumulative % of total amount recovered is plotted for each portion. Fraction 1, the first 105 mL volume of GPC eluate (portions 1–11a), contained about 90% of the fat and about 3% of the *p,p'*-TDE recovered. Fraction 2, the 106–185 mL volume (portions 11b–18) of the GPC eluate, contained about 10% of the fat, 100% of aldrin, and 97% of the *p,p'*-TDE recovered. In general, for all samples analyzed, more than 85% of the sample material was eluted in Fraction 1. This column has been used for 2 years and over 100 vegetable oil and associated by-product samples have been analyzed. No changes in column elution or separation characteristics have occurred in this time.

The Florisil technique described in *Supplemental Separation and Cleanup* provided additional separation of residues and hydrocarbon co-extractives and simplified residue determination for Florisil eluates 1 and 2. Florisil eluate 3, which contained no chromatographic responses, did not require additional cleanup or separation. Table 1 shows elution characteristics of various pesticide residues separated by this technique. Table 3 shows recoveries (90–103%) for 7 pesticide standards eluted through the supplemental Florisil columns. These data were obtained to augment previously reported recoveries (1) for the GPC and primary Florisil cleanup procedures.

The 3 recommended GC columns were selected because they gave different GC retention time patterns for residues in the 2 soybean deodorizer distillate samples. All 3 were

Table 3. Recoveries of 7 chlorinated pesticides from Florisil using eluants described under *Supplemental Separation and Cleanup*

Chemical	Added, μg	Rec., %
Aldrin	0.805	103
BHC, α-	0.500	90
DDT, *p,p'*-	2.45	101
Dieldrin	2.06	98
Heptachlor	0.730	91
Hexachlorobenzene	1.08	94
Lindane	0.555	98

necessary to support identifications and quantitations. Initial residue identifications were made with the OV-101 column, on which relative retention data are available for a large number of pesticide and industrial chemicals (3). This column was also suitable for use with the Hall 700A GC system used to support organohalogen residue identification. Documented retention time data exist for a smaller number of chemicals chromatographed with the more polar OV-225 column (3). This column did not resolve *p,p'*-TDE and *p,p'*-DDT (eluate 1B). The 1240 DA column appeared to be the most polar of the 3 columns. Unique properties of this column, which cause *p,p'*-TDE to elute later than *p,p'*-DDT, and α-BHC to elute later than aldrin, make it particularly useful for identification of these residues. It also provided the best separations of DDT analogs from sample co-extractives and other pesticides. Although relative retention time data are available for a small number of pesticide chemicals (8, 9), both relative and absolute retention times for certain pesticides are affected by the weight of column packing used in preparing the column (9). Heptachlor and aldrin (eluate 1A) were not resolved by this column.

Figures 3–6 show examples of chromatograms obtained from analysis of the 1976 and 1980 soybean deodorizer distillate samples by the method described here. The ^{63}Ni detector signals were attenuated to produce chromatograms with similar peak heights for easier comparison of eluate peak patterns. Chromatograms A (Figures 3–5) show responses obtained for 6 pesticides on each of the 3 recommended GC columns. Figure 3 (B, C, and D) shows OV-101 column chromatograms of eluates 1, 1A, and 1B of the 1976 soybean deodorizer distillate. Figures 4 (B, C, and D) and 5 (B, C, and D) show chromatograms for the same eluates for the OV-225 and SP 1240 DA columns, respectively. Conditions used with the OV-225 column are discussed below and are significantly different from those prescribed by the method. Figure 6 shows chromatograms of eluate 2B on the 3 columns.

Unidentified Materials

Several significant unidentified responses with greater than 10% FSD are present in the 1, 1A, and 1B eluate chromatograms of both soybean deodorizer distillate samples. These materials could not be identified by relative retention time data (3), but were believed to be halogenated because the same response patterns were obtained by a halogen-selective Hall 700A detector. These halogenated materials were present at about 0.5–2.0 ppm, based on comparison of the response to that for heptachlor epoxide.

Eluate 1 from both samples was analyzed by using a GC system equipped with a flame ionization detector to further characterize materials with GC retention times similar to those of pesticides. Figure 7B shows chromatograms obtained for eluate 1 of the 1976 sample. The electrometer signal was attenuated to give ½ FSD for about 5000 ng heptachlor epoxide for easier comparison of peak patterns. The 1980 sample chromatogram contained the same peaks. Responses were believed to be caused by nonhalogenated hydrocarbon co-extractives because they do not correspond to responses obtained for ^{63}Ni EC and Hall 700A detectors. These materials were estimated to be present at about 3% and 1% by weight (based on the response to heptachlor epoxide) for the 1976 and 1980 samples, respectively. The majority of them eluted earlier than aldrin on the OV-101 column. They eluted in eluate 1A of the supplemental Florisil elution system and were unaffected by treatment with alkali.

Several significant unidentified peaks were observed in eluates 2 and 2B of the 1980 sample ^{63}Ni EC chromatograms (Figure 6). The materials that produced these peaks were believed to be hydrocarbon co-extractives; they did not cause detector responses with the Hall 700A detector, but did cause responses with a flame ionization detector (Figure 7C). Eluates containing these materials could not be concentrated to the low volumes required for GC-MS confirmation. The 1976 sample was found to contain the same materials when the flame ionization detector was used. However, corresponding peaks were not observed in the ^{63}Ni EC chromatograms. Eluate 2 hydrocarbon co-extractives were estimated to be present at about 6 and 0.5% by weight (based on response to heptachlor epoxide) for the 1980 and 1976 samples, respectively. The hydrocarbon co-extractives generally eluted earlier than dieldrin on the OV-101 column, but interfered with resolution of dieldrin in the 1980 sample. Treatment of eluate 2B with alkali, as described in *Supplemental Separation and Cleanup*, completely eliminated these responses while leaving residues (dieldrin, endrin, and *p,p'*-dichlorobenzophenone) unaffected. Heptachlor epoxide, present in eluate 2A, was frequently degraded by this treatment.

Residue Determination

Table 4 lists residue determinations for 3 portions each of the 1976 and 1980 soybean deodorizer distillate samples analyzed by the described method. All quantitations reported in Table 4 were made by the supplemental Florisil elution procedure. Dieldrin and endrin (eluate 2B) were quantitated before the alkali saponification. Each portion was chromatographed on 1 of the 3 columns recommended for residue determination with a ^{63}Ni EC detector. Of 13 residues found in the 1976 soybean deodorizer distillate sample, 9 were also found in the 1980 soybean deodorizer distillate. Although residue levels determined by the 3 columns were generally equivalent, several inconsistent results were obtained. Values underlined in Table 4 were used to determine *accepted* levels because they corresponded to GC columns that provided acceptable chromatography and separation from interferences. The *accepted* level was the average if more than one determination was underlined in Table 4. OV-101 column determinations were selected as the *accepted* level for *cis*- and *trans*-chlordane because this column provided the best GC resolution for them (compare Figures 3D, 4D, and 5D). In many cases, elevated residue levels obtained on one or more columns suggested the presence of interferences, and therefore were not used to determine the *accepted* level of the corresponding residue. Alkali saponification of eluate 2B solutions removed interferences that elevated endrin (1976) and dieldrin (1980) levels. After this treatment, 2.4 ppm dieldrin (1980) and 0.28 ppm endrin (1976) were determined by the OV-101 and 1240 DA columns, respectively. These values compare favorably with those obtained by the other 2 columns before saponification. Pesticide *accepted* levels ranged from about 0.1 ppm for *p,p'*-DDT to 6.2 ppm for dieldrin in the 1976 sample, and from about 0.08 ppm for *p,p'*-DDE to 2.3 ppm for dieldrin in the 1980 sample.

Based on analysis of these 2 soybean deodorizer distillate samples, the limit of detection (5% FSD) with EC-GC was estimated to be 0.04 ppm heptachlor epoxide when 0.5 mg equivalent sample was injected under EC-GC conditions where 0.2 ng heptachlor epoxide caused ½ FSD. In most cases, residues determined by EC-GC at ≤0.3 ppm could not be confirmed by GC-MS.

The Hewlett-Packard ^{63}Ni EC detection system used in this work had a usable response range from about 10 to 10,000 p grams. This detector capability allowed 0.5 mg equivalent sample to be injected to achieve a 0.04 ppm detection limit

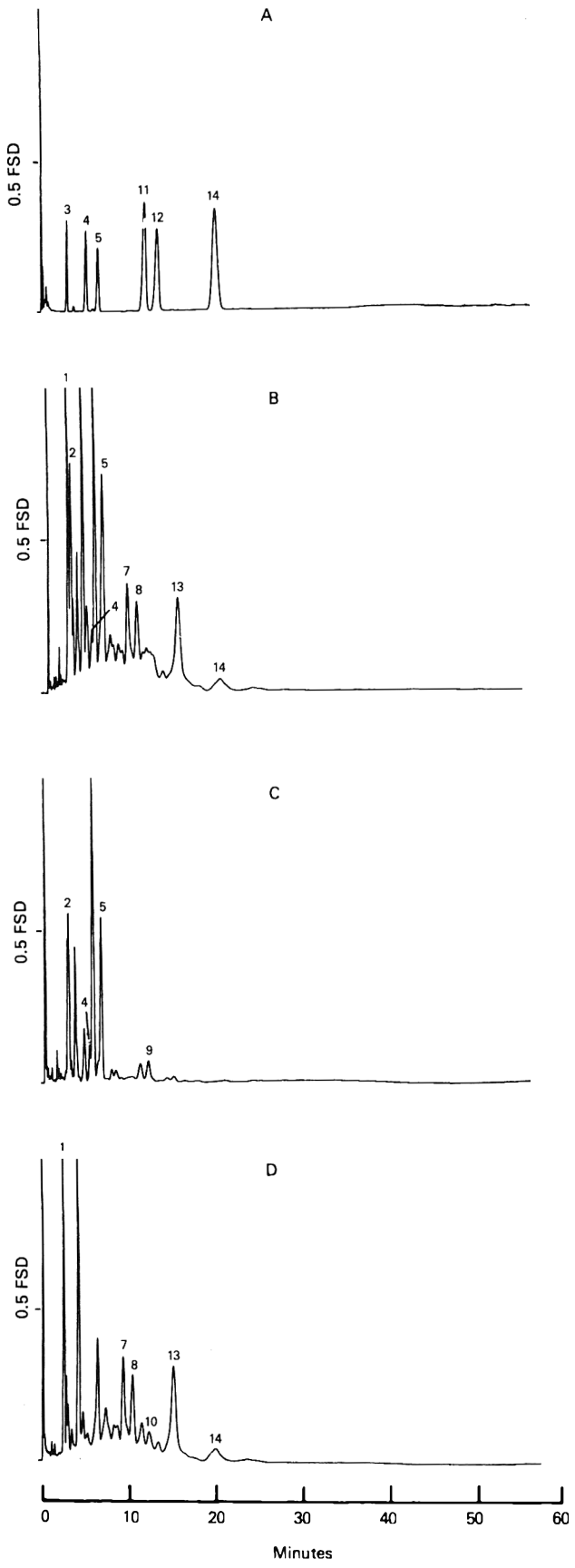

Figure 3. OV-101/electron capture (^{63}Ni) chromatograms of 1976 soybean deodorizer distillate Florisil eluates (0.5 mg) and pesticide standard containing 0.11 ng lindane, 0.15 ng heptachlor, 0.16 ng aldrin, 0.50 ng dieldrin, 0.38 ng endrin, and 1.00 ng p,p'-DDT; sensitivity, 0.5 ng heptachlor epoxide causes 50% FSD; GC column and parameters in Apparatus.

1, α-BHC; 2, hexachlorobenzene; 3, lindane; 4, heptachlor; 5, aldrin; 6, heptachlor epoxide; 7, *trans*-chlordane; 8, *cis*-chlordane; 9, *p,p'*-DDE; 10, *o,p'*-TDE; 11, dieldrin; 12, endrin; 13, *p,p'*-TDE; 14, *p,p'*-DDT. A, pesticide standard. B, Florisil eluate 1. C, Florisil eluate 1A. D, Florisil eluate 1B.

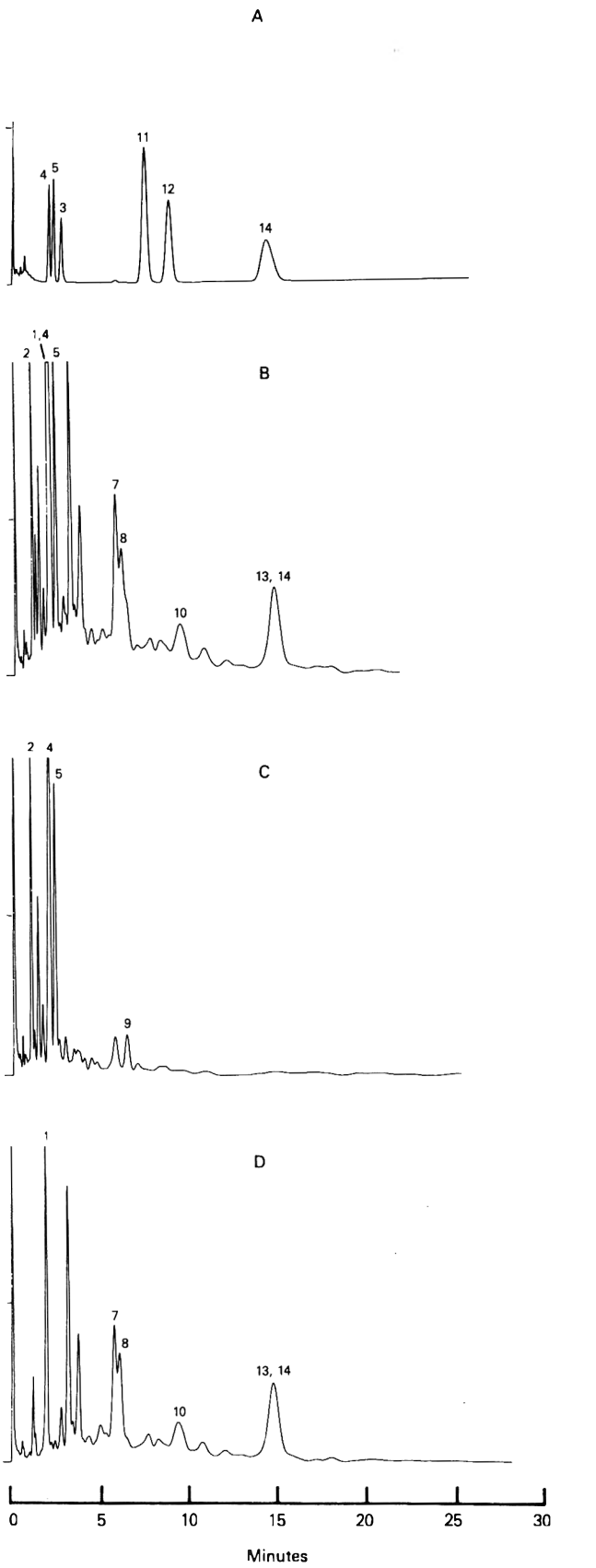

Figure 4. OV-225/electron capture (^{63}Ni) chromatograms of 1976 soybean deodorizer distillate Florisil eluates (0.15 mg) and pesticide standard containing 0.029 ng heptachlor, 0.032 ng aldrin, 0.022 ng lindane, 0.092 ng dieldrin, 0.076 ng endrin, and 0.32 ng p,p'-DDT; sensitivity, 0.1 ng heptachlor epoxide causes 50% FSD; GC column and parameters in Results and Discussion.

See Figure 3 for peak identifications. A, pesticide standard. B, Florisil eluate 1. C, Florisil eluate 1A. D, Florisil eluate 1B.

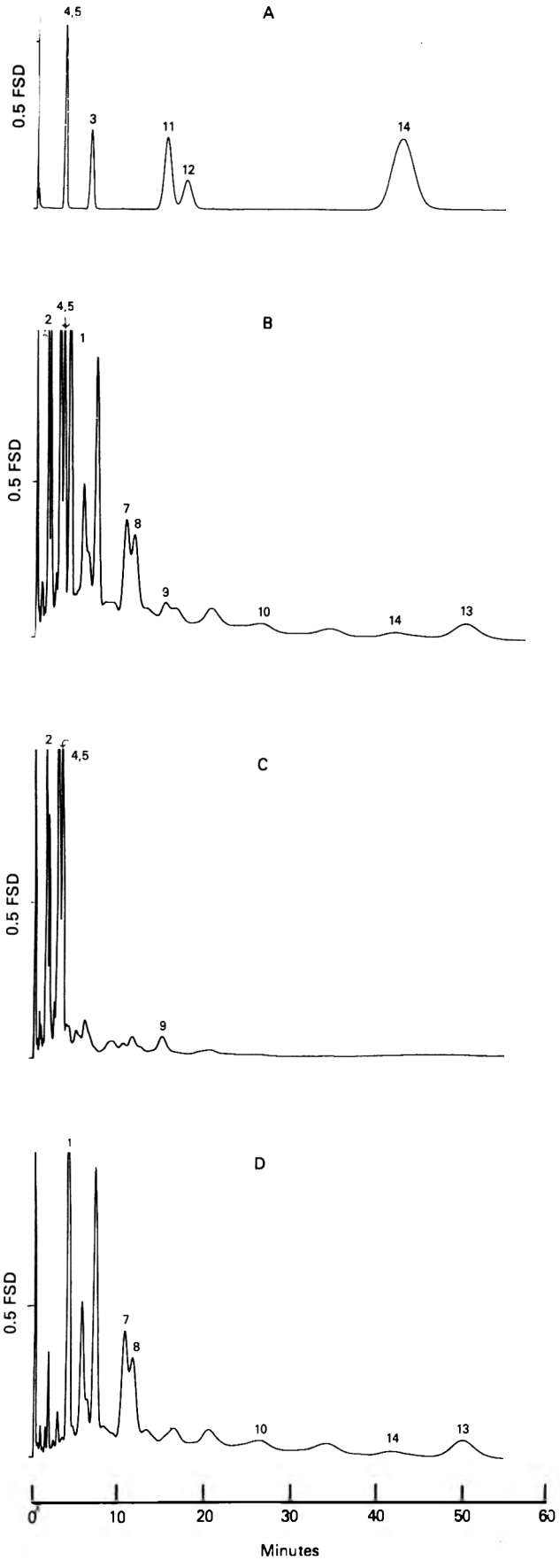

Figure 5. SP-1240 DA/electron capture (^{63}Ni) chromatograms of 1976 soybean deodorizer distillate Florisil eluates (0.5 mg) and pesticide standard containing 0.11 ng lindane, 0.15 ng heptachlor, 0.16 ng aldrin, 0.50 ng dieldrin, 0.38 ng endrin, and 2.00 ng p,p'-DDT; sensitivity, 0.5 ng heptachlor epoxide causes 50% FSD; GC column and parameters in Apparatus.

See Figure 3 for peak identifications. A, pesticide standard. B, Florisil eluate 1. C, Florisil eluate 1A. D, Florisil eluate 1B.

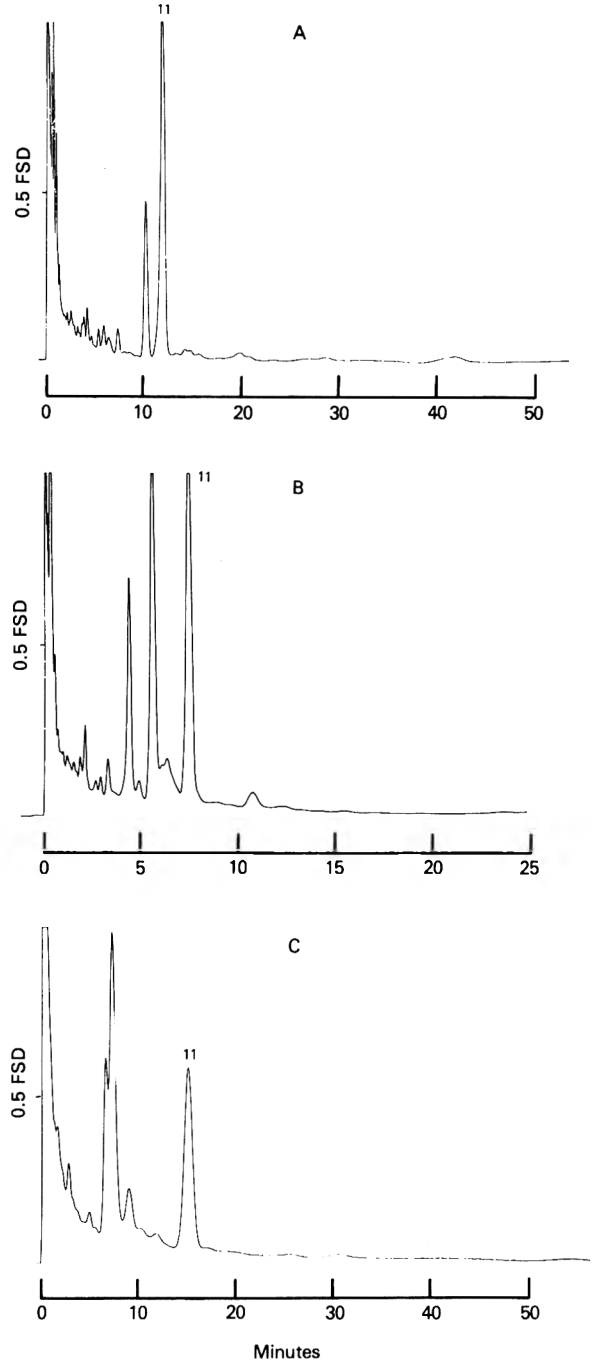

Figure 6. Electron capture (^{63}Ni) chromatograms of 1980 soybean deodorizer distillate Florisil eluate 2B (0.5 mg) obtained as follows:

A, OV-101 GC column; see Figure 3 for GC parameters and peak identifications. B, OV-225 GC column; see Figures 4 and 3 for GC parameters and peak identifications. C, SP-1240 DA GC column; see Figures 5 and 3 for GC parameters and peak identifications.

(heptachlor epoxide), thus eliminating column deterioration effects that had frequently occurred with larger (3 mg) sample weight equivalents. Correspondingly, the detector signal was adjusted to give ½ FSD for 0.2 rather than 1 ng heptachlor epoxide as specified in *AOAC* (2) and *PAM* methods (3).

Results (Table 4) and chromatograms (Figure 5) shown for the OV-225 GC column were obtained with a Varian 3700 gas chromatcgraph ^{63}Ni EC detection system. High purity (99.999%) nitrogen carrier gas maintained at a flow rate of 40 mL/min was used to meet the manufacturer's specifications and to obtain satisfactory chromatography. The carrier gas selection switch was placed in the "N_2 high" rather than the recommended "N_2 standard" position to obtain the optimum

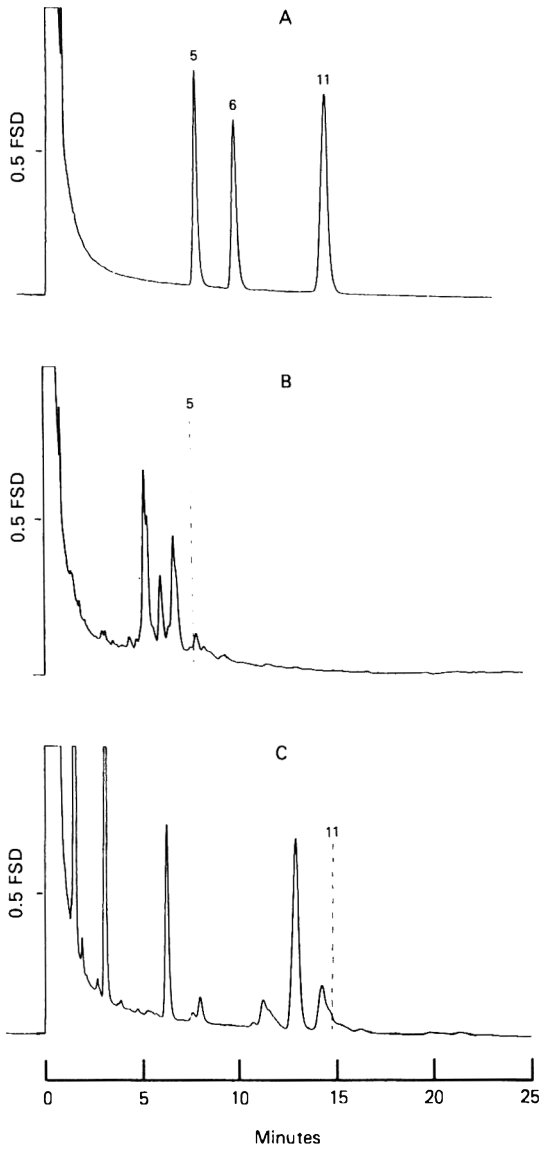

Figure 7. Flame ionization/OV-101 chromatograms of soybean deodorizer distillate Florisil eluates (0.5 mg) and pesticide standard containing 5.0 μg each of aldrin, heptachlor epoxide, and dieldrin; sensitivity, 5 μg heptachlor epoxide causes 60% FSD; GC parameters in Apparatus.

See Figure 3 for peak identifications. A, pesticide standard. B, 1976 sample Florisil eluate 1. C, 1980 sample Florisil eluate 2.

usable response range (10–1000 pg). The glass column used with the Varian system measured about 4.5 rather than 6 ft as stated. As a result, materials eluted from the column earlier than would be expected. The equivalent weight of sample injected averaged 0.15 mg, but was adjusted according to residue level so that no more than 500 pg of any pesticide would be injected for determination. The detector signal was adjusted to give ½ FSD for 50 pg heptachlor epoxide to compensate for the smaller sample equivalent weight injected. Figure 4 chromatograms were obtained after the detector signal was attenuated to give ½ FSD for 100 pg heptachlor epoxide for easier comparison of peak patterns as explained above.

Soybean deodorizer distillate samples were analyzed by the Hall 700A detector system to help support determinations. The limit of detection for this detector was estimated to be 0.2 ppm, based on the response to heptachlor epoxide. All ^{63}Ni EC detector responses observed for injections of Florisil eluates 1, 1A, and 1B were also observed for the Hall 700A detector. Several ^{63}Ni EC responses attributed to nonhalogenated hydrocarbon materials were not observed when the Hall 700A detector system was used to analyze Florisil eluates 2 and 2B from deodorizer distillate samples.

Residue Identity Confirmation

Because of the large number of residues present, as well as the presence of both halogenated and nonhalogenated unidentified materials in the soybean deodorizer distillate samples, confirmation of residues tentatively identified by EC-GC techniques was needed. GS-MS provided a single method of confirmation for all residues identified and present at ≥0.3 ppm.

GC-MS analysis of supplemental Florisil eluates (Table 4) confirmed the identities of 9 of 13 residues found in the 1976 soybean deodorizer distillate sample: dieldrin, heptachlor epoxide, aldrin, α-BHC, hexachlorobenzene, *cis*- and *trans*-chlordane, and *p,p'*-TDE. GC-MS techniques identified *o,p'*-TDE prior to quantitation by ^{63}Ni EC detection. GC-MS confirmations could not be obtained for endrin, *p,p'*-DDT, *p,p'*-DDE, or heptachlor, all of which were quantitated at ≤0.3 ppm. GC-MS identification of *p,p'*-DDT (<0.3 ppm) was not attempted. GC-MS analysis confirmed identities of 5 of 8 residues found in the 1980 sample: dieldrin, heptachlor epoxide, α-BHC, and *cis*- and *trans*-chlordane. Confirmations could not be obtained for aldrin, HCB, or *p,p'*-DDE, which were present at less than 0.3 ppm. Alkali saponification of all eluate 2B solutions was required before GC-MS confirmation. No attempts were made to quantitate any of the above residues by GC-MS.

Repetitive 2 s scans from 65 to 465 daltons obtained in the EI mode were suitable for confirmation of most pesticide residues, including aldrin. Aldrin was difficult to confirm because of the presence of large amounts of co-extractives in this portion of the chromatogram. Negative ion chemical ionization (NCI) yielded an abundant ion of 235 daltons for aldrin corresponding to $(C_5Cl_5)^-$. This information was used to supplement EI data obtained for this residue.

The identities of HCB, α-BHC, and dichlorobenzophenone, present at low levels, were best confirmed by CI. The NCI spectrum of HCB at a source temperature of 140°C consisted primarily of the 6 chlorine molecular ion isotope cluster. The NCI spectrum of α-BHC under these conditions did not contain a molecular ion. Principal ions in the NCI spectrum of α-BHC were chlorine cluster ions of 35 and 70 daltons and a fragment ion of 253 daltons corresponding to a loss of 1 chlorine from the molecular ion. Dichlorobenzophenone produced complementary positive ion and negative ion CI spectra. The chlorobenzoyl ion $(139)^+$ and the protonated molecular ion $(251)^+$ comprised the positive ion spectrum; the molecular ion $(250)^-$ was found in the negative spectrum. Operation of the ion source in the CI mode at a source temperature of 140°C increased the rate of source contamination, but it also improved the relative abundance of the molecular ion for many of these compounds, compared to source temperatures over 250°C.

Intralaboratory Trial

A 2-part intralaboratory trial was conducted to obtain additional pesticide recovery data, and to test the usefulness of this multi-column, multi-detector determinative procedure. Analyses for this study were performed by a chemist previously unfamiliar with the method. Table 5 shows pesticide recoveries for the peanut oil deodorizer distillate fortified with 4 pesticides. The alkali saponification and supplemental Florisil steps were not required. Determinations were made with a ^{63}Ni EC detector in conjunction with the 3 recommended columns, as well as a Hall 700A detector with an

Table 4. Organohalogen residues found in 2 soybean deodorizer distillate samples, quantitated by ^{63}Ni electron capture GC and 3 GC columns, and confirmed by capillary column GC-MS, instrument parameters, and GC columns described in *Method*

Residue	EC-GC determinations, ppm: EC-GC column[a,b] 1240 DA	OV-101	OV-225	Accepted level[b,c]	GC-MS[b] confirm.
1976 Sample					
Aldrin	1.0	0.92	0.82	0.87	yes
BHC, α-	1.2	1.1	1.2	1.2	yes
Chlordane, *cis*-	0.50	0.51	0.30	0.51	yes
Chlordane, *trans*-	0.50	0.59	0.67	0.59	yes
DDE, *p,p'*-	0.20	0.30	0.20	0.20	no
DDT, *p,p'*-	0.10	0.40	ND	0.10	ND
Dieldrin	6.2	6.2	6.5	6.3	yes
Endrin	0.20	0.36	0.60	0.28	no
Heptachlor	ND	0.10	1.5	0.10	no
Heptachlor epoxide	1.3	1.1	1.2	1.2	yes
Hexachlorobenzene	0.80	0.50	0.82	0.82	yes
TDE, *o,p'*-	0.30	0.50	0.70	0.30	yes
TDE, *p,p'*-	0.95	1.3	1.6	0.95	yes
1980 Sample					
Aldrin	0.25	0.10	0.10	0.10	no
BHC, α-	2.2	1.6	1.6	1.6	yes
Chlordane, *cis*-	0.30	0.30	0.30	0.30	yes
Chlordane, *trans*-	0.25	0.38	0.33	0.38	yes
DDE, *p,p'*-	0.050	0.090	0.10	0.05	no
DDT, *p,p'*-	NF	0.3	ND	NF	no
Dieldrin	2.2	3.3	2.5	2.4	yes
Endrin	NF	NF	NF	NF	ND
Heptachlor	ND	0.10	ND	0.10	ND
Heptachlor epoxide	1.0	1.1	1.1	1.10	yes
Hexachlorobenzene	0.20	0.30	0.30	0.30	no
TDE, *o,p'*-	NF	NF	NF	NF	ND
TDE, *p,p'*-	NF	NF	NF	NF	ND

[a]Underlined values were used for determining accepted level.
[b]NF, not found. ND, not determined.
[c]See *Results and Discussion* for explanation of accepted level.

Table 5. Intralaboratory study recoveries of pesticides from peanut oil deodorizer distillate sample with ^{63}Ni detector with 3 GC columns and Hall 700A detector with OV-101 column

Chemical	Added, ppm	Mean, ppm	Rec., %	CV (*N*)
BHC, α-[a]	1.04	1.13	108	1.4 (3)
Dieldrin	1.62	1.66	102	5.6 (4)
Heptachlor[a]	0.775	0.839	108	5.0 (3)
Heptachlor epoxide[b]	0.766	0.900	116	7.0 (3)

[a]Not determined with ^{63}Ni detector/OV-225 column GC system. α-BHC and heptachlor co-elute from this column.
[b]Not quantitated with ^{63}Ni detector/OV-225 column GC system. Retention time of peak in sample chromatogram was 10% greater than retention time of heptachlor epoxide standard injected.

Table 6. Intralaboratory study analysis of 1976 soybean deodorizer distillate with ^{63}Ni detection

Residue	Amt detd, ppm: Column OV-225	1240 DA	Mean[a]
Aldrin	0.92	0.80	0.86
BHC, α-	0.80	0.93	0.87
Chlordane, *cis*-	0.45	0.53	0.47[b]
Chlordane, *trans*-	0.64	0.73	0.64[b]
DDE, *p,p'*-	0.20	0.22	0.21
Dieldrin	ND[c]	5.7	5.7
Heptachlor epoxide	0.84	1.1	0.97
Hexachlorobenzene	0.91	0.75	0.83

[a]Average of GC determinations from a single analysis.
[b]Average of determinations on 3 GC columns. OV-101 determinations for *cis*- and *trans*-chlordane, 0.43 and 0.56 ppm, respectively, are included.
[c]ND, residue not determined.

OV-101 column. Average recoveries ranged from 102% for dieldrin to 116% for heptachlor expoxide. α-BHC and heptachlor could not be determined by the OV-225 column because they co-elute from it; however, they may be separated before GC determination by using the supplemental Florisil elution technique.

Table 6 shows the intralaboratory trial results for the analysis of the 1976 soybean deodorizer distillate. The analyst was requested to report residues present at levels ≥0.3 ppm. Alkali saponification and GC-MS steps were not used. The supplemental Florisil step, as well as the 3 recommended GC columns in conjunction with a ^{63}Ni detector, were used. The OV-101 column gave severe negative excursions for sample eluates and most residues could not be quantitated with this column. At first, malfunction of the gas chromatograph appeared to be the cause of the negative excursions. However, it later became apparent that when deodorizer distillate eluates were left at room temperature for several days, baseline and background GC responses were significantly elevated. Therefore, it is recommended that before EC analysis, all sample solutions be stored in the dark, and refrigerated when possible. One residue (*p,p'*-TDE) present at >0.3 ppm according to original analyses (Table 4) was not reported. Dieldrin (eluate 2B) was determined by using only the OV-225 GC column. In general, results reported in this study agree closely with those reported in Table 4 for this sample.

Conclusion

The method described provides satisfactory sample cleanup and residue separation for determination of nonpolar organohalogen pesticide residues in vegetable oils and associated refinery by-products. Analysis by this method of 2 soybean, 1 peanut, and 1 cottonseed oil deodorizer distillate samples, as well as other soybean and peanut oil refinery by-products and associated crude and completely refined oils, demon-

strated the presence of 14 organohalogen residues. More than 85% of sample material is separated from pesticide residues, and recoveries are more than 85% for organohalogen pesticides tested through the various steps of the method. Additional Florisil separation and alkali treatment techniques are described for separation of multiple organohalogen residues and hydrocarbon interferences that may be present in deodorizer distillate samples. Residues are determined by electron capture gas chromatography in conjunction with 3 GC columns of different polarities. The limit of detection for the method is 0.04 ppm based on the response of heptachlor epoxide. The identity of residues determined at ≥0.3 ppm can be confirmed by capillary column GC-MS using both EI and CI detection modes. Currently, only limited data exist on the incidence and levels of pesticide residues in vegetable oil refinery by-products. Data presented here indicate that certain by-products from the vegetable oil refining process may contain significant levels of organohalogen residues. It is hoped that this method will provide interested analysts with a means of obtaining additional data for this type of sample.

Acknowledgments

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Capillary Gas Chromatographic Determination of Naphthalene in Bran and Wheat Germ Products

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A method is described for determining naphthalene in bran and wheat germ products. The method involves distilling the naphthalene from a water–cereal mixture and then extracting the distillate with hexane. A 10 μL aliquot of the hexane extract is injected into a gas chromatograph equipped with a capillary column. Dodecan-1-ol is used as the internal standard. Mean recoveries, standard deviations (SD), and coefficients of variation (CV) for samples of unprocessed bran, unprocessed wheat germ, bran flakes breakfast cereal, and sultana bran breakfast cereal, spiked with naphthalene at various levels, were 1.0 ppm spike, mean 0.98, SD 0.09, CV 9.5%; 2.5 ppm spike, mean 2.37, SD 0.22, CV 9.4%; 5.0 ppm spike, mean 4.86, SD 0.19, CV 4.0%; 10 ppm spike, mean 9.25, SD 0.38, CV 4.1%. The mean recoveries exceeded 90% in each case. The detection limit for the method was about 0.5 ppm.

Our laboratory has received numerous food samples over the years from consumers complaining of unusual odors in their foods. One such recent complaint was associated with a bran sample which had a "moth ball" odor. The bran sample was found to contain naphthalene.

Naphthalene, a by-product of the coke-oven industry, is not usually found in food products; therefore, few methods are reported in the literature for its estimation in food material (1–3). Adachi (1) determined polymethylbiphenyl contaminants as well as naphthalene in several foods by gas chromatography–mass spectroscopy using a packed column. The extraction and cleanup procedures for isolation of the contaminants were time consuming. Polishchuk (2) determined naphthalene in crop samples by ultraviolet spectroscopy after naphthalene was isolated using a stream of hot air, while Neff and Anderson (3) determined naphthalene in oil-contaminated marine animals by ultraviolet spectroscopy after hexane extraction and Florisil cleanup procedures.

This paper describes a capillary gas chromatographic method for the determination of naphthalene in bran and wheat germ products. The method uses an initial distillation to isolate the naphthalene from the water–cereal mixture followed by extraction with hexane. The extract is then injected into a gas chromatograph equipped with a capillary column. The main advantage of capillary gas chromatography is the efficient separation of components of complex mixtures and the small probability of interference by these components on the quantitation of the analyte. The sensitivity of capillary gas chromatography is also excellent.

METHOD

Reagents

(a) *Hexane.*—Nanograde quality (Waters Associates).

(b) *Dodecan-1-ol internal standard solution.*—1000 μg dodecan-1-ol (Hopkin and Williams, Chadwell Heath, Essex, England)/mL hexane.

(c) *Naphthalene standard solutions.—(1) Stock solution.*—1000 μg naphthalene (Hopkin and Williams)/mL hexane. *(2) Standard solutions.*—Pipet 1.0, 2.0, 4.0, and 10.0 mL stock solution into separate 200 mL volumetric flasks, add 20 mL internal standard solution to each, and dilute to volume with hexane. Solutions contain 5, 10, 20, and 50

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μg naphthalene/mL, respectively, and 100 μg dodecan-1-ol internal standard/mL.

Apparatus

(a) *Gas chromatograph.*—Hewlett-Packard Model 5730A, with Model 18740B capillary column control, flame ionization detector (FID), and FID capillary jet. Injector port was modified for capillary column use and was lined with a split insert. Gas chromatograph was equipped with 50 m × 0.20–0.: 1 mm id flexible fused silica capillary column coated with si icone gum SE54 (Hewlett-Packard part No. 19091–60750). C olumn was used with the following operating parameters: c olumn pressure, 2.0 kg N/sq. cm split flow, 43 mL/min; septun purge flow, 2 mL/min; make-up gas flow, 20 mL N/min; ; ir and hydrogen flows for flame, 200 mL/min and 40 m _/min, respectively; column temperature, 150°C; injector te nperature, 300°C, and detector temperature, 350°C. Integra tion of peaks and processing of chromatograms was carried out by a Hewlett-Packard 3354 Laboratory Automation System.

(b) *Distillation apparatus.*—1 L round-bottom Quickfit flask, splash head SH7/12, jacketed coil condenser C6/12; and straight delivery adapter RA1/22. Straight delivery adapter penetrates 100 mL receiving volumetric flask to below neck, and the volumetric flask is partly immersed in beaker containing water and ice.

Determination

Weigh 20 g sample into 1 L round-bottom flask, add 500 mL water, several drops of silicone antifoam (Antifoam AF emulsion, Dow Corning), and a few boiling chips. Distill ca 80 mL into 100 mL receiving volumetric flask. Extract distillate in volumetric flask with 4 mL solution of 100 μg dodecan-1-ol/mL hexane by shaking vigorously 1 min. Transfer 2–3 mL hexane layer to 10 mL glass-stopper test tube, add a small quantity of anhydrous sodium sulfate to dry hexane, and inject 10 μL into gas chromatograph.

Calibration Curve and Calculation

Prepare calibration curve from the 4 naphthalene standard solutions. Plot ratio of naphthalene peak area to internal standard peak area against μg naphthalene/mL; use mean values of ≥3 injections of each standard. Obtain ratio of naphthalene peak area to internal standard peak area from sample chromatogram and read concentration of naphthalene in sample from calibration curve. Calculate naphthalene content of sample as follows:

$$\text{ppm Naphthalene} = \mu\text{g naphthalene/mL} \times 4\ \text{mL/wt sample (g)}$$

Results and Discussion

Ten samples were tested for naphthalene by the described procedure. These included 5 unprocessed brans, 2 unprocessed wheat germs, one wheat bran breakfast cereal, one bran flakes breakfast cereal, and one sultana bran breakfast cereal. These samples, which were all purchased from retail outlets, were found not to contain naphthalene (<0.5 ppm).

A sample of unprocessed bran which was the subject of a consumer complaint was found to contain 20 ppm naphthalene. It is likely that this complaint sample had at some time been stored near naphthalene "moth balls." The concentration of 20 ppm found in this sample is consistent with levels that can be detected by smell (4). Further confirmation that naphthalene was present in this sample was obtained by ultraviolet spectroscopy of the hexane extract of the distillate from this sample.

The recovery of naphthalene was determined after spiking 20 g samples with 1.0 or 2.0 mL solutions containing 10, 50, and 100 ppm naphthalene in hexane; this gives levels of 1.0, 2.5, 5.0, and 10 ppm naphthalene in the samples. After allowing the spiked samples to stand for 1–2 h to evaporate most of the hexane, the spiked samples were analyzed for naphthalene in the usual way. Mean recoveries, standard deviations (SD), and coefficients of variation (CV) for samples of unprocessed bran, unprocessed wheat germ, bran flakes breakfast cereal, and sultana bran breakfast cereal, spiked with naphthalene at various levels (5 spikes at each level), were 1.0 ppm spike, mean 0.98, SD 0.09, CV 9.5%; 2.5 ppm spike, mean 2.37, SD 0.22, CV 9.4%; 5.0 ppm spike, mean 4.86, SD 0.19, CV 4.0%; 10 ppm spike, mean 9.25, SD 0.38, CV 4.1%. The mean recoveries exceeded 90% in each case. Actual recovery data for samples are given in Table 1. Further confirmation that naphthalene was quantitatively recovered from the naturally contaminated complaint sample by the described distillation procedure was obtained by checking the naphthalene content of a second 80 mL distillate immediately following the first 80 mL distillate. No naphthalene was detected in the second distillate.

The calibration curve for naphthalene was linear from at least 5.0 to 50 ppm. Typical peak area ratios of naphthalene to internal standard were 0.065, 0.13, 0.26, and 0.65 for 5, 10, 20, and 50 ppm naphthalene standards which also contained 100 ppm internal standard. It was necessary to use an internal standard for quantitation because repeat injections of the same solution gave poor reproducibility of peak areas. The use of the internal standard overcame this problem because only ratios of peak areas are used in the quantitation, and not absolute values. The coefficient of variation for injection of the 10, 20, and 50 ppm naphthalene standards and spiked samples was between 2.0 and 3.5%. The detection limit for naphthalene was about 0.5 ppm for 20 g samples, representing an actual concentration of 2.5 ppm naphthalene in the analyte solutions. This detection limit corresponded to a peak height of 2 mm, at an attenuation 8 times less sensitive than the maximum. Although a measurable peak was obtained for a 1 ppm naphthalene standard (this corresponds to 0.2 ppm in a sample), it was found that 4 samples gave a small peak with a retention time identical to naphthalene. This peak was shown not to be naphthalene because the same peak was still present when a second 80 mL distillate was extracted and chromatographed subsequent to analysis of the first 80 mL distillate (naphthalene is quantitatively recovered in the first distillate). This observation indicated that a component of the sample was being continually distilled over. The size of the interfering peak (when present) represented a naphthalene content of about 0.2 ppm. The peak could not be attributed to any of the reagents, because reagent blank determinations gave no

Table 1. Recovery of naphthalene added to samples

Sample	Naphthalene, ppm		Av. rec., %
	Added	Recd	
Unprocessed bran	1.0, 2.5, 5.0, 10.0	0.95, 2.15, 5.11, 9.23	94
Unprocessed bran	1.0, 2.5, 5.0, 10.0	0.84, 2.2, 4.77, 9.30	90
Unprocessed wheat germ	1.0, 2.5, 5.0, 10.0	0.98, 2.29, 5.0, 8.72	94
Bran flakes breakfast cereal	1.0, 2.5, 5.0, 10.0	1.07, 2.66, 4.82, 9.20	100
Sultana bran breakfast cereal	1.0, 2.5, 5.0, 10.0	1.06, 2.55, 4.62, 9.80	99

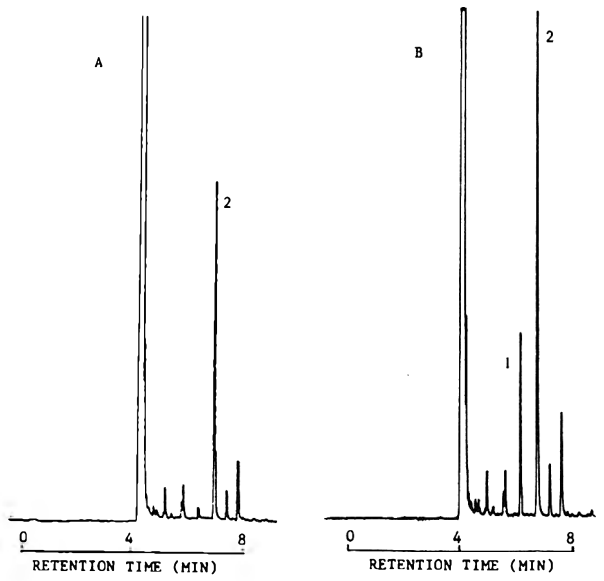

Figure 1. Gas chromatograms of uncontaminated bran sample (A) and bran sample spiked with naphthalene at 5 ppm (B). Naphthalene shown as peak (1) has a retention time of 6.68 min and internal standard, dodecan-1-ol, shown as peak (2) has retention time of 7.25 min.

peaks at or near the naphthalene or internal standard retention times.

The method was rapid, simple, and gave good recoveries. The distillation and extraction required only about ½ h and the gas chromatography required about 10 min per injection. The retention times for naphthalene and the internal standard were 6.68 min and 7.25 min, respectively. Typical chromatograms for a sample and a sample spiked at the 5 ppm level are given in Figure 1.

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Automated Sample Cleanup for Pesticide Multiresidue Determination. I. Evaluation of Solvent Partitioning Module

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An automated continuous flow procedure is described that improves the cost effectiveness and precision of AOAC methodology for multiresidue pesticide determinations in nonfatty foods. Individual modules capable of performing automated solvent partitioning and automated column chromatography were constructed and integrated into a continuous flow system. Data are presented comparing the recoveries and precision for the determination of 8 pesticides (aldrin, dieldrin, *p,p'*-DDT, ethion, heptachlor epoxide, lindane, parathion, and ronnel) partitioned from 2 food crops (spinach and tomatoes) by both the manual and automated procedures.

Monitoring the food supply for pesticide and industrial chemical residues is a major responsibility of state and federal regulatory agencies. Thousands of samples are analyzed each year to detect and remove from the marketplace products containing illegal and potentially harmful residues. Conventional analytical methodology makes this task extremely costly, in terms of both time and money.

In 1978, the Food and Drug Administration (FDA) established a task force to determine the feasibility of automating procedures for determining pesticide residues. The results of that study (1) indicated that automation of the extraction and cleanup steps of the current AOAC multiresidue method for nonfatty foods (2) would be most cost effective. In this report, we describe a portion of the continuous flow system that we have designed. This system contains 2 major components, a solvent partitioning module (SPM) and a column chromatography module. Experimental results obtained from the evaluation of the SPM with 8 pesticides in 2 food crops are presented.

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METHOD

Apparatus

(a) *Solvent partitioning module.*—Most of the components of the system were obtained from Technicon Industrial Systems (Tarrytown, NY). Some pieces of equipment were modified, while others were designed and assembled specifically for this application.

Sampler.—The mechanical arm of a large industrial sampler (Technicon No. 001-A002-01) was realigned to accept 50 mL sample tubes (25 × 150 mm), and the 10 min timers were replaced with 30 min Cycl-Flex timers (Eagle Signal Industrial Controls, Davenport, IA).

Proportioning pump.—A Technicon Pump III was fitted with a special air bar cam (Technicon No. 133-0045-01) that generates air segmentation at 1 s intervals.

Manifold.—The manifold configuration is shown in Figure 1. All pump tubing is Technicon Acidflex with the exception of the air and saline lines, which are Tygon, and the sample line, which is silicone. All transmission lines are Teflon. The mixing coils are made of 2 mm id chlorotrifluoroethylene tubing (Teleflex Fluoroplastics, Randolph, NJ); the first, third, and fourth coils have 17 turns each and an equivalent linear length of 42 in. (107 cm). The second mixing coil is a 68-turn

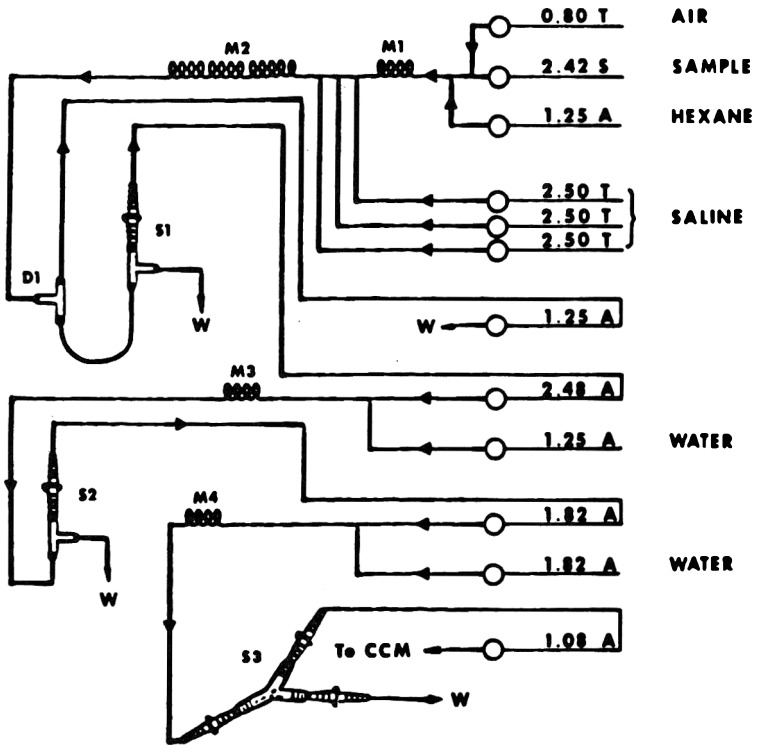

Figure 1. Flow diagram of manifold for solvent partitioning module. Numerical values refer to Technicon pump tube sizes in mL/min; symbols: T = Tygon, S = silicone, A = Acidflex, W = waste, CCM = column chromatography module.

coil fabricated from 288 in. (7.3 m) chlorotrifluoroethylene tubing. The polypropylene T-tube in front of the first phase separator is fitted with a Teflon insert (Technicon No. 021-0002-01). The first 2 phase separators (S1 and S2) are each fabricated from a polypropylene T-connector (3/16 in. od) joined to a polypropylene stepped connector (3/16 in. × 1/4 in. od). The tip of a glass Pasteur pipet is inserted through a nipple at the bottom leg of the T-connector so that the flared end of the glass tube is near the junction of the 2 connectors (Figure 2). The third phase separator (S3) is fabricated from one polypropylene Y-connector (5/16 in. od) and 3 polypropylene stepped connectors (3/16 in. × 1/4 in. od). All polypropylene connectors were obtained from Bel-Art Products, Pequannock, NJ.

(b) *Gas chromatograph.*—Tracor Model 550 equipped with constant current ^{63}Ni electron capture detector and 6 ft × 2 mm id glass column packed with 5% OV-101 on 80–100 mesh Chromosorb WHP. Nitrogen carrier gas had 30 mL/min flow rate. Column temperature (ca 200°C) was adjusted to permit elution of *p,p'*-DDT at 3.13 relative to chlorpyrifos and detector sensitivity was adjusted to provide ½ full-scale deflection for 0.3 ng chlorpyrifos.

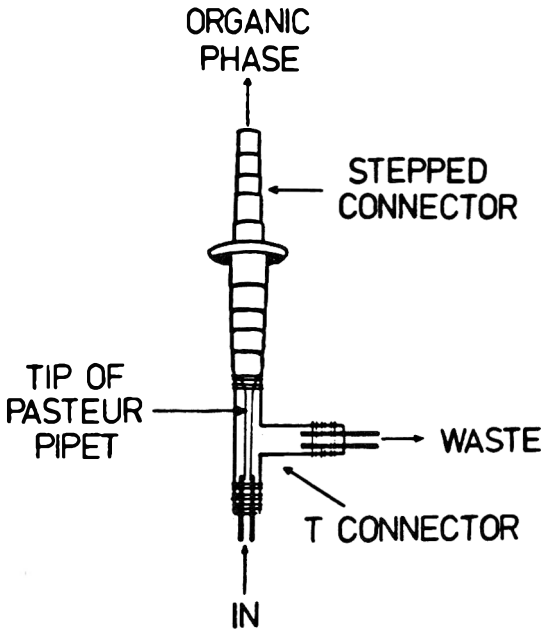

Figure 2. Detailed view of phase separator (S1 and S2 in Figure 1).

Reagents and Materials

(a) *Saline solution.*—Dilute 50 mL saturated NaCl solution to 3 L with water.

(b) *Solvents.*—Acetonitrile, hexane (Burdick & Jackson Laboratories, Muskegon, MI), distilled in glass, or equivalent.

(c) *Analytical pesticide standards.*—Aldrin, dieldrin, *p,p'*-DDT, ethion, heptachlor epoxide, lindane, parathion, and ronnel, obtained from Pesticides Analytical Standards Repository, Environmental Protection Agency, Research Triangle Park, NC.

(d) *Standard solutions.*—Stock solutions were prepared in hexane or acetone diluted with hexane. Injection solutions were diluted with hexane.

(e) *Spiking solutions.*—Individual solutions of pesticides were prepared in acetonitrile and mixed solution was diluted to volume with acetonitrile.

Procedure

Weigh 100 g homogeneous crop composite into high-speed blender jar, add 2.0 mL mixed spiking solution and 198 mL acetonitrile, and blend 2 min at high speed. Filter (with suction) through 12 cm Buchner funnel fitted with sharkskin paper into 500 mL suction flask. Transfer filtrate to 250 mL graduated cylinder and record volume. Transfer 50 mL aliquot to sample tube and load sample tube in tray of automated sampler. Automated SPM should be operating 30 min before

introduction of first sample, and sample line should be primed with acetonitrile–water (65 + 35). Collect extracts as they exit SPM. Chromatograph collected extracts on Florisil and quantitate eluates by gas chromatography.

Manually partition remaining filtered crude crop extract by standard procedures (2). Dilute organic layer to 100 mL with hexane and chromatograph 20 mL aliquot through Florisil. Quantitate eluates by gas chromatography.

SPM Operation

Crude, filtered acetonitrile crop extracts are aspirated by sampler and drawn into SPM during 16 min period, followed by 4 min purge of SPM with acetonitrile–water (65 + 35). Volume of extract aspirated into system during 16 min period is function of pump tube diameter, which is subject to manufacturing variability and mechanical fatigue. This volume (ca 40 mL) is periodically determined by measuring volume of extract remaining in sample tubes.

Sample stream is segmented with air to aid in mixing of phases. Hexane is introduced into sample stream, which then passes through first mixing coil, M1 (Figure 1). Acetonitrile is diluted with large volume of saline, and stream passes through mixing coil M2, debubbler D1, and first phase separator, S1. Lighter organic phase (which contains compounds of interest) is drawn off and washed with water, using mixing coil M3 and phase separator S2. Washing process is repeated using mixing coil M4; organic phase from third phase separator, S3 (which normally would be pumped to column chromatography module) is collected, in these experiments, for manual Florisil column chromatography.

Calculation of Equivalent Sample Weight

Calculations for automated system are based on AOAC method sec. **29.011(f)** where equivalent sample weight is given as $S \times (F/T) \times P/100$, and S = g sample taken, F = vol. filtrate, T = total vol. (mL water in sample + mL CH_3CN added − correction in mL for vol. contraction), P = mL petroleum ether extract, and 100 = mL petroleum ether into which residues were partitioned. For our calculations, S and T are as defined above. However, $P/100$ becomes percent hexane recovered by SPM and F is vol. (mL of filtrate aspirated by sample in 16 min). Data presented in this report (see *Results*) are based on experimentally obtained values of F = 40 mL and $P/100$ = 0.70.

Results and Discussion

Table 1 shows the data obtained for the determination of 8 pesticides in tomatoes and spinach. The results indicate excellent agreement between the automated and manual procedures, for both recovery and precision. The compounds chosen for these experiments included both organochlorine and organophosphate pesticides that have been studied collaboratively using the AOAC multiresidue procedure. The spiking levels chosen for these studies were very low, thus providing a difficult test for the system. In addition to the added pesticides, the spinach samples also contained incurred residues of *p,p'*-DDE and *p,p'*-DDT. Again, excellent precision and agreement with the manual procedure were observed.

Application of the automated continuous flow concept to the AOAC multiresidue pesticide procedure is atypical. The large sample size dictates that relatively large volumes be pumped in a relatively short time. Approximately 225 mL is pumped through the first phase separator in about 20 min, while typical automated applications have flow rates of <2 mL/min.

Table 1. Comparison of automated and manual solvent partitioning for determining 8 pesticides in tomatoes and spinach

	Automated partitioning[a]			Manual partitioning[b]		
Pesticide	Mean, ng/g	CV, %[c]	Rec., %	Mean, ng/g	CV, %[c]	Rec., %
			Tomatoes			
Aldrin	30.0	11.0	106	32.5	6.5	114
Dieldrin	57.4	6.3	103	57.5	3.7	103
p,p'-DDT	99.4	5.3	99	109.5	3.2	109
Ethion	199.6	5.5	92	230.0	3.1	106
Heptachlor epoxide	34.6	3.5	110	35.5	2.0	113
Lindane	19.2	4.2	120	17.0	8.2	106
Parathion	120.8	3.6	83	118.5	6.6	82
Ronnel	33.2	4.5	107	34.0	4.1	109
			Spinach			
Aldrin	28.2	5.3	99	29.5	7.2	104
p,p'-DDE[d]	54.6	2.8	—	61.0	4.6	—
p,p'-DDT	146.0	1.7	(91)[e]	150.0	4.7	(102)[e]
Dieldrin	59.8	4.9	107	59.5	1.2	107
Ethion	225.4	3.7	104	235.0	6.6	108
Heptachlor epoxide	35.4	3.2	112	35.0	8.1	111
Lindane	21.4	2.6	134	20.5	3.4	128
Parathion	137.4	3.7	95	137.5	5.7	95
Ronnel	34.0	2.9	109	31.5	11.2	101

[a]Five replicates.
[b]Two replicates.
[c]Coefficient of variation, %.
[d]Incurred residue.
[e]Recovery after correction for incurred residue.

Most of the phase separators commonly employed in automated systems are designed to operate with relatively low aqueous/organic phase ratios. In this application, we were confronted with aqueous/organic phase ratios as high as 9:1, and the organic phase had to be recovered. Furthermore, typical automated phase separations recover 60–80% of the desired phase. In this application, sensitivity is critical and it is necessary to maximize the recovery of the organic phase.

Early versions of our manifold design used 3 polypropylene Y's (¼ in. od) as the phase separators. This type of fitting can recover up to about 80% of the organic phase. Since our procedure calls for 3 phase separators in series, the final extract would contain only about 50% of the available organic phase. Therefore the process of phase separation in this system was studied. To visualize this, D&C Red No. 17 (a fat-soluble dye) was added to the organic phase reservoir. The smaller organic globules tended to rise and were taken off at the upper leg of the phase separator. The larger globules tended to sink and were drawn off with the aqueous waste. As a result of these observations, a new phase separator was designed and evaluated (Figure 2), which takes advantage of the high flow rates of the sample stream and the different affinities of the organic and aqueous phases for glass and polypropylene. The narrow diameter of the glass pipet tip produces small globules of organic phase that act as if they are shot from a cannon, and these globules are attracted to the hydrophobic surfaces of the polypropylene stepped connector. The aqueous phase is attracted to the hydrophilic surface of the glass pipet tip and flows over the lip and down the outside of the tube, eventually exiting through the side arm of the T-connector to waste. With properly adjusted flows, this new phase separator is capable of almost complete recovery of the organic phase, and is used in both the first and second phase separations. The third phase separator in the manifold is a polypropylene Y-tube that recovers approximately 80% of the organic phase without incorporating any

appreciable aqueous phase. We accept a 20% loss in organic phase recovery at this stage to ensure that water will not interfere with the subsequent Florisil column chromatography step. Organic phase recovery through the entire SPM is approximately 70%, and this value is periodically determined and used as a calibration factor in calculating residue levels.

Studies with pure solvents have shown that no materials that interfere with the determination of common pesticides by electron capture or flame photometric detection are leached from the system. Additionally, we have determined that sample blanks that are run immediately after spiked samples are free of contamination. Of course, at very high pesticide levels, some carryover might occur.

When completed, the system will sample a crude, filtered crop extract and provide the analyst with a purified extract suitable for electron capture and/or flame photometric gas chromatography. The system will be capable of processing 3 samples/h, an estimated 3-fold improvement over current procedures. The aliquot of crude, filtered crop extract used by the system will be equivalent to approximately 10 g sample, and the system will consume only one-fifth the quantity of solvents required by the manual procedure.

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Chlorinated Compounds and Phenols in Tissue, Fat, and Blood from Rats Fed Industrial Waste Site Soil Extract: Methods and Analysis

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A method was developed to analyze rat tissue, fat, and blood for some of the chlorinated compounds found in an extract of soil from an industrial waste site. Extraction with hexane and then with ethyl ether–hexane (1 + 1) was followed by concentration over steam, and gas chromatographic analysis with an electron capture detector. Volatile compounds were analyzed in a glass column coated with 6% SP-2100 plus 4% OV-11 on Chromosorb W. Semivolatile compounds, chlorinated compounds, and pesticides were analyzed in a 70 m glass capillary column coated with 5% OV-101. Phenols were analyzed in a glass column packed with 1% SP-1240 DA on Supelcoport. However, the most efficient means of separation was to use the same glass column for volatile compounds, a DB-5 fused silica capillary column for semivolatile compounds, pesticides, and phenols, and the same 1% SP-1240 DA glass column for separation of β-BHC and pentachlorophenol. Recoveries ranged from 86.3 ± 9.1% (mean ± standard deviation) to 105 ± 10.4%. Sensitivities for semivolatile chlorinated compounds, pesticides, and phenols were about 4 ng/g for fat, 1 ng/g for tissue, and 0.2 ng/mL for blood. Sensitivities for volatile compounds were about 4-fold higher (16, 4, and 0.8, respectively). Sensitivities for dichlorobenzenes and dichlorotoluenes were 8 ng/g for fat, 2 ng/g for tissue, and 0.4 ng/mL for blood.

Recent investigations of the toxicologic effects of the types of chemicals found in industrial waste sites (J. Silkworth, D. McMartin, R. Rej, C. Tumasonis, R. Narang, V. B. Stein, and L. Kaminsky, unpublished) have underscored the need for a method to determine these compounds and some of their metabolites in animal tissue. Scientists in our institute are studying the toxicity of soil from industrial waste sites after initial Soxhlet extraction with acetone–hexane (1 + 1) followed by benzene–methanol (1 + 1). The extracts are combined and evaporated to dryness. Rats are then fed doses of 0, 25, 75, or 150 mg of soil extract/kg/day for 20 days.

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Chemical analysis of the soil showed the compounds listed in Table 1. These types of compounds have been determined in air, soil, and water (1–3), but no method has been reported for their determination in animal tissue. We therefore developed a simple method to extract from tissue the types of chlorinated compounds found in industrial waste sites and some of their metabolites. The method uses extraction initially with hexane and then ethyl ether–hexane (1 + 1), concentration over steam, and gas chromatographic analysis with an electron capture detector. The samples tested were limited to tissue, fat, and blood, because neither urine nor feces was

Table 1. Compounds identified in soil and incorporated in spiking solution

Volatile compounds:
Carbon tetrachloride
Bromodichloromethane
Tetrachloroethylene
1,2-Dibromoethane
1,1,1,2-Tetrachloroethane
1,1,2,2-Tetrachloroethane
Chloroform
1,1,1- + 1,1,2-Trichloroethane
Semivolatile compounds and pesticides:
m,p,o-Dichlorobenzene
Hexachloroethane
α,α- + α,*o*- + α,*p*-Dichlorotoluene
2,4- + 2,5- + 2,6-, + 3,4-Dichlorotholuene
1,3,5 - + 1,2,3- + 1,2,4-Trichlorobenzene
α,α,α-Trichlorotoluene
C-46
1,2,3,5- + 1,2,4,5- + 1,2,3,4-Tetrachlorobenzene
Pentachlorobenzene
Hexachlorobenzene
α- + β- + γ- + δ-Hexachlorocyclohexane
Phenols
2,4- + 2,5-Dichlorophenol
2,4,6- + 2,4,5-Trichlorophenol
2,3,5,6- + 2,3,4,5-Tetrachlorophenol
Pentachlorophenol

collected. This procedure can most likely be applied to other animal tissues and probably to human tissue also.

Much work has been done on the metabolism of α-, β-, γ-, and δ-hexachlorocyclohexanes (α-, β-, γ-, and δ-BHC), hexachlorobenzene (HCB), and some of the other compounds of interest (4-9). Previous work on the metabolism of HCB (9) showed only pentachlorophenol present in measurable amounts in tissues. Other workers (6) have reported trichlorophenols as the major metabolites of trichlorobenzenes, while BHC (lindane) metabolism produces tri- and tetrachlorobenzenes and tri-, tetra-, and pentachlorophenols in liver, kidney, and blood (5). Because all metabolites other than the phenols were already contained in the soil extract, the only metabolites studied were phenols, which can hydrolyze to form conjugates such as glucuronides (6–7). To remove these bound metabolites, an acidic extraction must be carried out. Since this was not done, only free metabolites were measured in this study.

METHOD

Apparatus

(a) *Gas chromatograph.*—Hewlett-Packard Model 5880A (Avondale, PA) equipped with an electron capture detector (^{63}Ni) held at 300°C. The injector temperature was 200°C and argon–methane (10 + 90) was used as both the carrier and make-up gas.

(b) *Autosampler.*—Hewlett-Packard Model 7672 autosampler with 10 μL syringe.

(c) *Homogenizer.*—Super Dispax SDT Tissumizer (Tekmar Co., Cincinnati, OH).

(d) *Mixer.*—Vortex Genie mixer (Scientific Products, Evanston, IL).

(e) *Centrifuge.*—Dynac Model 0101 centrifuge (Parsippany, NJ).

(f) *Chromatographic column.*—100 mL pipets, cut in half.

(g) *Concentrator tubes.*—Graduated, 12 mL, 14/20 joint, K-288250 (Kontes Glass Co., Vineland, NJ).

(h) *Distillation column.*—Vigreaux distillation column with 14/20 joint, K-286700 (Kontes Glass Co.)

(i) *Packed glass GC column.*—6 ft × 2 mm id, packed with 6% SP-2100 + 4% OV-11 on Chromosorb W. Conditions: 40 to 225°C at 4°/min, held at 225°C for 5 min, with carrier gas flow of 30 mL/min.

(j) *Glass capillary column.*—70 m × 0.03 mm id, coated dynamically with 5% OV-101. Conditions: 40 to 225°C at 4°/min, held at 225°C for 5 min, gas flows of 20 mL/min for vent and 30 mL/min for make-up.

(k) *Fused-silica capillary columns—(1)* 25 m × 0.02 mm id DB-5 fused-silica, coated with SE-54. *(2)* 30 m × 0.02 mm id fused-silica capillary column coated with SE-54 (J. & W. Scientific, Inc., Cordova Park, CA). Conditions, see column (j).

(l) *Packed glass GC column.*—6 ft × 2 mm id glass column packed with 1% SP-1240 DA (Supelco, Inc., Bellefonte, PA). Conditions: 100 to 120°C at 4°/min, held at 120°C for 5 min, carrier gas flow 30 mL/min.

Reagents

(a) *Hexane.*—Pesticide grade (Baker Chemical Co., Phillipsburg, NJ).

(b) *Ethyl ether.*—Pesticide grade distilled before use (Mallinckrodt, Inc., St. Louis, MO).

(c) *Florisil.*—60–100 mesh, PR grade heated to 675°C overnight before use (Aldrich Chemical Co., Milwaukee, WI).

(d) *Sodium sulfate.*—Anhydrous, granular. Baked at 600°C overnight (Aldrich Chemical Co.)

Extraction and Cleanup

All compounds except phenols were extracted from tissues with hexane. Various concentrations of ethyl ether–hexane (10–90%) were then used to extract the phenols. A spiking solution (1 mg/mL) was prepared by dissolving 100 mg of each compound (Table 1) in 100 mL hexane and diluting to appropriate concentrations. Samples were fortified with 1μL of the spiking solution, and the extracts were analyzed to determine recoveries.

Microcolumns for cleanup were prepared by cutting 100 mL pipets in half, and each was filled with 1.6 g each of sodium sulfate above Florisil, over a glass wool plug. Each column was fortified with 1μL spiking solution and eluted with various concentrations of ethyl ether–hexane or ethyl ether–methanol to remove semivolatile compounds, pesticides, and phenols. Eluates were analyzed by gas chromatography to determine recoveries. In both the extraction and recovery steps, blanks were prepared and analyzed in the same manner as the samples.

Recovery Studies

Tissue, fat, and plasma were fortified with 25 μL of a 1 μg/mL (25 ng) spiking solution (Table 1) and carried through the procedure described below. Recoveries were determined in triplicate, and controls were analyzed at the same time.

Procedure

Place tissue samples (0.3–0.5 g) in ice until cold, then homogenize, centrifuge, and extract with two 2 mL portions of hexane. Take a 4 mL aliquot for determination of volatile compounds (10 μL used), mix with a vortex mixer, recentrifuge, and extract with two 2 mL portions of ethyl ether–hexane (1 + 1). Combine both extracts with the remainder of the aliquot taken for analysis of volatiles and concentrate the mixture to 1–2 mL over steam in a Kuderna-Danish tube fitted with a Vigreaux distillation column.

Extract blood (1–2 mL) in same manner but do not place in ice.

Quantitation

To determine amount of each compound in sample, determine areas of chromatographic peaks with integrator attached to gas chromatograph. Analyze standard solution in concentration range of samples after every fifth sample.

Detection Limits and Sensitivity

Detection limits were determined according to Hartman's (10) definition as the amount of sample which gives a response equal to twice the background level. Sensitivity was expressed in ppb (ng/g) for tissue and fat and in ng/mL for blood.

Results and Discussion

Gas Chromatography

The glass capillary column coated with 5% OV-101 was prepared in our laboratory by etching the column with gaseous HCl and coating it dynamically. A 30 m fused-silica capillary column coated with SE-54 was also tested but did not separate pentachlorophenol from β-BHC, or α,α,α-trichlorotoluene from C-46.

A similar fused-silica capillary column (DB-5) separated α,α,α-trichlorotoluene from C-46. This recently available column was actually the best for a broad range of separations,

using the same conditions as for the 5% OV-101 glass capillary column, because it separated volatile compounds, semivolatile compounds, pesticides, and phenols, but it was obtained late in the study and was not used for the analyses. The most convenient means of analyzing the mixture of compounds in Table 1 would be to use the DB-5 column for volatile compounds, semivolatile compounds, pesticides, and phenols and then the SP-1240 DA packed column for pentachlorophenol and β-BHC.

Typical chromatograms of the compounds of interest are shown for both the 3-column procedure (Figure 1) and the DB-5 column (Figure 2).

Extraction and Cleanup

The best solvent for elution of semivolatile compounds and pesticides was ethyl ether–hexane (10 + 90), while ethyl ether–methanol (1 + 1) was best for phenols. In each case, elution volume was about 10 mL, but no cleanup was needed. Hexane was concentrated 10:1 and found to be free of interferences (<1 ppb; Figure 3) when analyzed by gas chromatography on the 5% OV-101 glass capillary column. The blanks were analyzed and did not contain any interfering peaks either.

Recovery Studies

Recoveries of 25 ng volatile compounds, semivolatile compounds, pesticides, and phenols from tissues, fat, and blood ranged from 86.3 ± 9.1% (mean ± standard deviation) to 105 ± 10.4%. The data represent triplicate determinations averaged for all compounds in a given class (Table 2).

Detection Limits and Sensitivities

Detection limits were about 2 pg for the dichlorobenzenes and dichlorotoluenes and about 1 pg for the remaining compounds. Sensitivities for semivolatile compounds, pesticides, and phenols were 4 ng/g for fat, 1 ng/g for tissue, and 0.2 ng/mL for blood. Sensitivities for volatiles were about 4-fold higher (16, 4, and 0.8, respectively). Sensitivities for dichlorobenzenes and dichlorotoluenes were 8 ng/g for fat, 2 ng/g for tissue, and 0.4 ng/mL for blood.

Analysis of Samples

Liver and blood from rats fed the extract as described above were analyzed for volatile compounds, semivolatile compounds, and pesticides by the 3-column procedure (Tables 3 and 4). Because most metabolites are compounds already contained in the extract, the chlorinated phenols were the only metabolites of interest in liver and blood. Volatile compounds could not be detected in liver or blood samples and only small amounts of tetrachlorobenzene and BHCs were found in most of the samples, with α- and β-BHC present in the largest amounts as would be expected since α- and β-BHC are present in the largest amounts in the soil extract. Small amounts of 2,4,5-trichlorophenol, 2,3,5,6-tetrachlorophenol, and pentachlorophenol were present in both liver and blood, while 2,3,4,5-tetrachlorophenol was also present in blood. Attempts are being made to confirm the presence of phenols by mass spectrometry, although a number of samples of liver and blood were chromatographed on both the fused-silica capillary column coated with SE-54 and the glass packed column coated with SP-1240 DA on Supelcoport. The analysis of these samples confirmed the presence of the phenols mentioned above.

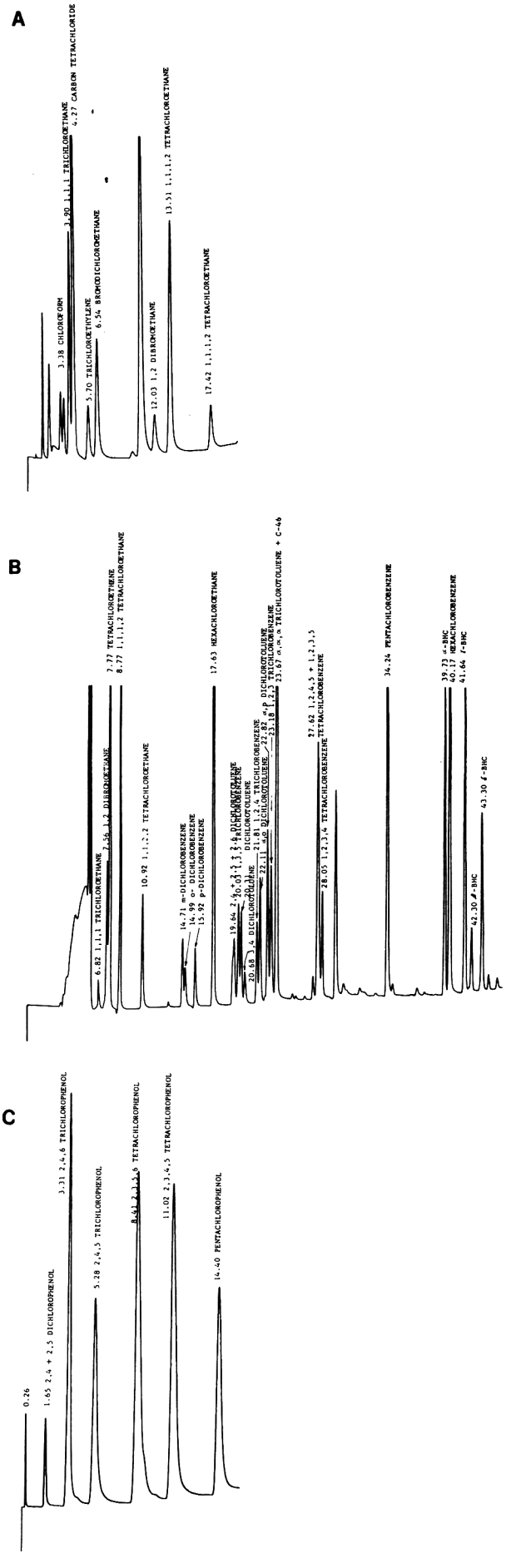

Figure 1. Typical chromatograms of (A) volatiles, (B) semivolatile compounds and pesticides, and (C) phenols by the 3-column gas chromatographic procedure (Table 2).

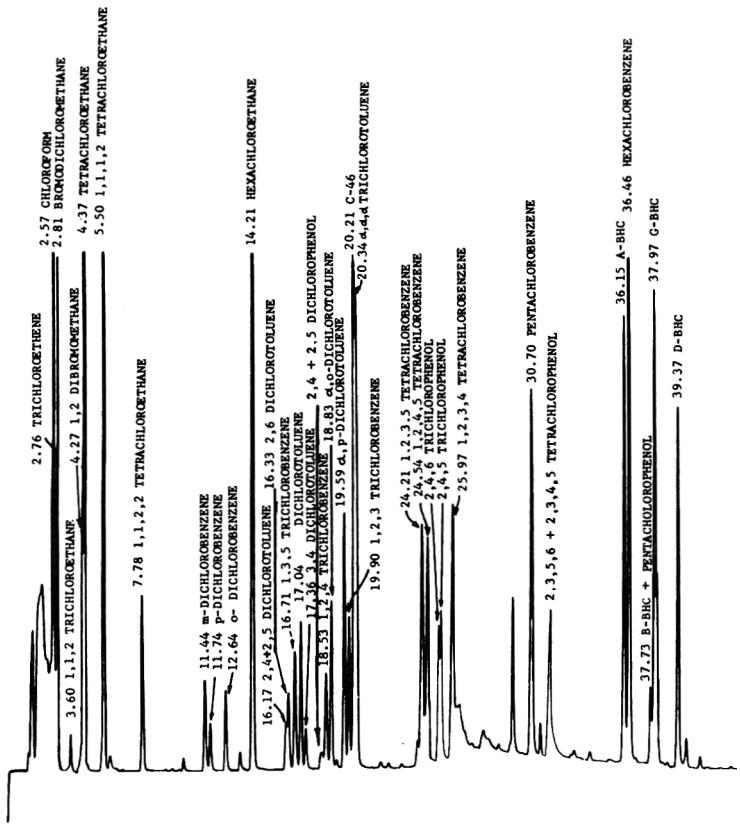

Figure 2. Gas chromatogram of spiking solution on DB-5 fused-silica capillary column, using conditions listed in Table 2 for semivolatile compounds and pesticides.

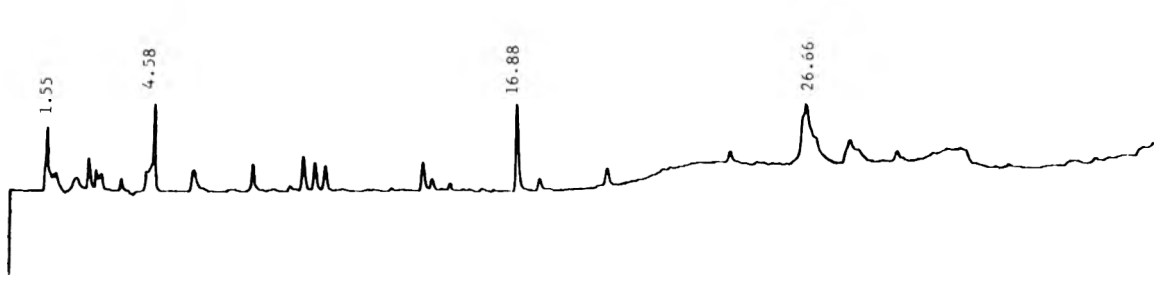

Figure 3. Gas chromatogram of hexane, concentrated 10:1 and found to be free of volatile compounds.

Table 2. Recoveries (mean ± standard deviation) of volatile compounds, semivolatile compounds, pesticides, and phenols from tissue, fat, and blood (25 ng)[a]

Tissue	Volatile compds	Semivolatile compds and pesticides	Phenols
Spleen	89.5 ± 6.7	101 ± 10	98.0 ± 5.9
Liver	92.0 ± 7.5	103 ± 6.1	105 ± 10.4
Kidney	91.6 ± 10.6	102 ± 9.8	92.3 ± 10.6
Fat	99.8 ± 15.6	86.3 ± 9.1	99.7 ± 11.6
Brain	96.7 ± 7.7	105 ± 7.4	101 ± 10.9
Blood	99.8 ± 5.8	99.0 ± 3.5	95.2 ± 7.0

[a]Recoveries represent triplicate determinations averaged in a given class.

Table 3. Results of liver analysis, in ppb (ng/g), from rats fed soil extract

Animal	1,2,4,5-TeCB	1,2,3,5-TeCB	1,2,3,4-TeCB	PCB	HCB	α-BHC	γ-BHC	β-BHC	δ-BHC	2,4,6-Trichlorophenol	2,3,5,6-Tetrachlorophenol	Pentachlorophenol
						25 mg/kg Extract						
156	—	—	—	—	1.6	3200	47	400	59	43	7.7	88
158	—	—	—	—	1.3	2500	17	330	16	21	3.9	14
167	2.5	—	4.1	—	2.3	2000	11	790	6.8	—	—	—
172	4.3	—	—	—	15	3200	17	600	19	38	6.3	35
178	—	—	—	—	—	3800	42	1100	77	—	8.3	66
185	—	34	—	14	96	3800	115	2500	12	56	12	169
187	—	—	—	—	27	4000	57	1100	99	26	9.8	23
201	—	—	—	—	12	3400	40	660	35	18	6.2	56
205	—	—	—	—	34	4900	46	970	68	71	10	62
211	—	—	—	—	12	4200	34	1000	12	47	12	52
213	—	—	—	—	47	5100	52	980	43	—	14	60
218	—	—	—	—	34	3700	34	1200	14	31	7.8	30
220	—	—	—	—	23	2600	87	9100	230	51	13	98
222	—	—	—	—	16	1600	22	430	12	18	3.5	59
224	—	—	—	—	20	3900	29	1000	21	18	11	11
233	—	—	—	—	27	4800	130	1400	310	52	30	219
240	—	—	—	—	25	6400	70	1900	140	52	17	35
257	—	—	—	—	20	2700	630	9100	20	29	11	73
260	170	270	—	—	190	4500	240	4400	120	21	14	115
						75 mg/kg Extract						
151	1.9	—	4.9	0.72	1.9	4600	220	2600	160	81	18	6.7
159	—	—	—	—	4.8	6600	27	2700	130	88	10	39
160	—	94	18	—	140	9100	160	4500	460	88	169	34
170	—	—	—	—	129	400	67	1100	56	110	21	34
174	4.7	—	13	—	16	16000	41	3700	41	124	67	67
175	4.5	—	9.5	—	43	8400	94	1800	312	67	20	22
180	4.4	—	12	—	29	11600	120	2000	100	224	55	88
184	2.3	—	1.1	—	—	6300	63	2500	12	79	27	6.6
186	—	—	—	—	210	15400	320	4400	510	175	32	76
188	—	—	—	—	40	13400	59	3600	77	113	40	59
206	—	—	—	—	67	17800	125	4300	74	111	52	69
217	—	—	—	—	44	11900	89	3500	160	80	29	48
221	—	—	—	—	41	7000	260	2500	2100	75	51	37
225	—	—	—	—	75	15700	78	4200	130	122	70	75
230	—	—	—	—	43	15600	1300	5000	33	136	119	121
234	—	—	—	—	45	7700	90	4000	20	50	47	6.1
242	—	—	—	—	67	10900	260	4000	4.7	132	66	49
258	78	125	—	—	87	19800	560	5500	530	179	103	247
263	83	55	57	—	230	19700	570	6500	280	92	128	67
						150 mg/kg Extract						
163	—	70	—	—	68	16900	750	5500	9	139	28	31
165	2.1	—	7.5	—	180	9900	550	6400	310	110	4.5	5.8
235	—	—	—	—	53	6800	260	5400	33	44	66	—
247	—	—	—	—	120	25900	360	8100	59	107	159	—
249	—	—	—	—	210	8300	1200	8400	930	154	152	9.7
181	—	—	—	—	—	—	—	—	—	102	72	6.6
243	—	—	—	—	—	—	—	—	—	279	114	63

Table 4. Results of blood analysis from rats fed soil extract (ng/mL)

Animal	HCB	α-BHC	γ-BHC	β-BHC	σ-BHC	2,4,5-Trichlorophenol	2,3,5,6-Tetrachlorophenol	2,3,4,5-Tetrachlorophenol	Pentachlorophenol
					25 mg/kg Extract				
156	3.6	1009	21	165	14	17	13	37	20
158	6.3	1154	11	—	—	—	—	—	—
167	1.4	443	3.3	62	6.0	4.3	4.0	94	5.2
172	2.1	616	7.5	90	5.0	5.6	3.2	152	8.2
178	—	476	12	86	7.5	7.2	—	38	12
182	—	191	28	35	9.0	11	3.7	25	19
185	2.2	460	16	105	13	3.5	5.5	30	2.9
187	—	117	4.3	33	—	3.0	2.8	83	3.6
201	—	228	9.7	47	5.1	4.8	3.3	7.7	5.8
205	14	184	7.1	42	14	4.4	3.8	13	3.5
211	53	258	20	67	8.9	4.5	3.4	40	2.6
213	1.7	122	—	36	—	2.1	2.5	27	2.2
218	5.4	317	—	74	—	6.6	6.6	10	17
220	8.5	272	21	58	22	3.6	3.8	16	11
224	2.1	293	12	100	4.1	6.6	6.7	15	6.4
233	3.2	370	34	57	17	4.0	3.5	9.0	9.8
240	2.0	531	7.1	93	5.0	7.5	6.8	10	6.3
246	—	—	—	95	—	4.1	3.9	4.6	8.5
257	—	—	—	64	—	16	17	27	42
260	—	340	—	60	7.5	3.2	2.8	37	24
					75 mg/kg Extract				
151	10	3660	119	556	64	15	24	36	17
159	2.9	758	12	105	11	7.8	6.3	67	6.8
160	5.0	1481	10	273	2.7	11	7.6	44	5.0
170	3.0	836	8.0	123	3.9	7.7	2.4	112	5.2
174	7.3	2132	24	283	3.6	9.8	7.4	85	6.0
175	5.4	1833	48	223	47	17	14	690	10
180	—	2353	53	448	24	13	28	27	4.8
184	24	728	12	105	—	3.5	5.5	30	2.9
186	4.6	695	23	173	13	9.6	11	49	—
188	9.2	905	9.6	201	—	9.2	14	103	3.7
214	3.9	516	15	154	11	8.9	8.5	27	1.2
217	7.4	1'99	80	337	26	22	13	46	19
221	24	1225	47	295	17	33	30	86	15
225	7.1	1238	14	336	1.0	12	15	35	1.6
230	3.7	1199	—	261	4.0	10	11	10	23
234	5.6	1885	29	402	2.5	11	14	61	2.0
239	5.8	1741	16	344	2.8	11	9.3	29	7.6
242	5.0	1792	31	321	12	14	26	33	7.1
258	—	1741	52	450	34	7.5	27	56	121
263	—	2472	54	599	19	11	31	217	35
					150 mg/kg Extract				
163	17.5	4245	23.0	660	23.0	25	37	39	18
165	14.0	4843	108	839	108	37	5	50	43
199	—	1747	23.7	420	23.7	13	36	44	6.8
235	—	2734	31.1	478	31.1	16	28	—	1.9

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Simple Spectrophotometric Method for Determination of Phosphine Residues in Wheat[1]

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A method has been developed for determination of phosphine residues in wheat, based on the reaction of phosphine with silver nitrate in aqueous solution to form an egg-yellow chromophore with an absorption maximum at 400 nm. At this wavelength, there is a linear relationship between absorbance and concentration of phosphine in the range 10–100 ng/mL. Phosphine-fumigated wheat is soaked in a known volume of $AgNO_3$ solution, and the absorbance of the filtrate is read against a blank at 400 nm. The method is sensitive, with lower detection and estimation limits of 0.008 and 0.01 μg PH_3, respectively. Recovery of added phosphine from a closed system was 85–100%. Accuracy for this method has been compared with that for the gas chromatographic method.

Phosphine (hydrogen phosphide, PH_3) has gained popularity as a commercial fumigant because it is simple to use and is highly toxic to insects. Phosphine was once thought to be a "no-residue" fumigant, but it has been shown that this is not true (1–4). Therefore, a simple, sensitive, and repeatable colorimetric method is needed for the determination of unchanged phosphine residue.

Earlier methods have included oxidation of phosphine. Muller (5) oxidized phosphine to phosphate by using acid potassium permanganate, and measured the phosphate colorimetrically as phosphomolybdate. The method of Bruce et al. (6) consisted of displacing phosphine from fumigated commodity in the presence of an equal amount of 10% sulfuric acid. The displaced phosphine was oxidized with bromine water to phosphate, which was measured by the method of Fiske and Subba Rao (7). Berck (8) removed phosphine from a closed system by exhaustive flushing with dry nitrogen. Phosphine was then trapped in chilled ethanolic mercuric chloride solution, and the hydrochloric acid released was determined potentiometrically. Nelson and Milum (9) used tubes of silica gel impregnated with silver nitrate; however, their method was not repeatable.

Gas chromatographic (GC) methods have been reported for determination of phosphine in air (10–12). Residues in wheat (3, 13) were determined by passing nitrogen through the commodity and collecting the desorbed phosphine in a trap cooled with Dry Ice; residues were quantitated by GC. Chakravarthy and Wainman (14) reported a GC method for determining phosphine in air. Muthu and Majumder (15) used a chromogenic column for determining phosphine in air; Kashi and Muthu (16) used a mixed indicator strip for phosphine detection. Nowicki (17) reported a screening method using GC with flame photometric detection for determining phosphine residues in wheat. Phosphine was measured as the sum of physically bound intact phosphine and that derived from residual aluminum phosphide. Recoveries varied with concentration; 67% was recovered at 0.1 ppm and 98% at 19 ppm. Phosphine concentrations as low as 0.04 ppm were easily determined in wheat.

The present work describes a simple spectrophotometric method for determination of phosphine residues in wheat. The method is based on the reaction that occurs when filter paper moistened with a drop of $AgNO_3$ solution is exposed to phosphine. The wet spot becomes egg-yellow color, and the periphery acquires a dark brown ring which slowly broadens towards the center. The egg-yellow color was thought to be due to silver trinitratophosphide. Adding dilute HNO_3 does not change the yellow spot, but with aqueous ammonia solution it immediately blackens (18). Under the conditions used in this work, the egg-yellow color is stable for more than 24 h and is not affected by either dilute HNO_3 or aqueous NH_3 solution.

The method consists of extracting phosphine residues by soaking 10 g wheat in 21 mL $AgNO_3$ solution for 1 h. The absorbance of the extract, after filtration and centrifugation, is measured at 400 nm. The absorbance of the crop control extract is similarly determined and subtracted from the absorbance of the extract of the fumigated wheat. The calibration line between concentration of phosphine and absorbance of the chromophore is obtained by passing a known volume of airborne phosphine through an aqueous solution containing 30 μg silver nitrate/mL. Reaction between phosphine and silver nitrate is allowed to proceed for 45 min when an egg-yellow chromophore is formed. The intensity of the chromophore bears a direct relationship to the concentration of phosphine.

METHODS

Apparatus and Reagents

(a) *Spectrophotometer.*—Bausch and Lomb Spectronic 21.

(b) *Tubes.*—5.5 cm × 11 mm id and 7.0 cm × 18 mm id with Bakelite screw cap with rubber septum.

(c) *Conical flask.*—With side arm fitted with rubber septum to permit injection and gas sampling.

(d) *Silver nitrate solution.*—Dissolve 75 mg $AgNO_3$ crystals (analytical grade, British Drug Houses) in 50 mL water to prepare stock solution. Prepare working solution by diluting 5 mL stock solution to 250 mL with water. Use 3 mL solution for each experiment.

(e) *Standard phosphine.*—Liberate phosphine from phostoxin tablet (Degesch America, Inc.) and store over 5% H_2SO_4 in gas buret.

Composition of gaseous mixture collected is 86% phosphine and 14% carbon dioxide. This serves as source for drawing phosphine with gas-tight microsyringe through rubber septum. Make further dilutions of phosphine with air in 100 mL gas-tight syringe after making corrections for 100% phosphine. Draw aliquots of diluted phosphine from syringe through rubber septum by using gas-tight microsyringe.

Preparation of Standard Curve

Pipet 3 mL aliquots of working standard silver nitrate solution into separate 6 mL tubes, and close with Bakelite screw-caps with gas-tight rubber septum. Inject air (50–200 μL) containing phosphine in range of 0.01–0.14 μg by using gas-tight microsyringe. Inject equal volume of air into 3 mL silver nitrate solution to serve as blank. Let tubes stand 45 min for formation of chromophore which has an absorption maximum at 400 nm (Figure 1). Measure absorbances in spectrophotometer at 400 nm against blank. Plot of absorbance vs concentration yields straight line over range 0.01–0.1 μg/mL PH_3 (Figure 2).

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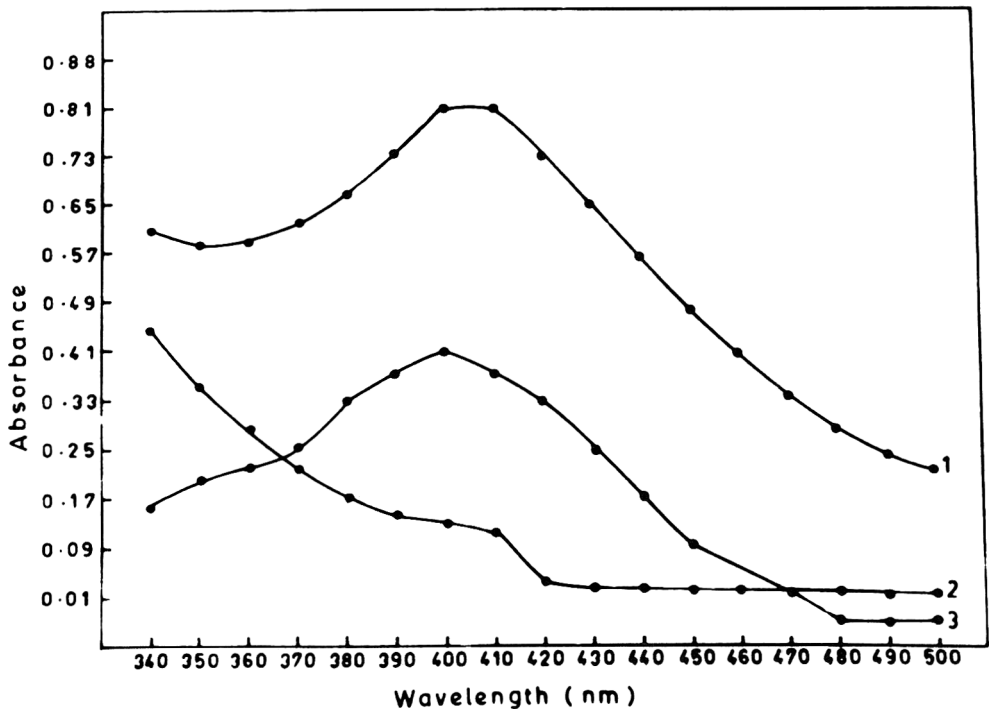

Figure 1. Absorption spectra of $AgNO_3$–PH_3 chromophore: 1, extract of PH_3-fumigated wheat; 2, extract of control wheat; 3, standard $AgNO_3$–PH_3 chromophore.

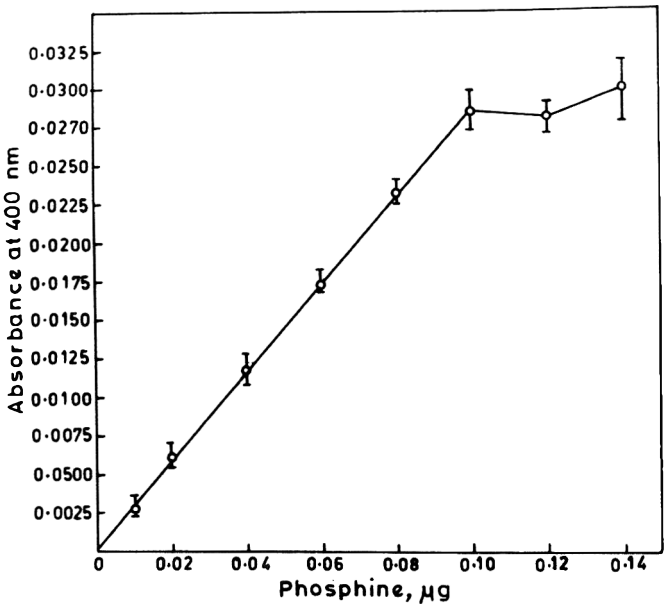

Figure 2. Relationship between phosphine (μg) and absorbance at 400 nm. Values plotted are means of 10 replicates. Standard deviation for each value also shown.

Recovery Study

First procedure.—Suspend 10 g wheat in 21 mL silver nitrate solution in 30 mL screw-cap tube with rubber septum. Inject known volume of phosphine into suspension. Let tube stand 1 h to allow extraction of wheat constituents as well as reaction of phosphine with silver nitrate. Filter extract through Whatman No. 1 paper, shake filtrate, and centrifuge at 3000 rpm for 25 min. Measure absorbance at 400 nm against silver nitrate solution as blank. Treat equal weight of wheat without phosphine similarly to serve as crop control.

Second procedure.—Fumigate 10 g wheat with different doses of phosphine in gas-tight 100 mL conical flask with side arm fitted with rubber septum to permit injection of phosphine. After 24 h fumigation, without disturbing either flask or contents, remove glass stopper of flask for 15 s to drive out free phosphine in headspace. Quickly add 21 mL silver nitrate solution to flask, and replace stopper tightly. Let extraction and color reaction proceed 1 h, with shaking at intervals. Filter extract, centrifuge filtrate, and read absorbance against silver nitrate as blank. Similarly run crop control. Also run blank experiments without wheat in precalibrated 100 mL conical flasks at every level of phosphine used to assess extent of absorption of added phosphine by glass, grease, and rubber septum. After 24 h, withdraw known volume of phosphine-borne air with gas-tight microsyringe, and analyze.

Third procedure.—Repeat fumigation of 10 g wheat at different doses of phosphine as under second procedure. Also carry out crop control and blank experiments. After 24 h fumigation, open stopper of flask, and quickly transfer fumigated wheat to 50 mL tube containing 21 mL silver nitrate solution. Tightly stopper tube and let stand 1 h with shaking at intervals. Read absorbance of extracts as under second procedure.

Fourth procedure.—Expose 500 g wheat to 0.75–0.95 mL phosphine for 2 weeks in 1 L flask fitted with rubber septum that permits injection and gas sampling. Inject equal amount of phosphine into another similar precalibrated empty flask to serve as control. At end of 2 weeks, determine concentration of phosphine in headspace over wheat and in control flask by withdrawing known volume of phosphine-borne air. Compute amount of phosphine taken up by 500 g wheat after 2 weeks exposure by subtracting headspace concentration from concentration in control flask.

Comparison with Gas Chromatographic Method

Wheat (250 g) was exposed to phosphine in the range 0.9 to 1.25 mL for 1 week in 1 L flask fitted with rubber septum. Phosphine concentration over the wheat was estimated by withdrawing 100 μL air from the flask and injecting an aliquot of this sample into 3 mL silver nitrate solution. The remaining

portion was analyzed by gas chromatography, using a Porapak column and flame photometric detector (Figure 3) (B. Chakravarthi, personal communication). Concentrations of phosphine in air were calculated by using the linear relationship between phosphine concentration and peak height.

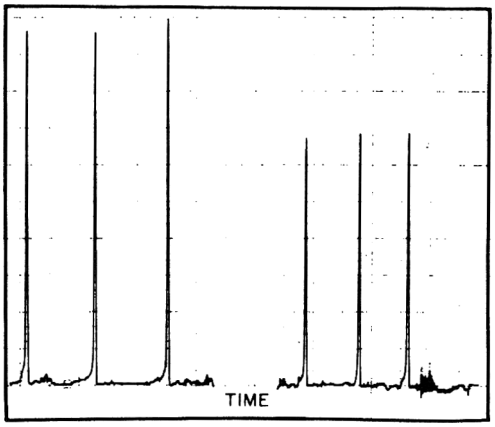

Figure 3. Gas chromatogram of phosphine. Conditions: 50 × 0.94 cm stainless steel column packed with 80–100 mesh Porapak Q; column temperature 50°C; retention time 30 s; nitrogen flow 40 mL/min.

Residue Analysis

One kg wheat was mixed with known weight of powdered Phosfume tablet (aluminum phosphide + a phosphoric ester + inorganic fillers + tableting agents) in 2 L flask which was closed with a greased gas-tight stopper. Fumigation was allowed to proceed for 2 weeks. In another experiment, 600 mg Phosfume tablet was packed in filter paper and placed at the bottom of a flask containing 1 kg wheat. Fumigation proceeded for 2 weeks.

At the end of 2 weeks, to determine the initial residue before airing, the stopper of the flask was opened, and wheat was poured into a 50 mL test tube that was previously calibrated so that 10 g sample of wheat could be quickly taken for extraction. This procedure avoided loss of phosphine residue that might occur during weighing. Then, 21 mL silver nitrate solution was immediately poured onto the grain in the test tube. The tube was tightly stoppered and allowed to stand for extraction for 1 h with occasional shaking. A corresponding crop control was similarly treated. The remaining fumigated wheat was spread in a tray and aired by exposure to the atmosphere. Samples were removed at intervals.

The experimental fumigations were done in duplicate. A minimum of 6 samples were drawn at random to get representative samples for residue determination.

Results

Residue Analysis

Absorbance of crop control extract analyzed by the described procedure ranged between 0.091 and 0.092, although a value of 0.132 was recorded with some control samples. Mud may be present in the grain, and although mud particles settle to the bottom during centrifugation, the mud pigment that has been dissolved in silver nitrate solution darkens the color of the extract. Therefore, it is advisable to remove the mud pieces from both the fumigated and control samples before weighing. It is important to use wheat from the same lot both for fumigation and for crop control because the absorbance of the extract may vary from lot to lot and from variety to variety.

Data in Table 1 represent the total residue due to free phosphine and that recovered from the unspent aluminum phosphide; data in Table 2 are residues due only to free phosphine. Samples of commercially fumigated wheat were also analyzed for phosphine residues. The complete history of the samples was not known except that the stocks were stored for a few years and were repeatedly fumigated with phosphine. Twenty grams of wheat was used for extraction, and an equal weight of unfumigated wheat was used as crop control. Due to the poor condition of the samples, the absorbance of the crop control was rather high, in the range of 0.40–0.42. Phosphine residues (ppm) in the 5 samples studied were (mean ± SD, n = 6) 0.1104 ± 0.0035, 0.0955 ± 0.0082, 0.1493 ± 0.0151, 0.072 ± 0.000, and 0.113 ± 0.011.

Another commercial fumigant was studied to determine the difference in residues due to the source of phosphine. Standard phosphine was liberated and collected over distilled water in a gas buret using about one-half a 3 g Phosfume tablet. Because there is no ammonium carbamate in the tablet, only phosphine is collected over water. Experiments on preparation of standard curve and recovery were also conducted using phosphine from this source. We found that there was no difference in values obtained with phosphine from these 2 different sources (Phosfume and Phostoxin).

Discussion

The minimum weight of phosphine needed to produce a detectable color is 0.008 μg. The lowest limit of phosphine that would produce a measurable chromophore is 0.01 μg. As shown in Figure 2, the linear relationship between absorbance at 400 nm and concentration of phosphine is obeyed between 0.01 and 0.1 μg; thereafter, the relationship ceases. As seen in Figure 1, the absorption spectra of the $AgNO_3$–PH_3 chromophore and that of $AgNO_3$-free PH_3 residue extracted from fumigated wheat are similar in all respects; that of the crop control shows no absorption at 400 nm. Although the chemical nature of the chromophore has not yet been established, the similarities in spectra suggest that phosphine is physically bound in/on the wheat grain, which, when replaced by $AgNO_3$ solution, reacts to form the chromophore. $AgNO_3$ has no absorption band at this wavelength.

Table 1. Phosphine residue (ppm) in experimentally fumigated wheat: Phosfume tablet powder mixed with wheat[a]

Time aired before sampling	Fumigation 1, 533.5 mg powder/kg	Fumigation 2, 256.4 mg powder/kg
Before airing	1.383 ± 0.0007	0.1009 ± 0.000016
2 h	0.2497 ± 0.0009	0.1469 ± 0.0008
5 h	0.1523 ± 0.0001	0.1179 ± 0.0001
24 h	0.0521 ± 0.0002	0.0572 ± 0.0003
3 days	0.0211 ± 0.0001	0.0212 ± 0.0002
4 days	detectable	0.0021 ± 0.0001
5 days	detectable	detectable
6 days	below detection	below detection

[a]Mean ± SD of 10 samples.

Table 2. Phosphine residue (ppm) in experimentally fumigated wheat: Phosfume tablet, 600mg/kg wheat, packed in filter paper and placed at bottom of flask

Time aired before sampling	Fumigation 1	Fumigation 2
Before airing	1.1910 ± 0.0009[a]	1.1530 ± 0.00001
2 h	0.1505 ± 0.000001	0.1454 ± 0.0000009
5 h	0.0868 ± 0.000001	0.0663 ± 0.00002
24 h	0.0378 ± 0.000002	0.0358 ± 0.000001
3 days	0.0327 ± 0.000001	0.0315 ± 0.000002
5 days	0.0342	0.02312
8 days	0.007	0.005

[a]Mean ± SD of 10 samples.

At concentrations of 0.08/μg and above, full development of the chromophore took place within 20 min. At lower concentrations the minimum time needed for full development was 45 min. Thereafter the intensity of color remains steady for 24 h (Figure 4).

Unlike solid or liquid pesticides whose recoveries can be demonstrated for a new method by recovering a known weight of pesticide added to substrate, recoveries of a gaseous fumigant are very difficult to establish. This is because fumigant may escape at every stage of sampling after a known weight has been applied to the substrate. Hence, experiments were carried out on recovering added phosphine from air and the maximum amount of phosphine residue on wheat by 4 different procedures to establish the soundness and applicability of the new method.

Recovery studies (Table 3) show that phosphine residues have been quantitatively extracted by soaking, and the wheat extractives have either hindered the full development of the chromophore or masked its color. By this method, 89–100% recovery of added phosphine was obtained.

The purpose of the second procedure (Table 4) was to extract only the phosphine remaining in/on wheat after allowing the free phosphine in the headspace to escape. It is likely that some of the phosphine sorbed on the grain might also escape when the stopper is opened for 15 s. Values of recovered phosphine for the 2 experimental fumigations (column 3) increased as phosphine added increased. If this is taken as an index of the amount of phosphine mixed indirectly with the wheat, these values indicate almost 100% recovery of phosphine fortified with wheat. So it has been possible to recover almost all phosphine held by wheat by the new method. For example, at a dose of 600 μg, 17.15 and 16.21 μg phosphine are held back by 10 g wheat, while at a dose of 12 μg, only 0.23 and 0.195 μg are held by the same weight of wheat. Values in Table 4 show that there is 94 to 99% recovery of added phosphine from the empty flask.

The purpose of the third procedure was to recover that amount of phosphine residing in/on wheat when the sample is transferred from the fumigation vessel to another container. Here not only free phosphine in the headspace, but also that amount of loosely bound phosphine on the grain would escape as the grains are disturbed during transfer. Values in Table 5 indicate higher phosphine recovered at a higher dose compared with less recovered phosphine at a lower dose. Four μg phosphine was held in wheat at 600 μg dosage, while only 0.033 μg was held at 12 μg dosage of 10 g wheat. This trend again indicates that it has been possible to recover all the phosphine from wheat by the new method. Within the limits of phosphine-holding capacity of wheat, 10 g wheat would hold only a definite amount of phosphine under a given set of conditions, including the dosage as observed by the second and third procedures, and this amount of phosphine has been completely recovered by the new method. It has been reported by Berck and Gunther (19, 20) that the amount of phosphine held back by wheat seeds is directly related to moisture content of the seeds. Wheat seeds of the same moisture content were used in all these recovery experiments, so different amounts of phosphine held back by 10 g wheat appear to be directly related to added phosphine, as shown in Tables 4 and 5. It is also interesting to note that samples soaked for 1 h, when re-extracted with silver nitrate solution, did not produce even traces of characteristic color.

By the fourth procedure, attempts were made to completely recover the amount of phosphine residue in/on wheat by computing the headspace concentration and the amount of added phosphine remaining after absorption by glass, grease, etc. Values in Table 6 indicate almost 100% recovery of phosphine residues on wheat. Here again, the residue on wheat decreases as added phosphine decreases. At a dose of 0.95 mL (1.14 mg) phosphine, phosphine residue on 500 g wheat is 705 μg, while at dose of 0.75 mL (0.900 mg) is only 537 μg. The values in the parentheses (last column) indicate that about 62% of the available phosphine has been retained by 500 g wheat at all 3 doses tried. Recovery of added phosphine from the control flask was 95–98%. Because it is not possible to make a known weight of gaseous fumigant settle as residue on grain and recover it by another method, these

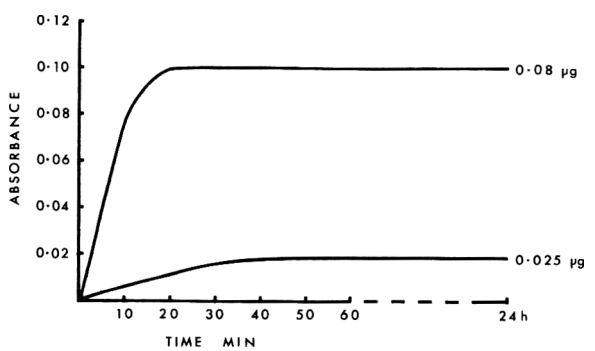

Figure 4. Stability of $AgNO_3$–PH_3 chromophore at PH_3 concentrations of 0.025 and 0.08 μg.

Table 3. Recovery of phosphine from wheat: first procedure

Injected, μg/100 μL air	Found, μg	Rec., %
0.024	0.0226 ± 0.003[a]	95
0.044	0.0393 ± 0.001	89
0.056	0.056 ± 0.0	100
0.066	0.0656 ± 0.0024	96

[a]Mean ± 10 replicate experiments.

Table 4. Recovery of phosphine from wheat: second procedure

Injected, μg	Found in control flask		Found, μg	
	μg	Rec., %	Fumigation 1	Fumigation 2
12	11.24 ± 0.55[a]	93.65	0.23 ± 0.0001[a]	0.195 ± 0.0002
30	29.60 ± 0.066	98.68	3.45 ± 0.0011	2.97 ± 0.011
60	59.92 ± 1.61	99.84	3.78 ± 0.0174	3.24 ± 0.011
240	215.70 ± 9.48	89.86	6.13 ± 0.011	6.29 ± 0.007
360	338.90 ± 3.45	94.14	14.00 ± 0.03	11.46 ± 0.007
600	591.20 ± 11.23	98.49	17.15 ± 0.02	16.21 ± 0.24

[a]Mean ± SD of 10 experiments.

Table 5. Recovery of phosphine from wheat: third procedure

Injected, μg	Found in control flask, μg	Found, μg
12	11.24 ± 2.37[a]	0.033 ± 0.0004
30	29.60 ± 2.22	0.550 ± 0.001
60	59.92 ± 5.36	0.637 ± 0.0002
240	215.70 ± 9.48	0.808 ± 0.009
360	338.90 ± 3.45	1.430 ± 0.001
600	591.20 ± 11.23	4.030 ± 0.0007

[a]Mean ± SD of 10 experiments.

Table 6. Computed recovery of phosphine from wheat: fourth procedure

Added, mL	Found in headspace, μg	Found in control flask		Found, μg
		μg	Rec., %	
0.95	419.60 ± 2.46	1125.00 ± 2.36[a]	98.68	705.40 (62.70)
0.80	358.66 ± 2.87	936.80 ± 2.98	97.63	578.14 (61.70)
0.75	318.00 ± 2.95	855.00 ± 3.06	95.00	537.00 (62.81)

[a]Mean ± SD of 6 replicates. Values in parentheses in last column indicate % uptake of available phosphine by wheat.

4 procedures indirectly establish good recoveries by the method developed.

In another trial, attempts were made to determine the phosphine residue on 10 g wheat by the second procedure at the doses shown by increasing the exposure time to 2 weeks instead of 24 h. Preliminary study indicated not much increase in phosphine residue. After 2 weeks exposure of 10 g wheat to 360 μg phosphine, amounts of phosphine retained as shown by recovery by the described method were 14.7 and 12.6 μg instead of 14.0 and 11.46 μg, respectively, for 24 h exposure time.

There is a clear difference between the color of the extract of fumigated wheat (50 L phosphine added to a 133 mL flask containing 10 g wheat) and that of the crop control. As time progresses after the first 2–3 h, the color of the chromophore remains steady while the color intensity due to crop extractives in crop control extract increases steadily.

As shown in Table 7, the repeatability, sensitivity, and soundness of the method compare favorably with that of the gas chromatographic method.

The purpose of carrying out the experimental fumigation studies was to determine whether the method developed could be applied for determining the total residue of phosphine. As aluminum phosphide powder was mixed with wheat, the residue that would arise from such fumigation is the sum of the residue recovered from unspent aluminum phosphide and that due to physically bound intact phosphine. The FAO/WHO tolerance level of phosphine in raw cereals, 0.1 ppm, is based on measurement of phosphine as the total of intact phosphine and that derived from any residual phosphides also present (21). In good fumigation practice, the aluminum phosphide tablet is covered in a paper pack and distributed in the stock to be fumigated so that the packs may be removed from the stocks after fumigation; however, the practice of mixing the aluminum phosphide tablets with the stock is still prevalent in some areas. In such cases, it is likely that a small percentage of the tablet mixed with grain becomes enveloped in paraffin wax and does not decompose readily during airing. The data in Table 1 show that the amount of total initial residue is directly related to the amount of unspent aluminum phosphide adhering to the sample. Residue levels of 1.38 and 0.10 ppm were found in samples from lots mixed with 533 and 256 mg phosphide, respectively, before airing. The residue leveled off to 0.021 ppm in both treatments by 3 days; thereafter, only detectable residue was observed in samples from the wheat treated with 533 mg phosphide, indicating that all unspent aluminum phosphide has been decomposed within 3 days. Increase in residue from 0.10 to 0.146 ppm after 2 h airing in samples of fumigation 2 is due only to more unspent phosphide powder adhering to the wheat seeds in the sample. By the sixth day of aeration, the residue fell below detectable limits. The remaining wheat was stored in a gas-tight container with a detector strip (22) placed on the wheat to detect traces of desorbed phosphine during storage. It has been observed that even after 30 days storage there was no change in the color of the detector strip, indicating that no phosphine was desorbed from wheat. The speed with which total residue has disappeared suggests either that not much of the free physically bound residue would arise by such mixing or that, due to larger levels of initial surface residue, initial desorption is very fast.

Table 7. Phosphine concentration (μg/mL) in headspace over wheat

Added, mL	This method	GC method
0.96	653.7 ± 1.88[a]	654.87 ± 2.12
1.04	707.4 ± 2.91	707.92 ± 3.29
1.25	849.0 ± 0.81	849.66 ± 5.54

[a]Mean ± SD of 6 replicate analyses.

Data presented in Table 2 represent the physically bound intact phosphine residue that would arise as a result of fumigation by using aluminum phosphide in paper pack. There is a close similarity in residue levels before airing in both fumigations. The fall of residue during airing is also regular and closely similar in samples of both trials for the first 3 days. By the eighth day, residue levels reached 0.007 and 0.005 ppm in wheat for fumigations 1 and 2, respectively. After 8 days of airing, the remaining wheat was stored in gas-tight containers with a detector strip placed on the grain. During the next 6 days there was a slow desorption of phosphine as the detector strip turned red (22); the area of the red band corresponded to 0.0165 and 0.0075 ppm in wheat of fumigations 1 and 2, respectively. On the 14th day, the stoppers of the containers were opened, 10 g samples were withdrawn for determination of the residue by the new method, which showed a level of 0.0195 and 0.009 ppm in the wheat of fumigations 1 and 2, respectively. Because the wheat was stored in gas-tight containers, the slowly desorbed phosphine, which would have otherwise escaped to the atmosphere in open pans, accumulated and was resorbed by the wheat, as indicated by the higher residue even after 14 days storage. The storage of the remaining wheat was continued for a further period of 30 days. Slow and regular development of the red band on the detector strip indicated a slow desorption of traces of phosphine. On the 23rd day, the residue levels by the strip method (22) corresponded to 0.004 and 0.001 ppm in wheat of fumigations 1 and 2, respectively. On that day, 10 g samples from both trials examined by the present method showed only a detectable color; increasing the soaking time to 24 h did not afford a measurable color, suggesting that the 10 g sample contained only a detectable amount of phosphine residue. It was possible to get a measurable characteristic color by increasing the sample size to 100 g and extracting with 150 mL $AgNO_3$ solution, but the extract of corresponding sample size crop control would not only have very high absorbance but also turbidity, which would vitiate the results. Although the residue was below the detection level of the new method, a slow desorption of phosphine from wheat was observed during storage for a further period of 15 days as indicated by detector strip.

The method can be used to determine as low as 0.001 ppm phosphine residue in wheat. Dumas (3) reported a desorbable residue level of 0.005 ppm in wheat after 17 days airing, using gas chromatography.

It was found by this method that residues of intact phosphine persist in wheat aired as in commercial practice for a considerable time; levels are higher in highly damaged and bad samples. Residue levels may also depend on the variety of wheat and also on the moisture content (18, 20). As evidenced by the residue data (Tables 1 and 2), the presence of an estimable amount of phosphine indicates that unchanged phosphine can remain in/on wheat in some loosely bound form for quite some time. Further evidence for such observations is visualized by its continuous evolution from the fumigated wheat, although in very small but detectable amounts. When such residue is left undisturbed during storage, part of it will be the source of nonvolatile phosphorus residues.

Sources of Errors and Limitations of Method

Leaky containers and syringes and turbidity were the only sources of error. It is imperative to use wheat of the same

variety and history, and preferably from the same lot as the crop control, otherwise the absorbance of the crop control would greatly affect the value of the residue. A sample size of more than 20 g is not advised because the high turbidity and absorbance of the crop control extract would invalidate residue value. Dilution of an extract with intense color by adding water to obtain an absorbance reading well within the Beer's Law limit would afford a lower final value compared to the absorbance within the Beer's Law curve, obtained without dilution of the extract of a smaller size sample.

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Validated Extraction and Cleanup Procedures for Polychlorinated Biphenyls and DDE in Human Body Fluids and Infant Formula

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As part of an epidemiology study, extraction methods and extract cleanup procedures were developed and validated for polychlorinated biphenyls (PCBs) and DDE, an ubiquitous metabolite of DDT, in human milk, blood serum, and infant formula. Studies included quantitative and reproducible recovery of total lipids, and reproducible and reasonably high recoveries of these chlorinated compounds from the human body fluids and infant formula, including levels of environmental health interest. An extensive quality control and assurance program was designed for use with these methods. Some validation work on serum was done using radiolabeled ^{14}C-Aroclor 1254. Dilution assays were developed to permit use of a constant procedure, which should minimize variability in results. Methods are based on selected organic solvent extraction and column chromatographic cleanup techniques and quantitation by electron capture gas chromatography (EC/GC). Using these extensively researched extraction and cleanup methods, the limits of detection for GC measurements were 10.0 and 2.00 ppb for PCBs and DDE, respectively, in milk and 4.00 and 0.80 ppb in serum.

2,2(4-Chlorophenyl)-1,1-dichloroethene (DDE), which is a metabolite of 2,2(4-chlorophenyl)-1,1,1-trichloroethane (DDT), and the industrial chemicals polychlorinated biphenyls (PCBs) are more soluble in fat than in aqueous systems. As a result they have become concentrated in the fatty tissues of birds, fish, and mammals. This process is known as biomagnification, or the food-chain concentration effect. Magnification factors of many thousands are common between water and fatty tissues. The effects of these chlorinated hydrocarbons on humans are not established, and there is grave concern about the potential health hazards of their entering the human food chain and becoming concentrated in the human body (1).

The determination of chemical residues such as PCBs in biological samples requires a rigorous sampling protocol to avoid sample contamination and loss of sample integrity. Thoroughly research methods are needed for extracting the residues of interest from the bulk sample, for removing co-extractables that can interfere in measurement, and for qualitative and quantitative determinations of the chemical residues of interest. Also, a quality assurance program (2) is necessary to ensure that when the method is applied to multiple samples, possibly in different laboratories, the results will be valid, accurate, and reliable. Sample extraction and cleanup are 2 of the most important steps for ensuring the reliability of the overall process. Studies generally involve the use of spikes or sample fortification with authentic chemical standards to measure recoveries through the extraction and cleanup steps and provide a basis on which to make quantitative estimates (3). It must be shown that the spiked chemicals equilibrate with the corresponding endogenous chemicals, or it must be empirically demonstrated that the recovery of exogenous spike is the same as the recovery of endogenous compound, over the full range of concentration levels. The dependence of both percentage recovery and its standard deviation on concentration must be determined and reported. Before unknown samples are analyzed, all proce-

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dures should be validated in terms of recovery, reproducibility, sensitivity, freedom from interference, and accuracy. Most important, the sample work-up procedures need to be validated for recovery, reproducibility, sensitivity, and freedom from interference (4). All of these critical steps must be described in sufficient detail to permit duplication by other analysts in other laboratories if the method is to be truly reliable.

This work describes the extensive validation efforts that were applied to human milk and blood serum and to formula to select or develop extraction methods that were adequately characterized for quantitative dependence of recoveries on analyte concentrations in the samples, and cleanup procedures that would effectively minimize gas chromatographic (GC) interferences while still providing compatibility with the need to process large numbers of samples rapidly. Studies included quantitative and reproducible recovery of total lipids and reproducible and reasonably high recoveries of PCBs and DDE from the samples. Both DDE (as an index of exposure to DDT) and polybrominated biphenyls (PBBs) were also of interest. Because these types of compounds are known to accumulate in fatty tissues, the methods proposed for extraction of breast milk and formula were first compared with other methods (5) to determine which procedure extracted lipids and pesticides most efficiently.

Quantitation methods for PCBs have been largely empirical because of the inability of the column to separate PCB mixtures into their individual components. Peak-by-peak methods (6, 7) have been devised which appear to be superior to earlier, more empirical methods; however, empirical procedures (8) continue to be in common use.

Experimental

Chemical and Reagents

(a) *Deionized water.*—Passed through Millipore filter system; each gallon of filtered water was shaken with 250 mL hexane (hexane was discarded).

(b) *Solvents.*—Hexane, methanol, isooctane, petroleum ether (low boiling), and acetone were distilled-in-glass quality obtained from Burdick & Jackson (Muskegon, MI).

(c) *Reagent alcohol.*—Ethanol–methanol–isopropanol (90 + 5 + 5), Fisher No. A-962 (Fisher Scientific Co., Pittsburgh, PA).

(d) *Anhydrous, granular sodium sulfate.*—10–15 lb was packed in 150 × 7 cm column with fritted glass disc, rinsed with ½ gal. petroleum ether and 3 lb ethyl ether, dried in hood overnight, then further dried in 130°C oven ca 24 h.

(e) *Florisil.*—60–100 mesh, PR grade, obtained from Floridin Co. (Berkeley Springs, WV), activated, and stored at 130°C. Elution patterns of standards were determined for each new lot and/or every 2 weeks, and elution volumes were adjusted as necessary.

Apparatus

(a) *Culture tubes.*—16 × 125 mm, 16 × 150 mm, and 25 × 150 mm, washed, and then rinsed with acetone and ethyl ether.

(b) *Glass wool.*—Each ½ lb was rinsed with 1 gal. petroleum ether and 3 lb ethyl ether. A portion of rinsed glass wool was rinsed with 100 mL petroleum ether which was concentrated to 10 mL, then injected into an electron capture gas chromatograph to check for interferences.

(c) *Squares of aluminum foil.*—Rinsed with petroleum ether. Dull side of foil was placed against sample (9).

(d) *Chromatographic columns.*—*(1)* 180 × 6 mm, with fritted glass disc in tip, and a 55 mL reservoir. *(2)* 180 × 10 mm, with fritted glass disc in tip, Teflon stopcock, and 90 mL reservoir (custom-made at Raltech Scientific Services).

Gas Chromatographic Conditions

Aliquots of reagent blanks, controls, spiked samples, and unknown samples were injected onto 2 gas chromatographs along with appropriate standards. An Aroclor 1254 standard was injected every 10–12 samples and a DDE standard was injected once per group of samples on instruments for PCB determination; on instruments for DDE determination, the number of standards was reversed. In addition, various concentrations of PCBs and DDE were prepared, and these standards were injected along with those generally used to quantitate results to check linearity of the detectors. Every 2–3 weeks, a mixed pesticide standard of lindane, heptachlor, aldrin, heptachlor epoxide, DDE, dieldrin, endrin, DDD, and DDT was run on both chromatographic systems, as were standards of hexachlorobenzene, hexachlorocyclohexane, oxychlordane, *o,p'*-DDT, chlordane, mirex, methoxychlor, toxaphene, β-nonachlor, Aroclor 1254, and Aroclor 1260, for future identification of any possible additional peaks discovered in the sample chromatograms. All standards and samples were prepared in isooctane to minimize problems of solvent evaporation.

The automatic sampling system for gas chromatographs used for DDE determinations was programmed to rinse injection syringe between sample injections to avoid carryover of DDE in high concentrations. PCBs and pesticides, such as DDE, were analyzed on Hewlett-Packard 5710A gas chromatographs equipped with 7671A or 7672A automatic samplers and ^{63}Ni electron capture detectors. Analyses for PBBs were performed on a Tracor 560 gas chromatograph equipped with a ^{63}Ni electron capture detector. Specific gas chromatographic conditions are given in Table 1.

Sample Processing

Milk and formula.—Properly collected samples (to be described elsewhere) from hospitals were shipped frozen to the analyzing laboratory. As soon as possible after arrival, samples were thawed overnight under refrigeration or immediately on ice. When necessary, these samples were sonicated until homogeneous, using a needle-tipped sonicator probe or a sonicator bath containing ice water. The probe was found to be effective, particularly for formula samples.

A 10 g sample was weighed into a 20 mL vial (if 10 g was not available, as large a sample as possible was weighed and analyzed as described under *Dilution Assay-Milk*). The vial was sealed with foil and a screw cap, and refrozen.

Serum.—Serum was thawed as described above. Samples were homogenized on a vortex mixer. If fibrin clots were present, the serum was centrifuged and only the supernatant was used. Five g serum was weighed into a 16 × 125 mm culture tube (if 5 g was not available, as large as sample as possible was weighed and analyzed as described under *Dilution Assay-Serum*). The tube was sealed with foil and a Teflon-lined screw cap, and refrozen.

Extraction and Cleanup

Milk and formula.—A modification of the AOAC hexane–ethanol method (**16.059**, ref. 10) was used. The processed and refrozen milk samples were thawed in the refrigerator overnight and were shaken to ensure homogeneity or sonicated if difficult emulsions formed. Controls were spiked with the analytes for recovery checks as needed. This milk was

Table 1. Gas chromatographic conditions for PCB, DDE, and PBB analysis

Analyte	Column[a]	Temperature, °C			Carrier gas flow rate,[b] mL/min
		Column	Inj. post	Detector	
PCBs	3% OV-1 on 80–100 mesh Supelcoport	200	250	300	34
DDE	1.5% SP2250/1.95% SP2401 on 100–120 mesh Supelcoport	200	250	300	34
PBBs	1% OV-101 on 80–100 mesh Supelcoport	220	250	350	63

[a]6 ft × 4 mm id glass, except for PBBs (2 ft glass).
[b]Carrier gas was mixture of argon–methane (95 + 5). When additional gas chromatographs were set up to run additional samples or new columns were packed, carrier gas flow was adjusted so *p,p'*-DDE had same retention time as on original chromatograph (±0.2–0.3 min).

transferred to a 45 mL vial with a foil-lined screw cap. Potassium oxalate (0.1 g) was added and the vial was swirled gently. Ten mL of 95% ethanol was added to the original 20 mL vial, which was then capped with a foil-lined screw top and shaken well. The contents were transferred to the 45 mL vial, which was capped and shaken manually for 30 s. Ten mL hexane–ether (1 + 1 v/v) was added to the 20 mL vial. The vial was capped, shaken well, and its contents were transferred to the 45 mL vial. The vial was covered and shaken manually for 1 min. Phases separated after the vial was centrifuged for approximately 10 min at 1000 rpm. If an emulsion formed which was not broken by centrifugation, sonication in a water bath and recentrifugation were necessary. The upper layer was transferred, using a disposable pipet, to a 125 mL separatory funnel containing 50 mL water and 3 mL saturated sodium chloride solution. The aqueous residue in the 45 mL vial was extracted twice more with 5 mL hexane–ethyl ether. Sonication and recentrifugation were used if necessary. The resulting organic extract phases were added to the separatory funnel. The combined extracts were mixed slowly with water and the phases were allowed to separate. The lower layer was discarded. The extract was washed twice more with 10 mL water. If emulsions formed, 2–3 mL saturated sodium chloride solution was added to the solvent layer.

A tightly packed 6 mm column with a fritted glass disc containing 6 g ether-rinsed sodium sulfate was prepared. Above this was a 58 mm diameter glass powder funnel with a rinsed glass wool plug containing 10 g ether-rinsed sodium sulfate. The organic phase was poured into the funnel and the eluate was collected in a 25 × 150 mm culture tube. The separatory funnel was rinsed twice with 5 mL hexane–ethyl ether which was added to the funnel. The funnel and column were rinsed with hexane–ethyl ether until 35–40 mL was collected. The column eluate was clear and was dried to 2–3 mL under a gentle stream of nitrogen in a 30–35°C water bath, and the residue was transferred to a 10 mL volumetric flask. The culture tube was rinsed with small quantities of hexane which were transferred to the 10 mL flask. The volume was adjusted to 10 mL with hexane.

For lipid analysis, a 5 mL aliquot was pipetted into an 8 mL vial which was at constant weight following heating in an 80°C oven and cooling in a desiccator. The solvent was evaporated from the sample vial under nitrogen, and the vial was placed successively in 40 and 80°C ovens until it reached a constant weight. The weight obtained was corrected for the aliquot taken and divided by the weight of the sample to obtain the percentage of lipid.

A micro-Florisil column of 4.0 g was wet-packed with hexane and topped with 1 cm ether-rinsed sodium sulfate. At least 25 mL hexane was allowed to flow through the system before sample application. Two mL lipid extract was applied to the column and rinsed in with 2 mL hexane. The column eluate was collected in a 50 mL graduated centrifuge tube into which 2 mL isooctane was added by pipet. Fraction one was eluted with 28 mL 1% methanol–hexane. A second fraction (20 mL 1% methanol–ethyl ether) was collected in a vial containing 2 mL isooctane. The first fraction was concentrated to 2 mL under nitrogen in a 30–35°C water bath. Necessary dilutions were made with isooctane. The second fraction was saved for future studies, stored at −20°C.

Dilution assay—milk.—Milk samples less than 10 g were analyzed by proportionately scaling down all reagents in the triple extraction procedure to the point where the eluate from the sodium sulfate column was transferred to a volumetric flask. These samples were diluted to 10 mL in petroleum ether. Additional samples weighing less than 10 g were diluted to 10 g weight with purified water (the water was monitored before use for possible interference in the analysis). Reagents used with the diluted samples were in proportions for the 10 g samples. Lipid content was determined using the hexane–ether–ethanol extraction method.

Serum.—Serum samples were thawed in the refrigerator overnight and homogenized on a vortex mixer. Controls were spiked with analytes for recovery checks as needed. Five mL methyl alcohol was added to the sample, which was vortex-mixed for 1 min. Five mL hexane–ethyl ether (1 + 1 v/v) was added to the culture tube, which was capped tightly and shaken at high speed on a horizontal shaker for 10–15 min. Phases in the tube separated after centrifuging for approximately 5 min at 2000 rpm. If an emulsion formed, the sample was sonicated and recentrifuged. The upper phase was transferred to a 16 × 150 mm culture tube. The aqueous residue was extracted twice more with 5 mL quantities of hexane–ethyl ether (1 + 1) by shaking at high speed on a horizontal shaker and recentrifuging. After removing the last extract, any remaining emulsion was sonicated and recentrifuged. Any resulting organic phase was transferred to the culture tube and the combined extract was concentrated to 1 mL under nitrogen in a 30–35° water bath. The concentrate was passed through a sodium sulfate column as in the milk and formula extraction (but without the funnel containing sodium sulfate), and collected in a 16 × 150 mm culture tube. The original tube was rinsed 3 times with 2 mL hexane–ethyl ether and the column was rinsed until 20–25 mL was collected. The sample was concentrated to 1 mL under nitrogen in a 30–35°C water bath and passed through a 4.0 g Florisil column as in the previous extraction. Two fractions were again collected.

Dilution assay—serum.—Serum samples of less than 5 g were analyzed by proportionately scaling down reagents used in the triple extraction procedure. Additional samples weighing less than 5 g were brought to 5 g with water previously checked for possible interference. Reagent quantities for the diluted samples were the same as those for a 5 g sample. Samples were analyzed using the hexane–ether–methanol extraction method.

Comparative Analyses of Fat in Milk and Formula

Modified Roese–Gottlieb extraction method for milk.—The milk sample was weighed into a tared 50 mL beaker. The

weighed sample was prepared for extraction by diluting it to a total volume of approximately 10 mL with deionized water. Three mL concentrated ammonium hydroxide was added, and the mixture was stirred and transferred to a Mojonnier tube. The beaker was washed once with 25 mL ethyl ether which was added to the Mojonnier tube. A 25 mL portion of hexane was added to the 50 mL beaker while the Mojonnier tube was stoppered and shaken for 30 s. Five drops of phenolphthalein indicator solution (1% in ethanol) was carefully added to the Mojonnier tube. The 25 mL hexane portion was transferred form the beaker to the Mojonnier tube, which was stoppered and shaken for 30 s. The sample was centrifuged for 15 s at 700 rpm, and then the extract was poured off into a dry, tared 150 mL beaker.

The 50 mL beaker and Mojonnier tube were washed a second time, first with ether, then with hexane, and the extraction was repeated. After recentrifugation, deionized water was added to the Mojonnier tube; the aqueous portion was just at the top of the narrow bend. The total ether–hexane portion was poured into the 150 mL beaker with the first portion. The solvents were evaported from the beaker overnight under a fan-vented fume hood. The remaining solvent-extracted fat was dried to constant weight at 105°C.

Acidic assay method for formula.—Formula samples were incompatible with the Roese–Gottlieb method and a new assay was developed. The sample was weighed into a 50 mL tared beaker and 2 mL reagent alcohol mixture and 10 mL hydrochloric acid (25 parts concentrated acid diluted with 1[illegible] parts deionized water) were added. The sample was heated on a water bath at 75–85°C for 30 min and stirred occasionally until totally clarified. Eight mL reagent grade alcohol was added to the hot sample and the mixture was stirred. The homogeneous sample was transferred to a Mojonnier tube. The sample was extracted as in the modified Roese–Gottlieb method with ether and hexane (2 extractions), and the extracts were combined in a 150 mL beaker.

Seventy mL fat-washing solution (450 mL 0.04N sodium hydroxide plus 450 mL reagent alcohol) was dispensed into a 500 mL separatory funnel. The fat extract was transferred into the funnel which was stoppered and shaken 4 or 5 times. The glass stopper and funnel neck were rinsed down into the funnel by using hexane. The sample completely separated after 10–15 min and the used fat-washing solution was drained off. The washed extract was drained back into the 150 mL beaker and the separatory funnel was rinsed with hexane. Solvents were evaporated from the beaker overnight under a fan-vented fume hood. The fat remaining in the beaker was dried to constant weight at 105°C.

Spiking Conditions

A series of stock solutions of PCB (Aroclor 1254), *p,p'*-DDE, and PBB (Firemaster FF-1) were used with dimethyl sulfoxide (DMSO) as the solvent. This procedure (3) has been shown to give a uniform, known (predictable) level of PCB incorporation suitable for providing a set of quality control samples. Standards were all serial dilutions of 3 stock solutions. Kirk micropipets were used for spiking samples. The reliability of the spiking procedure was checked in a preliminary study with pooled raw cow's milk. The pool was mixed well to ensure homogeneity, and 10 g samples were weighed and frozen until the analyses were performed. Samples were extracted according to the modified hexane–ether–ethanol procedure to determine levels of PCB, DDE, and PBB (spiked daily).

Lipid Analysis

Lipid content was also determined in the preliminary study on pooled raw cow's milk by extraction according to the modified hexane–ether–ethanol procedure. Additional samples were analyzed for lipid content by a modified Roese–Gottlieb method.

Lipid recovery values were also determined for 3 formulas: Enfamil®, Isomil®, and Similac® (all concentrates fortified with approximately 24 mg iron/L). These formulas were diluted 50:50 with purified water according to label directions. The samples were then analyzed for lipid content following hexane–ether–ethanol triple extraction and by the previously described acidic assay.

Quality Assurance Validation

Control pools of breast milk and blood serum were each mixed well to ensure homogeneity. Portions were retained as control material and other portions were spiked as pools according to the following schedule: for milk, 1 of 5 levels of DDE or PCB (50, 100, 200, 500, and 1000 ppb), 2 of the DDE levels containing 10 or 200 ppb PBB; for serum, 1 of 5 levels of DDE or PCB (10, 20, 50, 100, 200 ppb), 2 of the DDE levels containing 20 or 400 ppb PBB. Aliquots from each pool were weighed and frozen until analysis.

For breask milk validation, 2 groups of 10 control samples were analyzed initially; for blood serum validation, one group of 20 control samples was analyzed initially. Thereafter, for each matrix, groups of 10 samples, one from each spiking level, were analyzed. Spiked samples and reagent blanks were run with each set of analyses.

Data Analysis

Empirical quantitation methods based on packed GC columns were used in this work because we were primarily interested in relative PCB levels for comparison, and there was a need to minimize sample handling because large numbers of samples were to be processed for epidemiological purposes. In PBB-spiked samples, the peak height of the major component in the gas chromatograms was used to determine levels of PBBs. The heights of 2 peaks (designated 125 and 146) were combined for PCB estimation (Figure 1). This designation was based on assigning a retention time of 100 to *p,p'*-DDE. This choice was due to an apparent absence of non-PCB interference at these elution positions and the fact that the use of multiple peaks did not appreciably change the results. The amounts of detected compounds were corrected for various dilutions and divided by wet sample weights and/or lipid weights to express concentrations in parts per billion and/or parts per million. Levels of the compounds were corrected by using the mean percent accountability values determined as explained below. These accountability values, which were used to continuously monitor recoveries and drifts in detector response, are simply an expression of refined recoveries which account for background PCB levels and drifts in detector response. The latter problem could be of some importance also in the analysis of unknown residues.

The mean percent accountability factors used to correct the DDE and PCB values were determined in the following manner: All sample analytical runs included spiked samples. All matrices, with the exception of formula, were spiked at the levels used for quality assurance validation work. The first 5 times a matrix was analyzed, 4 spikes were included (2 DDE and 2 PCB) in addition to other assay control materials and samples. An accountability value was determined by subtracting the baseline control value (parts per billion) from the level determined for the spiked sample (parts per

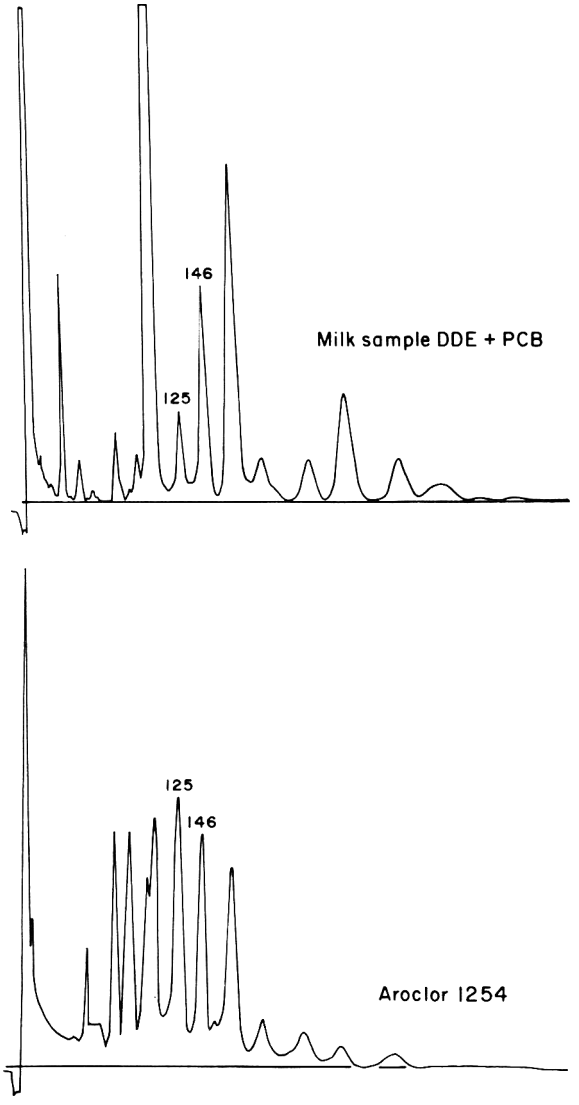

Figure 1. Gas chromatograms of PCB (Aroclor 1254) in standard mixture and human milk. GC conditions: 6 ft × 4 mm id column of 3% OV-1 at 220°C. Peak off-scale is DDE.

billion) and dividing by the spiking level for the sample used. After 10 accountability values had been determined for PCBs and DDE (2 values at each of the 5 spiking levels), these values were averaged and the resulting mean percent control samples was analyzed initially. Thereafter, for each matrix, the accountability factor was used to correct the values for PCBs and DDE in those assay runs used to generate the accountability factors. Each compound has its own set of accountability values and these values must lie between 60 and 120% to be acceptable. In succeeding assay runs, the spike alternated between PCBs and DDE, and rotated among the 5 spiking levels for each compound (see below). As each new accountability value was determined, it replaced a previously determined value for that compound and spiking level. Accountability values were monitored to ensure that accountability factors were not dependent on spiking levels. The values for control samples, quality assurance blind samples, and reagent blanks also helped determine if the assay run was valid.

Radiolabeled Spiked Serum Samples

A labeled PCB mixture was used to provide an accurate measure of the dependence of recovery on concentration in the sample. This study also provided a check on possible losses during the work-up procedure, such as the nitrogen concentration step. Human serum samples were spiked with approximately 20 000 dpm uniformly labeled ^{14}C-PCB (54% Cl) in 25 μL DMSO/5 g sample. These serum samples were also spiked with the quantities (duplicate samples at 0, 10, 20, 50, 100, 200 pbb) of unlabeled Aroclor 1254 along with reagent blanks.

Aliquots of the samples were weighed into Packard scintillation vials at several steps in the overall extraction and cleanup procedure, including the initial spiked serum, the aqueous phase and organic extract, material loaded on the column for cleanup, fractions 1 and 2 from the column, and the solution for GC analysis. Ten mL Packard INSTA-GEL was added and the samples were counted on a Packard Tri-Carb liquid scintillation counter (Model 2420) at the standard conditions for carbon-14 analysis. Samples were counted for 4000 counts or 10 min. A carbon-14 quench curve was also run. The paper tape counter output was processed to yield disintegrations per minute by using a Hewlett-Packard 3354 data system. The relationship between the external standard ratio and the counting efficiency was fitted to a second order equation to calculate counting efficiency for the samples.

Results and Discussion

Throughout this work considerable preliminary validation data were obtained using raw cow's milk. Those data will be documented in one or more internal publications for reference purposes. In this paper, only data from work with human milk or milk substitutes are described.

Milk Study

Several solvent systems were studied in validating the extraction procedure. These included hexane–acetone, petroleum ether–acetone, hexane–ethanol, hexane–isopropyl alcohol, benzene–methanol, and a modified version of the standard hexane–ethanol procedure, AOAC method **16.059.** The modified version, based on hexane–ether–ethanol extraction, was the only method that proved satisfactory with both lipid extractability and reproducibility of results. Reproducibility and reliability data from the modified hexane–ether–ethanol triple extraction method are presented in Table 2. Table 3 compares the lipid values obtained by the modified Roese–Gottlieb method with the modified triple extraction method. Table 4 summarizes PCB, PBB, DDE, and lipid data from the quality assurance work on human breast milk.

Table 2. Summary of reproducibility and reliability data for milk—modified hexane–ether–ethanol triple extraction method

	Found,[a] ppb				
Added	PBB	DDE	PCB	Std dev.	CV, %
Control	—	—	—	—	—
20 ppb PCB	—	—	20.3	4.93	24.3
40 ppb PCB	—	—	41.3	5.63	13.6
20 ppb DDE	—	16.1	—	2.23	13.8
50 ppb DDE	—	53.9	—	4.30	7.98
40 ppb PBB	28.7	—	—	6.93	24.2
100 ppb PBB	78.5	—	—	13.6	17.4

[a]Each value is the mean for 6 samples.

Use of trade names is for identification only and does not constitute endorsement by the Department of Health and Human Services.

Table 3. Comparison of modified triple extraction method with Roese-Gottlieb method for lipids in milk

Method	N	Mean % lipid[a]	Std dev.	CV, %
Modified hexane–ether–ethanol	30	3.47	0.14	4.06
Roese-Gottlieb	6	3.61	0.02	0.60

[a] $t = 2.417$, $P < 0.025$.

The data from the modified hexane–ether–ethanol procedure indicate that this method produces consistent lipid values that compare well with those from the Roese-Gottlieb method, which is a widely accepted lipid analysis method for this type of sample. Although a 2-tailed *t*-test showed that means were significantly different at the 0.05 confidence level, this may be due to method differences. The reproducibility of the lipid data indicates that the extraction portion of the procedure is well defined.

The data from the milk dilution assay study indicate, using a 2-way analysis of variance with the sample weight (2,5,7 or 10 g) as one factor and diluted or not as the other, that the dilution did not significantly affect ($P \leq 0.05$) lipid recovery. Somewhat lower values for the undiluted 2 g samples may have been due to difficulty in removing the small quantities of organic solvent from the small quantities of undiluted milk being extracted. When samples are diluted, a constant procedure can be applied, which should minimize variability.

Some data (Table 4) for these compounds show more variation due to GC variability. One consideration is the low levels of pesticides for which samples were assayed. The coefficient of variation was lower for each highly spiked level, indicating more reliable results when higher levels of compounds were present. Some small differences in recoveries could be due to a difference in solvent systems between GC standards (isooctane) and spiking standards (DMSO), although solubility should not be too great a factor at the levels used in the study. This was shown to be a minor consideration by preparing GC standards from the spiking standards and demonstrating nearly equal responses on the gas chromatographs for the same concentrations of pesticides. The large variation in the PBB data is explained by the increased difficulty in extraction and cleanup (9). The data obtained from the extensive breast milk quality assurance study demonstrate the ability of the analysts and the method to obtain reproducible results on a day-to-day basis. All coefficient of variation (CV) values were below 12% except for the low PBB spike level (15%); most values were less than 10%. These values were generated even though only one sample from each spiking level was analyzed with each analytical run.

Infant Formula Study

Summary data for the infant formula study are presented in Table 5. The acidic determination method yields results closest to product label claims. Although an analysis of variance indicated a highly significant difference ($P \leq 0.01$) between the methods, the hexane–ether–ethanol extractions produced relatively consistent results that were, however, low in all cases. About 85% of the label claim was recovered for Enfamil, while about 90% of the label claim was recovered for the other 2 formulas. Therefore, reproducible extraction of lipid in the formulas is possible using the method validated for breast milk, although results are not quantitative.

The major problem in analyzing infant formulas that have a soy protein base is formation of emulsions during extractions with organic solvents. Of the 3 formulas tested, Enfamil and Similac formed emulsions which did not break when

Table 4. Breast milk quality assurance study—data summary[a]

Added	No. of samples	Mean	Std dev.	Std error	CV, %	Range of values
50 ppb DDE	10	106	3.96	1.25	3.73	96.2–110
	10	2.96	0.0922	0.0292	3.11	2.79–3.09
10 ppb PBB	10	8.01	1.22	0.386	15.3	6.37–10.1
	10	0.224	0.0363	0.0115	16.2	0.172–0.280
100 ppb DDE	10	150	8.71	2.76	5.81	131–159
	10	4.21	0.186	0.0587	4.41	3.91–4.45
200 ppb DDE	10	228	12.2	3.86	5.36	204–242
	10	6.35	0.349	0.110	5.50	5.98–7.10
500 ppb DDE	10	509	52.7	16.7	10.4	442–598
	10	14.2	1.20	0.378	8.46	12.4–16.5
1000 ppb DDE	10	942	106	33.4	11.2	838–1199
	10	26.1	2.59	0.818	9.89	23.1–32.5
200 ppb PBB	10	191	18.0	5.70	9.44	161–215
	10	5.30	0.413	0.131	7.79	4.48–5.82
50 ppb PCB	10	84.9	7.77	2.46	9.15	75.7–97.4
	10	2.38	0.191	0.060	8.03	2.15–2.65
100 ppb PCB	10	122	5.90	1.87	484	114–131
	10	3.40	0.147	0.0466	4.33	3.11–3.65
200 ppb PCB	10	212	15.4	4.88	7.28	182–232
	10	6.04	0.373	0.118	6.17	5.45–6.49
500 ppb PCB	10	494	23.7	7.50	4.79	447–521
	10	13.6	0.428	0.135	3.13	12.8–14.1
1000 ppb PCB	10	939	58.9	18.6	6.28	830–1047
	10	26.1	1.40	0.443	5.36	23.7–28.6
Control DDE	19	69.12	8.16	1.87	11.8	53.4–82.8
	19	1.91	0.191	0.0439	10.0	1.58–2.33
PCB	18	47.1	4.48	1.06	9.51	37.4–58.0
	18	1.30	0.110	0.0258	8.42	1.13–1.53
All samples lipid	118	3.59	0.350	0.0322	9.75	3.19–3.79

[a] The first line for each compound summarizes the data on a whole milk basis (ppb); the second line summarizes the data on a lipid basis (ppm). Not corrected for endogenous levels of compounds.

Table 5. Infant formula study—data summary for lipid determinations[a]

	Lipid, %		
	Enfamil	Isomil	Similac
Label claim	7.5	7.2	7.22
Hexane–ether–ethanol triple extraction	6.41, 6.38	6.68, 6.34	6.53, 6.51
Acidic determination	7.22, 7.24	7.24, 7.12	7.10, 7.18

[a]All assays were performed on formulas diluted 50:50 with water. Data presented here have been doubled for comparison with the label claim for undiluted formula. The basis of the label claim is not known; other analyses were performed in duplicate.

centrifuged. Heating and/or freezing samples had little effect on the emulsions, and the addition of sodium sulfate often compounded the problem. However, sonicating samples in a water bath, followed by centrifuging, appeared to eliminate emulsions. It may be necessary to use this technique at any or all of the extraction steps in the hexane–ether–ethanol triple extraction method.

Another possible problem with the method is that the solution of lipid in 10 mL petroleum ether (or hexane) was not entirely homogeneous; some fluffy-looking material, presumably lipid, settled out of solution. The aliquots for lipid analysis were taken immediately after vigorously shaking the flask to obtain the most homogeneous sample possible. After drying, this material had the same color and consistency as the lipid material obtained from cow's milk.

Serum Study

Table 6 summarizes PCB, PBB, and DDE data from the most extensive validation study. Recovery values for the 10 ppb spiking levels for both Aroclor 1254 and DDE were only about 77%, which may be due to the low levels of these compounds. For spiking levels between 20 and 200 ppb, however, the recovery values for samples spiked with DDE ranged from 90 to 100% with no apparent relation between level and recovery. The recovery values for PCBs over this same range varied from 75 to 90%, also with no apparent pattern. Since the control pool of serum contained detectable levels of these compounds, recoveries were based on extracting the spikes added in DMSO and correcting for the compounds already in the serum. This could account for some of the increased variability in the data obtained, due to the problem of whether the recovery value of an added spike is representative of the recovery of the compound contained in the sample (3).

The data obtained for the extensive blood serum validation study demonstrate that the analysis procedure is reproducible as evidenced by the low CV values for DDE and PCB. All are below 10% and most are substantially lower than 10%. These values were generated even though only one sample from each spiking level was analyzed with each analytical run. However, the CV values for PBB levels are higher than desired. The low-level (20 ppb) results do indicate considerable variation; however, in the high-level group, the elimination of the highest value drops the CV to 8.01% and the mean to 326 ppb.

The ^{14}C-Aroclor 1254 spiking curve study indicated that recovery results do not depend on the quantity of PCBs present in the sample. The total radioactivity recovery values for the serum samples ranged from 90 to 100% for the entire procedure including the aqueous phase and subsequent fractions from the column. The results show that recovery is not dependent on spiking level. Recovery values for each step were never lower than 92%. Results again were corrected for sample weights taken and percentages of samples carried on to subsequent steps. The results indicated that from 1 to 3% of the PCBs remained in the aqueous residue of the serum and 1.5–6% were recovered in fraction 2 from the Florisil column. GC results were calculated by determining a parts per billion PCB level for the control serum spiked only with the ^{14}C-PCB (54%) and subtracting this value (15.2) from the level determined for the samples spiked with unlabeled Aroclor 1254. The resulting value was divided by the nominal spiking level to determine a percent recovery.

The data from the serum dilution study indicate that at the level of compounds (PCB or DDE) in the control pool (about 10 ppb) no difference is evident in the recovery between samples (2, 3, 4, or 5 g) that have been diluted in water to 5 g and those that were run by scaling the reagents proportionately to the sample size. Again, dilution is technically advantageous.

A number of investigations (11–18) have been made on PCBs in human milk. Mes et al. (19) were also concerned about the variation of PCB levels in human milk and its possible association with the analytical methodology. Using the extraction and cleanup methods described in this paper it is possible to make reproducible and reliable determinations of PCBs and DDE residues in human milk and blood serum at levels of interest from an environmental health viewpoint. In this regard, sample matrix interferences have been mini-

Table 6. Blood serum quality assurance study—data summary[a]

Added	No. of samples	Mean	Std. dev.	Std error	CV, %	Range of values
10 ppb DDE	10	18.9	0.806	0.255	4.27	17.2–20.0
20 ppb PBB	10	14.7	2.75	0.870	18.7	10.6–19.2
20 ppb DDE	10	26.3	1.49	0.470	5.65	23.7–28.5
50 ppb DDE	10	45.7	3.04	0.962	6.66	39.1–49.9
100 ppb DDE	10	103	7.49	2.37	7.27	89.5–113
200 ppb DDE	10	195	17.3	5.48	8.90	172–224
400 ppb PBB	10	343	59.8	18.9	17.4	294–499
10 ppb PCB	10	15.0	0.525	0.166	3.50	14.0–16.0
20 ppb PCB	10	23.1	0.943	0.298	4.07	21.8–24.7
50 ppb PCB	10	45.2	3.08	0.975	6.82	40.9–49.8
100 ppb PCB	10	75.6	3.78	1.20	5.00	70.0–80.8
200 ppb PCB	10	144	11.6	3.66	8.05	127–167
Control DDE	20	9.29	0.511	0.114	5.50	8.26–10.4
PCB	20	8.16	0.758	0.170	9.29	6.98–10.5

[a]Data are expressed on a whole serum basis (ppb). Not corrected for endogenous levels of compounds.

mized, and it is felt that the methodology should be easily transferable from one laboratory and analyst to another if the materials and methods are similarly controlled.

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Retention Indexes for Electron Capture Gas Chromatography on Programmed Temperature High Resolution Capillary Column

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A simple method and a series of compounds for a retention indexing study on a linear programmed capillary gas chromatograph are described. References are chlorobenzenes and decachlorobiphenyl, which show the same behavior in this system as Kovat's references (*n*-alkanes). This retention index system and gas chromatograph with cryogenic coolant apparatus and electron capture detection, is suitable for measuring the retention index of various compounds with characteristic detection ranging from low volatility to high molecular weight.

Since Kovats' Index was reported (1), this system has long been used for the registry of compounds separated by gas chromatography with flame ionization or mass spectrometry detection. Van Den Dool and Krats (2) devised a linear temperature programmed system on gas chromatography (LTPGC) with polyaromatic standards (Lee's reference), and successfully evaluated various types of PAHs in oil, coal, and environmental samples together with sulfur- and nitrogen-containing polycyclic aromatic hydrocarbons (3–6). However, *n*-paraffins (Kovat's reference) and PAHs (Lee's reference) are not suitable for compounds detected by electron capture detectors. ECD capillary analyses have become more popular and are being widely used and developed in the field of environmental GC analyses (7).

The electron capture detector is highly sensitive to many compounds found in environmental samples monitored on capillary GC. However, many are left as unknown components because of difficulty with GC-MS confirmation.

In the ECD-GC Index, typical pesticide homologs such as *p,p'*-DDE and aldrin, which usually have appropriate retention times, are often used as standards for compounds involved in pesticide and environmental health studies (8–10), but this technique does not supply significant chromatographic parameters between solutes and stationary phases.

An ECD Index compiled on capillary column GC with cryogenic coolant system would give useful fingerprints not only for qualitative analyses of low volatile compounds, but also higher boiling mass molecules. This also helps to analyze by comparison the ECD-Index results of compounds profiled by different sample sources and analytical laboratories in monitoring studies.

This report represents a new ECD-Retention Index system using chlorinated compounds such as chlorobenzene series and decachlorobiphenyl (i.e., Chlorophenyl Index, CP-Index) with capillary linear temperature programmed gas chromatography and electron capture detection.

Experimental

Reagents and Apparatus

(a) *Chlorobenzene standards.*—1,3,5-trichlorobenzene,[1] 1,2,4-trichlorobenzene (Tokyo Kasei Kogyo Co.); 1,2,4,5-tetrachlorobenzene,[1] 1,2,3,4-tetrachlorobenzene (Yashima Pure Chemicals Co.); 1,2,3,5-tetrachlorobenzene (Aldrich Chemical Co.); 1,2,3,4,5-pentachlorobenzene (Yashima Pure Chemicals Co.); 1,2,3,4,5,6-hexachlorobenzene (HCB)[1] (Gas Chro Co.).

(b) *1,2,3,4,5,6,7,8-Octachloronaphthalene standard.*—OCN (Nihon Chromato Works Ltd).

(c) *2,3,4,5,6,2',3',4',5',6'-Decachlorobiphenyl standard.*—DCB[1] (Analabs).

(d) *Others.*—All reagents with special grades or analytical grades are commercially available.

(e) *Solvent.*—Each reagent was diluted with *n*-hexane (Wako Pure Chemicals Co, PCB analysis grade) to appropriate concentrations.

(f) *Gas chromatograph.*—Hewlett-Packard 5790A with electron capture detector (^{63}Ni, 15 mCi) with capillary splitless injection system and cryogenic oven with liquid carbon dioxide coolant. Column pressure 15–50 psi argon–methane

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[1]CP-Index references.

(95 + 5), initial temperature 50°C, initial time 0.5 min, program rate 2–10°/min rise to 300 or 330°C, and hold for several minutes in each experiment if necessary. Average linear gas velocities were 20–70 cm/s. Carrier gas argon–methane (95 + 5), makeup gas 50 mL nitrogen/min, injection 250°C, detector 350°C.

(g) *Columns and stationary phases.*—Flexible wallcoated capillary columns: SE-54 (1% vinyl, 5% phenyl methyl silicone) Fused Silica® (25 m × 0.3 mm id, Hewlett-Packard), SE-54 Diacot FS® (25 m × 0.25 mm id, Nihon Chromato Works Ltd, Japan), OV-1 Fused Silica (12 m × 0.25 mm id, Hewlett-Packard). Phase thickness: no data by supplier except SE-54 Fused Silica (0.17 μm). Cross-linked methyl silicone capillary columns: Ultra No. 2® column (25 m × 0.3 mm id, phase thickness 0.17 μm and 0.54 μm, Hewlett-Packard), 5% phenyl methyl silicone.

(h) *Data acquisition.*—Integrator (Shimadzu C-R1A and Hewlett-Packard 3390A).

(i) *Sample size.*—1 μL splitless injection.

Results and Discussion

Reference Standard Selection

A linear retention index system on LTPGC reported by Van Den Dool and Kratz (2) is based on the *n*-alkane retention index system by Kovats (1). This index scale is shown in the equation:

$$RI = 100 \times \frac{T(\text{substance} - T(z)}{T(z + 1) - T(z)} + 100z$$

where z is the number of carbon atoms and $T(z)$, $T(z+1)$ are retention times on *n*-alkanes.

In the Polyaromatic Hydrocarbons Index (PAH Index), naphthalene (RI = 200), phenanthrene (300), crysene (400) and picene (500) were selected by Lee et al. (3) as internal standards as proportional to the number of rings of PAH.

Although response of the electron capture detector for PAHs depends on the compound structure, for many PAHs, ECD has much weaker sensitivity than flame ionization (FID). The PAH-Index is of little use as a retention index for ECD-LTPGC on high resolution capillary columns. Because of typical sensitivity and selectivity of the ECD, electrochemical stability in compound structure should be considered when reference compounds are chosen for the ECD. Not only high molecular weight compounds, but also volatile, low molecular weight should be covered on the selection.

Accordingly, a series of halogenated compounds summarized in Figure 1 were selected and found to be appropriate references. These are electrochemically equivalent in their structures.

Retention Index Measurement

Figure 2 shows a typical chromatogram and retention times for a 2°C program rise on the SE-54 capillary column. A linear regression coefficient of the retention times of each compound (Retention Time-Cl_n) shows high correlation (approximately = 1) with number of chlorine atoms.

Table 1 indicates actual retention times for the program rates of reference compounds and linear regression coefficients on the SE-54 capillary column. At the range of 2°–4°C, the last compound, DCB, is eluted within the programmed stage. The relation between retention time and chlorine atom number confirms the theoretical implication by Van Den Dool and Kratz (2). Therefore, these reference compounds are appropriate for ECD Index system, i.e., 1,3,5-tri-CB (300), 1,2,4,5-tetra-CB (400), HCB (600), and DCB (1000); most compounds separated by capillary ECD can be registered by this Chlorophenyl-Index (CP-Index).

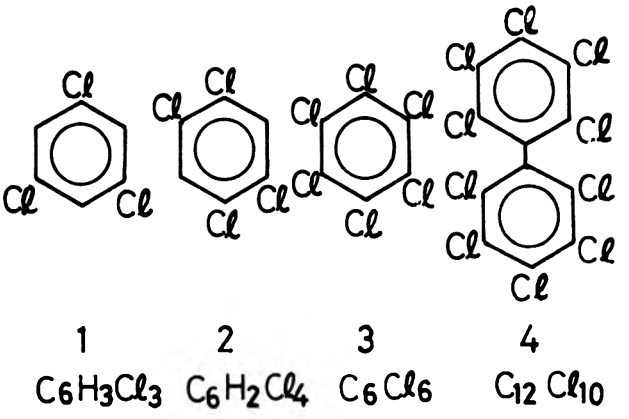

Figure 1. Series of reference compounds for ECD retention index: 1, 1,3,5-trichlorobenzene; 2, 1,2,4,5-tetrachlorobenzene; 3, HCB 4, DCB.

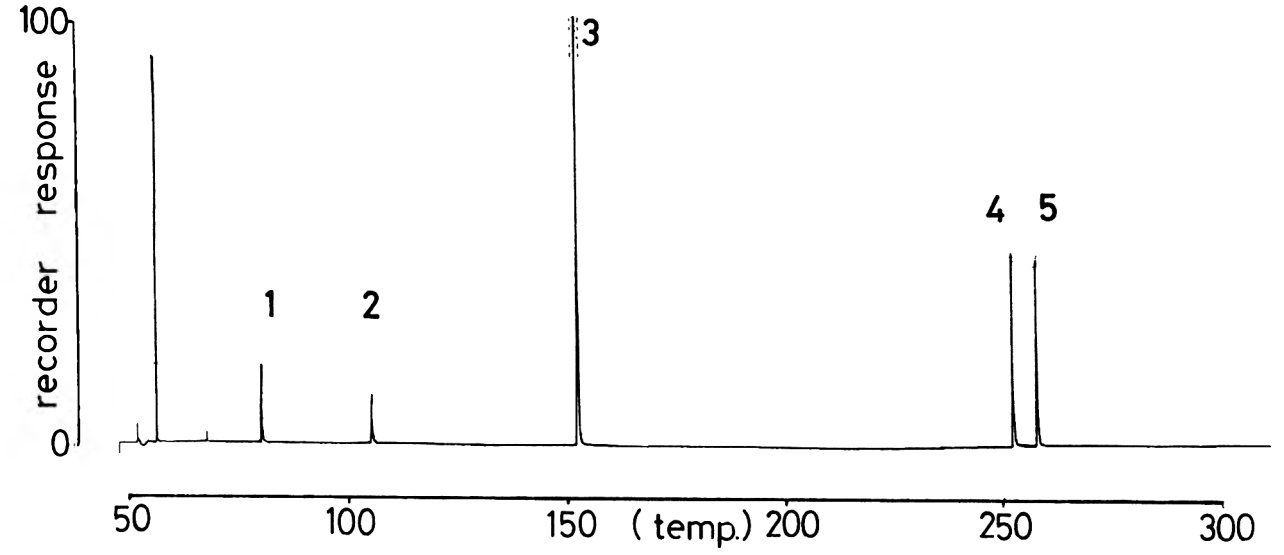

Figure 2. Typical ECD gas chromatogram on SE-54 capillary column chromatographed at 2°C rise: 1, 1,3,5-trichlorobenzene (10 pg); 2, 1,2,4,5-tetrachlorobenzene (10 pg); 3, HCB (35 pg); 4, OCN (25 pg); 5, DCB (25 pg).

Table 1. Retention data of reference compounds for ECD CP-Index on SE-54 capillary column[a]

Program temp. rate, °C/min	Retention time, min				Linear regression coefficient
	tri-CB	tetra-CB	HCB	DCB	
2	16.38	28.83	52.02	105.07	0.9996
4	12.29	19.27	31.53	58.81	0.9998
8	9.11	12.91	19.40	33.68	0.9998
10	8.22	11.37	16.69	28.61	0.9998

[a]Linear gas velocity $\mu = 20.0$ cm/s (50°C); column, 25 m × 0.3 mm id, fused silica.

Measurement of Retention Indexes

Chlorobenzene compounds series and OCN were measured under different analytical conditions, different suppliers, and different diameter columns by using these references. The results are summarized in Table 2. Retention indexes by CP-Index are reproducible, particularly at higher molecular weight. Correlation at the lower molecular weights may be a function of the programming rate and/or initial temperature for programming.

Shorter and longer retention times are associated with more "extended" molecules, as with the PAH-Index (4). The best reference compounds for retention indexing of lower and higher boiling compounds are currently under study.

Table 3 shows retention time regression to Cl_n on another stationary phase (methyl silicone OV-1). This also gave sufficient result as regression coefficient indicated in Table 3. These sequences are independent of polarity of the stationary phase.

Table 4 shows another relationship between retention time regression and reference compounds on cross-linked capillary columns with different film thickness and various programmed temperatures (2–10°C). Phase thickness of columns does not affect the relationship between retention time and Cl_n, that is, the index. Consequently, these reference compounds for the CP-Index gave sufficient reliability of indexing.

Application

Retention index study gives useful information for topological estimations (structure-compound-relation, SCR) and drug design studies (11, 12) as well as capacity factors for liquid chromatography and quantitative structure-activity relationship (QSAR) studies (13, 14). For example, in Table 2, the RI of penta-CB was 505 by calculation compared with 500 for the theoretical index.

This deviation originates from the unsymmetrical electrochemical structure by addition of one chlorine atom to 1,2,4,5-tetra-CB (400). Thus, by using this CP-Index, chemical structural information can be clarified.

Another application of the CP-Index is environmental study. Many components are extracted from environmental samples (15). Profiling these compounds by ECD CP-Index, including both knowns (16), supports the identification of compounds by Kovat's Index and PAH-Index from a GC-MS library research.

Table 2. Retention indexes of chlorobenzene congeners and OCN on SE-54 capillary column[a]

Compounds	SE-54(A)	SE-54(B)
1,3,5-tri-CB	*300*	*300*
1,2,4-tri-CB	325.48	322.91
1,2,3-tri-CB	344.60	341.06
1,2,4,5-tetra-CB	*400*	*400*
penta-CB	505.38	505.40
HCB	600	600
OCN	980.67	980.51
DCB	*1000*	*1000*
Linear reg. Coeff.	0.9998 (Cl_3—Cl_{10})	0.9998 (Cl_3—Cl_{10})

[a]A: HP Product ($\mu = 20.0$ cm/s) (25 m × 0.3 mm id).
B: Nihon Chromato Works Product ($\mu = 62.2$ cm/s) (25 m × 0.25 mm id).
Program rate at 8°/min.

Table 3. Retention data of reference compounds for ECD CP-Index on OV-1 capillary column[a]

Program temp. rate, °C/min	Retention time, min				Linear regression coefficient
	tri-CB	tetra-CB	HCB	DCB	
2	13.81	25.55	48.01	99.71	0.9995
4	10.35	16.98	28.83	55.25	0.9998
8	7.53	11.15	17.37	30.89	0.9999
10	6.72	9.69	14.77	25.72	0.9999

[a]Linear gas velocity $\mu = 29.4$ cm/s (50°C); column, 12 m × 0.25 mim id.

Table 4. Retention data of reference compounds for ECD CP-Index on cross-linked columns[a]

Program temp. rate, °C/min	Retention time, min				Linear regression coefficient
	Tri-CB	Tetra-CB	HCB	DCB	
Column Thickness 0.17 μm; Linear Gas Velocity 22.2 cm/s					
2	16.54	29.04	52.04	104.22	0.9996
4	12.34	19.27	31.35	58.09	0.9998
8	8.97	12.76	19.16	32.98	0.9998
10	8.11	11.22	16.44	27.64	0.9998
Column Thickness 0.54 μm; Linear Gas Velocity 20.4 cm/s					
2	27.61	42.06	66.72	120.51	0.9998
4	18.91	26.64	39.56	67.17	0.9998
8	12.85	16.97	23.79	38.43	0.9998
10	11.36	14.75	20.31	32.65	0.9998

[a]Column No. 2: 5% phenyl methyl silicone, 25 m × 0.3 mm id, initial column temp. 50°C, final column temp. 330°C.

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Potentiometric Determination of Chlorine in Microliter Water Samples

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A novel method has been developed to measure low concentrations (about 100 ppm) of chlorine in very small (1–5 μL) samples of water. The method uses a specially cast silicone rubber cap fitted over the tip of a combination electrode probe. The fabrication, calibration, and use of this modified commercial probe is described in detail.

In a recent study of mixing chlorine into water suspensions of wood pulp, we required a technique to measure chlorine concentrations of very small (1–5 μL) samples drawn from the suspension. The sample concentrations were also small, approximately 100 ppm. This posed a unique problem of chlorine concentration measurement in pulp bleaching; the problem may exist or arise in other industrial applications as well.

In pulp bleaching, the common method for measuring chlorine concentration is to react a known volume of the sample with an excess of acidified potassium iodide solution, and then titrate the resultant liberated iodine in solution with a standardized sodium thiosulfate solution (using a starch indicator) to complete the standard iodometric sequence (1). An alternative method of measuring the iodine concentration is to use a commercially available platinum–iodide combination electrode probe. These probes, however, require sample sizes of 100 mL or more.

When the iodine concentration in microliter-size samples is measured by titration, the method is slow and tedious. The indicators are not sufficiently distinctive to enable large dilutions, which would make the titration techniques easier. Other methods also appear to have shortcomings. For example, the OTA method is thought to be influenced by lignocellulose (2); another possible method, absorption spectrometry, lacks portability and requires optical alignment that is frequently not attainable at mill sites. Accordingly, to overcome these various shortcomings, we developed a novel technique to measure chlorine concentration in microliter-size samples by modifying a commercially available probe.

Principle of Measurement

The commercial probe used in our measurement is commonly used in pulp bleaching to measure dissolved chlorine in water solution. The aqueous sample of chlorine is first reacted with acidified potassium iodide to produce iodine as follows:

$$Cl_2 + 2I^- \rightarrow 2Cl^- + I_2 \qquad (1)$$

The probe contains a redox (platinum) electrode and an iodide (silver/silver iodide crystal) reference electrode (3). During measurement, the platinum electrode develops a potential that is governed by the relative levels of iodine and iodide in solution, according to the Nernst equation. Since potentials are measured directly, concentrations may be used in place of activities in this equation (4), and thus the potential may be given by:

$$E_1 = E_0^1 + \frac{S}{2}\log\frac{[I_2]}{[I^-]^2} \qquad (2)$$

where: E_1 = potential developed by the platinum electrode; E_0^1 = a constant; S = monovalent electrode slope (58 mV/decade at 20°C); $[I_2]$ = iodine concentration; $[I^-]$ = iodide concentration.

The iodide electrode on the probe develops a potential that depends on the iodide level in solution as follows:

$$E_2 = E_3 + S\log[I^-] \qquad (3)$$

where E_2 = potential developed by the iodide electrode; E_3 = a constant.

The potential between the 2 electrodes on the probe is thus related to the iodine concentration by the equation below:

$$E_0 = E_1 - E_2 = E_4 + \frac{S}{2}\log[I_2] \qquad (4)$$

where E_0 = measured potential; E_4 = a constant.

In practice, E_4 may be determined by measuring E_0 for a known iodine concentration. If the ionic strength and pH of the subject solution are similar to that of the known iodine solution, then the iodine concentration may be determined directly from equation *(4)*.

A limitation of the above theory should be noted. Iodine may also combine with the iodide ion to form the complex I_3^-. However, if the iodide concentration greatly exceeds the iodine concentration, the amount of iodide consumed in the

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reaction is negligible. When this is not the case, the measured potential will deviate from that predicted by equation *(4)*. This deviation becomes significant when the iodine concentration exceeds 20 ppm. For this reason, the procedure in the Orion Instruction Manual (3) should always be followed.

Experimental

Apparatus and Reagents

(a) *Cap.*—Modification involves fitting a cap over the Orion combination electrode (Model 97–70, Orion Research Corp., Cambridge, MA) probe to permit measurement of very small samples. Detailed dimensions of this cap are shown in Figure 1. Caps are prepared by casting Dow Corning RTV silicone rubber, prepared according to the manufacturer's specification, in the Plexiglas mold shown in Figure 2. The molten rubber is poured into this mold, then the insert is pressed into the mold and left in place until curing is complete. Approximately 20 such caps are needed for our measurement procedure.

(b) *pH millivolt meter.*—With 0.1 mV sensitivity.

(c) *Acid reagent.*—6.4M acetic acid and 1.8M sodium acetate.

(d) *Potassium iodide solution.*—0.5M KI and 0.038M Na_2CO_3.

(e) *Chloride-demand-free water.*—Distilled, deionized.

(f) *Standard potassium iodate solution.*—0.00281N.

(g) *Micropipet.*—Socorex Model No. 841 (1–5 μL).

Calibration

Calibration solutions.— Probe calibrations are carried out by using a standard potassium iodate solution to produce a known amount of iodine in solution according to the reaction below:

$$IO_3^- + 5I^- + 6H^+ \rightleftharpoons 3\,H_2O + 3I_2 \qquad (5)$$

Three standard solutions and a blank solution are prepared by pipetting 1 mL acid reagent and 1 mL potassium iodide solution into each of 4 glass-stopper 100 mL flasks. Then, 0.20, 1.00, and 5.00 mL standard iodate solution are pipetted into 3 of the flasks; the fourth is left as a blank (no iodate). All flasks are stoppered, mixed by swirling, and left to stand for at least 3 min. Chlorine-demand-free water is then added to each flask to bring the volume to 100 mL. Each flask is then stoppered and inverted several times to mix, and the contents are then poured into separate 150 mL glass-stopper conical flasks. The iodine concentrations of these solutions are 0.2, 1.0, and 5.0 mg/L, respectively. These (and other) iodine concentrations in this study may be referred to as equivalent chlorine concentrations because the iodine is produced from the chlorine in the sample by the equimolar reaction described by equation *(1)*.

Calibration without caps.—The details of this calibration are described in the Orion Instruction Manual (3). In essence, the probe is immersed in a standard solution of 1 ppm iodine, and the electrode is allowed to come to equilibrium as indicated by watching the electrode potential change. When it changes by less than 0.1 mV in 1 min, equilibrium has been reached. This usually takes about 10 min for an electrode that has conditioned surfaces. A typical response is shown in Figure 3. Once equilibrium is reached, the output is adjusted to read 0.0 mV by using the calibration control on the pH/mV meter. The electrode is now calibrated and can be used to measure the equivalent chlorine (iodine) concentrations of reacted samples.

Calibration with caps.—A cast rubber cap is fitted over the clean, dry electrode probe, and the cap reservoir holes are aligned over the electrode surfaces. Then 100 μL 0.20 mg/L equivalent chlorine standard is injected into the cap hole above the platinum electrode by using a syringe. A response curve in millivolts, similar to that shown in Figure 3, is obtained. This curve has a maximum value which is recorded. The cap is then removed, and the electrode is rinsed with distilled water and blotted dry with a paper tissue. This sequence is repeated 5 times, using a fresh silicone rubber cap each time. The maximum mV readings are averaged and plotted on the

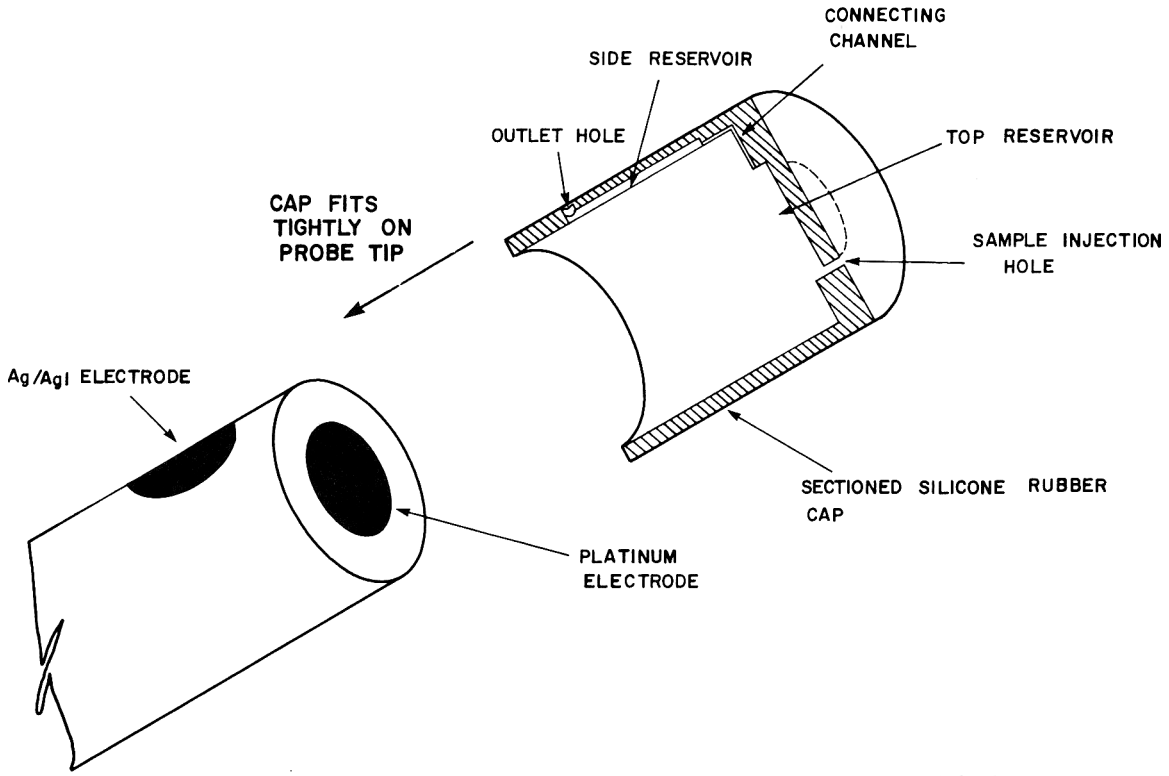

Figure 1. Cast silicone rubber cap (cross-section), and combination electrode probe over which cap is fitted.

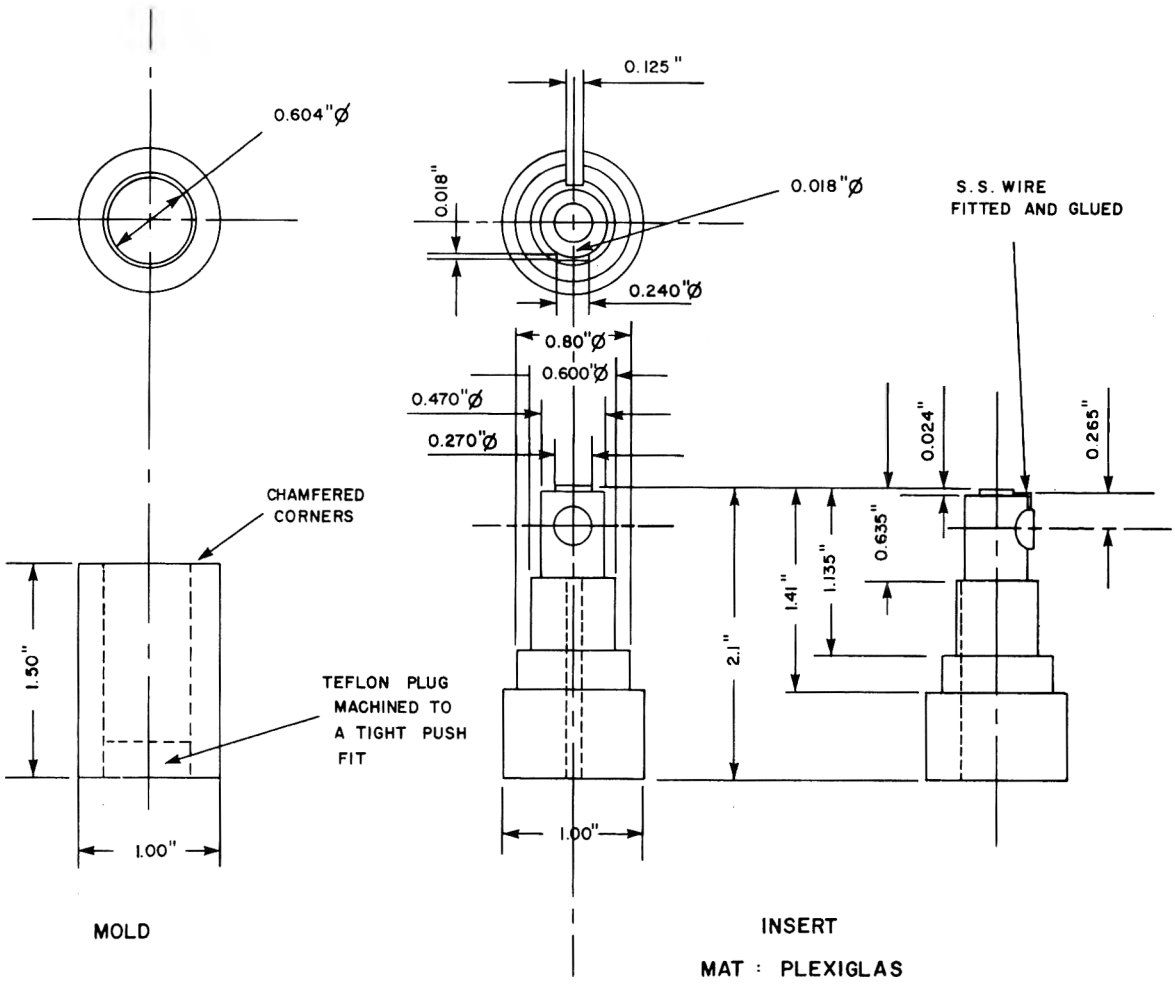

Figure 2. Working drawing of mold for casting silicone rubber caps.

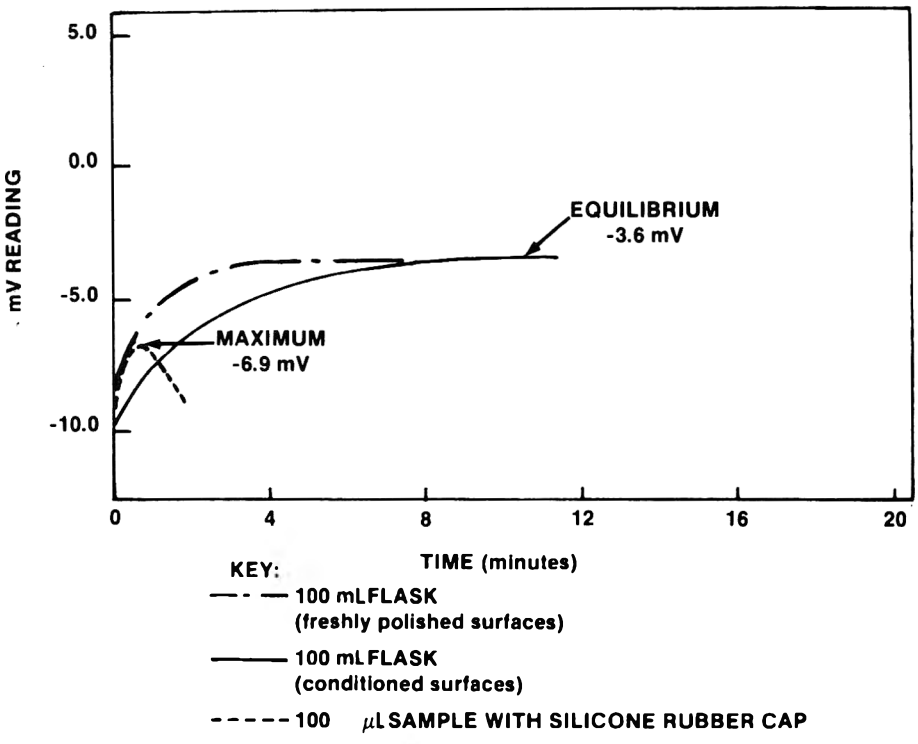

Figure 3. Typical electrode responses with and without silicone rubber caps in place over probe.

linear axis of semilogarithmic graph paper against the equivalent chlorine concentration of the standard. This procedure is repeated using the 2 remaining standard solutions (1.0 and 5.0 mg/L).

Calibration errors.—As shown in Figure 3, the mV response of the capped probe passes through a maximum, as opposed to the monotonic increase to an equilibrium value for the probe without a cap. We concluded that the maximum obtained using a cap is due to absorption of iodine by the cap. We found that this introduced a small random variation in the value of the maximum, but that these maximum values were otherwise reproducible. Typical values of maxima obtained and statistical parameters showing their reproducibility are given in Table 1. In this table, runs 1 and 2 had old electrode surfaces; run 3a had freshly polished surfaces; run 3b had the same electrode as 3a, but was restandardized to 0.0 mV in the 1 ppm solution between runs; run 4 had electrodes that had been conditioned in a 1 ppm iodine solution for several hours, then rinsed, dried, and let stand overnight.

The above tests were conducted using distilled, deionized water. Nevertheless, the blank samples showed some background iodine: 0.07 ppm for run 2, 0.03 ppm in runs 3 and 4, and 0.02 ppm in run 1. These background levels of iodine were added to the known concentrations in computing the calibration curves.

The regression lines fitted to the data of Table 1 are shown in Figures 4 and 5. Statistical parameters showing the good fit of the data are given in Table 2. It should be noted that the lines were fitted assuming that the standard deviation remained constant at all concentration levels, although this is clearly not the case in Table 1. However, we concluded that the accuracy of the measurements did not justify weighting the data to account for this change in standard deviation when making this logarithmic fit. Nevertheless, as is evident by the high correlation coefficients ($R^2 > 0.99$), good fits were obtained.

Procedure for Chlorine Measurement

An iodide solution is prepared to carry out the reaction given by equation *(1)* by mixing a 1 mL buffer solution and a 1 mL potassium iodide solution in a 100 mL glass-stopper flask. This solution is allowed to stand 1 min and then chlo-

Table 1. Summary of calibration data (mV response)

Run	Statistic	Equivalent chlorine concn, mg/L			
		Blank	0.2	1.0	5.0
1	$\bar{y}$	—	−22.86	−3.8	16.58
	s_y	—	0.63	0.41	0.41
	n	0	5	5	5
2	$\bar{y}$	−32.9	−20.6	−3.1	16.9
	S_y	1.22	2.09	1.06	1.97
	n	4	19	19	20
3a	$\bar{y}$	−38.2	−21.98	−1.11	20.64
	S_y	4.21	2.59	0.85	0.66
	n	11	11	11	11
3b	$\bar{y}$	−44.1	−23.3	−3.4	18.14
	S_y	4.18	2.58	1.34	1.09
	n	14	14	14	14
4	$\bar{y}$	−52.1	−24.98	−3.52	16.58
	S_y	4.26	1.36	0.88	0.65
	n	20	20	20	20

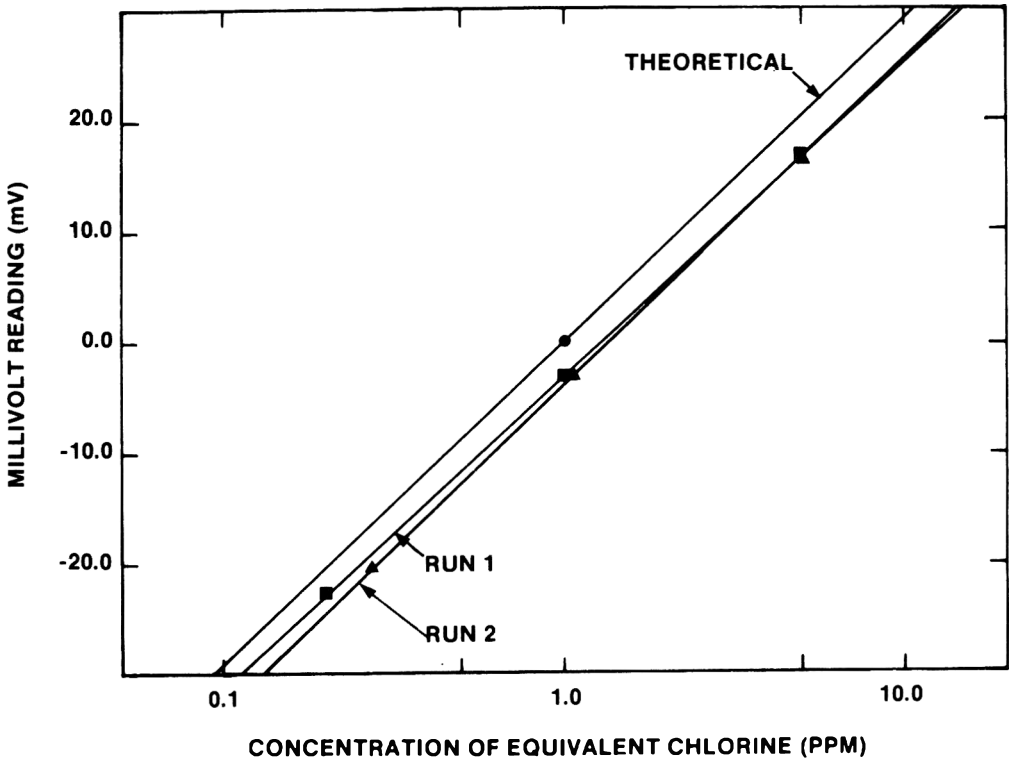

Figure 4. Calibration graph of data for probe with and without caps in place.

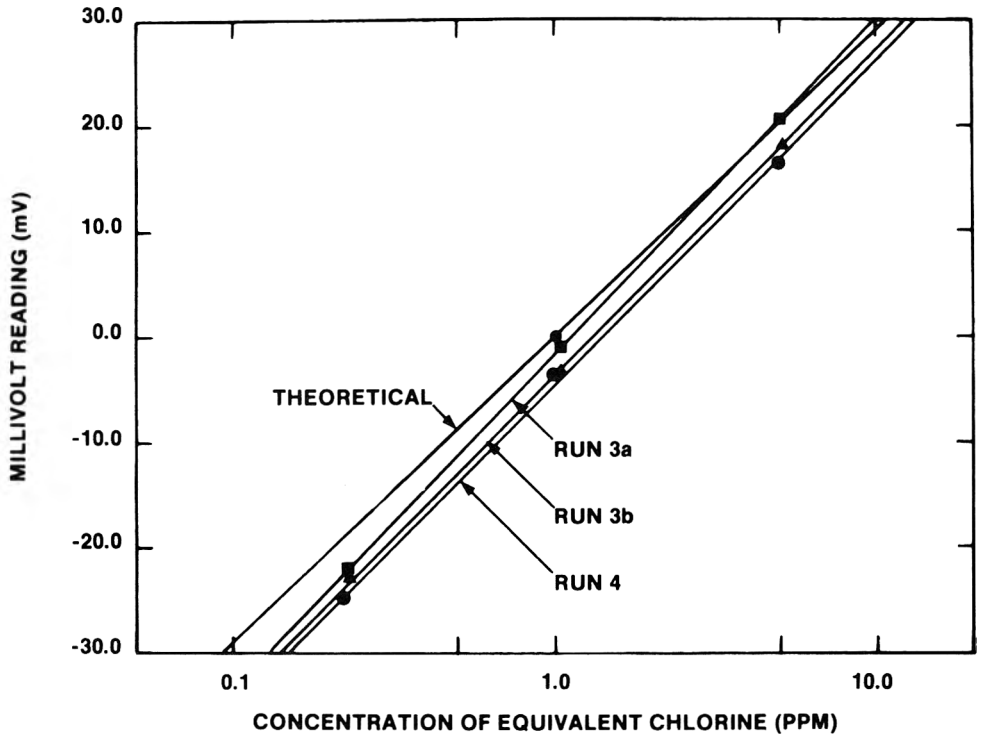

Figure 5. Calibration graph of data for probe with and without caps, for freshly polished electrode surfaces and a conditioned surface.

Table 2. Regression analysis data for calibration runs[a]

Run	slope, a	Intercept, b	S_y	R^2	S_a	S_b	DF
1	28.2	−3.12	0.48	0.999	9.123	0.216	13
2	29.4	−3.89	1.76	0.987	0.233	0.442	56
3a	31.8	−1.63	1.60	0.992	0.278	0.508	31
3b	30.9	−3.64	1.78	0.989	0.274	0.401	40
4	30.6	−4.5	1.12	0.996	0.145	0.262	58

[a]Equation fitted $y = a + b \log x$, where y = measurements, mV; x = equivalent chlorine concentration, ppm; S = standard deviation; R^2 = correlation coefficient; DF = degrees of freedom.

rine-demand-free water is added to volume. The flask is stoppered and mixed by inversion. A 100 μL portion of this solution is pipetted into a 150 mL glass test tube.

A microliter-size portion of sample is added to the test tube, and the solution is mixed. This solution is then injected into a fresh cap fitted over the probe, with holes aligned over the electrodes. The maximum mV potential is recorded as described in the calibration procedure. Several samples from the parent solution should be tested if possible. The replicated mV readings are averaged and an equivalent chlorine concentration, R, is determined from the calibration graph. The chlorine concentration of the original sample is determined by using the following formula:

$$\text{mg chlorine/L} = \frac{R \times (100 + \text{sample size, } \mu\text{L})}{(\text{sample size, } \mu\text{L})} \quad (6)$$

When the equivalent chlorine concentration, R, is less than 0.20 mg Cl_2/L, the apparent chlorine concentration measured for the blank cap should be subtracted from the measured equivalent chlorine concentration to determine R.

Concluding Remarks

We have used this novel method of chlorine measurement to determine the chlorine concentration of arrays of microliter-size samples in a study of microscale mixing of chlorine into pulp suspensions. The technique has proved useful, both in the laboratory and at mill sites where modest laboratory facilities exist.

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DRUGS

Simple Colorimetric Method for Determination of Metaproterenol Sulfate in Pharmaceuticals

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

Orciprenaline sulfate or metaproterenol sulfate (5-[1-hydroxy-2-[(1-methylethyl)amino]ethyl]-1,3-benzenediol sulfate) is a bronchodilator incorporated in tablet, injection, and syrup pharmaceutical preparations.

The *British Pharmacopoeia* (1) describes a nonaqueous titration method for the determination of metaproterenol sulfate reference standard. To determine the drug in tablet formulation, an ultraviolet assay (2) is specified. Beckett and Stenlake (3) proposed an ascending paper chromatographic method. Colorimetric methods (4, 5) have also been reported in the literature.

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

METHOD

Apparatus and Reagents

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

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

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

(d) *Trimethylamine solution.*—60% aqueous.

(e) *Ethyl alcohol*—96% v/v.

(f) *Standard metaproterenol sulfate solution.*—Dissolve 25 mg standard drug in 250 mL water for 100 ppm solution.

Preparation of Assay Solution

(a) *Tablets.*—Weigh 20 tablets and crush thoroughly. Dissolve portion equivalent to 25 mg metaproterenol sulfate in water and filter into 250 mL volumetric flask, wash filter, and dilute filtrate and washings to volume with water.

(b) *Syrup.*—Pipet syrup equivalent to 25 mg metaproterenol sulfate from thoroughly shaken formulation into 250 mL volumetric flask, and dilute to volume with water.

(c) *Injection.*—Pipet solution equivalent to 25 mg metaproterenol sulfate from thoroughly shaken injection into 250 mL volumetric flask and dilute to volume with water.

Standard Curve

Dilute standard metaproterenol sulfate solution to give concentrations of 1 to 8 μg/mL. Develop color and read absorbance of reaction product as described under *Assay*. Plot absorbance against concentration of metaproterenol sulfate to give straight line passing through origin. Beer's law is obeyed between 1 and 6 μg/mL. Molar absorptivities are 2.32×10^4, 2.75×10^4, and 2.75×10^4 L/mol./cm for dapsone, benzocaine, and *p*-nitroaniline, respectively.

Assay

With dapsone.—Pipet 2.5 mL dapsone working solution into each of series of 25 mL graduated flasks. Add 2 mL sodium nitrite solution and aliquots of standard working solution (0.25 to 1.75 mL). Concurrently prepare reagent blank. Dilute mixed solutions to volume with water after 2 min and measure absorbance at 420 nm against reagent blank. Determine concentration in test solution from standard curve, using same procedure detailed above.

With p-nitroaniline.—Carry out same procedure as detailed for dapsone.

With benzocaine.—Pipet 2.5 mL benzocaine working solution into each of series of 25 mL graduated flasks. Add 2 mL sodium nitrite followed by aliquots of standard working solution (0.25 to 1.75 mL). Add 2 mL ethyl alcohol. Let flasks stand 2 min. Then add 2.5 mL trimethylamine solution. Dilute mixed solutions to volume with water and measure absorbance at 435 nm against reagent blank.

Results and Discussion

The reactions reported make use of diazotization of dapsone, *p*-nitroaniline, or benzocaine followed by coupling with metaproterenol sulfate (Figure 1). In the case of benzocaine, this undergoes a bathochromic shift when it is made alkaline with trimethylamine. The color developed has been success-

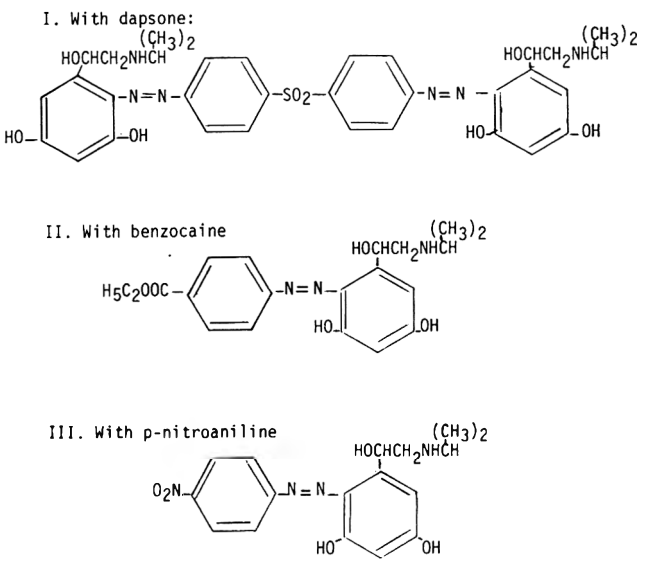

Figure 1. Probable structures of reaction products.

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fully used to determine metaproterenol sulfate from its formulations.

To study the reliability and recovery of the proposed method, we used a method of standard addition. A fixed weight of sample was taken and standard drug was added at 3 different levels. Each level was repeated 7 times. The total amount of drug was determined by the proposed method (Table 1).

Percent recovery was calculated as follows:

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

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

The proposed method has distinct advantages over similar methods reported in the literature. The present method is direct, fast, simple, sensitive, and accurate. The diazotization and coupling reactions are carried out at room temperature, excess nitrate ions need not be removed by addition of ammonium sulfamates, the color development and absorbance measurements are complete within 15 min. The high molar absorptivities indicate high sensitivity of the method. Percentage recoveries obtained are in the range 98–101%, indicating noninterferences of the other ingredients and the excipients in the determination of metaproterenol sulfate (Table 1). The method is also reproducible and precise as shown by results in Table 2. Therefore we recommend this method for routine quality control analysis of metaproterenol sulfate in formulations.

Acknowledgment

The authors are grateful to Messrs. German Remedies Ltd, Bombay, India, for the supply of reference standard, and to the UGC for a research grant.

Table 1. Reproducibility of colorimetric method for determining metaproterenol sulfate

Sample	Amt decld, mg	A, level: 1st	A, level: 2nd	A, level: 3rd	B, level: 1st	B, level: 2nd	B, level: 3rd	C, level: 1st	C, level: 2nd	C, level: 3rd	Decld %, BP
Tablet	20	97.5	99.7	99.57	102.0	95.0	99.8	100.6	99.45	101.3	90–110
Syrup	2	99.0	99.0	99.9	100.0	99.50	99.67	98.00	99.5	100.06	—
Injection	0.5	96.0	98.0	98.67	100.0	100.0	100.0	96.0	98.0	100.0	—

Table 2. Recovery of metaproterenol sulfate from 3 pharmaceutical preparations (3 analyses each)[a]

Sample	Amt decld, mg	Found, mg: A	Found, mg: B	Found, mg: C	Recd by addn method, %: A	Recd by addn method, %: B	Recd by addn method, %: C	SD: A	SD: B	SD: C	CV, %: A	CV, %: B	CV, %: C
MS tablet	20	20.04	20.3	19.8	99.78	98.50	101.00	0.630	0.460	0.640	1.95	1.33	1.85
MS syrup	2	2.01	2.01	2.0	99.50	99.71	100.00	0.076	0.066	0.057	2.37	1.92	1.61
M injection	0.5	0.51	0.5	4.99	98.8	100.0	100.20	0.021	0.016	0.029	2.49	2.02	2.40

[a]Three different treatments were used: A, diazotized dapsone; B, Benzocaine; C, *p*-nitroaniline.

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First Derivative Spectrophotometric Determination of Strychnine and Brucine in Nux Vomica Liquid Extract

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A simple, rapid, and accurate method is presented for determining strychnine and brucine in nux vomica liquid extract. The 2 alkaloids are directly extracted from the alkaline liquid extract with 0.1N H_2SO_4, and the liberated alkaloids are determined separately using first derivative spectrophotometry. The minimum strychnine and brucine concentrations detectable by means of this method are 3.0 μg/mL. The method has been successfully applied to the analysis of 3 batches of nux vomica liquid extract.

Most of the spectrophotometric methods published for the analysis of strychnine and brucine in nux vomica liquid galenicals (1–3) are based on the classical Vierordt's 2 wavelength method (4, 5). These methods (1–3) include different extraction techniques to liberate both alkaloids from nux vomica. Scott et al. (1) separated the 2 alkaloids by adsorption on alumina, cation exchange, and absorbance measurement at 255 and 264 nm; El-Masry and Wahbi (2) used the acid-dye technique as an extraction–purification method by complexing both alkaloids with methyl orange at pH 5.0, then extracting the yellow complex with $CHCl_3$, washing with NaOH, liberating the complexed alkaloids with 0.1N H_2SO_4, and measuring absorbance at 262 and 300 nm; the current *British Pharmacopoeia* (BP) (3) specifies a cleanup procedure to extract the 2 alkaloids in a pure form suitable for analysis using the 2 wavelengths 262 and 300 nm. The methods described are time consuming and require solving simultaneous equations. Also, the BP method uses a special extractor during the cleanup step, which may not be available in every control laboratory. Furthermore, in application of Vierordt's method, the amount of irrelevant absorption must be kept small. In practice, the last requirement places a considerable restric-

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tion on the application of the method, since the term "irrelevant absorption" must also include variations of the components which occur between batches. Thus, if the mixture has been prepared from batches of material that differ from the reference samples used to establish the assay coefficients, the over-all effect is equivalent to the introduction of irrelevant absorption. The results suffer accordingly (6).

Other methods, such as spectrophotometric determination of strychnine after destruction of brucine with $K_2S_2O_8/H_2SO_4$ (7), nitrating brucine and extracting strychnine (8), as well as colorimetric methods (9, 10), have been reported. These methods require certain reagents and their accuracy depends on critical adjustment of experimental conditions.

Recently, the application of the derivative technique to the study of absorption spectra from multicomponent preparations was found to enhance the specificity and precision of band measurement by removing UV interfering features of unknown and/or variable intensity (11).

This paper presents a simple, rapid, and accurate method for the direct determination of strychnine and brucine in nux vomica liquid extract. The method is based on the use of the first derivative spectrophotometry, using the zero crossing technique (11, 12).

Experimental

Apparatus and Reagents

(a) *Spectrophotometer.*—Perkin-Elmer Model 550S UV-VIS spectrophotometer and Hitachi Model 561 recorder. For recording first derivative (D_1) curve, set instrument as follows: λ initial, 330 nm; λ final, 220 nm; scan speed, 120 nm/min; chart speed, 60 nm/min; mode, D_1 (= $dA/d\lambda$); ordinate maximum and minimum, ± 0.2; response, 6.

(b) *Strychnine sulfate solution.*—100 mg (pure, Merck, Darmstadt, GFR)/100 mL 0.1N H_2SO_4.

(c) *Brucine solution.*—100 mg (pure, BDH, London, UK)/100 mL 0.1N H_2SO_4.

(d) *Nux vomica liquid extract.*—Batches A, B, and C (Lot No. 805, 389, and 918, respectively). Chemical Industries Development Laboratories, Pyramides Ave, Giza, Egypt.

(e) *Chloroform.*—Pure (Merck).

Strychnine and Brucine Calibration Graphs

Accurately transfer serial volumes of 1–2.5 mL strychnine sulfate and brucine solutions to separate 100 mL volumetric flasks and dilute to volume with 0.1N H_2SO_4. Measure D_1 of strychnine and brucine solutions against blank of 0.1N H_2SO_4 at 262.8 and 312 nm, respectively (Figure 1).

Nux Vomica Liquid Extract

Accurately transfer 2 mL liquid extract to 150 mL separatory funnel. Add 25 mL distilled water and 5 mL dilute NH_4OH solution, and extract with four 20 mL portions of $CHCl_3$. Extract combined $CHCl_3$ with four 20 mL portions of 0.1N H_2SO_4. Wash combined acid extract with 10 mL $CHCl_3$ (discard), dilute to 100 mL with 0.1N H_2SO_4 in 100 mL volumetric flask. Take 5 mL and dilute to 100 mL with 0.1N H_2SO_4. Measure D_1 value at 262.8 and 312 nm using 0.1N H_2SO_4 solution as blank.

Calculate concentration of strychnine and brucine from appropriate calibration graph.

Results and Discussion

Attempts to analyze strychnine and brucine by direct dilution of the liquid extract, using the first and second derivative modes (D_1 and D_2), were unsuccessful, due to heavy contributions of other constituents in the liquid extract (Figure 2).

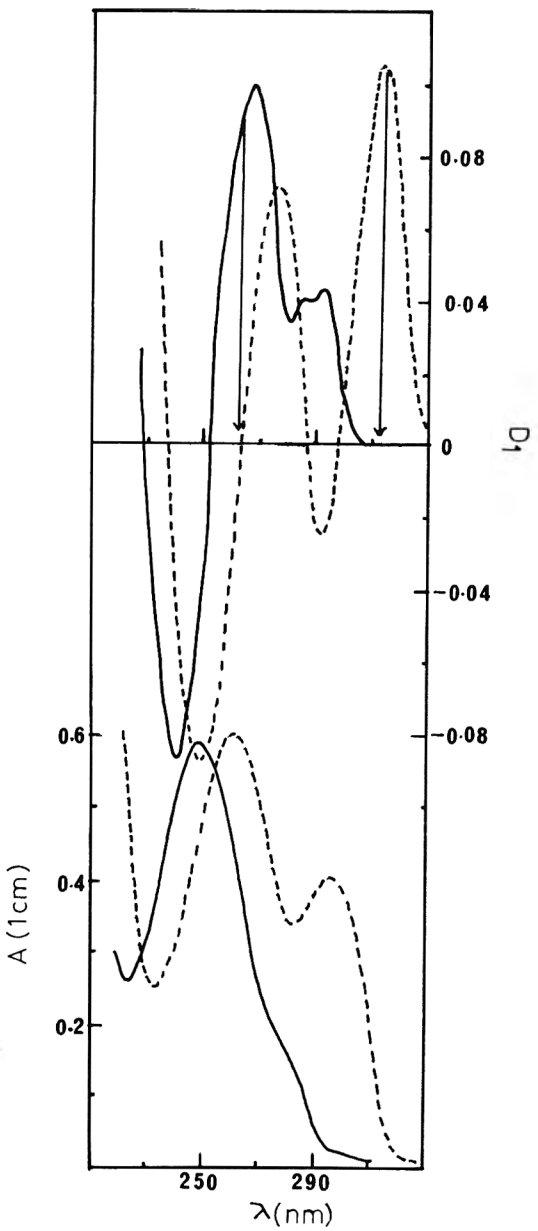

Figure 1. Absorption and first derivative spectra of 0.002% w/v each of strychnine sulfate (———) and brucine (----) in 0.1N H_2SO_4.

The D_1 spectrum of the liquid extract after a simple and rapid purification step (as in experimental) was superimposed with the D_1 spectrum of a synthetic mixture of strychnine and brucine (Figure 2b). Therefore, the D_1 mode was chosen and used to analyze strychnine and brucine in nux vomica liquid extract.

Figure 1 shows the zero order (A 1 cm) and the D_1 spectra of (2 mg%) strychnine sulfate and (2 mg%) brucine solutions in 0.1N H_2SO_4. It also shows, at 262.8 nm, that the D_1 value of brucine is equal to zero while strychnine appears to possess approximately maximum D_1 value at the same wavelength. The absolute value of the derivative of the zero order sum curves of the 2 alkaloids at the zero crossing point of brucine was used to quantitate strychnine. Brucine was determined by measuring its D_1 value at 312 nm (at this wavelength, strychnine has no contribution). The graphs obtained by plotting the D_1 value at the chosen wavelength against concentration of strychnine and brucine show a linear relationship within a considerable interval (1–2.5 mg%). The correlation coefficient was 0.999 for strychnine and brucine.

To prove the validity and applicability of the proposed method, 5 replicate assays of a synthetic mixture of the

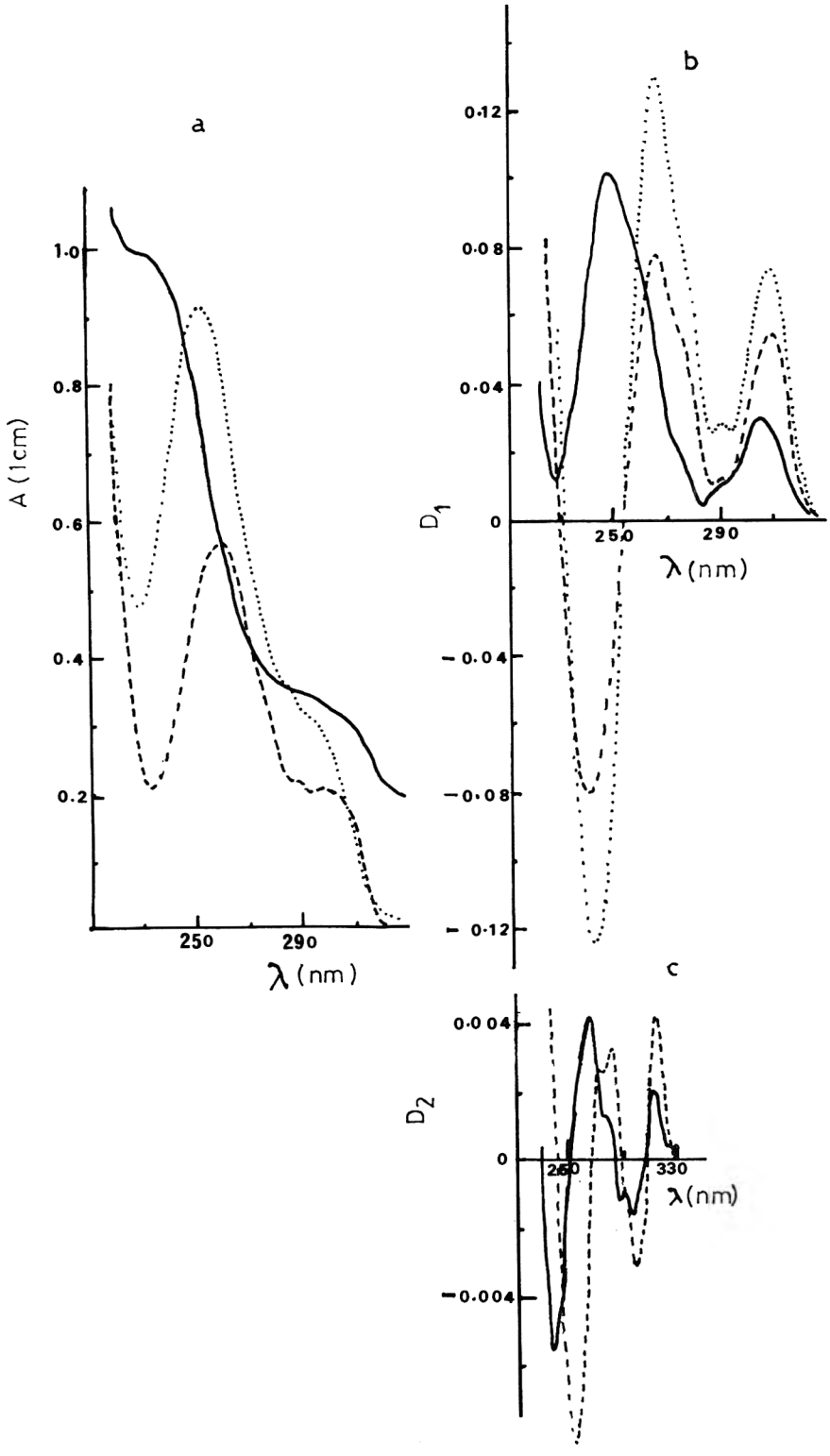

Figure 2. Absorption (a) and first (b) and second (c) derivative spectra of 0.1% v/v nux vomica liquid extract (———) and its purified liquid extract (. . . .) and synthetic mixture of 0.001% w/v strychnine sulfate and brucine (----) in 0.1N H_2SO_4.

alkaloids (in concentration range of 1–3 mg and 0.5–2 mg/100 mL 0.1N H_2SO_4 for strychnine and brucine, respectively) were carried out according to the above procedure. The mean percentage recovery (± standard deviations) for strychnine and brucine were 101.1 ± 1.8 and 100.4 ± 1.2, respectively.

The minimum strychnine and brucine concentrations detectable by this method correspond to 3.0 μg/mL.

Table 1 shows the results obtained by using the proposed and the BP (3) methods for determining strychnine in 3 different batches of nux vomica liquid extract. The concentration of brucine in each batch is also included. When a *t*-test at the 95% confidence level was applied to compare the results obtained for determination of strychnine by the proposed and BP (3) methods, the calculated *t*-value did not exceed the theoretical one. This indicates that there is no significant difference between the 2 mean percentages, confirming the high accuracy of the method. The variance ratio, *F*, also reveals that there is no significant difference between the precision of the methods.

Recovery experiments made on liquid extract (Batch A)

Table 1. Determination of strychnine and brucine (% w/v) in nux vomica liquid extract by proposed and BP (3) methods

Batch	Strychnine			Brucine
	Proposed		BP	Proposed
A	1.66, 1.62, 1.63, 1.60, 1.65		1.60, 1.57, 1.59, 1.57, 1.64	1.18, 1.17, 1.17, 1.17, 1.17
Mean ± SD	1.63 ± 0.0239		1.59 ± 0.0288	1.17 ± 0.0045
t		2.27 (2.31)[a]		
F		1.45 (6.39)		
B	1.40, 1.39, 1.39, 1.33, 1.32		1.37, 1.33, 1.32, 1.32, 1.33	1.19, 1.17, 1.17, 1.17, 1.17
Mean ± SD	1.37 ± 0.0378		1.33 ± 0.0207	1.17 ± 0.0089
t		1.66 (2.31)		
F		3.32 (6.39)		
C	1.52, 1.49, 1.51, 1.48, 1.48		1.50, 1.53, 1.52, 1.51, 1.51	1.19, 1.15, 1.16, 1.13, 1.14
Mean ± SD	1.50 ± 0.0182		1.51 ± 0.0114	1.15 ± 0.023
t		1.88 (2.31)		
F		2.54 (6.39)		

[a]Figures in parentheses are theoretical values.

spiked with 5 different concentrations of strychnine sulfate and brucine gave recoveries of 98.0–100.0 and 98.8–100.0%, respectively.

The results obtained show the high reliability and reproducibility of the method, which requires only simple extraction and much shorter analysis time compared with existing methods. The method can be applied for determining strychnine and brucine in nux vomica tincture and nux vomica acid and alkaline mixtures (13).

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DRUG RESIDUES IN ANIMAL TISSUES

Confirmatory Method for Sulfamethazine Residues in Cattle and Swine Tissues, Using Gas Chromatography–Chemical Ionization Mass Spectrometry

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A gas chromatographic (GC) method has been reported for the determination of sulfamethazine residues in cattle and swine tissues. The extracts from this procedure were found to be directly amenable to examination by gas chromatography–mass spectrometry (GC-MS), allowing positive confirmation of an apparent residue of sulfamethazine. Chemical ionization mass spectrometry (CIMS) was chosen as the MS technique because it generated an ion indicative of intact sulfamethazine and fragment ions indicative of the amine functionality and sulfanil moiety. Positive ion (PI) chemical ionization mass spectrometry was adequate by itself for a confirmatory technique. Negative ion (NI) chemical ionization mass spectrometry alone could not be used for the confirmatory analysis of sulfamethazine, but it did offer a means to check the quantitative data from the positive ion analyses and provided complementary confirmatory data. Satisfactory recoveries were obtained for sulfamethazine in swine and cattle tissues at the tolerance level of 0.1 ppm. Apparent sulfamethazine residues in control tissues were less than 0.01 ppm.

There are currently about 10 different sulfonamides in common use as antibacterials for large food-producing animals. The largest percentage of the sulfonamide market consists of sulfamethazine used in combination with antibiotics as feed supplements to promote growth and prevent diseases in cattle and swine. Much of the violative residue levels found by the U.S. Department of Agriculture in swine liver have been attributed to sulfamethazine, and have been determined essentially by the method of Tischler et al. (1) with minor modifications. Horwitz (2) and Matusik et al. (3) described the problems encountered with this method over the years, relating both to nonspecificity and varying background values. Hence, in our laboratories, a gas chromatographic (GC) method using an electron capture detector was developed (4). Excellent recovery was obtained for sulfamethazine from fortified cattle and swine tissues. Sulfamethazine was resolved from 6 other sulfonamides. This GC method and the GC-MS method of Suhre et al. (5) were collaboratively studied by Malanoski et al. (6), and both methods were adopted by AOAC. By modifying the GC parameters, Matusik et al. (3) showed that the GC method is applicable to the simultaneous determination of several metabolites identified by Paulson et al. (7) in swine tissues sacrificed 24 h after dosing with carbon-14 sulfamethazine.

For a confirmatory method of analysis, gas chromatography–mass spectrometry was chosen to incorporate the structural information generated by a mass spectrometer with the demonstrated resolving power of GC. Suhre et al. (5) reported using gas chromatography–electron impact mass spectrometry (GC-EIMS) for the determination of sulfamethazine in swine tissues. Garland et al. (8) reported using gas chromatography–chemical ionization mass spectrometry (GC-CIMS) to assay sulfadimethoxine in swine and cattle tissues. For quantitation, both of these methods use a stable isotope-labeled internal standard added to the tissue before extraction. Roach et al. (9) reported positive ion (PI) and negative ion (NI) chemical ionization mass spectra of a number of sulfonamides, and deduced the structural information generated by these compounds in both modes of analysis. One of the principal advantages of CIMS over EIMS is the generation of an intense $(M + H)^+$ ion indicative of the intact sulfonamide, whereas EIMS generates little or no molecular species for most of the sulfonamides. In addition, PICI generates a fragment ion that characterizes the amine functionality of the sulfonamide, and NICI generates a fragment ion that characterizes the sulfanil moiety. As a result of the high electron capture responses to most of the sulfonamides, their NICI responses are 100- to 1000-fold greater than are their PICI responses.

The objective of this work was to develop a GC-CIMS method to confirm the identity of apparent residues of sulfamethazine in cattle and swine tissues at levels of 0.1 ppm or greater. The method was to be designed as a complement to the GC-EC method of Manuel and Steller (4) so that the same extracts analyzed by GC-EC could be directly analyzed by GC-CIMS.

METHOD

Apparatus

(a) *Gas chromatograph.*—Finnigan Model 9610, or equivalent, suitable for on-column injections and fitted with 4 ft × 2 mm id glass column packed with 3% OV-1 on 60–80 mesh Gas-Chrom Q (Applied Science Div., Milton Roy Co. Laboratory Group, PO Box 440, State College, PA 16801). Operating conditions: injector 250°C; column oven 240°C; methane carrier gas at 15 mL/min; retention time for N^1-methyl derivative of sulfamethazine ca 2 min.

(b) *Mass spectrometer.*—Finnigan Model 4000 equipped with pulsed positive ion negative ion chemical ionization (PPINICI) accessory, or equivalent system. Operating conditions: GC-MS interface temperature 230°C; ion source temperature 250°C; methane source pressure 0.4 torr; conversion dynodes ± 3000 V; electron multiplier 900 V; preamplifier range 10^{-8} amp/V.

(c) *Data system.*—INCOS Model 2300, or equivalent. Operating conditions: positive ions monitored m/z 138^+, 166^+, and 293^+; dwell time for positive ions 210 ms/ion; negative ions monitored m/z 155^-, 156^-, and 157^-; dwell time for negative ions 80 ms/all 3 ions.

Reagents

(a) *Methane.*—UHP, 99.97% minimum purity (Matheson Gas Products, East Rutherford, NJ 07073).

(b) *Sulfamethazine standard solution.*—See reference 4.

(c) *Derivatizing reagent.*—See reference 4.

Procedure

Prepare tissue samples for GC-CIMS analysis using procedure of Manuel and Steller (4). Briefly, extract the finely ground tissue with acetone–chloroform (1 + 1), filter to separate solids, and add 1N HCl. Evaporate organic solvent,

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partition extract with hexane, and discard hexane phase. Buffer aqueous phase to pH 6 so that residue will partition into methylene chloride. Finally, evaporate methylene chloride and methylate sulfamethazine residue with diazomethane.

Results and Discussion

The PICI (CH_4) and NICH (CH_4) mass spectra obtained on 100 ng N^1-methylsulfamethazine are shown in Figures 1 and 2, and agree with those reported by Roach et al. (9). As expected from its excellent EC response, the response of N^1-methylsulfamethazine in NICI (CH_4) is at least 100-fold greater than its response in PICI (CH_4). In keeping with this response ratio, GC-NICI in the multiple ion detection (MID) mode exhibited a linear response range from 10 ng down to 5 pg; GC-PICI demonstrated linear response from 2.5 to 25 ng. However, the NICI (CH_4) mass spectrum of N^1-methylsulfamethazine characterizes only the sulfanil portion of the molecule. Because this moiety is common to many other sulfonamides from which the analyte is to be distinguished, the NICH (CH_4) data above are not adequate for confirmation. The PICI (CH_4) mass spectrum of N^1-methylsulfamethazine generates an $(M + H)^+$ ion at m/z 293^+, and ions indicative of the amine functionality of the sulfonamide at m/z 138^+ and 166^+. These PICI (CH_4) mass spectral data differentiate sulfamethazine from the other sulfonamides, and are adequate for a confirmatory method. Although NICI alone cannot be used for the confirmatory analysis of sulfamethazine, it offers a means to check the quantitative data from PICI analyses and provides additional confirmatory data that complement the PICI data. Because of its much greater response, only an 80 ms scan time is required to monitor all 3 negative ions, while a 210 ms scan time is spent on each of the positive ions. Therefore, for an instrument equipped with pulsed positive ion negative ion capability, only a small portion of the MID acquisition cycle is necessary to obtain the complementary negative ion data, which leaves the vast majority of time available for monitoring the positive ions.

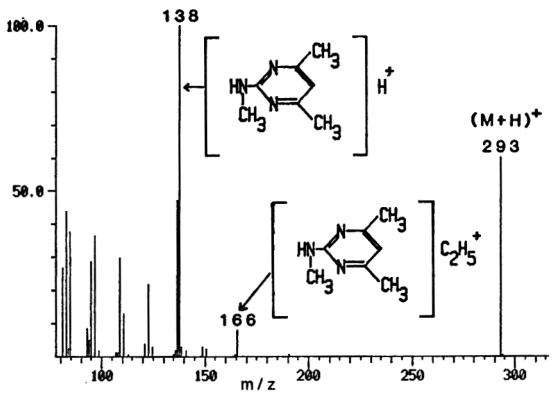

Figure 1. PICI (CH_4) mass spectrum of 100 ng N^1-methylsulfamethazine.

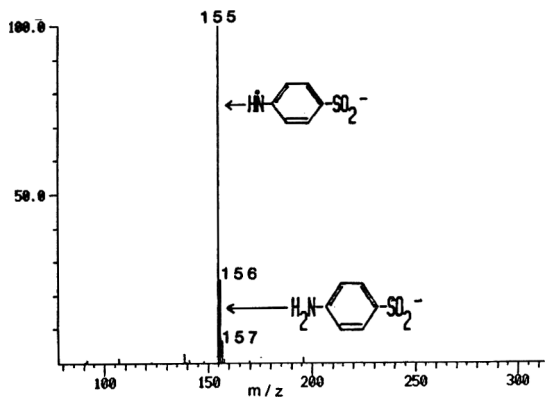

Figure 2. NICI (CH_4) mass spectrum of 100 ng N^1-methylsulfamethazine.

Figure 3 compares the MID analyses by GC-PICI (CH_4) of a 5 ng N^1-methylsulfamethazine standard (corresponding to 0.1 ppm sulfamethazine in the tissue), an extract of control swine liver, and an extract of swine liver fortified at 0.1 ppm sulfamethazine. Figure 4 presents the results from the MID analyses of the same samples by GC-NICI (CH_4). The results obtained on the other control and fortified tissues are similar. The elution of the analyte at the same retention time in the standard and the fortified tissue extracts, the absence of any significant interferences at the monitored ions in the control tissue extracts (apparent residues of <0.01 ppm), and the reproducibility of the analyte ion ratios between the standard and fortified tissue extracts confirm the identity of sulfamethazine in the fortified tissues.

Recovery values of sulfamethazine from swine and cattle tissues obtained from laboratory fortification studies are reported in Table 1. The good recoveries from both GC-PICI (CH_4) and GC-NICI (CH_4) analyses and the close agreement between both modes of analysis on the same sample add further confidence to the confirmatory capabilities of the method.

In conclusion, a confirmatory method using GC-CIMS has been developed for sulfamethazine residues in cattle and swine tissues at the 0.1 ppm level. While GC-PICI (CH_4) is

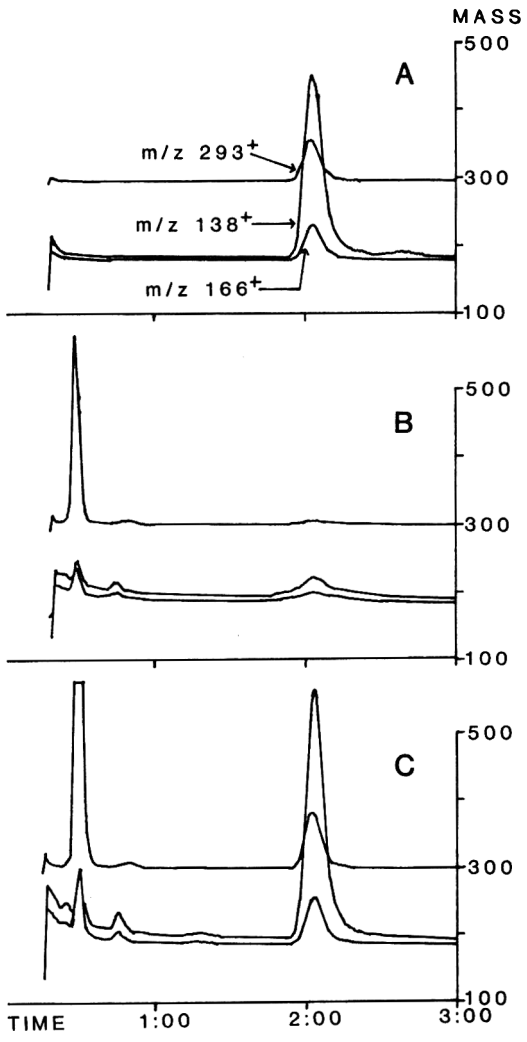

Figure 3. Ion traces from MID analyses in GC-PICI (CH_4) of A, 5 ng standard; B, control swine liver; and C, swine liver fortified at 0.1 ppm.

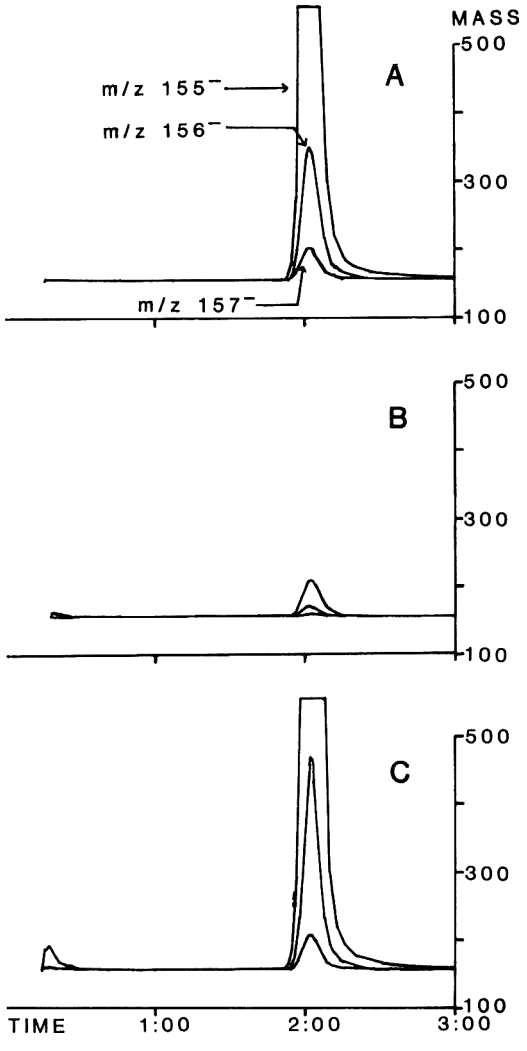

Figure 4. Ion traces from MID analyses in GC-NICI (CH_4) of A, 5 ng standard; B, control swine liver; and C, swine liver fortified at 0.1 ppm.

Table 1. Recovery of sulfamethazine from swine and cattle tissues

Tissue	Fortification level, ppm	% Rec., PICI		% Rec., NICI
		m/z 138⁺	m/z 293⁺	m/z 155⁻
Swine liver	0.1	138	124	114
Swine liver	0.1	135	126	112
Swine muscle	0.1	119	121	118
Swine muscle	0.1	89	91	92
Cattle liver	0.1	114	117	112
Cattle liver	0.5	110	124	98[a]
Cattle muscle	0.1	94	96	87
Cattle muscle	0.5	86	94	74[a]

[a]Detector response at m/z 155⁻ saturated. Response at m/z 157⁻ used for calculation.

adequate by itself to confirm violative residues (>0.1 ppm) in cattle and swine tissues, additional confirmatory data are readily obtainable from GC-NICI (CH_4), especially from mass spectrometers equipped with pulsed positive ion negative ion capability. Extracts prepared for analysis by the GC-EC method of Manuel and Steller (4), which was adopted official first action by AOAC (6), are analyzed directly by the GC-CIMS confirmatory procedure. Consequently, the need for additional sample preparation is avoided.

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CHEMICAL CONTAMINANTS MONITORING

Pesticide, Heavy Metal, and Other Chemical Residues in Infant and Toddler Total Diet Samples. (III). August 1976–September 1977

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The Total Diet Study has been conducted by the Food and Drug Administration, U.S. Department of Health and Human Services, since 1964 to monitor the diet of adults, and more recently the infant and toddler diet, for residues of pesticides and other chemicals. This report presents the residues found in 12 infant and toddler market baskets, i.e., items comprising the average 2-week diet of infants and toddlers, collected in 12 major cities in 4 geographical areas from August 1976 through September 1977. The analyses for each market basket were performed on composites of each of 11 food classes, and the results are presented in a variety of formats. In addition to chemical residues, the market baskets were also analyzed for 6 heavy metals: arsenic, lead, selenium, zinc, cadmium, and mercury. Results of recovery studies conducted with residue compounds of various types are also included in this report. The results for the adult market baskets for the same period are published as a separate report.

The Total Diet Program (1), conducted by the Food and Drug Administration (FDA), began as a surveillance program for fission products resulting from atmospheric tests of thermonuclear weapons in May 1961. The area of concern changed in 1964 to center on chemical residues in the dietary intake of the American consumer. The initial program, which focused on the 2-week diet of a 16- to 19-year-old male, continued for several years until August 1974, when 10 of the 30 adult market baskets were changed to represent the 2-week diet of the infant (6-month-old) and the toddler (2-year-old). The FDA's Office of Nutrition and Consumer Services, Bureau of Foods, used available U.S. Department of Agriculture 1965 survey data to calculate the average daily consumption of particular foods and food groups for infants and toddlers from 4 geographical areas of the United States, i.e., South, Northeast, North Central, and West.

After the food items were purchased at the grocery store, the items requiring cooking were prepared as if they were to be served and eaten at home. Each food was blended or chopped to a homogeneous consistency and then combined in its proper proportion with similar items to form a particular composite (Table 1). The composites were analyzed for organochlorine and organophosphorus pesticides; carbaryl; herbicides; the metals cadmium, selenium, zinc, mercury, lead, and arsenic; and industrial chemicals such as pentachlorobenzene and the polychlorinated biphenyls (PCBs). Methodology included established extraction and cleanup techniques followed by atomic absorption spectroscopy, fluorometry, gas chromatography, thin layer chromatography, and mass spectroscopy (2–9). Findings for the market baskets from June 1964 through July 1976 have been presented in earlier reports (1, 10–23). It must also be noted that previous infant and toddler reports (21, 23) were based on 10 market baskets per year; however, because of the additional 2 months in the present reporting period, it was necessary to increase the number of market baskets for this year to 12, which were collected in 12 widespread urban areas.

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Table 1. Commodity classes of infant and toddler foods analyzed for pesticides and other chemical residues, August 1976–September 1977

Key	Food class
I	Drinking water[a]
II	Whole milk, fresh[a]
III	Other dairy and substitutions, infant
	Other dairy and substitutions, toddler
IV	Meat, fish, and poultry, infant
	Meat, fish, and poultry, toddler
V	Grain and cereal products, infant
	Grain and cereal products, toddler
VI	Potatoes[a,b]
VII	Vegetables, infant
	Vegetables, toddler
VIII	Fruit and fruit juices, infant
	Fruit and fruit juices, toddler
IX	Oils and fats[a,c]
X	Sugar and adjuncts, infant
	Sugar and adjuncts, toddler
XI	Beverages[a,d]

Note: Use key above with Table 3.
[a]Because of similarity between infant and toddler diets, single determinations for certain classes of food were made and reported for both.
[b]No infant composites for western region.
[c]No infant composites for the north central, western, and southern regions.
[d]No infant composites for north central region.

Results

Table 2 lists the 39 compounds which accounted for the 445 residues reported for the infant composites and the 45 compounds which accounted for the 687 residues reported for the toddler composites. They are presented in decreasing order of frequency, together with the high and low amounts found. The distribution of these residues is shown in Table 3, which indicates the frequency of occurrence of each compound within each food class, and in Table 4, which presents a profile of each residue by food class. The total number of composites used to calculate each average figure shown in Table 4 was 12 in every instance (exceptions: 9 infant potato composites, 3 infant oil and fat composites, and 9 infant beverage composites), and because residues reported as "Trace" have no recorded numerical value, they were treated as zero in calculating the average values for the series. Table 5 shows the intake of pesticide and industrial chemical residues in terms of μg/kg body wt/day, and Table 6 shows the intake of 6 metals in terms of μg/day (mg/day in the case of zinc). For comparative purposes, the findings for the 1976 fiscal year are also shown. If a comparison is to be made with the 2 previous infant and toddler reports, it should be noted that this report is based on 12 market baskets, whereas the first and second reports were each based on 10 market baskets.

The most frequently found residues and their average levels are discussed below for each of the 11 food classes. No corrections were made as a result of the recovery study.

I. Drinking Water

Infant and Toddler.—Tap water was collected within the same areas as the respective market baskets and, in addition

Table 2. Chemical residues found in 117 infant and 132 toddler food composites from 12 United States cities—August 1976–September 1977

Chemical found	No. of composites with residues	No. of positive composites with residues reported as trace[a]	Range,[b] ppm
Infant			
Zinc	104	0	0.1 –41.5
Lead	66	0	0.01 – 0.44
Cadmium	64	0	0.001– 0.063
Selenium	37	0	0.01 – 0.29
Dieldrin	20	16	0.001– 0.003
p,p'-DDE	20	4	0.001– 0.009
α-BHC	19	18	0.001
Malathion	17	1	0.002– 0.050
Hexachlorobenzene	13	9	0.001– 0.006
Heptachlor epoxide	11	11	
Arsenic[c]	11	0	0.01 – 0.16
Mercury	10	0	0.001– 0.026
Chlorpropham	5	0	0.003– 0.234
Diazinon	4	3	0.002
Lindane	4	2	0.006– 0.010
Oxychlordane	3	2	0.001
Aroclor 1254	3	3	
Pentachlorobenzene	3	2	0.005
p,p'-Methoxychlor	2	2	
Parathion	2	1	0.001
Toxaphene	2	1	0.280
Ethion	2	2	
Dicloran	2	0	0.005– 0.046
Tecnazene	2	1	0.010
PCNB	2	1	0.006
Pentachloroaniline	2	0	0.004– 0.022
Pentachloroanisole	2	1	0.005
Pentachlorothioanisole	2	1	0.007
Chlordane	1	1	
Carbaryl	1	1	
Phosalone	1	0	0.009
PCB	1	1	
Tetrachlorobenzene	1	0	0.003
Tetrachloroaniline	1	0	0.001
Tetrachloroanisole	1	1	
Tetrachlorothioanisole	1	0	0.001
Tetrachloroanisidine	1	0	0.003
p,p'-DDT	1	0	0.002
p,p'-TDE	1	0	0.002
TOTAL	445	85	
Toddler			
Zinc	122	0	0.1 –37.5
Cadmium	87	0	0.001– 0.058
Lead	75	0	0.01 – 0.25
α-BHC	40	27	0.001– 0.003
Dieldrin	39	19	0.001– 0.004
Selenium	39	0	0.01 – 0.34
p,p'-DDE	29	4	0.001– 0.235
Malathion	27	3	0.003– 0.820
Heptachlor epoxide	27	14	0.001– 0.003
Hexachlorobenzene	27	17	0.001– 0.006
Lindane	18	9	0.001– 0.006
Mercury	18	0	0.001– 0.040
Arsenic[c]	16	0	0.02 – 0.33
Oxychlordane	13	9	0.001
Pentachlorobenzene	10	2	0.001– 0.010
Diazinon	9	5	0.001– 0.003
Pentachloroanisole	9	3	0.003– 0.009
PCNB	8	2	0.002– 0.015
Pentachloroaniline	8	1	0.002– 0.025
Pentachlorothioanisole	8	1	0.001– 0.010
Toxaphene	7	3	0.068– 0.184
Chlorpropham	7	0	0.012– 0.453
Aroclor 1254	6	6	
Parathion	5	3	0.002– 0.003
Dicloran	4	0	0.003– 0.059
Fonofos	3	0	0.001– 0.004
Endosulfan II	3	1	0.001– 0.002
p,p'-DDT	3	0	0.002– 0.044
Ethion	2	1	0.001
Tecnazene	2	1	0.011
Endosulfan I	2	1	0.001
Chlordane	1	1	
p,p'-Methoxychlor	1	1	
Phosalone	1	0	0.039
PCP	1	0	0.035
PCB	1	1	
trans-Nonachlor	1	0	0.003
Tetrachlorobenzene	1	0	0.003
Tetrachloroaniline	1	0	0.001
Tetrachloroanisole	1	1	
Tetrachlorothioanisole	1	0	0.001
Tetrachloroanisidine	1	0	0.003
Endosulfan sulfate	1	0	0.002
β-BHC	1	0	0.002
p,p'-TDE	1	0	0.178
TOTAL	687	136	

[a]Chemicals detected by a specific analytical methodology were confirmed qualitatively and reported as trace when present at concentrations below the limit of quantitation. The limit of quantitation varies with residue and food class.
[b]Range is for positive composites found in quantifiable amounts and does not include positive composites reported as trace.
[c]Calculated as arsenic trioxide (As_2O_3).

to being analyzed alone, was used in preparation of other items requiring addition of or dilution with water. A single analysis of tap water was performed for each of the 12 urban areas represented, and the results are reported for both the infant and the toddler diets. Low levels of zinc and cadmium were found, and a single composite contained lead at the 0.04 ppm level.

II. Whole Milk, Fresh

Infant and Toddler.—Because this food class was common to both the infant and toddler diets, one analysis was reported for both. Zinc was found in all 12 composites in concentrations that ranged from 1.8 to 4.8 ppm and averaged 3.85 ppm. Although dieldrin and α-BHC were reported for 8 and 10 composites, respectively, they were found mostly at the trace level. Residues of *p,p'*-DDE, which averaged 0.0012 ppm for the 12 composites examined, were found in 4 composites at levels ranging from 0.001 to 0.009 ppm and in one composite at the trace level. Hexachlorobenzene, with an overall average level of 0.0003 ppm in fresh whole milk, was found in 4 composites. Selenium, which averaged 0.007 ppm for the 12 composites examined, was found in 4 composites, and both cadmium and lead were each found in one composite at a low level. Trace level averages were reported for the other pesticide residues found in this food class: heptachlor epoxide, *p,p'*-methoxychlor, and lindane. Industrial chemicals were found in 2 composites at the trace level; one was identified as the PCB Aroclor 1254®, and the other was an undefined PCB.

III. Other Dairy and Substitutions

Infant.—The infant and toddler composites for this food class were analyzed separately because of the differences in these diets. The highest level of an organochlorine pesticide was 0.002 ppm, reported for both *p,p'*-DDE and dieldrin; residues of heptachlor epoxide, *p,p'*-methoxychlor, α-BHC, hexachlorobenzene, and lindane were also reported at the trace level. All 6 metals were found at relatively low levels with the following averages for the 12 composites examined: zinc, 5.37 ppm; lead, 0.018 ppm; arsenic, 0.005 ppm; mercury, 0.0031 ppm; selenium, 0.008 ppm; and cadmium, 0.0018 ppm. The organophosphorus pesticide malathion was also found in one composite at the trace level.

Toddler.—By contrast, the toddler composites of this food class showed higher average residue levels than the infant composites and additional compounds. More positive com-

Table 3. Frequency of occurrence of chemical residues, by food class, in infant and toddler food composites—August 1976–September 1977

Chemical	Food class[a] I	II	III	IV	V	VI	VII	VIII	IX	X	XI
Number of Occurrences in Infant Foods											
Zinc	5	12	12	12	11	8	12	11	3	10	8
Lead	1	1	5	8	9	8	9	8	2	7	8
Cadmium	6	1	4	8	11	9	10	5	3	2	5
Selenium	0	4	5	11	12	1	2	0	1	1	0
Dieldrin	0	8	4	6	0	1	0	0	1	0	0
p,p'-DDE	0	5	3	7	0	1	2	0	2	0	0
α-BHC	0	10	5	3	0	0	0	0	1	0	0
Malathion	0	0	1	0	12	0	0	1	3	0	0
Hexachlorobenzene	0	4	3	2	0	0	1	0	3	0	0
Heptachlor epoxide	0	4	4	2	0	1	0	0	0	0	0
Arsenic	0	0	2	2	7	0	0	0	0	0	0
Mercury	0	0	2	2	1	2	0	1	0	2	0
Chlorpropham	0	0	0	0	0	5	0	0	0	0	0
Diazinon	0	0	0	0	4	0	0	0	0	0	0
Lindane	0	1	1	0	2	0	0	0	0	0	0
Oxychlordane	0	0	0	3	0	0	0	0	0	0	0
Aroclor 1254	0	1	0	2	0	0	0	0	0	0	0
Pentachlorobenzene	0	0	0	1	0	0	0	0	2	0	0
p,p'-Methoxychlor	0	1	1	0	0	0	0	0	0	0	0
Parathion	0	0	0	0	1	0	0	0	1	0	0
Toxaphene	0	0	0	0	0	0	0	0	2	0	0
Ethion	0	0	0	0	0	0	0	2	0	0	0
Dicloran	0	0	0	0	0	1	0	1	0	0	0
Tecnazene	0	0	0	0	0	1	0	0	1	0	0
PCNB	0	0	0	0	0	0	0	0	2	0	0
Pentachloroaniline	0	0	0	0	0	0	0	0	2	0	0
Pentachloroanisole	0	0	0	0	0	0	0	0	2	0	0
Pentachlorothioanisole	0	0	0	0	0	0	0	0	2	0	0
Chlordane	0	0	0	0	0	0	1	0	0	0	0
Carbaryl	0	0	0	0	0	0	0	1	0	0	0
Phosalone	0	0	0	0	0	0	0	1	0	0	0
PCB	0	1	0	0	0	0	0	0	0	0	0
Tetrachlorobenzene	0	0	0	0	0	1	0	0	0	0	0
Tetrachloroaniline	0	0	0	0	0	1	0	0	0	0	0
Tetrachloroanisole	0	0	0	0	0	1	0	0	0	0	0
Tetrachlorothioanisole	0	0	0	0	0	1	0	0	0	0	0
Tetrachloroanisidine	0	0	0	0	0	1	0	0	0	0	0
p,p'-DDT	0	0	0	1	0	0	0	0	0	0	0
p,p'-TDE	0	0	0	1	0	0	0	0	0	0	0
Number of Occurrences In Toddler Foods											
Zinc	5	12	12	12	11	11	12	12	12	11	12
Cadmium	6	1	6	6	11	11	12	6	12	10	6
Lead	1	1	6	8	9	10	12	11	5	7	5
α-BHC	0	10	12	7	0	0	2	0	1	8	0
Dieldrin	0	8	11	12	0	2	1	0	5	0	0
Selenium	0	4	9	11	10	0	2	0	2	1	0
p,p'-DDE	0	5	8	11	0	1	1	0	3	0	0
Malathion	0	0	0	0	12	0	0	0	12	3	0
Heptachlor epoxide	0	4	10	10	0	1	0	0	2	0	0
Hexachlorobenzene	0	4	8	6	0	1	0	0	7	1	0
Lindane	0	1	2	3	0	1	2	0	0	9	0
Mercury	0	0	0	9	0	3	0	0	5	0	1
Arsenic	0	0	1	10	4	0	0	0	0	1	0
Oxychlordane	0	0	6	7	0	0	0	0	0	0	0
Pentachlorobenzene	0	0	0	1	0	1	0	0	8	0	0
Diazinon	0	0	0	1	4	0	0	1	2	1	0
Pentachloroanisole	0	0	0	0	0	1	0	0	8	0	0
PCNB	0	0	0	0	0	0	0	0	8	0	0
Pentachloroaniline	0	0	0	0	0	0	0	0	8	0	0
Pentachlorothioanisole	0	0	0	0	0	0	0	0	8	0	0
Toxaphene	0	0	0	0	0	0	0	0	7	0	0
Chlorpropham	0	0	0	0	0	7	0	0	0	0	0
Aroclor 1254	0	1	1	2	0	0	0	0	1	1	0
Parathion	0	0	0	0	1	0	0	0	4	0	0
Dicloran	0	0	0	0	0	0	0	4	0	0	0
Fonofos	0	0	0	0	0	0	0	0	3	0	0
Endosulfan II	0	0	1	0	0	0	1	1	0	0	0
p,p'-DDT	0	0	0	3	0	0	0	0	0	0	0
Ethion	0	0	0	0	0	0	0	2	0	0	0
Tecnazene	0	0	0	0	0	1	0	0	1	0	0
Endosulfan I	0	0	1	0	0	0	1	0	0	0	0
Chlordane	0	0	0	0	0	0	0	0	1	0	0
p,p'-Methoxychlor	0	1	0	0	0	0	0	0	0	0	0
Phosalone	0	0	0	0	0	0	0	1	0	0	0
PCP	0	0	0	0	0	0	0	0	0	1	0
PCB	0	1	0	0	0	0	0	0	0	0	0
trans-Nonachlor	0	0	0	1	0	0	0	0	0	0	0
Tetrachlorobenzene	0	0	0	0	0	1	0	0	0	0	0
Tetrachloroaniline	0	0	0	0	0	1	0	0	0	0	0
Tetrachloroanisole	0	0	0	0	0	1	0	0	0	0	0
Tetrachlorothioanisole	0	0	0	0	0	1	0	0	0	0	0
Tetrachloroanisidine	0	0	0	0	0	1	0	0	0	0	0
Endosulfan sulfate	0	0	0	0	0	0	0	1	0	0	0
β-BHC	0	0	0	1	0	0	0	0	0	0	0
p,p'-TDE	0	0	0	1	0	0	0	0	0	0	0

[a]See Table 1 for description of food class.

posites and higher average levels were reported for *p,p'*-DDE, heptachlor epoxide, dieldrin, α-BHC, hexachlorobenzene, and lindane. In addition, low levels of oxychlordane, endosulfan I, and endosulfan II were reported for the toddler composites. Both the numbers of positive composites and the average levels of the metals, zinc, lead, arsenic, and cadmium for the toddler composites were similar to those for the infant composites. Mercury was found in 2 infant composites at the 0.011 and 0.026 ppm levels, but none was reported for the toddler composites; however, a much higher incidence and level of selenium were found in the toddler composites. The industrial chemical Aroclor 1254 was found only once in this food class in one toddler composite. The toddler composites did not contain malathion residues.

IV. Meat, Fish, and Poultry

Infant.—These composites contained a higher number of residues than any other food class in the infant diet. Slightly more than half were metal residues with the following overall average concentrations: zinc, 17.88 ppm; selenium, 0.097 ppm; lead, 0.024 ppm; arsenic, 0.016 ppm; and low levels of cadmium and mercury. The overall average levels of the organochlorine pesticide residues were very low with the exception of *p,p'*-DDE, which was found in 7 composites at levels ranging from 0.002 to 0.007 ppm and averaged 0.0021 ppm for the 12 composites examined. Two industrial chemicals, Aroclor 1254 and pentachlorobenzene, were found at trace levels.

Toddler.—The residues detected most frequently and at the highest level were found in the toddler composites for the meat, fish, and poultry food class. All composites contained zinc residues that ranged from 15.0 to 37.5 ppm and averaged 30.02 ppm. Eleven composites contained selenium residues, which ranged from 0.10 to 0.34 ppm and averaged 0.203 ppm for the 12 composites examined. Arsenic was found in 10 composites at levels ranging from 0.03 to 0.33 ppm with an overall average level of 0.143 ppm in this food class. Eight composites contained lead concentrations that ranged from 0.01 to 0.13 ppm with an average level of 0.037 ppm for the 12 composites examined. Low levels of mercury and cadmium were found in 9 and 6 composites, respectively. Among the organochlorine pesticide residues, the highest levels, which ranged from 0.001 to 0.235 ppm, were reported for *p,p'*-DDE. In addition to the same organochlorine pesticide residues reported for the infant composites, the toddler composites also contained low levels of lindane, *trans*-nonachlor, and β-BHC. A trace of the organophosphorus compound diazinon was reported for one toddler composite, and traces of the same 2 industrial chemicals found in the infant composites were also reported.

V. Grain and Cereal Products

Infant.—All 12 grain and cereal product composites contained selenium residues at levels ranging from 0.02 to 0.29 ppm and malathion residues at levels ranging from 0.004 to

Table 4. Levels of chemical residues found by food class, infant and toddler Total Diet--August 1976-September 1977

Residue	Overall Average in Infant Composites, [a] ppm	Positive Infant Composites: Total Number	Positive Infant Composites: Number Reported as Trace	Positive Infant Composites: Range, [b] ppm	Overall Average in Toddler Composites, [a] ppm	Positive Toddler Composites: Total Number	Positive Toddler Composites: Number Reported as Trace	Positive Toddler Composites: Range, [b] ppm
I. Drinking Water								
Zinc	0.18	5	0	0.1 - 0.9	0.18	5	0	0.1 - 0.9
Lead	0.003	1	0	0.04	0.003	1	0	0.04
Cadmium	0.002	6	0	0.001 - 0.008	0.002	6	0	0.001 - 0.008
II. Whole Milk, Fresh								
Zinc	3.85	12	0	1.8 - 4.8	3.85	12	0	1.8 - 4.8
Dieldrin	0.0001	8	7	0.001	0.0001	8	7	0.001
Hexachloro-benzene	0.0003	4	2	0.001 - 0.003	0.0003	4	2	0.001 - 0.003
Heptachlor epoxide	T	4	4		T	4	4	
p,p'-DDE	0.0012	5	1	0.001 - 0.009	0.0012	5	1	0.001 - 0.009
α-BHC	T	10	10		T	10	10	
Selenium	0.007	4	0	0.02	0.007	4	0	0.02
PCB	T	1	1		T	1	1	
Cadmium	0.0002	1	0	0.003	0.0002	1	0	0.003
PCB (as Aroclor 1254)	T	1	1		T	1	1	
Lindane	T	1	1		T	1	1	
p,p'-Methoxychlor	T	1	1		T	1	1	
Lead	0.001	1	0	0.01	0.001	1	0	0.01
III. Other Dairy and Substitutions								
Zinc	5.37	12	0	3.8 - 7.5	5.86	12	0	4.7 - 7.1
Heptachlor epoxide	T	4	4		0.009	10	2	0.001 - 0.002
p,p'-Methoxychlor	T	1	1					
α-BHC	T	5	5		0.0011	12	3	0.001 - 0.002
p,p'-DDE	0.0002	3	2	0.002	0.0020	8	2	0.001 - 0.011
Dieldrin	0.0002	4	3	0.002	0.0017	11	3	0.002 - 0.004
Lead	0.018	5	0	0.02 - 0.07	0.014	6	0	0.01 - 0.05
Hexachloro-benzene	T	3	3		0.0003	8	6	0.001 - 0.003
Arsenic	0.005	2	0	0.03	0.003	1	0	0.04
Selenium	0.008	5	0	0.01 - 0.02	0.027	9	0	0.01 - 0.07
Cadmium	0.0018	4	0	0.001 - 0.016	0.0022	6	0	0.001 - 0.012
Oxychlordane					0.0001	6	5	0.001
Endosulfan II					0.0001	1	0	0.001
Mercury	0.0031	2	0	0.011 - 0.026				
Malathion	T	1	1					
Lindane	T	1	1		T	2	2	
Endosulfan I					0.0001	1	0	0.001
PCB (as Aroclor 1254)					T	1	1	
IV. Meat, Fish, and Poultry								
Cadmium	0.0047	8	0	0.001 - 0.019	0.0029	6	0	0.001 - 0.015
Zinc	17.88	12	0	10.8 - 32.5	30.02	12	0	15.0 - 37.5
Selenium	0.097	11	0	0.06 - 0.20	0.203	11	0	0.10 - 0.34
Lead	0.024	8	0	0.01 - 0.08	0.037	8	0	0.01 - 0.13
Mercury	0.0010	2	0	0.005 - 0.007	0.0075	9	0	0.005 - 0.016
Heptachlor epoxide	T	2	2		0.0004	10	6	0.001 - 0.002
Arsenic	0.016	2	0	0.03 - 0.16	0.143	10	0	0.03 - 0.33
Hexachloro-benzene	T	2	2		T	6	6	
PCB (as Aroclor 1254)	T	2	2		T	2	2	
p,p'-TDE	0.0002	1	0	0.002	0.0148	1	0	0.178
Lindane					0.0001	3	2	0.002
β-BHC					0.0002	1	0	0.002
Dieldrin	0.0001	6	5	0.001	0.0014	12	4	0.001 - 0.004
p,p'-DDE	0.0021	7	0	0.002 - 0.007	0.0256	11	0	0.001 - 0.235
α-BHC	0.0001	3	2	0.001	T	7	7	
Oxychlordane	0.0001	3	2	0.001	0.0002	7	4	0.001
Pentachloro-benzene	T	1	1		T	1	1	
p,p'-DDT	0.0002	1	0	0.002	0.0043	3	0	0.002 - 0.044
trans-Nonachlor					0.0002	1	0	0.003
Diazinon					T	1	1	
V. Grain and Cereal Products								
Arsenic	0.026	7	0	0.01 - 0.10	0.014	4	0	0.02 - 0.08
Selenium	0.162	12	0	0.02 - 0.29	0.131	10	0	0.04 - 0.24
Diazinon	0.0002	4	3	0.002	0.0005	4	1	0.001 - 0.003
Lead	0.068	9	0	0.03 - 0.26	0.039	9	0	0.01 - 0.12
Mercury	0.0001	1	0	0.001				
Cadmium	0.0232	11	0	0.015 - 0.041	0.0204	11	0	0.014 - 0.035
Zinc	14.36	11	0	8.7 - 23.5	10.42	11	0	7.3 - 24.0
Malathion	0.0165	12	0	0.004 - 0.050	0.0121	12	0	0.003 - 0.042
Lindane	0.0013	2	0	0.006 - 0.010				
Parathion	0.0001	1	0	0.001	T	1	1	

Continued

Table 4. (cont'd)

VI. Potatoes								
Cadmium	0.0344	9	0	0.014 - 0.058	0.0317	11	0	0.013 - 0.058
Zinc	2.53	8	0	0.8 - 5.7	3.03	11	0	1.8 - 5.7
Lead	0.036	8	0	0.01 - 0.07	0.039	10	0	0.01 - 0.11
Dieldrin	T	1	1		0.0001	2	1	0.001
Selenium	0.006	1	0	0.05				
Chlorpropham	0.0668	5	0	0.003 - 0.234	0.0872	7	0	0.012 - 0.453
Mercury	0.0024	2	0	0.010 - 0.012	0.0052	3	0	0.010 - 0.040
Tecnazene	0.0011	1	0	0.010	0.0009	1	0	0.011
Tetrachloro-anisidine	0.0003	1	0	0.003	0.0002	1	0	0.003
Tetrachloro-anisole	T	1	1		T	1	1	
Lindane					0.0003	1	0	0.004
Pentachloro-benzene					T	1	1	
Heptachlor epoxide	T	1	1		T	1	1	
Dicloran	0.0051	1	0	0.046				
p,p'-DDE	0.0002	1	0	0.002	0.0002	1	0	0.002
Tetrachloro-aniline	0.0001	1	0	0.001	0.0001	1	0	0.001
Tetrachloro-thioanisole	0.0001	1	0	0.001	0.0001	1	0	0.001
Tetrachloro-benzene	0.0003	1	0	0.003	0.0002	1	0	0.003
Hexachloro-benzene					T	1	1	
Pentachloro-anisole					T	1	1	
VII. Vegetables								
Cadmium	0.0197	10	0	0.007 - 0.058	0.0210	12	0	0.008 - 0.047
Zinc	4.62	12	0	2.3 - 16.0	4.54	12	0	3.2 - 7.5
p,p'-DDE	0.0004	2	1	0.005	T	1	1	
Chlordane	T	1	1					
Endosulfan II					T	1	1	
Dieldrin					T	1	1	
Lead	0.085	9	0	0.01 - 0.26	0.103	12	0	0.04 - 0.25
Hexachloro-benzene	T	1	1					
Selenium	0.002	2	0	0.01 - 0.02	0.004	2	0	0.02 - 0.03
Endosulfan I					T	1	1	
Lindane					0.0002	2	1	0.002
α-BHC					0.0003	2	0	0.001 - 0.003
VIII. Fruit and Fruit Juices								
Lead	0.063	8	0	0.02 - 0.22	0.064	11	0	0.02 - 0.11
Dicloran	0.0004	1	0	0.005	0.0093	4	0	0.003 - 0.059
Mercury	0.0009	1	0	0.011				
Cadmium	0.0055	5	0	0.001 - 0.025	0.0060	6	0	0.003 - 0.027
Carbaryl	T	1	1					
Endosulfan sulfate					0.0002	1	0	0.002
Zinc	1.27	11	0	0.6 - 2.4	1.06	12	0	0.5 - 3.0
Phosalone	0.0007	1	0	0.009	0.0032	1	0	0.039
Malathion	0.0002	1	0	0.002				
Ethion	T	2	2		0.0001	2	1	0.001
Endosulfan II					0.0002	1	0	0.002
Diazinon					T	1	1	
IX. Oils and Fats								
Cadmium	0.0627	3	0	0.062 - 0.063	0.0329	12	0	0.017 - 0.046
Zinc	27.33	3	0	23.5 - 32.5	13.11	12	0	5.2 - 18.0
p,p'-DDE	0.0023	2	0	0.003 - 0.004	0.0008	3	0	0.001 - 0.005
Malathion	0.0210	3	0	0.010 - 0.041	0.1016	12	1	0.003 - 0.820
Selenium	0.023	1	0	0.07	0.007	2	0	0.04
Pentachloro-benzene	0.0017	2	1	0.005	0.0024	8	0	0.001 - 0.010
Pentachloro-anisole	0.0017	2	1	0.005	0.0023	8	2	0.003 - 0.009
Pentachloro-aniline	0.0087	2	0	0.004 - 0.022	0.0076	8	1	0.002 - 0.025
Lead	0.010	2	0	0.01 - 0.02	0.022	5	0	0.01 - 0.12
Hexachloro-benzene	0.0023	3	1	0.001 - 0.006	0.0021	7	1	0.001 - 0.006
Toxaphene	0.0933	2	1	0.280	0.0468	7	3	0.068 - 0.184
Parathion	T	1	1		0.0004	4	2	0.002 - 0.003
Dieldrin	0.0010	1	0	0.003	0.0004	5	3	0.002 - 0.003
Tecnazene	T	1	1		T	1	1	
PCNB	0.0020	2	1	0.006	0.0027	8	2	0.002 - 0.015
Pentachloro-thioanisole	0.0023	2	1	0.007	0.0026	8	1	0.001 - 0.010
α-BHC	T	1	1		T	1	1	
Fonofos					0.0006	3	0	0.001 - 0.004
Heptachlor epoxide					0.0002	2	1	0.003
Chlordane					T	1	1	
Diazinon					T	2	2	
Mercury					0.0018	5	0	0.001 - 0.006
PCB (as Aroclor 1254)					T	1	1	

Continued

Table 4. (cont'd)

				X. Sugar and Adjuncts				
Zinc	4.06	10	0	0.1 - 41.5	4.48	11	0	1.4 - 16.5
Selenium	0.002	1	0	0.02	0.002	1	0	0.03
Cadmium	0.0003	2	0	0.001 - 0.003	0.0067	10	0	0.003 - 0.021
Lindane					0.0014	9	3	0.001 - 0.006
Lead	0.023	7	0	0.01 - 0.13	0.040	7	0	0.02 - 0.14
Mercury	0.0004	2	0	0.001 - 0.004				
α-BHC					0.0004	8	6	0.002 - 0.003
PCB (as Aroclor 1254)					T	1	1	
Arsenic					0.002	1	0	0.03
Malathion					0.0012	3	2	0.015
PCP					0.0029	1	0	0.035
Hexachloro-benzene					T	1	1	
Diazinon					0.0002	1	0	0.002
				XI. Beverages				
Zinc	0.40	8	0	0.1 - 0.9	0.23	12	0	0.1 - 0.9
Cadmium	0.0009	5	0	0.001 - 0.004	0.0027	6	0	0.001 - 0.016
Lead	0.082	8	0	0.01 - 0.44	0.017	5	0	0.01 - 0.08
Mercury					0.0001	1	0	0.001

NOTE: T = trace.
[d]Average values are based on the total number of composites examined; trace residues, if present, were treated as zero in calculating averages. Thus, an average value of "T" can be well below the detection limit of a method for a specific compound. If the average value was less than 0.0001 ppm, it is reported as "T".
[D]Range is for positive composites found in quantifiable amounts and does not include positive composites reported as trace.

0.050 ppm. Zinc and cadmium were found in 11 composites and averaged 14.36 and 0.0232 ppm, respectively, for the 12 composites examined. Lead, ranging from 0.03 to 0.26 ppm, was found in 9 composites with an overall average level of 0.068 ppm in this food class, and arsenic, ranging from 0.01 to 0.10 ppm, was found in 7 composites. In addition to malathion, 2 other organophosphorus compounds, diazinon and parathion, were reported at low levels. The only organochlorine pesticide, lindane, was found in 2 composites at the 0.006 and 0.010 ppm levels.

Toddler.—Generally, the same residues reported for the infant composites in this food class were also found in the toddler composites, but at lower levels. The exceptions were the absence of mercury and lindane residues in the toddler composites; however, mercury was found in only one infant composite, at the 0.001 ppm level.

VI. Potatoes

Infant.—The nonmetal residue found at the highest level in the potato composites was chlorpropham, which ranged from 0.003 to 0.234 ppm in 5 composites and averaged 0.0668 ppm for the 9 composites examined. The overall average levels of the metals in potatoes were led by zinc with the highest average, 2.53 ppm, followed by the averages for lead, 0.036 ppm, and cadmium, 0.0344 ppm. Each of the following organochlorine pesticide residues was found in only one composite: dieldrin, heptachlor epoxide, dicloran, and *p,p'*-DDE. The remaining residues, each of which was found in a single composite, were associated with the organochlorine fungicide tecnazene, either as a breakdown product or as a by-product in its manufacture.

Toddler.—In this food class, the differences between the toddler composites and the infant composites were minimal. Traces of the industrial chemical pentachlorobenzene and the fungicide pentachloroanisole were found only in the toddler composites; selenium and dicloran were found only in the infant composites.

VII. Vegetables

Infant.—The metals were the predominant residues found in the infant composites of this food class. Zinc, lead, and cadmium, each found in 9 or more of the composites, averaged 4.62, 0.085, and 0.0197 ppm, respectively, for the 12 composites examined. Selenium was found in 2 composites at the 0.01 and 0.02 ppm levels. Chlordane, *p,p'*-DDE, and hexachlorobenzene accounted for the remaining residues.

Toddler.—All toddler composites in the vegetable food class contained zinc, lead, and cadmium residues at relatively the same overall average levels as the infant composites, and 2 infant and 2 toddler composites contained selenium; however, the toddler selenium residue levels were slightly higher. The remaining residues, all found at very low levels, were *p,p'*-DDE, endosulfan I, endosulfan II, lindane, α-BHC, and dieldrin. Residues of hexachlorobenzene or chlordane were not found in the toddler composites.

VIII. Fruit and Fruit Juices

Infant.—Eleven infant fruit composites contained zinc residues at levels ranging from 0.6 to 2.4 ppm, 8 contained lead residues at levels ranging from 0.02 to 0.22 ppm, and 5 contained cadmium at levels ranging from 0.001 to 0.025 ppm. Three organophosphorus residues were detected at very low levels in just a few composites: phosalone, malathion, and ethion. The carbamate pesticide carbaryl was reported in one composite at the trace level.

Toddler.—The toddler fruit composites contained the same metal residues as the infant composites with one exception: The toddler composites contained no mercury residues, whereas one infant composite contained mercury at the 0.011 ppm level. The organochlorine pesticide dicloran was found in 4 toddler composites at levels ranging from 0.003 to 0.059 ppm, compared with a single infant composite that contained dicloran at the 0.005 ppm level. The fruit and fruit juices class was the only food class in the toddler diet in which dicloran was found. The other organochlorine pesticide residues detected included endosulfan II and endosulfan sulfate. Although no malathion residues were reported, 3 other organophosphorus compounds were detected: Phosalone was found in one composite at the 0.039 ppm level; ethion was found in 2 composites, at the 0.001 ppm level in one and at the trace level in the other; and diazinon was found in one composite at the trace level. The fruit composites were also the only toddler composites that contained residues of endosulfan sulfate, phosalone, and ethion.

IX. Oils and Fats

Infant.—The specific infant composites for this food class consisted primarily of peanut butter and were prepared from market baskets originating in only one of the 4 geographical

Table 5. Intake of pesticide and industrial chemical residues in infant and toddler Total Diet Studies—FY 1976 vs FY 1977

Compound	Intake, μg/kg body wt/day			
	Infant		Toddler	
	FY 76	FY 77	FY 76	FY 77
Aldrin	ND	ND	ND	ND
Dieldrin	0.0249	0.0405	0.0412	0.0423
Endrin	ND	ND	0.0007	ND
Total	0.0249	0.0405	0.0419	0.0423
DDE	0.0682	0.0999	0.0985	0.3316
DDT	T	0.0010	0.0046	0.0481
TDE	ND	0.0010	0.0018	0.1682
Total	0.0682	0.1019	0.1049	0.5479
Endosulfan I	0.0011	ND	ND	0.0004
Endosulfan II	0.0045	ND	ND	0.0012
Endosulfan sulfate	0.0368	ND	ND	0.0008
Total	0.0424	ND	ND	0.0024
Heptachlor epoxide	0.0001	0.0133	0.0057	0.0182
Heptachlor	ND	ND	ND	ND
Total	0.0001	0.0133	0.0057	0.0182
BHC	0.0055	0.0314	0.0132	0.0253
Captan	ND	ND	ND	ND
Carbaryl	T	ND	ND	ND
Carbophenothion	ND	ND	ND	ND
Chlordane	T	ND	0.0100	ND
Chlorpropham	0.0321	0.0505	0.3942	0.2251
2,4-D	ND	ND	0.0058	ND
DCPA	ND	ND	ND	ND
Diazinon	0.0053	0.0140	0.0030	0.0071
Dicloran	0.0230	0.0132	0.0342	0.0891
Dicofol	ND	ND	ND	ND
Ethion	T	0.0011	ND	0.0010
Fonofos	T	ND	0.0002	0.0007
Hexachlorobenzene	0.0009	0.0382	0.0042	0.0219
Leptophos	ND	ND	ND	ND
Lindane	0.0049	0.0059	0.0096	0.0081
Malathion	0.0865	0.0643	0.1488	0.2097
Methoxychlor	T	0.0066	T	0.0026
trans-Nonachlor	ND	ND	0.0050	0.0021
Oxychlordane	0.0008	0.0011	T	0.0051
Parathion	T	0.0112	0.0013	0.0005
Parathion-methyl	ND	ND	ND	ND
PCNB	ND	0.0014	0.0007	0.0029
PCP	T	ND	0.0162	0.0060
Pentachloroaniline	ND	0.0053	0.0064	0.0079
Pentachloroanisole	ND	0.0012	0.0003	0.0028
Pentachlorobenzene	ND	0.0013	0.0013	0.0026
Pentachlorothioanisole	ND	0.0016	0.0004	0.0027
Perthane	T	ND	ND	ND
o-Phenylphenol	ND	ND	T	ND
Phosalone	ND	0.0115	ND	0.0425
Polychlorinated biphenyls (PCBs)	T	0.0253	ND	0.0301
Ronnel	ND	ND	0.0022	ND
Tecnazene	0.0019	0.0009	0.0074	0.0026
Tetrachloroaniline	ND	ND	ND	0.0002
Tetrachloroanisidine	ND	0.0002	ND	0.0007
Tetrachloroanisole	ND	0.0001	ND	T
Tetrachlorobenzene	ND	0.0016	ND	0.0007
Tetrachlorothioanisole	ND	ND	ND	0.0002
Toxaphene	T	0.0683	0.0127	0.0443

Note: ND = not detected; T = trace (below the limits of quantitation; detected and verified, but not quantifiable).

regions represented in this study. Thus the residues reported for the infant oil and fat composites reflect the analysis of peanut butter from 3 market baskets collected within a single geographic area. All 3 composites contained zinc residues, which ranged from 23.5 to 32.5 ppm and averaged 27.33 ppm. All 3 composites also contained cadmium, which ranged from 0.062 to 0.063 ppm; 2 composites contained lead at the 0.01 and 0.02 ppm levels; and one composite contained selenium at the 0.07 ppm level. The organochlorine pesticide toxaphene was found in 2 composites, at the 0.280 ppm level in one and at the trace level in the other; organochlorine compounds found at lower levels included hexachlorobenzene in all 3 composites, and *p,p'*-DDE, dieldrin, and α-BHC. Malathion was found in all 3 composites at levels ranging from 0.010 to 0.041 ppm. Parathion was detected in one composite at the trace level. The organochlorine fungicide PCNB, together with associated compounds (8), was detected in 2 composites.

Toddler.—Each toddler oil and fat composite contained about half the amount of peanut butter as each infant composite; the other half consisted of other items, such as gravies, oils, and mayonnaise. Except for a few additional compounds, the residues in these composites were similar to those found in the infant oil and fat composites and were detected either at the same or lower levels. Malathion, found in all 3 infant composites at levels ranging from 0.010 to 0.041 ppm, was reported for all 12 toddler composites at levels ranging from 0.003 to 0.820 ppm in 11 composites and at the trace level in one. The additional residues included mercury, found in 5 composites at levels ranging from 0.001 to 0.006 ppm, and heptachlor epoxide, chlordane, diazinon, fonofos, and Aroclor 1254, all found at low levels.

X. Sugar and Adjuncts

Infant.—Only metal residues were reported for these infant composites. Zinc, found in 10 composites at levels ranging from 0.1 to 41.5 ppm, averaged 4.06 ppm for the 12 composites examined. Lead was found in 7 composites and averaged 0.023 ppm for the 12 composites examined. Selenium, mercury, and cadmium were found in a few composites at low levels.

Toddler.—The metal residues found in the toddler composites included zinc, selenium, and lead at approximately the same levels as in the infant composites. Cadmium, on the other hand, was found in 10 composites at levels ranging from 0.003 to 0.021 ppm, and arsenic was found in one composite at the 0.03 ppm level. Organochlorine pesticide residues found at low levels included lindane, α-BHC, hexachlorobenzene, and PCP. Two organophosphorus compounds, malathion and diazinon, were reported at low levels, and one composite contained a trace of the industrial chemical Aroclor 1254.

XI. Beverages

Infant.—The only residues found in the infant beverage composites were metals: Eight composites contained zinc at levels ranging from 0.1 to 0.9 ppm, 8 composites contained lead at levels ranging from 0.01 to 0.44 ppm, and 5 composites contained cadmium at low levels.

Toddler.—Zinc, lead, and cadmium were also found in the toddler composites in this food class; zinc and cadmium were found more frequently and lead was found less frequently than in the infant composites. A single composite contained mercury at the 0.001 ppm level.

Discussion

This is the third report in the infant and toddler series; however, any comparison with the 2 previous reports should be made with the understanding that these results are based on 12 market baskets, whereas 10 market baskets were collected for each of the other 2 reporting periods. Thus the total numbers in this report are not appropriate for direct compar-

Table 6. Dietary intakes of metals in infant and toddler Total Diet Studies—FY 1976 vs FY 1977

Metal	Intake, μg/day			
	Infant		Toddler	
	FY 76	FY 77	FY 76	FY 77
Arsenic[a]	0.4	4.6	12.3	24.7
Cadmium	12.3	5.8	14.2	7.7
Lead	26.9	22.1	30.1	27.8
Mercury	0.6	1.0	0.8	1.1
Selenium	10.8	15.1	45.0	46.3
Zinc[b]	8.2	4.3	9.5	7.8

[a]Values calculated as arsenic trioxide (As_2O_3).
[b]Values are mg/day.

Table 7. Recovery data for residues found in infant and toddler Total Diet samples—August 1976–September 1977

Residue	Type of food composites	Spike level, ppm	Range of unfortified level, ppm[a]	Range of total found, ppm[a,b]	Number of recovery attempts
p,p'-TDE	fatty	0.01	—[c]	0.006 – 0.008 (0.0066)	4
	nonfatty	0.01	—	0.005 – 0.009 (0.0070)	5
Aroclor 1248	fatty	0.05	—	0.024 – 0.039 (0.033)	3
	nonfatty	0.05	—	0.024 – 0.048 (0.037)	4
Ethion	fatty	0.01	—	0.005 – 0.006	2
	nonfatty	0.01	—	0.008	1
Aroclor 1242	fatty	0.05	—	0.023 – 0.034	2
	nonfatty	0.05	—	0.025 – 0.055 (0.043)	3
Ronnel	fatty	0.005	—	0.004 – 0.005	2
	nonfatty	0.005	—	0.003 – 0.005 (0.0040)	4
p,p'-DDE	fatty	0.005	0.002	0.006	2
		0.10	0.001– 0.002	0.069 – 0.089	2
	nonfatty	0.10	—	0.070 – 0.122 (0.097)	3
Toxaphene	fatty	0.20	0 – 0.093	0.010 – 0.226	2
	nonfatty	0.20	—	0.122	1
Diazinon	fatty	0.005	—	0.004 – 0.005	2
	nonfatty	0.005	—	0.003 – 0.006	2
p,p'-DDT	fatty	0.02	—	0.013 – 0.019	2
	nonfatty	0.02	—	0.013 – 0.024 (0.019)	3
Hexachloro-1,3-butadiene	fatty	0.001	—	0.001 – 0.002	2
	nonfatty	0.001	—	0.001 – 0.002 (0.0012)	3
Pentachlorobenzene	fatty	0.001	—	0.0003– 0.0007	2
	nonfatty	0.001	—	0.0007– 0.0014 (0.0011)	3
Pentachloroanisole	fatty	0.002	—	0.001 – 0.002	2
	nonfatty	0.002	—	0.001 – 0.003 (0.0022)	3
Endosulfan I	fatty	0.005	—	0.002 – 0.004	2
	nonfatty	0.005	—	0.003 – 0.007 (0.0047)	3
p,p'-Methoxychlor	fatty	0.01	—	0 – 0.11	2
	nonfatty	0.01	—	0.007 – 0.010 (0.0083)	3
Fonofos	fatty	0.005	—	0.002 – 0.003	2
	nonfatty	0.005	—	0.002 – 0.004 (0.0036)	3
MCPA	fatty	0.04	—	0.016 – 0.036 (0.026)	4
	nonfatty	0.04	—	0.021 – 0.037 (0.031)	7
2,4,5-T	fatty	0.04	0 – 0.003	0.010 – 0.055 (0.032)	5
	nonfatty	0.04	0 – 0.003	0.016 – 0.049 (0.040)	8
2,4-D	fatty	0.04	—	0.023 – 0.024	2
	nonfatty	0.04	—	0.005 – 0.040 (0.026)	5
PCP	fatty	0.02	0.002– 0.005 (0.002)	0.014 – 0.025 (0.020)	3
		0.04	0 – 0.004 (0.001)	0 – 0.019 (0.006)	4
	nonfatty	0.02	0 – 0.003 (0.001)	0.013 – 0.024 (0.017)	3
		0.04	0 – 0.003 (0.001)	T – 0.029 (0.015)	6
Carbaryl	nonfatty	0.20	—	0 – 0.20 (0.150)	17
o-Phenylphenol	nonfatty	0.40	—	0 – 0.40 (0.230)	17
Arsenic	fatty	0.26	0 – 0.33 (0.043)	0.16 – 0.59 (0.292)	8
	nonfatty	0.26	0 – 0.04 (0.004)	0.22 – 0.34 (0.272)	15
Cadmium	fatty	0.20	0 – 0.106 (0.016)	0.161 – 0.338 (0.203)	13
	nonfatty	0.20	0 – 0.049 (0.014)	0.155 – 0.297 (0.211)	21
Lead	fatty	0.20	0 – 0.05 (0.008)	0.12 – 0.35 (0.210)	13
	nonfatty	0.20	0 – 0.12 (0.044)	0.15 – 0.34 (0.238)	21
Mercury	fatty	0.04	0 – 0.011 (0.002)	0.038 – 0.057 (0.050)	11
	nonfatty	0.04	0 – 0.004 (0.000)	0.022 – 0.070 (0.047)	18

Table 7. (cont'd)

Residue	Type of food composites	Spike level ppm	Range of unfortified level, ppm[a]	Range of total found ppm[a,b]	Number of recovery attempts
Selenium	fatty	0.20	0 – 0.34 (0.072)	0.10 – 0.57 (0.238)	9
	nonfatty	0.20	0 – 0.14 (0.012)	0.06 – 0.32 (0.196)	15
Zinc	fatty	25	1.8 –37.5 (10.7)	21.3 –60.0 (32.6)	13
	nonfatty	25	0 – 7.4 (2.4)	18.0 –31.0 (23.7)	23

Note: T = trace.
[a]The numbers in parentheses represent average levels.
[b]These values are uncorrected for background.
[c]Dash means none found in blank.

ison; however, percentages and frequencies of occurrence can be used for cursory comparisons with corresponding data in the other reports.

Infant

The infant composites contained a total of 445 residues with the following distribution: 65.6% or 292 were metals; 28.3% or 126 were pesticides, consisting of 99 organochlorine, 26 organophosphorus, and one carbaryl residues; 3.4% or 15 were fungicides; and 2.7% or 12 were residues of other types, of which 7 were industrial chemicals, and 5 were herbicides.

The highest total of metal residues was found in the grain and cereal products food class. Residues of 4 of the 6 metals occurred most frequently in the grain-cereal composites; these included 12 residues of selenium, which was found at the highest level in this food class. The meat-fish-poultry composites were the source of a large proportion of the metals and contained arsenic at a higher level than any other food class. Zinc, lead, and cadmium were found in all food classes; arsenic was found in only the dairy, the meat-fish-poultry, and the grain-cereal composites; all food classes except water, fruit-fruit juices, and beverages contained selenium; and a few mercury residues were reported in each of the dairy, meat-fish-poultry, grain-cereal, potato, fruit-fruit juices, and sugar food classes at low levels. The fewest number of metal residues were found in the water, the beverage, and the oil-fat composites.

The pesticide residues were found predominantly in 3 food classes: The whole milk composites contained 33, the dairy composites contained 22, and the meat-fish-poultry composites contained 25. The most frequently occurring pesticides were dieldrin and *p,p'*-DDE, each of which accounted for 20 residues. Of the total 125 pesticide residues, 26 were organophosphorus compounds; 17 of these, attributed to malathion, diazinon, and parathion, were found in the grain and cereal products food class. No pesticide residues were reported for the water, the sugar, and the beverage food classes.

The fungicides were found only in the potato and the oil-fat composites; the herbicides were found only in the potato composites. Industrial chemicals were found in the whole milk, the meat-fish-poultry, and the oil-fat composites; PCB or pentachlorobenzene residues or both were detected at low levels in these food classes.

Toddler

The toddler composites contained a total of 687 residues with the following distribution: 52% or 357 were metals; 38.7% or 266 were pesticides; 5.8% or 40 were fungicides; and 3.5% or 24 were residues of other types, of which 17 were industrial chemicals and 7 were herbicides.

The highest number of metal residues was found in the meat-fish-poultry composites, followed closely by the grain-cereal composites. Three heavy metals, zinc, cadmium, and lead, were found in all food classes. Every food class except water, potatoes, fruit-fruit juices, and beverages contained selenium. Mercury was found in only the meat-fish-poultry, the potato, the oil-fat, and the beverage food classes. Sixteen composites contained arsenic residues: 10 in the meat-fish-poultry food class, 4 in the grain-cereal food class, and one each in the dairy and the sugar food classes.

Most of the pesticide residues were found in the dairy and the meat-fish-poultry composites, and all but one of these 122 residues were organochlorine compounds. No organochlorine pesticide residues were found in the grain and cereal products; however, 17 organophosphorus residues, which included malathion, diazinon, and a single trace of parathion, were reported for this food class. The oil-fat composites contained 47 pesticide residues, which were almost evenly divided between organochlorine and organophosphorus compounds. Of the organochlorine pesticide residues, toxaphene and hexachlorobenzene were found most frequently in the oil-fat composites. Malathion, which was detected in all the grain-cereal composites, was also found in every oil-fat composite. Residues of *p,p'*-DDT and the related compounds *p,p'*-DDE and *p,p'*-TDE were found mainly in the meat-fish-poultry composites; *p,p'*-DDE residues, which occurred most frequently, were found in 11 composites. Two fruit-fruit juice composites contained the only ethion residues, which were found at low levels.

The fungicide PCNB and related compounds were found in 8 oil-fat composites. And although PCNB was not detected in the potato composites, they did contain single low-level residues of tecnazene and related compounds. The potato food class also contained the only residues of the herbicide chlorpropham, which was found in 7 composites.

Two industrial chemicals were detected in the toddler composites. Pentachlorobenzene or PCB residues were found in 17 composites. Of the 10 residues of pentachlorobenzene reported, 8 were found in the oil-fat composites; 6 of the 7 PCB residues were identified as Aroclor 1254 and the other was undefined.

Recovery studies, in which composites were fortified with known compounds of each residue type, were performed with each market basket. The ranges of the background or unfortified levels and the ranges of the levels for the total compounds recovered are shown in Table 7.

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Pesticide, Metal, and Other Chemical Residues in Adult Total Diet Samples. (XIII). August 1976–September 1977

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Since 1964, the Food and Drug Administration has reported residues of pesticides and other chemicals in the average diet of the United States' heartiest eater, the young adult male, through the Total Diet Study. This report is the thirteenth in the series and includes results of analyses for 25 market basket samples collected from August 1976 through September 1977 in 20 different cities throughout 4 geographical areas. All food items were divided into 12 different composite food classes, and 25 composites, 1 from each market basket, were prepared and analyzed for each food class. Averages and ranges of pesticide and chemical residues are presented in various formats, together with results for the following heavy metals: zinc, cadmium, lead, mercury, arsenic, and selenium. Individual items making up the dairy composites and the meat-fish-poultry composites were analyzed separately for 4 market baskets and are presented as a part of this report. Recovery studies of pesticides and chemicals added to each of the various food classes are also included.

Since 1964, the residues of pesticides and other chemicals found in the average diet of the United States' heartiest eater, the young adult male, have been reported by the Food and Drug Administration under the Total Diet Program. This program, sometimes called the Market Basket Study, has undergone many changes to provide the most meaningful surveillance data within the scope of the program. A significant change in program format occurred in August 1974 when one-third of the adult market baskets were replaced with market baskets representing the infant and toddler diets. Other changes included basket composition, area sampled, and frequency of sampling. The results for the 2 previous reporting periods, each of which began August 1 and ended July 31, were based on 20 adult market baskets and 10 infant and toddler market baskets. In order to have the reporting period correspond to the fiscal year, the ending date for this and subsequent reporting periods was extended to September 30, and the beginning date for all subsequent reporting periods was changed from August 1 to October 1. Because of the additional 2 months in the present reporting period, which extends from August 1, 1976, through September 30, 1977, the number of market baskets for this series was increased from 30 to 37—25 adult and 12 infant and toddler market baskets. The results for the 12 infant and toddler market baskets collected during this period are published in a separate report. Amounts and types of residues found from June 1964 through July 1976 can be found in earlier reports (1–15).

The adult market baskets, which represent the basic 2-week diet of a 16- to 19-year-old male, were collected in each of 4 different geographic areas with the specific diet of the particular region determining the composition of the market basket. The various food items were prepared separately in the manner in which they would normally be served and eaten at home, and then each item was placed into one of 12 composite food classes, which are listed in Table 1. Each composite class, which contained foods with similar characteristics, was analyzed for pesticides, herbicides, fungicides, industrial chemicals, and heavy metals. Methodology included atomic absorption spectroscopy, fluorometry, polarography, gas chromatography, thin layer chromatography, mass spectrometry, and established extraction and cleanup techniques (16–23).

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Table 1. Commodity composites of adult foods analyzed for pesticide and chemical residues, August 1976–September 1977

Key	Food class
I	Dairy products
II	Meat, fish, and poultry
III	Grain and cereal products
IV	Potatoes
V	Leafy vegetables
VI	Legume vegetables
VII	Root vegetables
VIII	Garden fruits
IX	Fruits
X	Oils, fats, and shortening
XI	Sugar and adjuncts
XII	Beverages (including drinking water)

Results

The reader should be aware of the extended time period for this reporting period in which 25 market baskets were collected, compared with 20 market baskets in previous reporting periods. A total of 1605 residues were reported for this series of composites; they included 51 different pesticides, herbicides, fungicides, and industrial chemicals and 6 heavy metals. Table 2 lists these compounds and metals in decreasing order of frequency. The frequency of occurrence of each residue by food class is presented in Table 3. The total number of positive composites, the range of the positive findings, and the average for all composites analyzed are given in Table 4 for each residue within each food class. The total number of composites used to calculate each average value was 25 in every instance, and because residues reported as "Trace" have no recorded numerical value, they were treated as zero in calculating the average values for the series. Some average values, therefore, are well below the detection limits of the method for a particular compound. Table 5 shows the intake of pesticide and industrial chemical residues in terms of μg/kg body wt/day, and Table 6 shows the intake of 6 metals in terms of μg/day. For comparative purposes, the findings for the 1976 fiscal year are also shown in Table 5. The data for individual items from the dairy composites and from the meat-fish-poultry composites of 4 market baskets are given in Tables 7 and 8, respectively. Recovery studies of standards added to various composites were conducted, and the results are included in Table 9.

The residues most frequently found and their maximum levels are discussed below for each of the 12 food classes. These findings have not been corrected as a result of the recovery study.

I. Dairy Products

Organochlorine pesticides and metals accounted for all of the residues reported in this food class. The highest average pesticide level in dairy products was 0.0012 ppm for *p,p'*-DDE, which ranged from 0.001 to 0.008 ppm in the positive composites. The pesticide α-BHC was found in 23 composites, mostly at the trace level, and averaged 0.0002 ppm for the 25 composites examined. Other pesticide residues and their overall average levels in the dairy composites included 0.0006 ppm dieldrin, 0.0007 ppm *p,p'*-methoxychlor, and 0.0002 ppm heptachlor epoxide. Traces of oxychlordane, hexachlorobenzene, and lindane were also reported. All composites in this food class contained zinc residues, which ranged from 3.7 to 6.2 ppm and averaged 4.94 ppm. In addition, 13 composites contained selenium, which ranged from 0.01 to 0.04 ppm; the average level for all 25 composites was 0.012 ppm. Overall average levels for cadmium and lead in dairy products were 0.0025 and 0.010 ppm, respectively, and mercury was found in 2 composites at the 0.007 and 0.008 ppm levels. The dairy composites were also analyzed for arsenic, but none was found.

II. Meat, Fish, and Poultry

The 25 composites in this food class contained 285 residues. Residues of *p,p'*-DDE and dieldrin were found in every composite and ranged from 0.001 to 0.014 ppm and from 0.001 to 0.005 ppm, respectively. Toxaphene, with an overall average level of 0.0076 ppm in the meat-fish-poultry composites, was reported twice, at the 0.026 and 0.163 ppm levels. Ten com-

Table 2. Chemical residues found in 300 food composites, adult Total Diet—August 1976–September 1977

Chemical found	No. of composites with residues	No. of positive composites with residues reported as trace[a]	Range,[b] ppm
Zinc	298	0	0.2 –37.0
Cadmium	224	0	0.001– 0.126
Lead	195	0	0.01 – 0.92
Selenium	87	0	0.01 – 0.33
Dieldrin	83	22	0.001– 0.012
α-BHC	66	50	0.001– 0.014
Mercury	59	0	0.001– 0.050
p,p'-DDE	58	10	0.001– 0.019
Arsenic[c]	45	0	0.02 – 0.83
Malathion	44	3	0.003– 0.115
Lindane	38	24	0.001– 0.010
Heptachlor epoxide	36	19	0.001– 0.003
Hexachlorobenzene	33	29	0.001– 0.002
Diazinon	26	11	0.001– 0.008
Dicloran	23	2	0.001– 0.109
Chlorpropham	20	0	0.007– 1.260
Oxychlordane	20	15	0.001
Pentachloroaniline	18	5	0.001– 0.007
Endosulfan II	18	6	0.001– 0.011
Endosulfan I	16	4	0.001– 0.005
Endosulfan sulfate	16	5	0.002– 0.020
Parathion	15	5	0.002– 0.009
Pentachlorobenzene	15	10	0.001– 0.003
Tecnazene	13	3	0.001– 0.281
PCNB	12	7	0.001– 0.006
Pentachloroanisole	12	8	0.001
Pentachlorothioanisole	11	8	0.001– 0.002
p,p'-DDT	11	3	0.002– 0.007
Toxaphene	10	2	0.026– 0.597
Aroclor 1254	10	9	0.05
Ethion	8	2	0.002– 0.260
DCPA	7	2	0.002– 0.006
p,p'-TDE	6	4	0.002– 0.011
Captan	5	0	0.013– 0.127
Tetrachloroaniline	4	0	0.003– 0.036
2-Chloroethyl linoleate	4	0	0.060– 1.140
β-BHC	4	3	0.013
p,p'-Methoxychlor	3	0	0.003– 0.008
Parathion-methyl	3	1	0.002– 0.017
trans-Nonachlor	3	0	0.002– 0.008
2-Chloroethyl palmitate	3	0	0.015– 0.200
Chlordane	2	2	
Dicofol	2	0	0.005– 0.026
Perthane	2	2	
PCBs	2	2	
Tetrachlorobenzene	2	0	0.010– 0.055
Tetrachloroanisidine	2	0	0.002– 0.003
δ-BHC	2	1	0.002
Endrin	1	1	
o-Phenylphenol	1	0	0.075
Phosalone	1	0	0.004
PCP	1	0	0.016
Aroclor 1242	1	1	
Leptophos	1	0	0.012
Heptachloronorbornene	1	1	
Tetrachloroanisole	1	0	0.003
Tetrachlorothioanisole	1	0	0.001
TOTAL	1605	282	

[a]Chemicals detected by a specific analytical methodology were confirmed qualitatively and reported as trace when present at concentrations below the limit of quantitation. The limit of quantitation varies with residue and food class.

[b]Range is for positive composites found in quantifiable amounts and does not include positive composites reported as trace.

[c]Calculated as arsenic trioxide (As_2O_3).

Table 3. Frequency of occurrence chemical residues by food class, adult Total Diet—August 1976–September 1977

Chemical	Food class[a]											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
	Number of occurrences											
Zinc	25	25	23	25	25	25	25	25	25	25	25	25
Cadmium	12	16	25	24	25	14	23	24	13	23	20	5
Lead	10	16	15	14	13	25	18	23	21	11	18	11
Selenium	13	24	24	4	5	6	3	3	1	2	2	0
Dieldrin	16	25	1	9	0	0	0	24	0	8	0	0
α-BHC	23	19	0	0	0	0	0	5	0	5	14	0
Mercury	2	24	4	6	2	2	3	3	2	7	4	0
p,p'-DDE	14	25	0	3	8	0	4	0	0	4	0	0
Arsenic	0	25	15	1	0	0	2	0	1	1	0	0
Malathion	0	0	25	0	0	0	0	0	0	15	4	0
Lindane	3	8	2	0	2	0	1	7	0	1	14	0
Heptachlor epoxide	12	17	0	2	0	0	0	1	0	4	0	0
Hexachlorobenzene	5	17	0	0	0	0	0	0	0	10	1	0
Diazinon	0	1	11	0	4	0	4	2	2	2	0	0
Dicloran	0	0	0	3	9	2	0	2	7	0	0	0
Chlorpropham	0	0	1	18	0	0	0	1	0	0	0	0
Oxychlordane	5	15	0	0	0	0	0	0	0	0	0	0
Pentachloroaniline	0	1	0	0	0	0	1	0	0	15	1	0
Endosulfan II	0	0	0	1	6	0	0	8	3	0	0	0
Endosulfan I	0	0	0	0	8	0	0	7	1	0	0	0
Endosulfan sulfate	0	0	0	3	6	0	0	5	2	0	0	0
Parathion	0	0	0	0	7	2	2	4	0	0	0	0
Pentachlorobenzene	0	0	0	1	0	0	0	0	0	13	1	0
Tecnazene	0	0	1	5	1	0	1	3	0	1	1	0
PCNB	0	0	0	0	0	0	0	0	0	12	0	0
Pentachloroanisole	0	0	0	0	0	0	0	0	0	12	0	0
Pentachlorothioanisole	0	0	0	0	0	0	0	0	0	11	0	0
p,p'-DDT	0	10	0	0	1	0	0	0	0	0	0	0
Toxaphene	0	2	0	0	0	0	0	4	0	4	0	0
PCB (as Aroclor 1254)	0	7	0	0	0	0	0	0	0	3	0	0
Ethion	0	0	0	0	0	0	1	0	7	0	0	0
DCPA	0	0	0	0	3	0	3	1	0	0	0	0
p,p'-TDE	0	4	0	1	0	0	0	0	0	1	0	0
Captan	0	0	0	0	0	0	0	0	5	0	0	0
Tetrachloroaniline	0	0	0	4	0	0	0	0	0	0	0	0
2-Chloroethyl linoleate	0	0	1	0	0	0	0	0	0	3	0	0
β-BHC	0	0	0	0	1	0	1	2	0	0	0	0
p,p'-Methoxychlor	3	0	0	0	0	0	0	0	0	0	0	0
Parathion-methyl	0	0	0	0	3	0	0	0	0	0	0	0
trans-Nonachlor	0	3	0	0	0	0	0	0	0	0	0	0
2-Chloroethyl palmitate	0	0	0	0	0	0	0	0	0	3	0	0
Chlordane	0	0	0	1	1	0	0	0	0	0	0	0
Dicofol	0	0	0	0	0	0	0	0	2	0	0	0
Perthane	0	0	0	0	1	0	0	0	1	0	0	0
PCB (nonspecific)	0	0	1	0	0	0	0	0	0	1	0	0
Tetrachlorobenzene	0	0	0	1	0	0	0	1	0	0	0	0
Tetrachloroanisidine	0	0	0	2	0	0	0	0	0	0	0	0
δ-BHC	0	0	0	0	0	0	0	2	0	0	0	0
Endrin	0	0	0	0	0	0	0	1	0	0	0	0
o-Phenylphenol	0	0	1	0	0	0	0	0	0	0	0	0
Phosalone	0	0	0	0	0	0	0	0	1	0	0	0
PCP	0	0	1	0	0	0	0	0	0	0	0	0
PCB (as Aroclor 1242)	0	0	0	0	0	0	0	0	1	0	0	0
Leptophos	0	0	0	0	0	0	0	0	1	0	0	0
Heptachloronorbornene	0	1	0	0	0	0	0	0	0	0	0	0
Tetrachloroanisole	0	0	0	1	0	0	0	0	0	0	0	0
Tetrachlorothioanisole	0	0	0	1	0	0	0	0	0	0	0	0

[a]See Table 1 for description of food class.

posites contained *p,p'*-DDT, which averaged 0.0013 ppm for all 25 composites. Other organochlorine pesticides found at low levels included α-BHC, heptachlor epoxide, hexachlorobenzene, oxychlordane, lindane, *p,p'*-TDE, *trans*-nonachlor, pentachloroaniline, and heptachloronorbornene. The organophosphorus compound diazinon was reported for one composite at the trace level. The highest average level of any residue for the entire Market Basket series of 300 composites was reported for zinc in this food class; zinc levels in the positive composites ranged from 23.0 to 37.0 ppm, and the overall average level was 30.71 ppm. Arsenic was found in all 25 composites in concentrations which ranged from 0.07 to 0.83 ppm and averaged 0.240 ppm. Selenium, which averaged 0.168 ppm for the 25 composites examined, was found in 24 composites. Lead, which ranged from 0.01 to 0.10 ppm, and cadmium, which ranged from 0.002 to 0.008 ppm, were each found in 16 composites. One industrial chemical, a polychlorinated biphenyl (PCB) reported as Aroclor 1254®, was found in 6 composites at trace levels and in one composite at the 0.05 ppm level.

III. Grain and Cereal Products

Two organophosphorus pesticides were the predominant nonmetal residues in these composites. Malathion, reported for all 25 composites, ranged from 0.006 to 0.115 ppm and averaged 0.0246 ppm. Eleven composites contained diazinon, 6 with trace levels and the other 5 with concentrations that ranged from 0.001 to 0.003 ppm; the overall average level of diazinon in grain and cereal products was 0.0004 ppm. Other residues found in single composites included chlorpropham, dieldrin, PCP, tecnazene, and *o*-phenylphenol. Five metals with the following overall average levels for this food class were found in 15 or more of the composites: zinc, 7.84 ppm; selenium, 0.139 ppm; lead, 0.030 ppm; cadmium, 0.0205 ppm;

Table 4. Levels of chemical residues found by food class, adult Total Diet—August 1976–September 1977

Residue	Overall average,[a] ppm	Positive composites		
		Total number	Number reported as trace	Range,[b] ppm
I. Dairy Products				
Zinc	4.94	25	0	3.7 – 6.2
Selenium	0.012	13	0	0.01 – 0.04
Cadmium	0.0025	12	0	0.001– 0.030
Oxychlordane	T	5	5	
Lindane	T	3	3	
α-BHC	0.0002	23	19	0.001– 0.002
p,p'-DDE	0.0012	14	4	0.001– 0.008
Heptachlor epoxide	0.0002	12	9	0.001– 0.003
Hexachlorobenzene	T	5	5	
Mercury	0.0006	2	0	0.007– 0.008
Dieldrin	0.0006	16	8	0.001– 0.003
Lead	0.010	10	0	0.01 – 0.06
p,p'-Methoxychlor	0.0007	3	0	0.003– 0.008
II. Meat, Fish, and Poultry				
Zinc	30.71	25	0	23.0 –37.0
Dieldrin	0.0017	25	5	0.001– 0.005
α-BHC	T	19	18	0.002
Lead	0.024	16	0	0.01 – 0.10
p,p'-DDT	0.0013	10	3	0.002– 0.007
p,p'-TDE	T	4	3	0.002
Pentachloroaniline	0.0001	1	0	0.003
Arsenic	0.240	25	0	0.07 – 0.83
Selenium	0.168	24	0	0.08 – 0.33
Heptachlor epoxide	0.0006	17	6	0.001– 0.002
Cadmium	0.0029	16	0	0.002– 0.008
Lindane	0.0001	8	7	0.003
trans-Nonachlor	0.0005	3	0	0.002– 0.008
Diazinon	T	1	1	
p,p'-DDE	0.0057	25	0	0.001– 0.014
Mercury	0.0185	24	0	0.006– 0.050
Hexachlorobenzene	T	17	16	0.002
Oxychlordane	0.0002	15	10	0.001
PCB (as Aroclor 1254)	0.002	7	6	0.05
Toxaphene	0.0076	2	0	0.026– 0.163
Heptachloronorbornene	T	1	1	
III. Grain and Cereal Products				
Cadmium	0.0205	25	0	0.007– 0.030
Zinc	7.84	23	0	5.1 –21.2
Diazinon	0.0004	11	6	0.001– 0.003
Chlorpropham	0.0058	1	0	0.146
Dieldrin	0.0001	1	0	0.003
PCB	T	1	1	
Malathion	0.0246	25	0	0.006– 0.115
Lead	0.030	15	0	0.02 – 0.11
Mercury	0.0009	4	0	0.001– 0.014
o-Phenylphenol	0.0030	1	0	0.075
Tecnazene	0.0001	1	0	0.003
Selenium	0.139	24	0	0.01 – 0.27
Arsenic	0.020	15	0	0.02 – 0.08
Lindane	T	2	1	0.002
2-Chloroethyl linoleate	0.0024	1	0	0.060
PCP	0.0006	1	0	0.016
IV. Potatoes				
Zinc	4.77	25	0	1.5 –10.5
Lead	0.031	14	0	0.02 – 0.21
Tecnazene	0.0145	5	1	0.004– 0.281
Dicloran	0.0003	3	1	0.003– 0.005
Tetrachloroanisidine	0.0002	2	0	0.002– 0.003
Arsenic	0.001	1	0	0.02
Endosulfan I	0.0001	1	0	0.003
Cadmium	0.0443	24	0	0.012– 0.111
Dieldrin	0.0004	9	5	0.001– 0.006
Selenium	0.004	4	0	0.01 – 0.04
Endosulfan sulfate	0.0006	3	0	0.004– 0.007
Heptachlor epoxide	T	2	1	0.001
Tetrachloroanisole	0.0001	1	0	0.003
Chlordane	T	1	1	
Chlorpropham	0.1193	18	0	0.007– 1.260
Mercury	0.0019	6	0	0.002– 0.011
Tetrachloroaniline	0.0021	4	0	0.003– 0.036
p,p'-DDE	0.0002	3	2	0.005
Tetrachlorobenzene	0.0022	1	0	0.055
Tetrachlorothioanisole	T	1	0	0.001
p,p'-TDE	T	1	1	
Pentachlorobenzene	T	1	1	

Table 4. (cont'd)

V. Leafy Vegetables				
Zinc	3.18	25	0	1.8 –22.0
Dicloran	0.0026	9	1	0.001– 0.032
Parathion	0.0012	7	1	0.002– 0.009
Selenium	0.004	5	0	0.01 – 0.04
Parathion-methyl	0.0008	3	1	0.002– 0.017
β-BHC	0.0005	1	0	0.013
Cadmium	0.0459	25	0	0.010– 0.107
p,p'-DDE	0.0021	8	1	0.002– 0.019
Endosulfan sulfate	0.0022	6	2	0.009– 0.020
Diazinon	0.0006	4	1	0.003– 0.007
Mercury	0.0002	2	0	0.002– 0.004
p,p'-DDT	T	1	0	0.002
Lead	0.019	13	0	0.02 – 0.08
Endosulfan I	0.0006	8	3	0.002– 0.003
Endosulfan II	0.0004	6	2	0.002– 0.004
DCPA	0.0002	3	1	0.002
Lindane	T	2	1	0.001
Tecnazene	T	1	0	0.001
Perthane	T	1	1	
Chlordane	T	1	1	
VI. Legume Vegetables				
Zinc	8.88	25	0	4.9 –36.5
Selenium	0.007	6	0	0.01 – 0.04
Parathion	0.0002	2	1	0.004
Lead	0.176	25	0	0.03 – 0.39
Dicloran	0.0009	2	0	0.011– 0.012
Cadmium	0.0051	14	0	0.001– 0.050
Mercury	0.0004	2	0	0.001– 0.010
VII. Root Vegetables				
Zinc	2.78	25	0	1.2 – 8.0
Diazinon	0.0005	4	1	0.003– 0.006
Mercury	0.0019	3	0	0.001– 0.044
Cadmium	0.0267	23	0	0.002– 0.126
p,p'-DDE	0.0006	4	1	0.002– 0.008
DCPA	0.0004	3	1	0.003– 0.006
Lead	0.046	18	0	0.01 – 0.29
Selenium	0.010	3	0	0.01 – 0.18
Arsenic	0.002	2	0	0.02 – 0.03
Parathion	T	2	1	0.002
Tecnazene	T	1	0	0.001
Ethion	0.0002	1	0	0.005
Pentachloroaniline	T	1	1	
Lindane	T	1	0	0.002
β-BHC	T	1	1	
VIII. Garden Fruits				
Zinc	2.25	25	0	0.8 – 5.0
Lead	0.141	23	0	0.03 – 0.92
Lindane	0.0008	7	3	0.002– 0.010
Toxaphene	0.0420	4	0	0.060– 0.597
Mercury	0.0007	3	0	0.001– 0.013
Cadmium	0.0140	24	0	0.001– 0.033
Endosulfan II	0.0010	8	2	0.001– 0.011
α-BHC	0.0015	5	0	0.001– 0.014
Parathion	0.0003	4	2	0.002– 0.005
Tecnazene	0.0003	3	1	0.002– 0.006
Dieldrin	0.0033	24	2	0.001– 0.012
Endosulfan I	0.0009	7	1	0.001– 0.005
Endosulfan sulfate	0.0007	5	2	0.002– 0.012
Selenium	0.003	3	0	0.01 – 0.04
Dicloran	0.0003	2	0	0.001– 0.006
δ-BHC	T	2	1	0.002
Tetrachlorobenzene	0.0004	1	0	0.010
Endrin	T	1	1	
Diazinon	0.0003	2	1	0.008
Chlorpropham	0.0003	1	0	0.008
Heptachlor epoxide	T	1	1	
β-BHC	T	2	2	
DCPA	0.0001	1	0	0.003
IX. Fruits				
Zinc	1.19	25	0	0.5 – 3.1
Dicloran	0.0162	7	0	0.003– 0.109
Endosulfan II	0.0004	3	2	0.010
Diazinon	0.0002	2	0	0.001– 0.003
Lead	0.046	21	0	0.01 – 0.18
Ethion	0.0109	7	2	0.002– 0.260
Mercury	0.0007	2	0	0.008– 0.010
Endosulfan sulfate	0.0001	2	1	0.002
Cadmium	0.0058	13	0	0.001– 0.031
Captan	0.0099	5	0	0.013– 0.127
Dicofol	0.0012	2	0	0.005– 0.026
Selenium	0.002	1	0	0.06

Table 4. (cont'd)

IX. Fruits (continued)				
Leptophos	0.005	1	0	0.012
Endosulfan I	0.0001	1	0	0.003
Arsenic	0.001	1	0	0.02
Perthane	T	1	1	
Phosalone	0.0002	1	0	0.004
PCB (as Aroclor 1242)	T	1	1	
X. Oils, Fats, and Shortening				
Zinc	4.49	25	0	3.6 – 7.0
Pentachloroaniline	0.0016	15	3	0.001– 0.007
Pentachloroanisole	0.0002	12	8	0.001
Hexachlorobenzene	0.0001	10	7	0.001
α-BHC	0.0001	5	2	0.001
Cadmium	0.0112	23	0	0.002– 0.050
Pentachlorobenzene	0.0003	13	8	0.001– 0.003
Lead	0.016	11	0	0.01 – 0.08
Dieldrin	0.0007	8	2	0.001– 0.004
Toxaphene	0.0039	4	2	0.046– 0.051
Malathion	0.0110	15	3	0.003– 0.084
PCNB	0.0004	12	7	0.001– 0.006
Pentachlorothioanisole	0.0002	11	8	0.001– 0.002
Mercury	0.0026	7	0	0.003– 0.017
p,p'-DDE	0.0006	4	2	0.002– 0.012
Heptachlor epoxide	T	4	2	0.001
PCB (as Aroclor 1254)	T	3	3	
Arsenic	0.002	1	0	0.06
Tecnazene	T	1	1	
2-Chloroethyl linoleate	0.0603	3	0	0.180– 1.140
Selenium	0.004	2	0	0.02 – 0.07
p,p'-TDE	0.0004	1	0	0.011
Lindane	T	1	1	
2-Chloroethyl palmitate	0.0094	3	0	0.015– 0.200
Diazinon	0.0003	2	1	0.008
PCB	T	1	1	
XI. Sugar and Adjuncts				
Zinc	2.27	25	0	0.9 – 5.0
Lindane	0.0006	14	8	0.001– 0.004
Mercury	0.0011	4	0	0.004– 0.009
Pentachlorobenzene	T	1	1	
Cadmium	0.0059	20	0	0.001– 0.025
α-BHC	0.0002	14	11	0.001– 0.002
Selenium	0.002	2	0	0.02 – 0.03
Pentachloroaniline	T	1	1	
Lead	0.028	18	0	0.01 – 0.08
Malathion	0.0008	4	0	0.003– 0.008
Tecnazene	T	1	0	0.001
Hexachlorobenzene	T	1	1	
XII. Beverages				
Zinc	0.46	25	0	0.2 – 1.4
Lead	0.012	11	0	0.01 – 0.05
Cadmium	0.0004	5	0	0.001– 0.005

Note: T = trace.

[a]Average values are based on the 25 composites examined; trace residues, if present, were treated as zero in calculating averages. Thus, an average value of "T" can be well below the detection limit of a method for a specific compound. If the average value was less than 0.0001 ppm, it is reported as "T".

[b]Range is for positive composites found in quantifiable amounts and does not include positive composites reported as trace.

and arsenic, 0.020 ppm. Four composites contained mercury at levels ranging from 0.001 to 0.014 ppm. Two industrial chemicals were found in one composite each: a PCB at the trace level and 2-chloroethyl linoleate, a residue reported for the first time in these studies, at the 0.060 ppm level (20).

IV. Potatoes

The widespread use of chlorpropham on potatoes is quite evident from the 18 positive composites reported for this herbicide. Chlorpropham residue levels ranged from 0.007 to 1.260 ppm and averaged 0.1193 ppm for all 25 composites. Tecnazene was found in 5 composites, one with a trace level and 4 with concentrations that ranged from 0.004 to 0.281 ppm; the overall average level of tecnazene in potatoes was 0.0145 ppm. Residues of similar compounds reported for this food class included tetrachloroaniline, tetrachloroanisidine, tetrachloroanisole, and tetrachlorothioanisole (19). Dieldrin was found in 9 composites, 5 with trace levels and the remaining 4 with concentrations ranging from 0.001 to 0.006 ppm. Other pesticides found in 3 or fewer composites included dicloran, *p,p'*-DDE, heptachlor epoxide, endosulfan I, endosulfan sulfate, chlordane, and *p,p'*-TDE. Of the 6 metals reported, zinc was found most frequently, in all 25 composites, and at the highest levels, which ranged from 1.5 to 10.5 ppm and averaged 4.77 ppm. The other metal residues and their average levels for the 25 composites examined were 0.0443 ppm cadmium, 0.031 ppm lead, 0.0019 ppm mercury, 0.004 ppm selenium, and 0.001 ppm arsenic. Pentachlorobenzene at the trace level and tetrachlorobenzene at the 0.055 ppm level were also found, each in a single composite.

V. Leafy Vegetables

These composites exhibited a variety of residues. Several organochlorine pesticides were reported for this food class. Dicloran residues were found in 9 composites, one with a trace level and 8 with concentrations that ranged from 0.001

Table 5. Intake of pesticide and industrial chemical residues (μg/kg body wt/day)—FY 1976 vs FY 1977

Compound	FY 76	FY 77
Aldrin	ND	ND
Dieldrin	0.0405	0.0226
Total	0.0405	0.0226
DDE	0.0545	0.0394
DDT	0.0074	0.0057
TDE	0.0008	0.0012
Total	0.0627	0.0463
Heptachlor epoxide	0.0055	0.0074
Heptachlor	ND	ND
Total	0.0055	0.0074
Endosulfan I	0.0028	0.0026
Endosulfan II	0.0029	0.0031
Endosulfan sulfate	0.0058	0.0042
Total	0.0115	0.0099
Hexachlorobenzene	0.0019	0.0018
PCNB	0.0004	0.0010
Tecnazene	0.0159	0.0346
Pentachloroaniline	0.0020	0.0004
trans-Nonachlor	0.0004	0.0020
Oxychlordane	0.0017	0.0025
BHC	0.0130	0.0105
Diazinon	0.0039	0.0061
Malathion	0.1278	0.1540
Lindane	0.0026	0.0038
Methoxychlor	0.0086	0.0078
Chlorpropham	0.0875	0.3098
Parathion	0.0009	0.0016
Parathion-methyl	ND	0.0006
Perthane	0.0027	T
Dicloran	0.0316	0.0557
Toxaphene	T	0.0802
Dicofol	0.0095	0.0040
Ethion	0.0051	0.0180
o-Phenylphenol	T	0.0044
PCP	0.0174	0.0009
DCPA	0.0011	0.0005
Phosalone	0.0014	0.0005
Captan	0.0102	0.0305
Leptophos	0.0005	0.0015
PCBs	T	0.0164

Note: ND = not detected; T = trace (below the limits of quantitation; detected and verified, but not quantifiable).

to 0.032 ppm; the average dicloran level for the 25 composites examined was 0.0026 ppm. Eight composites contained *p,p'*-DDE, 7 with concentrations that ranged from 0.002 to 0.019 ppm and one with a trace level; the average *p,p'*-DDE level for all 25 composites was 0.0021 ppm. Endosulfan I, endosulfan II, and endosulfan sulfate were all found in leafy vegetables with overall average levels in this food class of 0.0006, 0.0004, and 0.0022 ppm, respectively. Lesser amounts of lindane, β-BHC, *p,p'*-DDT, Perthane®, and chlordane were also found.

Organophosphorus residues included parathion, which averaged 0.0012 ppm for all 25 composites and was found at levels ranging from 0.002 to 0.009 ppm in 6 composites and at the trace level in one composite; and diazinon and parathion-methyl, with overall average levels in this food class of 0.0006 and 0.0008 ppm, respectively. DCPA was found in 3 composites, at the trace level in one and at the 0.002 ppm level in the other two. Tecnazene was found in a single composite. Three metals were reported for 63 of the 131 residues found in this food class; zinc and cadmium, found in all 25 composites, averaged 3.18 and 0.0459 ppm, respectively, and lead, found in 13 composites, averaged 0.019 ppm for the 25 composites examined. Two other metal residues occurred less frequently; selenium, ranging from 0.010 to 0.040 ppm, was found in 5 composites, and mercury was found in 2 composites at the 0.002 and 0.004 ppm levels.

VI. Legume Vegetables

In this food class, the only nonmetals reported were dicloran and parathion; each was found in only 2 composites at low levels. Among the metals, zinc and lead were found in every composite and averaged 8.88 and 0.176 ppm, respectively. Cadmium, which averaged 0.0051 ppm for all 25 composites, was found in 14 composites. Selenium and mercury were both found less frequently and at low levels.

VII. Root Vegetables

Very low levels of ethion, parathion, and diazinon were found in one, 2, and 4 composites, respectively. Residues of *p,p'*-DDE, which averaged 0.0006 ppm for all 25 composites, were found in 4 composites, and lindane and β-BHC, each in a single composite, were also reported. The organochlorine herbicide DCPA was reported for 3 composites, and the fungicides tecnazene and pentachloroaniline were each found in one composite. All 6 metals were found in this food class: the 3 most abundant metal residues, found in 18 or more composites, and their overall average levels for the 25 composites examined were zinc, 2.78 ppm; cadmium, 0.0267 ppm; and lead, 0.046 ppm; the metal residues found less frequently were selenium, ranging from 0.01 to 0.18 ppm in 3 composites; mercury, ranging from 0.001 to 0.044 ppm in 3 composites; and arsenic, reported at the 0.02 and 0.03 ppm levels in 2 composites.

VIII. Garden Fruits

Of the 23 residues found in the garden fruit composites, 13 were organochlorine pesticides. Dieldrin ranging from 0.001 to 0.012 ppm, was found in 24 of the 25 composites; the average level for the 25 composites examined was 0.0033 ppm. Although toxaphene residues were reported for only 4

Table 6. FY 77 daily intakes of metals by food class

	Arsenic[a]		Cadmium		Lead		Mercury		Selenium		Zinc	
Food class	μg/day	% Total intake	μg/day	% Total intake	μg/day	% Total intake	μg/day	% Total intake	μg/day	% Total intake	μg/day	% Total intake
I. Dairy products	0.00	0.0	1.87	5.1	7.16	9.0	0.45	7.1	8.78	7.9	3713	20.6
II. Meat, fish, and poultry	62.8	87.7	0.75	2.0	6.28	7.9	4.62	73.1	44.0	39.7	8057	44.8
III. Grain and cereal products	8.2	11.5	8.36	22.7	12.2	15.4	0.37	5.9	55.1	49.8	3416	19.0
IV. Potatoes	0.13	0.2	7.04	19.1	6.09	7.7	0.29	4.6	0.57	0.5	757	4.2
V. Leafy vegetables	0.00	0.0	2.52	6.8	1.09	1.4	0.01	0.2	0.24	0.2	193	1.1
VI. Legume vegetables	0.00	0.0	0.39	1.1	13.0	16.4	0.03	0.5	0.50	0.5	503	2.8
VII. Root vegetables	0.07	0.1	12.2	33.0	1.58	2.0	0.07	1.1	0.30	0.3	97	0.5
VIII. Garden fruits	0.00	0.0	1.03	2.8	10.4	13.1	0.05	0.8	0.24	0.2	166	0.9
IX. Fruits	0.17	0.2	1.12	3.0	9.67	12.2	0.16	2.5	0.56	0.5	258	1.4
X. Oils, fats, and shortening	0.18	0.2	0.81	2.2	1.14	1.4	0.18	2.8	0.27	0.2	327	1.8
XI. Sugar and adjuncts	0.00	0.0	0.49	1.3	2.32	2.9	0.09	1.4	0.17	0.2	186	1.0
XII. Beverages (including drinking water)	0.00	0.0	0.32	0.9	8.32	10.5	0.00	0.0	0.00	0.0	316	1.8
Total	71.6	99.9[b]	36.9	100	79.3	99.9[b]	6.32	100	110.7	100	17989	99.9[b]

[a]Values for arsenic are calculated as arsenic trioxide (As_2O_3).
[b]Does not total 100 because of rounding error.

Table 7. Pesticide and industrial chemical residues in individual commodities of the dairy product composites of 4 market basket samples, adult Total Diet—August 1976–September 1977

	Commodity[a,b]									
Residue found	Milk (4)	Evaporated milk (4)	Ice cream (4)	Cottage cheese (4)	Processed cheese (4)	Natural cheese (4)	Butter (4)	2% milk (1)	Skim milk (3)	Ice milk (2)
Hexachlorobenzene										
Times found	2	3	3	1	4	4	4	1		1
Range, ppm	T	T–0.001	T–0.001	T	T–0.003	T–0.001	T–0.004	T	T	T
α-BHC										
Times found	2	4	4	4	4	4	4	1	1	1
Range, ppm	T	T–0.002	T–0.001	T	0.002–0.009	0.001–0.004	0.004–0.012	T		T
p,p'-DDE										
Times found	2	2	2	1	4	3	3	1		1
Range, ppm	T	T–0.004	0.002	0.001	0.001–0.020	T–0.004	0.004–0.017	T		0.001
Dieldrin										
Times found	3	2	3	3	4	4	4			1
Range, ppm	T	0.001–0.002	0.001–0.004	0.001–0.002	0.003–0.017	T–0.014	0.012–0.026			T
Heptachlor epoxide										
Times found	2	3	2	2	4	3	4			1
Range, ppm	T	T–0.001	0.002–0.003	T–0.001	0.002–0.014	0.004–0.008	0.003–0.015			T
Oxychlordane										
Times found	1				4	2	2			
Range, ppm	T				T–0.003	0.002	0.003–0.006			
p,p'-Methoxychlor										
Times found	1	1			1		1			
Range, ppm	T	0.018			0.032		0.011			
Lindane										
Times found		1	2		1	1	1			1
Range, ppm		0.002	T		T	T	T			T
Dicofol										
Times found			1							
Range, ppm			0.014							
PCB (as Aroclor 1254)										
Times found							1			
Range, ppm							0.03			
trans-Methoxychlor										
Times found					1					
Range, ppm					T					
p,p'-DDT										
Times found						1				
Range, ppm						0.003				

Note: T = trace.
[a]No residues were reported for nonfat dry milk (4) and buttermilk (1).
[b]Numbers in parentheses show the number of times the commodity was analyzed.

composites, they ranged from 0.060 to 0.597 ppm and averaged 0.0420 ppm for all 25 composites. After dieldrin, the most frequently reported pesticides were endosulfan II, found in 6 composites at levels ranging from 0.001 to 0.011 ppm and in 2 composites at the trace level; endosulfan I, found in 6 composites at levels ranging from 0.001 to 0.005 ppm and in one composite at the trace level; and endosulfan sulfate, found in 3 composites at levels ranging from 0.002 to 0.012 ppm and in 2 composites at the trace level. Lindane, with an overall average level of 0.0008 ppm in garden fruits, was found in 4 composites at levels ranging from 0.002 to 0.010 ppm and in 3 composites at trace levels; α-BHC, which averaged 0.0015 ppm for all 25 composites, was found in 5 composites. The remaining pesticide residues were reported in only one or 2 composites each. Parathion, diazinon, the herbicides chlorpropham and DCPA, and tecnazene, the only fungicide, were all reported at low levels. The metals zinc, cadmium, and lead were all found in 23 or more composites, whereas selenium and mercury were reported in only 3 composites each.

IX. Fruits

This food class contained the only residues of the fungicide captan, which was found in 5 composites at levels ranging from 0.013 to 0.127 ppm and averaged 0.0099 ppm for the 25 composites examined. The organophosphorus residues included ethion, found in 5 composites at levels ranging from 0.002 to 0.260 ppm and in 2 composites at trace levels; diazinon, found in 2 composites at the 0.001 and 0.003 ppm levels; and leptophos and phosalone, each found in a single composite at a low level. The highest overall average level reported for an organochlorine pesticide in fruits was 0.0162 ppm for dicloran, which was found in 7 composites at levels ranging from 0.003 to 0.109 ppm. Dicofol was found in 2 composites, at the 0.005 and 0.026 ppm levels, and endosulfan I, endosulfan II, and endosulfan sulfate were reported at lower levels. Perthane was found twice in this Market Basket series—once in a leafy vegetable composite and once in a fruit composite—in both instances at the trace level. All 6 metals were reported for this food class in a wide frequency range: High zinc levels that averaged 1.19 ppm were reported for all 25 composites; selenium and arsenic were each found in a single composite at the 0.06 and 0.02 ppm levels, respectively. An industrial chemical PCB (reported as Aroclor 1242) was also found at the trace level in a single composite.

X. Oils, Fats, and Shortening

Although these composites were a distant second to the meat-fish-poultry composites in total number of residues, they contained the greatest number of compounds reported for any food class. The industrial chemicals were very much

nical residues in individual commodities of the meat-fish-poultry composites of 4 market basket samples, adult Total Diet— August 1976

Commodity[a]											
Pork chops (4)	Bacon (4)	Chicken (4)	fish fillet (4)	Tuna (3)	Salmon (1)	Luncheon meat (4)	frank-furters (4)	Liver (4)	Eggs (4)	Ham (4)	Round steak (4
	2 T		3 T–0.005		1 0.003	3 T–0.001	4 T–0.002	2 T–0.001	2 T–0.001	1 T	2 T
	3 T–0.002		4 T–0.001		1 0.004	4 T–0.002	4 0.001–0.002	2 T			1 0.003
1 0.002	4 0.004–0.039	4 0.001–0.007	3 0.011–0.034	2 0.012–0.015		4 0.004–0.033	4 0.002–0.026	2 0.003	2 0.004–0.011	1 0.005	2 0.006–0.0
1 T	3 0.002–0.007	4 0.001–0.004	1 0.002	2 T–0.002	1 T	4 0.002–0.004	4 0.002–0.007	2 T–0.004	3 T–0.004	1 0.002	1 0.002
2 T–0.002	3 T–0.002	2 T				4 0.001–0.003	4 0.001–0.003	2 0.002–0.003	2 T–0.001	1 T	1 0.001
						4 T–0.002	4 T–0.002	1 0.003		1 0.001	1 0.001
3 T–0.004	2 T					3 0.001–0.003	2 0.001		1 0.008		
2 T	3 T	3 T	3 0.035–0.070		1 T	2 T	3 T		2 T	1 T	3 T
1 0.584	1 1.26								1 0.49		
	2 0.005–0.042		3 0.003–0.051	2 0.013–0.033		3 0.005–0.010	1 0.030			1 0.012	

o,p'-DDT			
Times found			1
Range, ppm			0.019
p,p'-TDE			
Times found	1		3
Range, ppm	0.006		0.004–0.010
p,p'-Methoxychlor			
Times found			
Range, ppm			
Endrin			
Times found		1	
Range, ppm		0.001	
Ronnel			
Times found			
Range, ppm			
trans-Nonachlor			
Times found			
Range, ppm			

Note: T = trace.

[a]Number in parentheses indicates the number of times the item was analyzed.

1 0.004			1 0.003	1 0.008	
				1 0.026	
	1 0.002				
1 T					
			1 0.002		

TABLE 9. Recovery data for residues found in adult Total Diet samples, August 1976–September 1977

Range of Blank Level, ppm[a]	Range of Total Residue Found, ppm[a,b]	No. of Recovery Attempts	Residue	Type of Food Composites	Spike Level, ppm	Range of Blank Level, ppm[a]	Range of Tota Residue Found ppm[a,b]
0 - 0.001 (0.0007)	0.007 - 0.012 (0.0088)	7	Hexachloro-1,3-butadiene	Fatty	0.001	—	0.0003 - 0.(
0 - 0	0.06 - 0.12 (0.089)	4		Nonfatty	0.001	0 - 0.0005 (0.0001)	0.001 - 0.0((0.001)
0 - 0.001 (0.0001)	0.006 - 0.011 (0.008)	14	Endosulfan I	Fatty	0.005	—	0.004 - 0.0((0.004)
0 - 0.001 (0.0001)	0.077 - 0.120 (0.094)	10		Nonfatty	0.005	—	0.004 - 0.0((0.0047)
—[c]	0.003 - 0.007 (0.005)	4	PCP	Fatty	0.02	0 - 0.009 (0.002)	T - 0.026 (0.008)
—	0.003 - 0.011 (0.008)	8			0.04	0 - 0.008 (0.002)	T - 0.029 (0.016)
—	0.003 - 0.005 (0.004)	3		Nonfatty	0.02	0 - 0.004 (0.001)	0.004 - 0.0((0.012)
—	0.002 - 0.005 (0.0039)	6			0.04	0 - 0.007 (0.002)	0.002 - 0.0((0.021)
0 - 0.003 (0.001)	0.030 - 0.063 (0.046)	4	2,4,5-T	Fatty	0.02	—	0.009 - 0.0((0.014)
0 - 0.008 (0.002)	0.069 - 0.088 (0.080)	5			0.04	0 - 0.001 (0.0001)	0 - 0.043 (0.028)
—	0.003 - 0.006 (0.004)	6		Nonfatty	0.02	—	0.006 - 0.0((0.010)
0 - 0.009 (0.001)	0.034 - 0.068 (0.052)	10			0.04	—	0.009 - 0.0((0.030)
0 - 0.010 (0.002)	0.070 - 0.109 (0.091)	8	2,4-D	Fatty	0.04	0 - 0.017	0.008 - 0.0(
—	0.116 - 0.162 (0.133)	3		Nonfatty	0.04	—	0.021 - 0.0((0.028)
—	0.130 - 0.218 (0.171)	6	2,4-DB	Fatty	0.04	—	0 - 0.019 (0.007)
—	0.003 - 0.005 (0.004)	3		Nonfatty	0.04	—	0 - 0.043 (0.018)
—	0.004 - 0.005 (0.004)	6	Silvex	Fatty	0.04	—	0.023
—	0.012 - 0.021 (0.016)	4		Nonfatty	0.04	—	0.015 - 0.0((0.026)
—	0.014 - 0.240 (0.018)	8	MCPA	Fatty	0.04	—	0.008 - 0.0((0.023)
0 - 0.001 (0.0003)	0.0004 - 0.0015 (0.0008)	3		Nonfatty	0.04	—	0.009 - 0.0((0.029)
0 - 0.0005	0.0009 - 0.0017 (0.0010)	6	Carbaryl	Nonfatty	0.20	—	0.05 - 0.20 (0.173)
0 - 0.001 (0.0002)	0.001 - 0.003 (0.002)	3	o-Phenyl-phenol	Nonfatty	0.40	—	0.10 - 0.40 (0.303)
—	0.0020 - 0.0022 (0.0020)	6	PCB (as Aroclor 1242)	Fatty	0.10	—	0.041 - 0.0(
—	0.003 - 0.004 (0.003)	3		Nonfatty	0.10	0 - 0.016 (0.003)	0.064 - 0.1((0.081)
—	0.005 - 0.006 (0.005)	6	PCB (as Aroclor 1248)	Fatty	0.05	0 - 0.010 (0.003)	0.023 - 0.0((0.033)
—	0.009 - 0.013 (0.011)	3		Nonfatty	0.05	--	0.024 - 0.0((0.036)
—	0.006 - 0.010 (0.008)	6	Arsenic	Fatty	0.26	0 - 0.22 (0.060)	0.20 - 0.46 (0.310)
				Nonfatty	0.26	0 - 0.03 (0.003)	0.12 - 0.34 (0.244)

Residue	Type of Food Composites	Spike Level, ppm	Range of Blank Level, ppm[c]	Range of Total Residue Found, ppm[a,b]	No. of Recovery Attempts
Selenium	Fatty	0.20	0 - 0.20 (0.066)	0.08 - 0.38 (0.228)	14
	Nonfatty	0.20	0 - 0.18 (0.014)	0.07 - 0.27 (0.165)	33
Zinc	Fatty	25.0	3.70 - 5.50 (4.80)	17.0 - 31.2 (26.47)	17
	Nonfatty	25.0	0.05 - 20.0 (3.61)	12.5 - 36.0 (24.30)	51
Mercury	Fatty	0.04	0 - 0.025 (0.004)	0.033 - 0.067 (0.048)	21
	Nonfatty	0.04	0 - 0.009 (0.001)	0.037 - 0.061 (0.047)	43
Cadmium	Fatty	0.20	0.001 - 0.030 (0.006)	0.157 - 0.208 (0.191)	18
	Nonfatty	0.20	0.001 - 0.090 (0.018)	0.116 - 0.300 (0.202)	44
Lead	Fatty	0.20	0.005 - 0.06 (0.021)	0.17 - 0.31 (0.214)	17
	Nonfatty	0.20	0.01 - 0.13 (0.034)	0.13 - 0.30 (0.216)	38

NOTE: T = trace.

[a] Numbers in parentheses represent average residue levels.
[b] These values are uncorrected for background.
[c] Dash means none found in blank.

in evidence: Pentachlorobenzene was reported in 13 of the fat composites, 8 with trace levels and the other 5 with concentrations ranging from 0.001 to 0.003 ppm. PCBs were found in 4 composites at trace levels—3 PCB residues were identified as Aroclor 1254; the fourth was undefined. This Market Basket series marks the first reporting of 2 more industrial chemicals: 2-Chloroethyl linoleate and 2-chloroethyl palmitate were found in 3 composites at levels ranging from 0.180 to 1.140 ppm and from 0.015 to 0.200 ppm (20), respectively. The fungicide PCNB was detected in 12 composites with 7 at trace levels; in addition, related compounds such as pentachloroaniline, pentachloroanisole, and pentachlorothioanisole were also reported (19). The organophosphorus residue malathion was present in 15 composites in concentrations ranging from 0.003 to 0.084 ppm; the average malathion level for the 25 composites examined was 0.0110 ppm. The most noteworthy among the 8 organochlorine compounds was toxaphene, found in 4 composites, in 2 at concentrations of 0.046 and 0.051 ppm and 2 at trace levels; hexachlorobenzene, found mostly at trace levels, in 10 composites; and dieldrin, α-BHC, and *p,p'*-DDE, all present at low levels, in at least 4 composites each. Residues of all 6 metals were found in this food class; zinc and cadmium were found in almost all composites, and 11 composites contained lead.

XI. Sugar and Adjuncts

Lindane and α-BHC were found in 14 composites each; for both compounds, the majority of the residues were present at trace levels and the rest were detected at low levels. Malathion, at levels ranging from 0.003 to 0.008 ppm in 4 composites, and a trace of pentachlorobenzene in one composite were also reported. Residues of 5 metals were found in this food class; no arsenic was reported. Except for zinc, which averaged 2.27 ppm in the composites examined, the other 4 metals detected had low averages.

XII. Beverages

The beverage composites contained the fewest number of residues reported; only 41 low-level metal residues were found.

Discussion

A total of 1605 residues were reported for 300 composites with the following distribution: 908 metals (56.6%), 578 pesticides (36%), 57 fungicides (3.6%), 35 industrial chemicals (2.2%), and 27 herbicides (1.6%).

Because composites from 25 market baskets, instead of the usual 20, were analyzed for this reporting period, the results from this report can be compared only on a percentage basis with those from the preceding adult Total Diet report. The metals and the pesticides were a few percentage points lower and the herbicides were just slightly higher in the current study. The most sizable increase occurred with the fungicides, which comprised about 0.75% of the total residues in the last report and 3.6% in this series. The industrial chemicals also increased from about 0.5% in the last report to the current value of 2.2%.

Zinc was the residue most frequently reported, with levels ranging from 0.2 ppm in a beverage composite to 37.0 ppm in one meat-fish-poultry composite. The meat-fish-poultry composites were also the source for the highest levels of selenium, arsenic, and mercury. The highest cadmium level was reported in root vegetables, and the highest lead level was found in garden fruits.

Pesticide residues were found most frequently in the meat-fish-poultry composites and next most often, with about half

that number altogether, in the dairy composites and also in the garden fruits. Dieldrin, α-BHC, and *p,p'*-DDE accounted for most of the organochlorine residues, whereas malathion and diazinon were the organophosphorus residues most often detected. Except for parathion and dicloran, present in 2 composites each, the metals were the only residues found in legume vegetables.

Most of the fungicide residues were found in the oils-fats-shortening composites and were identified as tecnazene, PCNB, and related compounds. A look at the potato composite results reveals 18 herbicide residues, of which all were identified as chlorpropham and detected in concentrations ranging from 0.007 to 1.260 ppm. The industrial chemicals as a group were found primarily in the oils-fats-shortening and meat-fish-poultry composites; however, the majority of the PCB findings were centered in the meat-fish-poultry composites. The individual items making up the dairy composites and the meat-fish-poultry composites from 4 of the market baskets collected in this series were analyzed for organochlorine and organophosphorus residues; the results are given in Tables 7 and 8. These particular commodities were chosen in an attempt to identify the source of residues which are generally reported at high levels in their respective composites.

Recovery studies were also performed for all classes of chemicals sought throughout the entire sampling period. An attempt was made to alter the sample matrix as well as the level of fortification to demonstrate the overall effectiveness of the methods employed. For each recovery attempt, fortified and unfortified composites were analyzed simultaneously and the amount of recovery was calculated. Data from these recovery studies are presented in Table 9.

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Pesticide, Heavy Metal, and Other Chemical Residues in Infant and Toddler Total Diet Samples. (IV). October 1977–September 1978

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The Food and Drug Administration has conducted the Total Diet Study since 1964. The program originally focused on the average total diet of the young adult male but was eventually expanded to include the average total diets of infants and toddlers. This report is the fourth in the infant and toddler series and represents residues found in market basket samples, i.e., items comprising the average 2-week diet of infants and toddlers, collected in 10 different cities throughout 4 geographic areas of the United States from October 1977 through September 1978. The results are based on analyses of food composites representing 11 food classes into which all the food items were separated. Averages and ranges are reported for residues of pesticides, herbicides, fungicides, and selected metals and industrial chemicals. Recovery results are also included for various representative compounds added to a variety of food classes. The results for the adult market basket samples for the same reporting period are presented in a separate report.

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The Food and Drug Administration (FDA), U.S. Department of Health and Human Services, has been monitoring the nation's diet for residues of pesticides and other chemical residues since 1964 through the Total Diet Program (1). For several years, the program was concerned with the average 2-week diet of the young adult male, as determined by FDA's Office of Nutrition and Consumer Services, Bureau of Foods. Beginning in August 1974, one-third of the adult market basket samples were replaced by samples representing the infant and toddler diets. The 10 infant and toddler market basket samples of this report were purchased in grocery stores in 10 different cities throughout 4 geographical areas of the United States, i.e., South, Northeast, North Central, and West. Those items requiring cooking were prepared by a dietitian in the manner in which a consumer would prepare and serve them at home. Each food item was blended or chopped to a homo-

geneous consistency and then combined in the proper proportion with other similar items to form a particular composite (Table 1). Each composite was analyzed for organochlorine, organophosphorus, and carbamate pesticides and for herbicides and selected metals and industrial chemicals. Methodology included atomic absorption spectroscopy, flurometry, gas chromatography, thin layer chromatography, mass spectroscopy, and established extraction and cleanup techniques (2–10). Results for market basket samples from June 1964 through September 1977 are presented in earlier reports (1, 11–26).

Results

The 98 infant composites for the present reporting period contained 417 residues of 42 compounds; 95 were detected at the trace level. The 110 toddler composites for the same period contained 606 residues of 48 compounds; 122 were detected at the trace level. All of these compounds are listed in Table 2 in order of decreasing frequency of occurrence, with the number of residues found at the trace level and the range of the findings listed for each compound. Table 3 shows the frequency of occurrence of each compound within each food class. Table 4 gives a more detailed presentation of the residues detected in each food class. Each average value in Table 4 is based on the total number of composites examined for the corresponding food class; because residues reported as "Trace" have no recorded numerical value, they were treated as zero in calculating the average value for each residue within each food class. Table 5 shows the intake of pesticide and industrial chemical residues in terms of μg/kg body wt/day, and Table 6 shows the intake of 6 metals in terms of μg/day (mg/day in the case of zinc). Residues and the levels at which they were reported are discussed below for each of the 11 food classes. No corrections were made on the basis of the recovery study results.

I. Drinking Water

Infant and Toddler.—Tap water samples were collected from the same areas as the market baskets with a single determination made and reported for both the infant and toddler diets. The tap water was also used throughout the market basket analyses in the preparation of items requiring dilution or addition of water. Zinc residues, ranging from 0.1 to 0.4 ppm, were found in 3 of the 10 water samples. Lead residues were reported for 2 water samples at the 0.020 and 0.030 ppm levels.

Table 1. Commodity classes of infant and toddler foods analyzed for pesticides and other chemical residues, October 1977–September 1978

Key	Food class
I	Drinking water[a]
II	Whole milk, fresh[a]
III	Other dairy and substitutions, infant
	Other dairy and substitutions, toddler
IV	Meat, fish, and poultry, infant
	Meat, fish, and poultry, toddler
V	Grain and cereal products, infant
	Grain and cereal products, toddler
VI	Potatoes[a,b]
VII	Vegetables, infant
	Vegetables, toddler
VIII	Fruit and fruit juices, infant
	Fruit and fruit juices, toddler
IX	Oils and fats[a,c]
X	Sugar and adjuncts, infant
	Sugar and adjuncts, toddler
XI	Beverages[a,d]

Note: Use key with Table 3.
[a]Because of similarity between infant and toddler diets, single determinations for certain classes of food were made and reported for both.
[b]No infant composites for western region.
[c]No infant composites for the north central, western, and southern regions.
[d]No infant composites for north central region.

II. Whole Milk, Fresh

Infant and Toddler.—In this food class the composites from each market basket were analyzed once and reported for both the infant and toddler diets. Residues of 4 metals—zinc, lead, selenium, and cadmium—were found in these composites at comparatively low levels. Zinc was found in all 10 composites at an average level of 3.23 ppm; lead and cadmium were found in 3 composites each, and selenium was found in one composite at the 0.04 ppm level. The fungicide hexachlorobenzene was found in 5 composites at the trace level. All the other residues, including α-BHC, *p,p'*-DDE, dieldrin, heptachlor epoxide, oxychlordane, and methoxychlor, were organochlorine pesticides found mostly at the trace level.

III. Other Dairy and Substitutions

Infant.—The variation in infants' and toddlers' diets becomes evident with these composites. Residues reported at very low levels included the organochlorine pesticides α-BHC, dieldrin, heptachlor epoxide, lindane, oxychlordane, and *p,p'*-DDE, and the fungicide hexachlorobenzene. Residues of 5 metals—zinc, lead, cadmium, selenium, and mercury—were reported for these infant composites; all except zinc were found at the lowest levels in this food class. Zinc was found in all 10 composites, and lead, cadmium, and selenium were found in 6, 3, and 2 composites, respectively. Methoxychlor, at the 0.007 ppm level, was found in a single infant composite; however, it was not found in the toddler composites of this food class.

Toddler.—The metal residues found in the toddler composites were very similar to those found in the infant composites with one exception: Selenium residues were found in 6 of the 10 composites although the levels of these residues were the lowest reported for selenium in the toddler diet. Organochlorine pesticide residues were found more frequently and at higher levels than in the infant composites of this food class. The most frequently reported were α-BHC, found in all 10 composites with an average level of 0.0012 ppm, dieldrin, found in 9 composites with an overall average level of 0.0015 ppm, and heptachlor epoxide, which averaged 0.0006 ppm for the 10 composites examined. Other organochlorine pesticide residues included oxychlordane, found at the trace level in 6 composites, and *p,p'*-DDE, found in 6 composites at low levels. The fungicide hexachlorobenzene was found in 8 composites at the trace level, and the industrial chemical pentachlorobenzene was also reported at the trace level for a single toddler composite.

IV. Meat, Fish, and Poultry

Infant.—These composites are generally among the highest both in total number of residues and the levels detected. Of the 5 metal residues reported, zinc and selenium were found in all composites and averaged 20.63 and 0.111 ppm, respectively. Other metal residues included cadmium, lead, and mercury. Residues of 6 organochlorine pesticides were reported. The most significant of these was *p,p'*-DDE, found in 8 composites and averaging 0.0021 ppm for the 10 composites examined. Dieldrin was found in 7 composites and the others, found in a few composites each, included oxychlordane, α-BHC, heptachlor epoxide, and *p,p'*-DDT. The fungicide hexachlorobenzene and 3 industrial chemicals—a

Table 2. Chemical residues found in 98 infant and 110 toddler food composites from 10 United States cities— October 1977–September 1978

Chemical found	No. of composites with residues	No. of positive composites with residues reported as trace[a]	Range,[b] ppm
Infant			
Zinc	88	0	0.1 –33.1
Lead	51	0	0.005– 0.51
Cadmium	50	0	0.001– 0.084
Selenium	30	0	0.02 – 0.22
Dieldrin	22	14	0.001– 0.009
p,p'-DDE	22	12	0.001– 0.009
α-BHC	19	18	0.001
Hexachlorobenzene	17	13	0.003– 0.008
Malathion	15	2	0.008– 0.667
Heptachlor epoxide	12	11	0.001
Arsenic[c]	11	0	0.01 – 0.12
Mercury	9	0	0.001– 0.008
Oxychlordane	7	7	
Diazinon	6	4	0.001– 0.002
Chlorpropham	4	1	0.003– 0.020
Pentachloroaniline	4	0	0.005– 0.029
Pentachlorobenzene	4	0	0.002– 0.012
Lindane	3	2	0.003
Parathion	3	2	0.001
Toxaphene	3	0	0.112– 0.149
Dicloran	3	1	0.027– 0.059
PCNB	3	0	0.002– 0.008
Pentachloroanisole	3	0	0.002– 0.010
Pentachlorothioanisole	3	0	0.004– 0.021
Methoxychlor	2	0	0.002– 0.007
Carbaryl	2	2	
Ethion	2	1	0.001
Phosalone	2	0	0.008– 0.015
Fenitrothion	2	1	0.001
Endosulfan sulfate	2	0	0.001– 0.004
p,p'-DDT	2	0	0.002– 0.003
Chlordane	1	0	0.020
Endrin	1	0	0.001
Perthane	1	0	0.008
DCPA	1	0	0.018
Tecnazene	1	1	
PCB	1	1	
Aroclor 1254	1	1	
2-Chloroethyl palmitate	1	0	0.014
2-Chloroethyl linoleate	1	0	0.113
Tetrachloroanisidine	1	1	
Endosulfan II	1	0	0.002
TOTAL	417	95	
Toddler			
Zinc	98	0	0.1 –36.5
Lead	64	0	0.003– 0.38
Cadmium	64	0	0.001– 0.146
Selenium	37	0	0.01 – 0.38
α-BHC	36	23	0.001– 0.003
Dieldrin	29	11	0.001– 0.007
Hexachlorobenzene	29	20	0.001– 0.010
p,p'-DDE	24	7	0.001– 0.016
Malathion	20	0	0.001– 0.430
Heptachlor epoxide	18	12	0.001– 0.002
Mercury	17	0	0.001– 0.016
Oxychlordane	16	14	0.001
Arsenic[c]	16	0	0.01 – 0.43
Pentachlorobenzene	13	4	0.002– 0.008
Lindane	11	5	0.001– 0.003
Pentachloroaniline	11	0	0.002– 0.018
PCNB	10	2	0.001– 0.006
Diazinon	9	4	0.001– 0.002
Pentachloroanisole	9	1	0.002– 0.011
Pentachlorothioanisole	9	1	0.001– 0.013
Toxaphene	7	0	0.050– 0.250
Parathion	6	2	0.001– 0.002
Chlorpropham	6	1	0.004– 0.241
Dicloran	6	0	0.002– 0.044
2-Chloroethyl linoleate	4	0	0.040– 0.496
Carbaryl	3	3	
Ethion	3	1	0.001– 0.002
Tecnazene	3	3	
Chlordane	2	0	0.010– 0.028
Fonofos	2	0	0.002
PCB	2	2	
Aroclor 1254	2	2	
2-Chloroethyl palmitate	2	0	0.011– 0.057
Endosulfan II	2	0	0.001– 0.004
Endosulfan sulfate	2	0	0.004– 0.005
p,p'-DDT	2	0	0.002– 0.013
Methoxychlor	1	0	0.002
Ronnel	1	1	
DCPA	1	1	
Phosalone	1	0	0.011
Fenthion	1	1	
Aroclor 1242	1	0	0.05
Aroclor 1260	1	0	0.06
Fenitrothion	1	0	0.002
Tetrachlorobenzene	1	0	0.009
Tetrachloroanisidine	1	1	
Endosulfan I	1	0	0.004
p,p'-TDE	1	0	0.003
TOTAL	606	122	

[a]Chemicals detected by a specific analytical methodology were confirmed qualitatively and reported as trace when present at concentrations below the limit of quantitation. The limit of quantitation varies with residue and food class.
[b]Range is for positive composites found in quantifiable amounts and does not include positive composites reported as trace.
[c]Calculated as arsenic trioxide (As_2O_3).

polychlorinated biphenyl (PCB) (identified as Aroclor 1254®), 2-chloroethyl linoleate, and 2-chloroethyl palmitate—were also reported.

Toddler.—These 10 composites contained a total of 100 residues, of which 46 were metals. Zinc and selenium levels were the highest reported for the series; both metals were found in all 10 composites with zinc levels ranging from 27.6 to 36.5 ppm and selenium levels ranging from 0.07 to 0.38 ppm. The greatest number of findings and the highest level of arsenic were also reported for these composites; arsenic was found in 9 composites at levels ranging from 0.02 to 0.43 ppm. Seven composites contained mercury at levels ranging from 0.001 to 0.016 ppm. Lead and cadmium were also found. The organochlorine pesticide residues included *p,p'*-DDE, found in 9 composites at levels ranging from 0.001 to 0.016 ppm, α-BHC, dieldrin, oxychlordane, and heptachlor epoxide. Five industrial chemicals were reported: a PCB (calculated as Aroclor 1260), found in one composite at the 0.06 ppm level; a second PCB (calculated as Aroclor 1254), found in 2 composites at the trace level; pentachlorobenzene, found in one composite at the 0.002 ppm level; 2-chloroethyl linoleate, found in 2 composites at the 0.040 and 0.180 ppm levels; and 2-chloroethyl palmitate, found in a single composite at the 0.011 ppm level. Single residues of the fungicides tetrachlorobenzene and hexachlorobenzene were also reported.

V. Grain and Cereal Products

Infant.—Metals accounted for 45 of the 68 residues reported for the infant composites of this food class; the remaining residues included most of the organophosphorus pesticide residues reported for all of the composite groups. Although the malathion levels for this food class were not the highest reported, malathion residues were found in 10 composites at levels ranging from 0.008 to 0.082 ppm. Diazinon and fenitrothion residues were found in 6 and 2 composites, respectively, at low levels. Of the metal residues, the selenium and arsenic levels found in 10 and 7 infant composites, respectively, were the highest reported for infant composites in any food class. Residues of cadmium and zinc were found in all 10 infant grain and cereal composites at moderate levels, and 8 composites contained lead. Only a few organochlorine pesticide residues were reported, and a trace of a nonspecific PCB was found in one composite.

Toddler.—Metal residues also dominated the toddler composites of this food class. Zinc, lead, and arsenic were found

Table 3. Frequency of occurrence of chemical residues, by food class, infant and toddler Total Diet—October 1977–September 1978

Chemical	Food class[a] I	II	III	IV	V	VI	VII	VIII	IX	X	XI
Number of Occurrences in Infant Foods											
Zinc	3	10	10	10	10	7	10	10	3	9	6
Lead	2	3	6	6	8	1	6	8	2	5	4
Cadmium	0	3	3	8	10	8	9	3	2	1	3
Selenium	0	1	2	10	10	0	3	0	3	1	0
Dieldrin	0	5	5	7	0	3	0	0	2	0	0
p,p'-DDE	0	6	2	8	1	1	3	0	1	0	0
α-BHC	0	9	8	2	0	0	0	0	0	0	0
Hexachlorobenzene	0	5	4	4	0	1	0	0	3	0	0
Malathion	0	0	0	0	10	0	1	1	3	0	0
Heptachlor epoxide	0	4	4	3	0	0	0	0	1	0	0
Arsenic	0	0	0	0	7	0	1	1	0	1	1
Mercury	0	0	1	1	0	1	2	2	1	0	1
Oxychlordane	0	3	2	2	0	0	0	0	0	0	0
Diazinon	0	0	0	0	6	0	0	0	0	0	0
Chlorpropham	0	0	0	0	0	3	1	0	0	0	0
Pentachloroaniline	0	0	0	0	0	1	0	0	3	0	0
Pentachlorobenzene	0	0	0	0	0	1	0	0	3	0	0
Lindane	0	0	2	0	1	0	0	0	0	0	0
Parathion	0	0	0	0	0	0	0	3	0	0	0
Toxaphene	0	0	0	0	0	0	0	0	3	0	0
Dicloran	0	0	0	0	0	2	0	1	0	0	0
PCNB	0	0	0	0	0	0	0	0	3	0	0
Pentachloroanisole	0	0	0	0	0	0	0	0	3	0	0
Pentachlorothioanisole	0	0	0	0	0	0	0	0	3	0	0
Methoxychlor	0	1	1	0	0	0	0	0	0	0	0
Carbaryl	0	0	0	0	1	0	0	1	0	0	0
Ethion	0	0	0	0	0	0	0	2	0	0	0
Phosalone	0	0	0	0	0	0	0	2	0	0	0
Fenitrothion	0	0	0	0	2	0	0	0	0	0	0
Endosulfan sulfate	0	0	0	0	0	1	0	0	0	1	0
p,p'-DDT	0	0	0	1	1	0	0	0	0	0	0
Chlordane	0	0	0	0	0	0	0	0	1	0	0
Endrin	0	0	0	0	0	0	0	0	1	0	0
Perthane	0	0	0	0	0	0	0	1	0	0	0
DCPA	0	0	0	0	0	0	1	0	0	0	0
Tecnazene	0	0	0	0	0	1	0	0	0	0	0
PCB (nonspecific)	0	0	0	0	1	0	0	0	0	0	0
PCB (as Aroclor 1254)	0	0	0	1	0	0	0	0	0	0	0
2-Chloroethyl palmitate	0	0	0	1	0	0	0	0	0	0	0
2-Chloroethyl linoleate	0	0	0	1	0	0	0	0	0	0	0
Tetrachloroanisidine	0	0	0	0	0	1	0	0	0	0	0
Endosulfan II	0	0	0	0	0	0	0	0	0	1	0
Number of Occurrences in Toddler Foods											
Zinc	3	10	10	10	10	10	10	10	10	10	5
Lead	2	3	6	6	8	0	10	9	6	10	4
Cadmium	0	3	3	4	10	10	9	4	10	8	3
Selenium	0	1	6	10	10	0	1	2	2	4	1
α-BHC	0	9	10	8	0	0	0	0	0	9	0
Dieldrin	0	5	9	7	0	3	3	0	2	0	0
Hexachlorobenzene	0	5	8	6	0	1	0	0	9	0	0
p,p'-DDE	0	6	6	9	0	1	1	0	1	0	0
Malathion	0	0	0	0	10	0	0	1	8	1	0
Heptachlor epoxide	0	4	9	4	0	0	0	0	1	0	0
Mercury	0	0	1	7	0	2	2	0	3	1	1
Oxychlordane	0	3	6	7	0	0	0	0	0	0	0
Arsenic	0	0	0	9	3	1	0	1	1	1	0
Pentachlorobenzene	0	0	1	1	0	1	0	0	10	0	0
Lindane	0	0	1	2	0	0	0	0	0	8	0
Pentachloroaniline	0	0	0	0	0	1	0	0	10	0	0
PCNB	0	0	0	0	0	0	0	0	10	0	0
Diazinon	0	0	0	0	7	0	0	1	1	0	0
Pentachloroanisole	0	0	0	0	0	0	0	0	9	0	0
Pentachorothioanisole	0	0	0	0	0	0	0	0	9	0	0
Toxaphene	0	0	0	0	0	0	0	0	7	0	0
Parathion	0	0	0	0	0	0	4	1	1	0	0
Chlorpropham	0	0	0	0	0	4	2	0	0	0	0
Dicloran	0	0	0	0	0	0	1	4	0	1	0
2-Chloroethyl linoleate	0	0	0	2	1	0	0	0	1	0	0
Carbaryl	0	0	0	0	1	0	0	1	0	1	0
Ethion	0	0	0	0	0	0	0	3	0	0	0
Tecnazene	0	0	0	0	0	1	1	0	1	0	0
Chlordane	0	0	0	1	0	0	0	0	1	0	0
Fonofos	0	0	0	0	0	0	0	0	2	0	0
PCB (nonspecific)	0	0	0	0	1	0	0	0	1	0	0
PCB (as Aroclor 1254)	0	0	0	2	0	0	0	0	0	0	0
2-Chloroethyl palmitate	0	0	0	1	0	0	0	0	1	0	0
Endosulfan II	0	0	0	0	0	0	1	1	0	0	0
Endosulfan sulfate	0	0	0	0	0	1	1	0	0	0	0
p,p'-DDT	0	0	0	2	0	0	0	0	0	0	0
Methoxychlor	0	1	0	0	0	0	0	0	0	0	0
Ronnel	0	0	0	0	1	0	0	0	0	0	0
DCPA	0	0	0	0	0	0	1	0	0	0	0
Phoslaone	0	0	0	0	0	0	0	1	0	0	0
Fenthion	0	0	0	0	1	0	0	0	0	0	0
PCB (as Aroclor 1242)	0	0	0	0	0	0	0	0	1	0	0
PBC (as Aroclor 1260)	0	0	0	1	0	0	0	0	0	0	0
Fenitrothion	0	0	0	0	1	0	0	0	0	0	0
Tetrachlorobenzene	0	0	0	1	0	0	0	0	0	0	0
Tetrachloroanisidine	0	0	0	0	0	1	0	0	0	0	0
Endosulfan I	0	0	0	0	0	0	0	1	0	0	0
p,p'-TDE	0	0	0	1	0	0	0	0	0	0	0

[a]See Table 1 for description of food class.

at lower levels than in the infant composites of this food class, whereas cadmium and selenium were found at about the same level. The levels of the organophosphorus pesticide residues diazinon, malathion, and fenitrothion were about the same as in the infant composites; however, a trace each of 2 different organophosphorus residues—fenthion and ronnel—were also reported for the toddler composites. A trace of a nonspecific PCB was found in one composite, and another industrial chemical, 2-chloroethyl linoleate, was detected in one composite at the 0.214 ppm level. No organochlorine pesticide residues were found in the toddler grain and cereal composites.

VI. Potatoes

Infant.—Residues of the herbicide chlorpropham, which is generally associated with the treatment of potatoes, were detected in 3 composites; one contained a trace and the other 2, 0.009 and 0.020 ppm. Organochlorine pesticide residues were each found in 3 or fewer composites and included dicloran, dieldrin, *p,p'*-DDE, and endosulfan sulfate. Residues of the fungicides hexachlorobenzene, pentachloroaniline, tecnazene, and tetrachloroanisidine and the industrial chemical pentachlorobenzene were found in one composite each. Cadmium residues were found in 8 composites and included the highest level reported for the infant composites in any food class. The other metal residues reported included zinc, found at low levels in 7 composites, and lead and mercury, each found in a single composite.

Toddler.—The toddler potato composites differed little from their infant counterparts with one exception: Four toddler composites contained chlorpropham residues which ranged from 0.009 to 0.241 ppm, with an average of 0.0330 ppm for the 10 composites examined, compared to the corresponding average level of 0.0036 ppm for the infant composites. Two toddler composites contained low levels of mercury and a single composite contained 0.02 ppm arsenic. No dicloran or lead residues were reported for the toddler potato composites.

VII. Vegetables

Infant.—Of the 37 residues reported for the infant vegetable composites, 31 were metals. Low-level zinc, cadmium, and lead residues accounted for most of these, with only a few composites containing low levels of selenium, mercury, or arsenic. The remaining residues included one organochlorine pesticide, *p,p'*-DDE; 2 herbicides, chlorpropham and DCPA; and one organophosphorus pesticide, malathion. No arsenic or malathion residues were found in the toddler composites of this food class.

Toddler.—Residues of 5 metals were reported for the toddler vegetable composites. Of these the most significant were

Table 4. Levels of chemical residues found by food class, infant and toddler Total Diet--October 1977-September 1978

Residue	Positive Infant Composites: Overall Average in Infant Composites,[a] ppm	Total Number	Number Reported as Trace	Range,[b] ppm	Positive Toddler Composites: Overall Average in Toddler Composites,[a] ppm	Total Number	Number Reported as Trace	Range,[b] ppm
I. Drinking Water								
Zinc	0.06	3	0	0.1 - 0.4	0.06	3	0	0.1 - 0.4
Lead	0.005	2	0	0.02 - 0.03	0.005	2	0	0.02 - 0.03
II. Whole Milk, Fresh								
Zinc	3.23	10	0	2.0 - 4.5	3.23	10	0	2.0 - 4.5
Hexachlorobenzene	T	5	5		T	5	5	
α-BHC	T	9	9		T	9	9	
Dieldrin	T	5	5		T	5	5	
p,p'-DDE	0.0007	6	4	0.003 - 0.004	0.0007	6	4	0.003 - 0.004
Lead	0.013	3	0	0.01 - 0.09	0.013	3	0	0.01 - 0.09
Selenium	0.004	1	0	0.04	0.004	1	0	0.04
Oxychlordane	T	3	3		T	3	3	
Heptachlor epoxide	T	4	4		T	4	4	
Cadmium	0.0020	3	0	0.001 - 0.017	0.0020	3	0	0.001 - 0.017
Methoxychlor	0.0002	1	0	0.002	0.0002	1	0	0.002
III. Other Dairy and Substitutions								
Heptachlor epoxide	T	4	4		0.0006	9	5	0.001 - 0.002
Dieldrin	T	5	5		0.0015	9	1	0.001 - 0.003
Lead	0.014	6	0	0.005 - 0.06	0.016	6	0	0.01 - 0.05
Zinc	5.15	10	0	3.5 - 6.5	5.16	10	0	3.1 - 7.6
α-BHC	T	8	8		0.0012	10	3	0.001 - 0.003
Cadmium	0.0041	3	0	0.001 - 0.038	0.0030	3	0	0.002 - 0.025
Selenium	0.005	2	0	0.02 - 0.03	0.016	6	0	0.01 - 0.04
Lindane	T	2	2		T	1	1	
Methoxychlor	0.0007	1	0	0.007				
Hexachlorobenzene	T	4	4		T	8	8	
Oxychlordane	T	2	2		T	6	6	
Mercury	0.0001	1	0	0.001	0.0002	1	0	0.002
p,p'-DDE	T	2	2		0.0014	6	1	0.001 - 0.006
Pentachlorobenzene					T	1	1	
IV. Meat, Fish, and Poultry								
p,p'-DDE	0.0021	8	3	0.001 - 0.009	0.0044	9	1	0.001 - 0.016
Oxychlordane	T	2	2		0.0002	7	5	0.001
Dieldrin	0.0004	7	3	0.001	0.0008	7	3	0.001 - 0.003
Hexachlorobenzene	0.0003	4	3	0.003	0.0007	6	5	0.007
Cadmium	0.0100	8	0	0.005 - 0.024	0.0034	4	0	0.005 - 0.013
Lead	0.027	6	0	0.01 - 0.08	0.024	6	0	0.003 - 0.13
Selenium	0.111	10	0	0.05 - 0.19	0.200	10	0	0.07 - 0.38
Zinc	20.63	10	0	12.4 - 33.1	31.08	10	0	27.6 - 36.5
PCB (as Aroclor 1254)	T	1	1		T	2	2	
α-BHC	0.0001	2	1	0.001	T	8	8	
Heptachlor epoxide	T	3	3		0.0003	4	2	0.001 - 0.002
2-Chloroethyl linoleate	0.0113	1	0	0.113	0.0220	2	0	0.040 - 0.180
2-Chloroethyl palmitate	0.0014	1	0	0.014	0.0011	1	0	0.011
p,p'-DDT	0.0003	1	0	0.003	0.0015	2	0	0.002 - 0.013
Mercury	0.0006	1	0	0.006	0.0044	7	0	0.001 - 0.016
Arsenic					0.131	9	0	0.02 - 0.43
PCB (as Aroclor 1260)					0.006	1	0	0.06
Pentachlorobenzene					0.0002	1	0	0.002
Tetrachlorobenzene					0.0009	1	0	0.009
p,p'-TDE					0.0003	1	0	0.003
Lindane					0.0001	2	1	0.001
Chlordane					0.0028	1	0	0.028
V. Grain and Cereal Products								
Diazinon	0.0003	6	4	0.001 - 0.002	0.0006	7	3	0.001 - 0.002
Malathion	0.0252	10	0	0.008 - 0.082	0.0239	10	0	0.004 - 0.104
Carbaryl	T	1	1		T	1	1	
Arsenic	0.037	7	0	0.01 - 0.12	0.005	3	0	0.01 - 0.02
Cadmium	0.0241	10	0	0.010 - 0.062	0.0251	10	0	0.013 - 0.050
Lead	0.097	8	0	0.03 - 0.39	0.052	8	0	0.02 - 0.20
Selenium	0.156	10	0	0.09 - 0.22	0.188	10	0	0.08 - 0.29
Zinc	12.88	10	0	4.3 - 23.3	8.94	10	0	4.3 - 12.4
Fenitrothion	0.0001	2	1	0.001	0.0002	1	0	0.002
Lindane	0.0003	1	0	0.003				
p,p'-DDT	0.0002	1	0	0.002				
p,p'-DDE	T	1	1					
PCB (nonspecific)	T	1	1		T	1	1	
2-Chloroethyl linoleate					0.0214	1	0	0.214
Fenthion					T	1	1	
Ronnel					T	1	1	
VI. Potatoes								
Dicloran	0.0107	2	0	0.027 - 0.059				
Cadmium	0.0320	8	0	0.009 - 0.084	0.0384	10	0	0.009 - 0.084
Zinc	2.78	7	0	2.0 - 4.4	3.08	10	0	1.2 - 4.6
Pentachlorobenzene	0.0002	1	0	0.002	0.0002	1	0	0.002
Hexachlorobenzene	T	1	1		T	1	1	
Chlorpropham	0.0036	3	1	0.009 - 0.020	0.0330	4	1	0.009 - 0.241

Continued

Table 4. (cont'd)

VI. Potatoes								
Pentachloroaniline	0.0006	1	0	0.005	0.0005	1	0	0.005
p,p'-DDE	T	1	1		T	1	1	
Tecnazene	T	1	1		T	1	1	
Tetrachloroanisidine	T	1	1		T	1	1	
Dieldrin	0.0002	3	1	0.001	0.0002	3	1	0.001
Endosulfan sulfate	0.0004	1	0	0.004	0.0005	1	0	0.005
Lead	0.002	1	0	0.02				
Mercury	0.0002	1	0	0.002	0.0004	2	0	0.002
Arsenic					0.002	1	0	0.02
VII. Vegetables								
p,p'-DDE	0.0014	3	1	0.006 - 0.008	0.0007	1	0	0.007
Cadmium	0.0158	9	0	0.008 - 0.031	0.0178	9	0	0.006 - 0.038
Lead	0.028	6	0	0.01 - 0.10	0.132	10	0	0.04 - 0.38
Zinc	3.98	10	0	2.9 - 5.7	4.38	10	0	3.2 - 5.8
Chlorpropham	0.0003	1	0	0.003	0.0011	2	0	0.004 - 0.007
DCPA	0.0018	1	0	0.018	T	1	1	
Selenium	0.010	3	0	0.03 - 0.04	0.003	1	0	0.03
Malathion	T	1	1					
Mercury	0.0005	2	0	0.002 - 0.003	0.0005	2	0	0.002 - 0.003
Arsenic	0.006	1	0	0.06				
Parathion					0.0003	4	1	0.001
Dieldrin					0.0004	3	1	0.002
Tecnazene					T	1	1	
Endosulfan II					0.0001	1	0	0.001
Endosulfan sulfate					0.0004	1	0	0.004
Dicloran					0.0002	1	0	0.002
VIII. Fruit and Fruit Juices								
Parathion	0.0001	3	2	0.001	T	1	1	
Lead	0.047	8	0	0.02 - 0.12	0.052	9	0	0.03 - 0.09
Zinc	0.70	10	0	0.2 - 1.2	0.81	10	0	0.4 - 1.5
Ethion	0.0001	2	1	0.001	0.0003	3	1	0.001 - 0.002
Malathion	T	1	1		0.0001	1	0	0.001
Cadmium	0.0038	3	0	0.003 - 0.018	0.0162	4	0	0.002 - 0.146
Carbaryl	T	1	1		T	1	1	
Dicloran	T	1	1		0.0091	4	0	0.011 - 0.044
Arsenic	0.004	1	0	0.04	0.002	1	0	0.02
Phosalone	0.0023	2	0	0.008 - 0.015	0.0011	1	0	0.011
Mercury	0.0003	2	0	0.001 - 0.002				
Perthane	0.0008	1	0	0.008				
Endosulfan I					0.0004	1	0	0.004
Endosulfan II					0.0004	1	0	0.004
Diazinon					T	1	1	
Selenium					0.009	2	0	0.02 - 0.07
IX. Oils and Fats								
Toxaphene	0.1320	3	0	0.112 - 0.149	0.0727	7	0	0.050 - 0.250
Hexachlorobenzene	0.0050	3	0	0.003 - 0.008	0.0025	9	1	0.001 - 0.010
Pentachlorobenzene	0.0060	3	0	0.003 - 0.012	0.0026	10	3	0.002 - 0.008
Pentachloroanisole	0.0047	3	0	0.002 - 0.010	0.0035	9	1	0.002 - 0.011
PCNB	0.0060	3	0	0.002 - 0.008	0.0025	10	2	0.001 - 0.006
Pentachloroaniline	0.0147	3	0	0.006 - 0.029	0.0078	10	0	0.002 - 0.018
Pentachlorothioanisole	0.0100	3	0	0.004 - 0.021	0.0037	9	1	0.001 - 0.013
Malathion	0.3057	3	0	0.022 - 0.667	0.1286	8	0	0.006 - 0.430
Cadmium	0.0420	2	0	0.060 - 0.066	0.0417	10	0	0.024 - 0.056
Lead	0.017	2	0	0.02 - 0.03	0.019	6	0	0.01 - 0.05
Selenium	0.067	3	0	0.04 - 0.11	0.015	2	0	0.04 - 0.11
Zinc	25.73	3	0	21.8 - 28.3	16.28	10	0	10.6 - 23.6
Dieldrin	0.0047	2	0	0.005 - 0.009	0.0009	2	0	0.002 - 0.007
Heptachlor epoxide	0.0003	1	0	0.001	T	1	1	
Endrin	0.0003	1	0	0.001				
Chlordane	0.0067	1	0	0.020	0.0010	1	0	0.010
p,p'-DDE	0.0013	1	0	0.004	0.0003	1	0	0.003
Mercury	0.0027	1	0	0.008	0.0009	3	0	0.002 - 0.004
Tecnazene					T	1	1	
2-Chloroethyl palmitate					0.0057	1	0	0.057
2-Chloroethyl linoleate					0.0496	1	0	0.496
Diazinon					0.0001	1	0	0.001
Fonofos					0.0004	2	0	0.002
Arsenic					0.002	1	0	0.02
PCB (as Aroclor 1242)					0.005	1	0	0.05
Parathion					0.0002	1	0	0.002
PCB (nonspecific)					T	1	1	
X. Sugar and Sugar Adjuncts								
Zinc	1.15	9	0	0.3 - 7.0	4.91	10	0	1.5 - 18.7
Lead	0.014	5	0	0.01 - 0.04	0.044	10	0	0.02 - 0.09
Selenium	0.002	1	0	0.02	0.010	4	0	0.02 - 0.04
Arsenic	0.001	1	0	0.01	0.002	1	0	0.02
Endosulfan II	0.0002	1	0	0.002				
Endosulfan sulfate	0.0001	1	0	0.001				
Cadmium	0.0002	1	0	0.002	0.0074	8	0	0.002 - 0.013
α-BHC					0.0012	9	3	0.001 - 0.003
Lindane					0.0013	8	3	0.001 - 0.003
Carbaryl					T	1	1	
Dicloran					0.0005	1	0	0.005
Malathion					0.0001	1	0	0.001
Mercury					0.0004	1	0	0.004

Continued

Table 4. (cont'd)

XI. Beverages								
Zinc	0.53	6	0	0.2 - 1.3	0.15	5	0	0.1 - 0.4
Lead	0.120	4	0	0.02 - 0.51	0.023	4	0	0.01 - 0.12
Cadmium	0.0053	3	0	0.001 - 0.021	0.0080	3	0	0.009 - 0.050
Arsenic	0.003	1	0	0.02				
Mercury	0.0003	1	0	0.002	0.0002	1	0	0.002
Selenium					0.006	1	0	0.06

NOTE: T = trace.

[a] Average values are based on the total number of composites examined; trace residues, if present, were treated as zero in calculating averages. Thus, an average value of "T" can be well below the detection limit of a method for a specific compound. If the average value was less than 0.0001 ppm, it is reported as "T".

[b] Range is for positive composites found in quantifiable amounts and does not include positive composites reported as trace.

the 10 lead residues, ranging from 0.040 to 0.380 ppm, the highest level reported for toddler composites in any food class. Residues of the other metals—zinc, cadmium, selenium, and mercury—were all detected at relatively low levels. Although a trace of malathion was found in the infant composites, none was found in the toddler composites; however, 4 composites contained residues of a different organophosphorus pesticide, parathion, at levels ranging from trace to 0.001 ppm. Residues of 5 organochlorine pesticides were reported: Dieldrin was found in 3 composites at levels ranging from trace to 0.002 ppm; *p,p'*-DDE, endosulfan II, endosulfan sulfate, and dicloran were found at low levels in one composite each. The toddler composites contained residues of 6 compounds that were not detected in the infant composites.

Table 5. Intake of pesticide and industrial chemical residues in infant and toddler Total Diet Studies—FY 1977 vs FY 1978

	Intake, μg/kg body wt/day			
	Infant		Toddler	
Compound	FY 77	FY 78	FY 77	FY 78
Aldrin	ND	ND	ND	ND
Dieldrin	0.0405	0.0446	0.0423	0.0386
Total	0.0405	0.0446	0.0423	0.0386
Endrin	ND	0.0002	ND	ND
DDE	0.0999	0.0876	0.3316	0.0876
DDT	0.0010	0.0029	0.0481	0.0129
TDE	0.0010	ND	0.1682	0.0028
Total	0.1019	0.0905	0.5479	0.1033
Endosulfan I	ND	ND	0.0004	0.0050
Endosulfan II	ND	0.0002	0.0012	0.0055
Endosulfan sulfate	ND	0.0007	0.0008	0.0035
Total	0.0000	0.0009	0.0024	0.0140
Heptachlor	ND	ND	ND	ND
Heptachlor epoxide	0.0133	0.0230	0.0182	0.0188
Total	0.0133	0.0230	0.0182	0.0188
BHC	0.0314	0.0340	0.0253	0.0285
Captan	ND	ND	ND	ND
Carbaryl	ND	0.0876	ND	0.0495
Chlordane	ND	0.0051	ND	0.0236
Chlorpropham	0.0505	0.0066	0.2251	0.0343
DCPA	ND	0.0201	ND	0.0004
Diazinon	0.0140	0.0020	0.0071	0.0066
Dicloran	0.0132	0.0155	0.0851	0.1058
Dicofol	ND	ND	ND	ND
Ethion	0.0011	0.0032	0.0010	0.0042
Fonofos	ND	ND	0.0007	0.0005
Hexachlorobenzene	0.0382	0.0116	0.0219	0.0145
Leptophos	ND	ND	ND	ND
Lindane	0.0059	0.0028	0.0081	0.0046
Malathion	0.0643	0.3305	0.2097	0.2985
Methoxychlor	0.0066	0.0289	0.0026	0.0080
Nitrofen	ND	ND	ND	ND
Oxychlordane	0.0011	0.0051	0.0051	0.0078
Parathion	0.0112	0.0046	0.0005	0.0027
Parathion-methyl	ND	ND	ND	ND
PCNB	0.0014	0.0045	0.0029	0.0026
PCP	ND	ND	0.0060	ND
Pentachloroaniline	0.0053	0.0035	0.0079	0.0036
Pentachloroanisole	0.0012	0.0039	0.0028	0.0068
Pentachlorobenzene	0.0013	0.0046	0.0026	0.0049
Pentachlorothioanisole	0.0016	0.0074	0.0027	0.0037
Perthane	ND	0.0123	ND	ND
o-Phenylphenol	ND	ND	ND	ND
Phosalone	0.0115	0.0365	0.0425	0.0136
Polychlorinated biphenyls (PCBs)	0.0253	0.0113	0.0301	0.0985
Ronnel	ND	ND	ND	T
Tecnazene	0.0009	T	0.0026	0.0006
Tetrachloroaniline	ND	ND	0.0002	ND
Tetrachloroanisidine	0.0002	T	0.0007	0.0001
Tetrachloroanisole	0.0001	ND	T	ND
Tetrachlorobenzene	0.0016	ND	0.0007	0.0083
Toxaphene	0.0683	0.0983	0.0443	0.0590

Note: ND = not detected; T = trace (below the limits of quantitation; detected and verified, but not quantifiable).

VIII. Fruit and Fruit Juices

Infant.—The only infant composites that contained any of 3 organophosphorus pesticide residues—parathion, phosalone, and ethion—were those of this food class: Phosalone was found in 2 composites at the 0.008 and 0.015 ppm levels; parathion and ethion were reported at lower levels. A trace of a fourth organophosphorus pesticide, malathion, was also found in one of the 10 composites. Residues of 5 metals—lead, zinc, cadmium, arsenic, and mercury—were all found at low levels; no selenium residues were reported. A trace of the carbamate pesticide carbaryl was found in one composite, and the only Perthane® residue reported for any composite group was found in an infant composite in this food class.

Toddler.—Residues of 5 organophosphorus compounds—parathion, ethion, malathion, phosalone, and diazinon—were found in the toddler composites of this food class. Of these residues, phosalone, found in one composite, was reported at the highest level, 0.011 ppm. All of the organophosphorus pesticides detected as residues in the toddler composites except diazinon were also found in the infant composites of this food class. Residues of 5 metals were found in these toddler composites. Two composites contained selenium, a metal not found in the infant composites. Cadmium residues were found in 4 toddler composites at levels ranging from 0.002 to 0.146 ppm, which was the highest cadmium level reported for any composite group. Dicloran was reported for both the infant and the toddler composites in this food class; however, the 4 dicloran residues found in these toddler composites at levels ranging from 0.011 to 0.044 ppm were the highest reported for toddler composites in any food class. Residues of endosulfan I and endosulfan II were found only in the toddler composites, in one composite each.

IX. Oils and Fats

Infant.—Since only the northeast region had a specific infant diet for this food class, results were reported for 3

Table 6. Dietary intakes of metals in infant and toddler Total Diet Studies—FY 1977 vs FY 1978

	Intake, μg/day			
	Infant		Toddler	
Metal	FY 77	FY 78	FY 77	FY78
Arsenic[a]	5	2	24	18
Cadmium	6	6	8	11
Lead	24	25	29	35
Mercury	0.9	0.2	1.0	0.7
Selenium	15	18	46	52
Zinc[b]	4	5	8	9

[a] Values calculated as arsenic trioxide (As_2O_3).

[b] Values are mg/day.

market basket samples rather than 10. Residues of 6 organochlorine pesticides were found: Three composites contained toxaphene at levels ranging from 0.112 to 0.149 ppm; the other organochlorine pesticide residues were dieldrin, heptachlor epoxide, endrin, chlordane, and *p,p'*-DDE. The organochlorine fungicide PCNB was found in all 3 composites at levels ranging from 0.002 to 0.008 ppm, together with the associated compounds hexachlorobenzene, pentachloroaniline, pentachlorothioanisole, pentachlorobenzene, and pentachloroanisole. Residues of zinc and selenium were found at moderately high levels in all 3 composites. Malathion was the only organophosphorus pesticide detected. Residues of malathion were found in all 3 composites at levels ranging from 0.022 to 0.667 ppm, which was the highest malathion level reported for any composite group.

Toddler.—Since all regions had toddler diets for this food class, results were reported for 10 market basket samples. These composites contained 118 residues of 26 compounds, the highest number of compounds and the highest number of residues reported for any composite group. Most of these residues were fungicides: 58 residues were PCNB or one of its related compounds, and each of these compounds was found in almost every composite, except for tecnazene, which was found in one composite at the trace level. Pentachloroaniline, found in 10 composites at levels ranging from 0.002 to 0.018 ppm and averaging 0.0078 ppm, was the fungicide reported at the highest level. Residues of all 6 metals were reported at relatively low levels; the most frequently reported were zinc, cadmium, and lead. Residues of the organophosphorus pesticide malathion were found in 8 composites at levels ranging from 0.006 to 0.430 ppm, which was the highest malathion level reported for toddler composites in any food class. The toddler composites in this food class were the only composites that contained fonofos, which was found twice at the 0.002 ppm level. Diazinon and parathion were also found in one composite each. Residues of 5 organochlorine pesticides were reported: Toxaphene residues were found in 7 composites at levels ranging from 0.050 to 0.250 ppm; dieldrin residues were found in 2 composites; and residues of heptachlor epoxide, chlordane, and *p,p'*-DDE were found in one composite each. Residues of 4 industrial chemicals were also found in one composite each: Two were PCBs and 2 were 2-chloroethyl esters of fatty acids.

X. Sugar and Adjuncts

Infant.—Five metals accounted for 17 of the 19 residues reported for the infant composites of this food class. Zinc and lead residues, found at low levels in 9 and 5 composites, respectively, occurred the most frequently. Two organochlorine pesticide residues, endosulfan II and endosulfan sulfate, were found in one composite each; however, neither pesticide was detected in the toddler composites of this food class.

Toddler.—The toddler composites of this food class contained low levels of 17 residues of 2 isomers of the organochlorine pesticide BHC: α-BHC and lindane. All 10 composites contained residues of zinc and lead and 8 composites also contained cadmium; all of these 28 metal residues were found at relatively low levels. Residues of 3 other metals—selenium, arsenic, and mercury—and single findings of the carbamate pesticide carbaryl and the organophosphorus pesticide malathion were also reported. The following residues found in these toddler composites were not detected in the infant composites of this food class: α-BHC, lindane, dicloran, carbaryl, malathion, and mercury.

XI. Beverages

Infant.—The only residues reported for the 7 infant beverage composites were the metals zinc, lead, cadmium, arsenic, and mercury. The highest lead level reported for any composite group was found in these infant composites; 4 of the 7 infant beverage composites contained lead residues at levels ranging from 0.02 to 0.51 ppm.

Toddler.—The 10 toddler beverage composites also contained residues of 5 metals; however, instead of arsenic, a single residue of selenium was reported at the 0.06 ppm level. Lead residues were found at slightly lower levels than in the infant beverage composites.

Discussion

This is the fourth report in the infant and toddler series. The 10 market basket samples collected for the present reporting period, October 1977 through September 1978, included 3 market baskets each from the north central and northeastern regions and 2 each from the southern and western regions. Since some regions do not have a specific infant diet for all of the 11 food classes, results are reported for analyses of 98 infant composites and 110 toddler composites.

Infant

The 98 infant composites contained a total of 417 residues, compared with 306 and 301 residues, respectively, for the first and second reports in the infant and toddler series. The third report in the series cannot be compared with the others since 2 additional market basket samples were included for the third reporting period. The increase in the total number of residues in the present report is due to the increase in metal, pesticide, and fungicide residues. Of these, the latter showed the most significant increase, primarily because of improved instrumentation, which allowed the identification and quantitation of impurities and metabolities of parent compounds.

Although 17 of the 32 fungicide residues were hexachlorobenzene, the highest fungicide level found, 0.029 ppm, was reported for pentachloroaniline. (In previous reports, hexachlorobenzene was classified as an organochlorine pesticide rather than a fungicide; nevertheless, 15 of the 32 fungicide residues reported for these infant composites were compounds other than hexachlorobenzene, and that number is still substantially higher than those reported for the infant composites in the first and second reports of the infant and toddler series, i.e., 5 fungicides and one fungicide, respectively.)

The total number of metal residues, which represent 57.3% of all the residues, increased by about 20% over each of the first 2 reporting periods, with zinc, lead, and cadmium accounting for 189 of the 239 residues. The highest zinc level was found in the 10 meat-fish-poultry composites, which contained zinc residues at levels ranging from 12.4 to 33.1 ppm. Lead residues found in the infant composites ranged from 0.005 to 0.51 ppm; the highest lead level reported, which was abnormally high, was found in a beverage composite. The highest level of selenium was found in a grain-cereal composite.

Most of the 133 pesticide residues were found at relatively low levels. The organochlorine pesticide residue reported at the highest level was toxaphene, which was found in 3 oil-fat composites at levels ranging from 0.112 to 0.149 ppm. Residues of dieldrin and *p,p'*-DDE were found in 22 composites each, although all of them were reported at low or trace levels. Single findings of endrin in an oil-fat composite and Perthane

data for residues found in infant and toddler Total Diet samples--October 1977-September 1978

pe of od mposite	Spike Level, ppm	Range of Blank Level, ppm[1]	Range of Total Found, ppm[a,b]	No. of Recovery Attempts
tty	0.003	—[c]	0.002	1
nfatty	0.003	—	0.002 - 0.003 (0.0026)	3
tty	0.003	0.003	0.004	1
	0.03	0 - 0.002	0.020 - 0.026 (0.024)	3
nfatty	0.003	0 - 0.001	0.002 - 0.006 (0.0034)	4
	0.03	0 - 0.001	0.019 - 0.032 (0.026)	3
nfatty	0.005	—	0.003 - 0.005 (0.0040)	3
tty	0.002	—	0.001 - 0.002 (0.0014)	4
nfatty	0.002	—	0.001 - 0.002 (0.0016)	8
tty	0.004	0 - 0.0004 (0.0001)	0.003 - 0.004 (0.0035)	4
nfatty	0.004	—	0.003 - 0.006 (0.0037)	8
tty	0.010	—	0.003 - 0.010 (0.0055)	4
nfatty	0.010	—	0.007 - 0.017 (0.0107)	8
tty	0.002	—	0.002	1
nfatty	0.002	—	0.002	1
tty	0.020	0.002	0.021	1
nfatty	0.020	—	0.021	1
tty	0.040	—	0.022 - 0.030 (0.025)	3
nfatty	0.040	—	0.026 - 0.034 (0.030)	3
tty	0.02	0 - 0.001	0.013 - 0.016 (0.014)	3
nfatty	0.02	—	0.010 - 0.020 (0.015)	3
tty	0.04	0 - 0.003 (0.0007)	0.003 - 0.022 (0.013)	4
	0.08	0 - 0.003 (0.0008)	0.013 - 0.040 (0.028)	5
nfatty	0.04	0 - 0.004 (0.0006)	0.007 - 0.028 (0.020)	10
	0.08	0 - 0.002 (0.0013)	0.016 - 0.031 (0.026)	3

Residue	Type of Food Composite	Spike Level, ppm	Range of Blank Level, ppm[1]	Range of Total Found, ppm[a,b]	No. of Recove Attemp
Silvex	Nonfatty	0.40	—	0.179 - 0.283 (0.231)	2
Carbaryl	Nonfatty	0.20	—	0.08 - 0.24 (0.189)	17
o-Phenylphenol	Nonfatty	0.40	—	0.16 - 0.40 (0.290)	17
Arsenic	Fatty	0.26	—	0.23 - 0.31 (0.269)	6
	Nonfatty	0.26	—	0.23 - 0.31 (0.268)	9
Cadmium	Fatty	0.20	0 - 0.025 (0.005)	0.177 - 0.233 (0.202)	10
	Nonfatty	0.20	0 - 0.146 (0.029)	0.140 - 0.317 (0.212)	19
Lead	Fatty	0.20	0 - 0.05 (0.018)	0.20 - 0.40 (0.245)	10
	Nonfatty	0.20	0 - 0.20 (0.044)	0.17 - 0.45 (0.235)	19
Mercury	Fatty	0.02	0 - 0.002 (0.0007)	0.015 - 0.025 (0.022)	4
		0.04	0 - 0.002 (0.0008)	0.038 - 0.044 (0.041)	5
	Nonfatty	0.04	—	0.035 - 0.045 (0.040)	11
		0.06	0 - 0.005 (0.001)	0.048 - 0.072 (0.062)	6
Selenium	Fatty	0.20	—	0.18 - 0.24 (0.204)	5
	Nonfatty	0.20	0 - 0.022 (0.038)	0.16 - 0.41 (0.222)	14
Zinc	Fatty	30	2.0 - 33.2 (9.87)	32.2 - 63.9 (40.0)	9
	Nonfatty	30	0 - 12.4 (4.06)	20.5 - 43.2 (33.24)	20

NOTE: T = trace.

[a] Numbers in parentheses represent average levels.

[b] These values are uncorrected for background.

[c] Dash means none found in blank.

in a fruit-fruit juice composite were reported for the infant composites although neither of these compounds was found in the toddler composites. Of the 133 pesticide residues found, 30 were organophosphorus compounds and half of these were malathion. Most of the malathion residues were found in grain-cereal composites; however, 3 malathion residues were found in oil-fat composites at levels ranging from 0.022 to 0.667 ppm. All 3 of the parathion residues and both of the ethion residues were found in the fruit-fruit juice composites. Residues of the carbamate pesticide carbaryl were found in a single composite at the trace level in each of 2 food classes.

Eight residues of 5 industrial chemicals were reported for the infant composites: Four residues were pentachlorobenzene; 2 were PCBs—one found in a meat-fish-poultry composite and the other found at the trace level in a grain-cereal composite; and 2 were 2-chloroethyl esters of fatty acids—both found in meat-fish-poultry composites. Five residues of 2 herbicides, chlorpropham and DCPA, were also found in the infant composites.

Toddler

As in the infant composites, the total number of residues found in the toddler composites far exceeded the numbers found in earlier reports. The 110 toddler composites contained a total of 606 residues, compared to 477 and 473 residues, respectively, for the first and second reports in the infant and toddler series. The increase can be attributed to the greater number of metal and fungicide residues detected as a result of improved techniques and instrumentation, as stated earlier.

Most of the 73 fungicide residues were reported at low levels and were identified as metabolites or impurities of the compound PCNB. These included hexachlorobenzene, pentachloroaniline, tecnazene, pentachloroanisole, and pentachlorothioanisole, which were found primarily in the oil-fat composites. A single tetrachlorobenzene residue was found in a meat-fish-poultry composite; however, tetrachlorobenzene was not reported for the infant diet.

The 296 metal residues reported represent 48.8% of all the residues. Besides the normally high levels of zinc, there were 64 lead residues with the highest level found in a vegetable composite and 64 cadmium residues with the highest level found in a fruit-fruit juice composite. Arsenic, selenium, and mercury were each found at the highest reported levels in meat-fish-poultry composites, which contained more metal residues than any other composite group.

The total number of 205 pesticide residues did not differ significantly from the number reported for the first 2 infant and toddler reports in the series; 158 of these residues were organochlorine pesticides, 44 were organophosphorus pesticides, and 3 were residues of the carbamate pesticide carbaryl found in 3 different food classes at the trace level. Although numerous residues were reported for several organochlorine pesticides, they were generally found at a very low level; these included α-BHC, dieldrin, *p,p'*-DDE, heptachlor epoxide, oxychlordane, lindane, dicloran, and others. The organochlorine pesticide residue reported at the highest level was toxaphene, which was found exclusively in the 7 oil-fat composites at levels ranging from 0.050 to 0.250 ppm. The 20 malathion residues, which were found primarily in the grain-cereal composites and oil-fat composites at levels ranging from 0.001 to 0.430 ppm, accounted for approximately half of the 44 organophosphorus pesticide residues reported. In all, 9 organophosphorus compounds were detected and most of them were found in the grain-cereal composites. Exceptions included 4 parathion residues found in the vegetable composites and the 3 ethion residues found in the fruit-fruit juice composites.

Organophosphorus compounds detected in the toddler diet but not in the infant diet included ronnel, fenthion, and fonofos. The remaining 32 residues consisted of 7 industrial chemicals and 2 herbicides. The industrial chemicals were 2-chloroethyl linoleate and 2-chloroethyl palmitate (2-chloroethyl esters of fatty acids), pentachlorobenzene, and 4 PCBs—Aroclor 1242, Aroclor 1254, Aroclor 1260, and one nonspecific PCB. The herbicides were chlorpropham, found in the potato and vegetable composites, and DCPA, found in a single vegetable composite. Neither Aroclor 1242 nor Aroclor 1260 was found in the infant composites.

Recovery studies, in which composites were fortified with known compounds of each residue type, were performed with each market basket. The ranges of the background or unfortified levels and the ranges of the levels for the total compounds recovered are shown in Table 7.

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Pesticide, Metal, and Other Chemical Residues in Adult Total Diet Samples. (XIV). October 1977–September 1978

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Residues of pesticides, herbicides, fungicides, and selected industrial chemicals and metals have been reported since 1964 through the Total Diet Study conducted by the Food and Drug Administration. This report, which is the fourteenth in the series, includes data for the adult diet market basket samples collected from October 1977 through September 1978 in 20 cities throughout 4 geographical areas. All items from each market basket were separated into 12 food classes for analysis, and the averages and ranges of the residues found are presented in various formats. In addition to the 240 composites analyzed, the individual items making up the dairy composites and the meat-fish-poultry composites were analyzed separately for 4 market baskets. The results of a recovery study in which composites were fortified with the more commonly reported residue compounds are also presented as a part of this report. Results for the infant and toddler Total Diet market baskets for the same period are published in a separate report.

This report is the fourteenth in the Total Diet Study series conducted by the Food and Drug Administration (FDA) since 1964. The program was originally concerned with the diet of the young adult male, as determined by FDA's Office of Nutrition and Consumer Services; however, one-third of the 30 market basket samples collected annually after August 1, 1974, were reformulated to represent the diets of the infant (6-month-old) and the toddler (2-year-old). The adult market basket samples, which represent the basic 2-week diet of a 16- to 19-year-old male, were collected within 4 different geographical areas of the United States, i.e., South, Northeast, North Central, and West, with the specific diet of each region determining the composition of the market basket. The various food items were prepared separately as they would be prepared in a home prior to serving, separated into 12 food classes (Table 1), and blended and combined to form a homogeneous composite for analysis. The composites were analyzed by established methodologies (1–8) for residues of organochlorine, organophosphorus, and carbaryl pesticides, herbicides, fungicides, and certain metals and industrial chemicals. The types, amounts, and distribution of the various residues found in market basket samples collected from June 1964 through September 1977 are presented in earlier reports (9–25). This report includes results for market basket samples collected and analyzed from October 1977 through September 1978. The results for the infant and toddler market basket samples for the same period are reported separately.

Table 1. Classes of adult food composites analyzed for pesticides and other chemical residues, October 1977–September 1978

Key	Food class
I	Dairy products
II	Meat, fish, and poultry
III	Grain and cereal products
IV	Potatoes
V	Leafy vegetables
VI	Legume vegetables
VII	Root vegetables
VIII	Garden fruits
IX	Fruits
X	Oils, fats, and shortening
XI	Sugar and adjuncts
XII	Beverages (including drinking water)

Results

A total of 1357 residues, represented by 61 different pesticides, metals, herbicides, fungicides, and industrial chemicals, were reported for this series of 20 market baskets. Table 2 lists these residues in decreasing order of frequency. Table 3 shows their frequency distribution within each food class, and Table 4 shows the levels at which these residues were reported within each food class. The calculation of each average level given in Table 4 was based on the levels found in all 20 composites, the number which was analyzed for each food class. "Trace" values were treated as zero in these calculations. For some residues, therefore, the average value was well below the detection limit of the method used. Table 5 shows the intake of pesticide and industrial chemical residues in terms of μg/kg body wt/day, and Table 6 shows the intake of 6 metals in terms of μg/day (mg/day in the case of zinc).

The more significant residues within each food class are discussed below with respect to residue type and level detected.

I. Dairy Products

Most of the 20 dairy composites contained trace residues of α-BHC, hexachlorobenzene, and heptachlor epoxide. The organochlorine pesticide reported at the highest level was *p,p'*-DDE, found in 5 of 11 positive composites at levels ranging from 0.001 to 0.021 ppm; the *p,p'*-DDE level averaged 0.0017 ppm for all 20 composites. Other organochlorine pesticides included dieldrin, found in 4 composites, and *p,p'*-methoxychlor, found in 3 composites, with overall averages of 0.0003 and 0.0004 ppm, respectively; trace amounts of oxychlordane in 3 composites and lindane in one composite were also reported. Zinc, found in all 20 composites, ranged from 3.0 to 5.2 ppm, and selenium, found in 12 composites, averaged 0.017 ppm for all 20 composites. Lead was found in 3 composites with 0.20 ppm reported as the highest level. Six composites contained low levels of cadmium, and one composite contained mercury. No arsenic residues were reported.

II. Meat, Fish, and Poultry

These composites consistently contained the highest number and levels of residues. Of the 12 organochlorine pesticides reported, *p,p'*-DDE, found in all 20 composites, was the most prevalent residue and averaged 0.0104 ppm. More than half of the composites contained the following residues: dieldrin, α-BHC, hexachlorobenzene, heptachlor epoxide, and oxychlordane. Residues of *p,p'*-DDT, lindane, *trans*-nonachlor, *trans*-chlordane, and *p,p'*-TDE were found in a few composites. Toxaphene, reported for 2 composites, was found in one at the trace level and in the other at the 0.469 ppm level, which was the highest level reported for this residue in any food class. Five industrial chemicals were found in the meat, fish, and poultry composites: A polychlorinated biphenyl (PCB) reported as Aroclor 1254® was found in one composite at the 0.05 ppm level and in 4 composites at trace levels; pentachlorobenzene was found in 2 composites and traces of another PCB reported as Aroclor 1260, 2-chloroethyl palmitate, and 2-chloroethyl linoleate (5, 6) were found in one composite each.

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Table 2. Chemical residues found in 240 food composites, adult Total Diet—October 1977–September 1978

Chemical found	No. of composites with residues	No. of positive composites with residues reported as trace[a]	Range,[b] ppm
Zinc	237	0	0.1 –41.2
Cadmium	178	0	0.001 – 0.139
Lead	153	0	0.01 – 0.40
Selenium	82	0	0.01 – 0.45
α-BHC	56	41	0.001 – 0.018
Dieldrin	53	11	0.001 – 0.008
Hexachlorobenzene	49	40	0.001 – 0.002
p,p'-DDE	48	11	0.001 – 0.103
Arsenic[c]	46	0	0.01 – 0.37
Mercury	42	0	0.001 – 0.027
Malathion	39	2	0.003 – 0.054
Heptachlor epoxide	29	17	0.001 – 0.005
Lindane	22	13	0.001 – 0.005
Pentachlorobenzene	22	12	0.001 – 0.005
Pentachloroaniline	20	4	0.001 – 0.024
Pentachloroanisole	19	14	0.001 – 0.003
Diazinon	17	6	0.001 – 0.005
Endosulfan II	17	3	0.001 – 0.006
Chlorpropham	16	0	0.003 – 0.375
PCNB	16	12	0.001 – 0.005
Pentachlorothioanisole	16	9	0.001 – 0.006
Dicloran	14	2	0.001 – 0.118
Oxychlordane	14	9	0.001 – 0.002
Endosulfan sulfate	14	2	0.002 – 0.014
Endosulfan I	13	1	0.001 – 0.006
Parathion	12	4	0.002 – 0.014
p,p'-DDT	10	2	0.003 – 0.014
Tecnazene	8	2	0.001 – 0.029
2-Chloroethyl linoleate	7	0	0.075 – 0.523
Aroclor 1254	6	5	0.05
p,p'-Methoxychlor	5	1	0.002 – 0.011
DCPA	5	1	0.002 – 0.011
2-Chloroethyl palmitate	5	1	0.011 – 0.060
Tetrachloroanisidine	5	2	0.001 – 0.003
Dicofol	4	0	0.005 – 0.016
Toxaphene	4	2	0.173 – 0.469
Ethion	4	0	0.001 – 0.002
Chlorpyrifos	4	1	0.006 – 0.012
Fonofos	4	1	0.001 – 0.008
β-BHC	4	1	0.001 – 0.010
Carbaryl	3	1	0.050
Phosalone	3	0	0.007 – 0.066
Tetrachloroaniline	3	2	0.013
Tetrachlorothioanisole	3	2	0.004
δ-BHC	3	0	0.001 – 0.003
p,p'-TDE	3	0	0.002 – 0.007
Captan	2	0	0.002 – 0.045
Chlordane	2	2	
Perthane	2	0	0.008 – 0.023
o-Phenylphenol	2	0	0.050 – 0.200
Pentachlorobenzonitrile	2	0	0.001 – 0.005
Endrin	1	0	0.002
trans-Chlordane	1	0	0.001
trans-Nonachlor	1	0	0.004
Aroclor 1242	1	1	
Aroclor 1260	1	1	
Aroclor 1016	1	1	
Tetrachlorobenzene	1	0	0.003
2-Chloroethyl laurate	1	0	0.190
2-Chloroethyl myristate	1	0	0.080
2-Chloroethyl caprate	1	0	0.030
TOTAL	1357	242	

[a]Chemicals detected by a specific analytical methodology were confirmed qualitatively and reported as trace when present at concentrations below the limit of quantitation. The limit of quantitation varies with residue and food class.
[b]Range is for positive composites found in quantifiable amounts and does not include positive composites reported as trace.
[c]Calculated as arsenic trioxide (As_2O_3).

Residues of all 6 metals were found throughout most of the composites, with the highest levels of zinc (41.2 ppm), arsenic (0.37 ppm), and mercury (0.027 ppm) reported for this food class. All composites contained selenium residues, which ranged from 0.14 to 0.32 ppm, and cadmium and lead were each found in 13 composites. The 2 organophosphorus pesticides malathion and ethion were found in one composite each.

III. Grain and Cereal Products

Malathion was the most significant residue reported for this food class. Malathion residues, ranging from 0.007 to 0.054 ppm, were found in all 20 composites and averaged 0.0224 ppm. Other organophosphorus residues, diazinon and chlorpyrifos, were found at low levels in some composites. The only organochlorine residues were α-BHC and lindane, each found in a few composites at low levels. Selenium, which was found at the highest level in these grain and cereal composites, ranged from 0.06 to 0.45 ppm and averaged 0.202 ppm for all 20 composites. Zinc levels were high and averaged 8.52 ppm for the 20 composites examined. Lead, which ranged from 0.02 to 0.18 ppm in 17 composites, averaged 0.055 ppm for all the composites examined. Cadmium and arsenic averaged 0.0263 and 0.029 ppm, respectively, and mercury, which averaged 0.0014 ppm for all 20 composites, was found in 5 composites. The 7 industrial chemical residues reported were identified as 2-chloroethyl esters of fatty acids and originated in spices (5, 6).

IV. Potatoes

The use of the organochlorine herbicide chlorpropham on potatoes was evident from the 13 positive composites that contained levels ranging from 0.006 to 0.375 ppm; chlorpropham averaged 0.0619 ppm for all 20 composites. The organochlorine fungicide tecnazene was found in 6 composites at levels ranging from a trace to 0.029 ppm; 4 tecnazene metabolites were also detected. The organochlorine pesticides reported mostly at low levels included dicloran, heptachlor epoxide, dieldrin, endosulfan sulfate, chlordane, and *p,p'*-DDE. Residues of all 6 metals were reported: Zinc and cadmium were found in all 20 composites; lead, ranging from 0.01 to 0.29 ppm, was found in 14 composites; and selenium, arsenic, and mercury were each found in fewer than half of the composites. A trace of one organophosphorus residue, fonofos, was also found in a single composite.

V. Leafy Vegetables

Although there were 11 different organochlorine pesticide residues reported in this food class, all of these except *p,p'*-DDE and endosulfan sulfate were detected in 3 or fewer composites with none at very high levels. Parathion was found in 7 composites at levels ranging from trace to 0.010 ppm, diazinon was found in 4 composites at levels ranging from trace to 0.003 ppm, and only a trace of a third organophosphorus pesticide, malathion, was reported. Low levels of 2 organochlorine fungicides, pentachloroaniline and pentachlorobenzonitrile, were reported. The organochlorine herbicide DCPA was found in 3 composites at levels ranging from 0.002 to 0.011 ppm. The highest level of cadmium reported for any food class was found in leafy vegetables; all 20 leafy vegetable composites contained cadmium at levels ranging from 0.012 to 0.139 ppm. Zinc, also detected in all 20 composites, ranged from 1.4 to 3.2 ppm. Lead, mercury, and selenium were each found in a few composites at low levels.

VI. Legume Vegetables

Metals accounted for almost all of the residues reported for this food class. Although zinc was found at relatively moderate levels in all 20 composites with an overall average of 7.29 ppm, lead levels ranged from 0.05 to 0.40 ppm in 19 composites with an overall average of 0.162 ppm—the highest average lead level for any food class. Low levels of cadmium, selenium, arsenic, and mercury were also reported for this food class. The legume vegetable composites also contained 8 nonmetal residues: 5 organochlorine pesticides, all found

Table 3. Frequency of occurrence of chemical residues by food class, adult Total Diet—October 1977–September 1978

	Food class[a]											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Chemical	Number of occurrences											
Zinc	20	20	20	20	20	20	20	20	20	20	20	17
Cadmium	6	13	20	20	20	13	19	19	7	16	15	10
Lead	3	13	17	14	10	19	13	18	13	8	17	8
Selenium	12	20	20	7	2	9	4	4	0	2	1	1
α-BHC	18	16	1	0	0	0	0	7	0	1	13	0
Dieldrin	11	17	0	3	1	0	0	14	2	5	0	0
Hexachlorobenzene	10	18	0	0	1	0	0	0	0	19	1	0
p,p'-DDE	11	20	0	2	10	1	2	0	0	1	1	0
Arsenic	0	20	14	2	0	1	3	2	1	1	1	1
Mercury	1	16	5	2	3	3	3	1	2	5	1	0
Malathion	0	1	20	0	1	0	0	0	1	10	6	0
Heptachlor epoxide	10	16	0	3	0	0	0	0	0	0	0	0
Lindane	1	3	1	0	0	0	0	5	0	0	12	0
Pentachlorobenzene	0	2	0	0	0	0	0	0	0	19	1	0
Pentachloroaniline	0	0	0	0	1	0	0	0	0	18	1	0
Pentachloroanisole	0	0	0	0	0	0	0	0	0	18	1	0
Diazinon	0	0	9	0	4	0	0	2	2	0	0	0
Endosulfan II	0	0	0	0	3	1	0	7	6	0	0	0
Chlorpropham	0	0	0	13	0	0	1	1	1	0	0	0
PCNB	0	0	0	0	0	0	0	0	0	15	1	0
Pentachlorothioanisole	0	0	0	0	0	0	0	0	0	16	0	0
Dicloran	0	0	0	5	3	0	0	0	6	0	0	0
Oxychlordane	3	11	0	0	0	0	0	0	0	0	0	0
Endosulfan sulfate	0	0	0	2	5	1	0	5	1	0	0	0
Endosulfan I	0	0	0	0	3	1	0	7	2	0	0	0
Parathion	0	0	0	0	7	1	0	3	1	0	0	0
p,p'-DDT	0	6	0	0	2	0	1	0	1	0	0	0
Tecnazene	0	0	0	6	0	0	0	2	0	0	0	0
2-Chloroethyl linoleate	0	1	2	0	0	0	0	0	0	4	0	0
PCB (as Aroclor 1254)	0	5	0	0	0	0	0	0	0	1	0	0
p,p'-Methoxychlor	4	0	0	0	0	0	0	1	0	0	0	0
DCPA	0	0	0	0	3	0	2	0	0	0	0	0
2-Chloroethyl palmitate	0	1	2	0	0	0	0	0	0	2	0	0
Tetrachloroanisidine	0	0	0	5	0	0	0	0	0	0	0	0
Dicofol	0	0	0	0	0	0	0	1	3	0	0	0
Toxaphene	0	2	0	0	0	0	0	1	0	1	0	0
Ethion	0	1	0	0	0	0	0	0	3	0	0	0
Chloropyrifos	0	0	3	0	0	0	0	1	0	0	0	0
Fonofos	0	0	0	1	0	0	0	0	0	3	0	0
β-BHC	0	0	0	0	1	0	0	3	0	0	0	0
Carbaryl	0	0	0	0	0	0	0	1	2	0	0	0
Phosalone	0	0	0	0	0	0	0	0	3	0	0	0
Tetrachloroaniline	0	0	0	3	0	0	0	0	0	0	0	0
Tetrachlorothioanisole	0	0	0	3	0	0	0	0	0	0	0	0
δ-BHC	0	0	0	0	0	0	0	3	0	0	0	0
p,p'-TDE	0	1	0	0	1	1	0	0	0	0	0	0
Captan	0	0	0	0	0	0	0	0	2	0	0	0
Chlordane	0	0	0	1	1	0	0	0	0	0	0	0
Perthane	0	0	0	0	0	0	0	0	2	0	0	0
o-Phenylphenol	0	0	0	0	0	0	0	0	2	0	0	0
Pentachlorobenzonitrile	0	0	0	0	1	0	0	1	0	0	0	0
Endrin	0	0	0	0	0	0	0	1	0	0	0	0
trans-Chlordane	0	1	0	0	0	0	0	0	0	0	0	0
trans-Nonachlor	0	1	0	0	0	0	0	0	0	0	0	0
PCB (as Aroclor 1242)	0	0	0	0	0	1	0	0	0	0	0	0
PCB (as Aroclor 1260)	0	1	0	0	0	0	0	0	0	0	0	0
PCB (as Aroclor 1016)	0	0	0	0	0	1	0	0	0	0	0	0
Tetrachlorobenzene	0	0	0	1	0	0	0	0	0	0	0	0
2-Chloroethyl laurate	0	0	1	0	0	0	0	0	0	0	0	0
2-Chloroethyl myristate	0	0	1	0	0	0	0	0	0	0	0	0
2-Chloroethyl caprate	0	0	1	0	0	0	0	0	0	0	0	0

[a]See Table 1 for description of food class.

at low levels in one composite each; 2 industrial chemicals, Aroclor 1242 and Aroclor 1016, found only in this food class in one composite each; and the organophosphorus compound parathion, found in a single composite at the 0.004 ppm level.

VII. Root Vegetables

In root vegetables, as in the previous food class, the metals dominated the residues reported. All 6 metals were detected at low levels; zinc, cadmium, and lead were found in more than half the composites, and arsenic, selenium, and mercury were limited to just a few. Two organochlorine herbicides, DCPA and chlorpropham, and 2 organochlorine pesticides, *p,p'*-DDE and *p,p'*-DDT, were also found at very low levels.

VIII. Garden Fruits

The garden fruit composites contained residues of 13 various organochlorine pesticides. Of these, the predominant residue was dieldrin, which was found at levels ranging from 0.001 to 0.008 ppm in 13 composites and at the trace level in one composite; the average level of dieldrin for all the composites examined was 0.0022 ppm. Parent compounds and metabolites were among the remaining organochlorine residues: Endosulfan I and II were each found in 7 composites, and 5 composites contained the sulfate form. α-BHC, found in 7 composites, and δ-BHC, found in 3 composites, were each reported at their highest levels, 0.018 and 0.003 ppm, respectively; 5 composites contained lindane and 3 contained β-BHC at low levels. Organochlorine pesticide residues each

Table 4. Levels of chemical residues found by food class, adult Total Diet—October 1977–September 1978

Residue	Overall average,[a] ppm	Positive composites		
		Total number	Number reported as trace	Range,[b] ppm
I. Dairy Products				
Zinc	4.11	20	0	3.0 – 5.2
Hexachlorobenzene	T	10	10	
Dieldrin	0.0003	11	7	0.001– 0.002
p,p'-DDE	0.0017	11	6	0.001– 0.021
Oxychlordane	T	3	3	
Cadmium	0.0020	6	0	0.001– 0.019
Mercury	0.0001	1	0	0.002
α-BHC	T	18	18	
Heptachlor epoxide	T	10	10	
p,p'-Methoxychlor	0.0004	4	1	0.002– 0.004
Selenium	0.017	12	0	0.01 – 0.06
Lead	0.014	3	0	0.01 – 0.20
Lindane	T	1	1	
II. Meat, Fish, and Poultry				
Arsenic	0.168	20	0	0.01 – 0.37
Lead	0.031	13	0	0.02 – 0.10
Selenium	0.225	20	0	0.14 – 0.32
α-BHC	0.0001	16	14	0.001– 0.002
p,p'-DDE	0.0104	20	1	0.001– 0.103
Dieldrin	0.0016	17	2	0.001– 0.004
PCB (as Aroclor 1254)	0.002	5	4	0.05
Cadmium	0.0070	13	0	0.002– 0.042
Mercury	0.0091	16	0	0.004– 0.027
Zinc	30.02	20	0	18.1 –41.2
Hexachlorobenzene	0.0002	18	15	0.001– 0.002
Heptachlor epoxide	0.0007	16	7	0.001– 0.005
p,p'-DDT	0.0008	6	2	0.003– 0.006
Oxychlordane	0.0004	11	6	0.001– 0.002
2-Chloroethyl palmitate	0.0005	1	0	0.011
Lindane	T	3	3	
trans-Chlordane	T	1	0	0.001
Pentachlorobenzene	T	2	1	0.001
PCB (as Aroclor 1260)	T	1	1	
Ethion	T	1	0	0.001
2-Chloroethyl linoleate	0.0046	1	0	0.091
trans-Nonachlor	0.0002	1	0	0.004
p,p'-TDE	0.0004	1	0	0.007
Toxaphene	0.0234	2	1	0.469
Malathion	0.0004	1	0	0.008
III. Grain and Cereal Products				
Cadmium	0.0263	20	0	0.011– 0.040
Selenium	0.202	20	0	0.06 – 0.45
Diazinon	0.0005	9	5	0.001– 0.005
Arsenic	0.029	14	0	0.01 – 0.11
2-Chloroethyl caprate	0.0015	1	0	0.030
2-Chloroethyl linoleate	0.0278	2	0	0.126– 0.430
2-Chloroethyl palmitate	0.0030	2	1	0.060
Mercury	0.0014	5	0	0.003– 0.008
Zinc	8.52	20	0	5.4 –11.0
Malathion	0.0224	20	0	0.007– 0.054
Lead	0.055	17	0	0.02 – 0.18
2-Chloroethyl myristate	0.0040	1	0	0.080
2-Chloroethyl laurate	0.0095	1	0	0.190
Chlorpyrifos	0.0008	3	1	0.006– 0.009
α-BHC	T	1	1	
Lindane	0.0001	1	0	0.002
IV. Potatoes				
Cadmium	0.0432	20	0	0.023– 0.078
Zinc	4.00	20	0	1.5 –11.7
Dicloran	0.0005	5	2	0.001– 0.006
Arsenic	0.004	2	0	0.03 – 0.04
Tetrachloroanisidine	0.0003	5	2	0.001– 0.003
Dieldrin	0.0002	3	1	0.002– 0.003
Chlordane	T	1	1	
Lead	0.046	14	0	0.01 – 0.29
Heptachlor epoxide	0.0003	3	0	0.001– 0.003
Chlorpropham	0.0619	13	0	0.006– 0.375
Tecnazene	0.0020	6	1	0.001– 0.029
Selenium	0.008	7	0	0.01 – 0.05
Endosulfan sulfate	0.0004	2	0	0.003– 0.005
Mercury	0.0004	2	0	0.004– 0.005
Tetrachlorobenzene	0.0002	1	0	0.003
Tetrachlorothioanisole	0.0002	3	2	0.004
Fonofos	T	1	1	
Tetrachloroaniline	0.0006	3	2	0.013
p,p'-DDE	0.0001	2	1	0.002

Table 4. (cont'd)

V. Leafy Vegetables				
Cadmium	0.0517	20	0	0.012– 0.139
Zinc	2.16	20	0	1.4 – 3.2
p,p'-DDE	0.0017	10	1	0.001– 0.008
Endosulfan sulfate	0.0018	5	0	0.004– 0.014
DCPA	0.0008	3	0	0.002– 0.011
Malathion	T	1	1	
Endosulfan I	0.0004	3	0	0.002– 0.004
Lead	0.020	10	0	0.01 – 0.08
Parathion	0.0012	7	2	0.002– 0.010
p,p'-DDT	0.0008	2	0	0.003– 0.014
Dicloran	0.0004	3	0	0.002– 0.004
Diazinon	0.0003	4	1	0.001– 0.003
β-BHC	0.0005	1	0	0.010
Endosulfan II	0.0002	3	1	0.001– 0.004
Mercury	0.0006	3	0	0.003– 0.005
Chlordane	T	1	1	
Pentachloroaniline	0.0002	1	0	0.003
Hexachlorobenzene	T	1	0	0.001
p,p'-TDE	0.0001	1	0	0.002
Selenium	0.004	2	0	0.03 – 0.05
Pentachlorobenzonitrile	0.0002	1	0	0.005
Dieldrin	T	1	0	0.001
VI. Legume Vegetables				
Cadmium	0.0054	13	0	0.001– 0.021
Zinc	7.29	20	0	5.6 –10.5
Selenium	0.010	9	0	0.01 – 0.03
Parathion	0.0002	1	0	0.004
Endosulfan I	0.0002	1	0	0.003
Endosulfan sulfate	0.0006	1	0	0.013
p,p'-DDE	0.0001	1	0	0.002
Lead	0.162	19	0	0.05 – 0.40
Arsenic	0.002	1	0	0.03
PCB (as Aroclor 1016)	T	1	1	
PCB (as Aroclor 1242)	T	1	1	
Endosulfan II	0.0002	1	0	0.005
Mercury	0.0011	3	0	0.005– 0.009
p,p'-TDE	0.0001	1	0	0.002
VII. Root Vegetables				
Arsenic	0.006	3	0	0.01 – 0.06
Lead	0.027	13	0	0.02 – 0.11
p,p'-DDE	0.0002	2	1	0.004
Selenium	0.004	4	0	0.02 – 0.03
p,p'-DDT	0.0002	1	0	0.003
Cadmium	0.0254	19	0	0.006– 0.045
Zinc	2.11	20	0	1.1 – 4.0
DCPA	0.0001	2	1	0.002
Mercury	0.0007	3	0	0.004– 0.005
Chlorpropham	0.0003	1	0	0.007
VIII. Garden Fruits				
Cadmium	0.0158	19	0	0.007– 0.068
Zinc	2.62	20	0	1.0 – 6.9
Endosulfan I	0.0010	7	1	0.001– 0.006
Endosulfan sulfate	0.0007	5	1	0.002– 0.005
Tecnazene	T	2	1	0.001
Selenium	0.006	4	0	0.01 – 0.06
Dicofol	0.0005	1	0	0.010
Lead	0.089	18	0	0.03 – 0.34
Dieldrin	0.0022	14	1	0.001– 0.008
Endosulfan II	0.0011	7	0	0.001– 0.006
Carbaryl	T	1	1	
Diazinon	0.0002	2	0	0.002
Arsenic	0.002	2	0	0.02 – 0.03
α-BHC	0.0014	7	2	0.001– 0.018
β-BHC	0.0002	3	1	0.001– 0.003
Lindane	0.0004	5	2	0.001– 0.005
Pentachlorobenzonitrile	T	1	0	0.001
Parathion	0.0004	3	2	0.008
Chlorpyrifos	0.0006	1	0	0.012
Mercury	0.0001	1	0	0.002
δ-BHC	0.0002	3	0	0.001– 0.003
Endrin	0.0001	1	0	0.002
Toxaphene	0.0086	1	0	0.173
Chlorpropham	0.0007	1	0	0.014
p,p'-Methoxychlor	0.0006	1	0	0.011
IX. Fruits				
Lead	0.045	13	0	0.02 – 0.21
Cadmium	0.0022	7	0	0.002– 0.025
Dicloran	0.0095	6	0	0.002– 0.118
Endosulfan II	0.0007	6	2	0.001– 0.005
Diazinon	0.0001	2	0	0.001
Dicofol	0.0014	3	0	0.005– 0.016

Table 4. (cont'd)

IX. Fruits (continued)				
Endosulfan sulfate	T	1	1	
Zinc	0.72	20	0	0.3 – 1.3
Perthane	0.0016	2	0	0.008– 0.023
Dieldrin	0.0002	2	0	0.001– 0.002
Ethion	0.0002	3	0	0.001– 0.002
o-Phenylphenol	0.0125	2	0	0.050– 0.200
Endosulfan I	0.0003	2	0	0.003
Malathion	0.0002	1	0	0.004
Arsenic	0.005	1	0	0.10
Captan	0.0024	2	0	0.002– 0.045
p,p'-DDT	0.0002	1	0	0.004
Chlorpropham	0.0002	1	0	0.003
Carbaryl	0.0050	2	0	0.050
Phosalone	0.0053	3	0	0.007– 0.066
Parathion	0.0007	1	0	0.014
Mercury	0.0002	2	0	0.001– 0.004
X. Oils, Fats, and Shortening				
Cadmium	0.0158	16	0	0.006– 0.034
Zinc	5.22	20	0	4.0 –10.3
Hexachlorobenzene	0.0003	19	14	0.001– 0.002
PCNB	0.0004	15	11	0.001– 0.005
Fonofos	0.0005	3	0	0.001– 0.008
Arsenic	0.002	1	0	0.03
Malathion	0.0057	10	0	0.006– 0.026
Lead	0.020	8	0	0.02 – 0.16
Pentachloroanisole	0.0005	18	13	0.001– 0.003
Pentachlorobenzene	0.0009	19	10	0.001– 0.005
Pentachloroaniline	0.0032	18	4	0.001– 0.024
Pentachlorothioanisole	0.0008	16	9	0.001– 0.006
Dieldrin	0.0007	5	0	0.002– 0.004
α-BHC	T	1	1	
Selenium	0.002	2	0	0.01 – 0.04
2-Chloroethyl linoleate	0.0454	4	0	0.075– 0.523
Mercury	0.0014	5	0	0.004– 0.009
Toxaphene	T	1	1	
2-Chloroethyl palmitate	0.0035	2	0	0.020– 0.050
p,p'-DDE	0.0003	1	0	0.006
PCB (as Aroclor 1254)	T	1	1	
XI. Sugar and Adjuncts				
Cadmium	0.0137	15	0	0.002– 0.069
Zinc	2.04	20	0	0.6 – 3.8
α-BHC	0.0006	13	5	0.001– 0.003
p,p'-DDE	T	1	1	
Pentachlorobenzene	T	1	1	
Pentachloroanisole	T	1	1	
PCNB	T	1	1	
Lead	0.051	17	0	0.02 – 0.15
Malathion	0.0025	6	1	0.003– 0.019
Lindane	0.0005	12	7	0.001– 0.005
Arsenic	0.001	1	0	0.02
Hexachlorobenzene	T	1	1	
Pentachloroanillne	T	1	0	0.001
Selenium	0.002	1	0	0.03
Mercury	0.0001	1	0	0.002
XII. Beverages				
Lead	0.018	8	0	0.02 – 0.08
Cadmium	0.0036	10	0	0.002– 0.013
Selenium	0.001	1	0	0.02
Zinc	0.34	17	0	0.1 – 0.9
Arsenic	0.001	1	0	0.02

Note: T = trace.

[a]Average values are based on the 20 composites examined; trace residues, if present, were treated as zero in calculating averages. Thus, an average value of "T" can be well below the detection limit of a method for a specific compound. If the average value was less than 0.0001 ppm, it is reported as "T".

[b]Range is for positive composites found in quantifiable amounts and does not include positive composites reported as trace.

found in one composite included 0.173 ppm toxaphene, 0.011 ppm *p,p'*-methoxychlor, and 0.010 ppm dicofol. This food class contained the only endrin residue, which was found in one composite at the 0.002 ppm level. The remaining residues reported for garden fruits included 3 organophosphorus pesticides, diazinon, chlorpyrifos, and parathion; 2 organochlorine fungicides, tecnazene and pentachlorobenzonitrile; one organochlorine herbicide, chlorpropham; and a trace of the carbamate carbaryl. All 6 metals were detected at relatively low levels with zinc, cadmium, and lead accounting for the greatest number of residues.

IX. Fruits

The greatest number of organophosphorus pesticides were found in these fruit composites. This food class was the only one that contained phosalone, which was found in 3 composites at levels ranging from 0.007 to 0.066 ppm. Three composites contained ethion at levels ranging from 0.001 to 0.002 ppm; parathion was found in one composite at the 0.014 ppm level and low levels of diazinon and malathion were also reported. The organochlorine pesticide residues reported for this food class included Perthane®, which was found only twice in the entire Market Basket series in 2 of these fruit

Table 5. Intake of pesticide and industrial chemical residues in adult Total Diet Studies—FY 1977 vs FY 1978

Compound	Intake, μg/kg body wt/day FY 77	FY 78
Aldrin	ND	ND
Dieldrin	0.0226	0.0170
Total	0.0226	0.0170
DDE	0.0394	0.0607
DDT	0.0057	0.0084
TDE	0.0012	0.0014
Total	0.0463	0.0705
Endosulfan I	0.0026	0.0025
Endosulfan II	0.0031	0.0034
Endosulfan sulfate	0.0042	0.0049
Total	0.0099	0.0108
α-BHC	0.0105	0.0091
Captan	0.0305	0.0080
Chlordane	0.0011	0.0014
Chlorpropham	0.3098	0.1437
DCPA	0.0005	0.0008
Diazinon	0.0061	0.0044
Dicloran	0.0557	0.0328
Dicofol	0.0040	0.0051
Ethion	0.0180	0.0008
Heptachlor epoxide	0.0074	0.0077
Hexachlorobenzene	0.0018	0.0039
Lindane	0.0038	0.0024
Malathion	0.1540	0.1423
Methoxychlor	0.0078	0.0071
trans-Nonachlor	0.0020	0.0008
Oxychlordane	0.0025	0.0025
Parathion	0.0016	0.0038
Parathion-methyl	0.0006	ND
PCBs	0.0164	0.0269
PCNB	0.0010	0.0007
PCP	0.0009	ND
Pentachloroaniline	0.0010	0.0007
Pentachloroanisole	0.0004	0.0010
Perthane	T	0.0048
o-Phenylphenol	0.0044	0.0380
Phosalone	0.0005	0.0174
Tecnazene	0.0346	0.0049
Toxaphene	0.0802	0.1071

Note: ND = not detected; T = trace (below the limits of quantitation; detected and verified, but not quantifiable).

Table 6. Dietary intakes of metals in adult Total Diet Studies—FY 1977 vs FY 1978

Metal	Intake, μg/day FY 77	FY 78
Arsenic[a]	71.6	59.1
Cadmium	36.9	30.9
Lead	79.3	95.1
Mercury	6.3	3.4
Selenium	110.7	156.2
Zinc[b]	18.0	16.8

[a]Values calculated as arsenic trioxide (As_2O_3).
[b]Values are mg/day.

composites. Six composites contained dicloran at levels ranging from 0.002 to 0.118 ppm with an average level of 0.0095 ppm for the 20 composites examined. Six composites contained endosulfan II at levels ranging from a trace to 0.005 ppm, and dicofol was found in 3 composites at levels ranging from 0.005 to 0.016 ppm. Other residues reported for this food class included dieldrin, endosulfan I, endosulfan sulfate, and *p,p'*-DDT. The 2 fungicides captan and *o*-phenylphenol were found only in this food class. Zinc and lead were the predominant metal residues with cadmium, mercury, and arsenic accounting for the remainder. This food class was the only one for which no residues of selenium were reported.

Table 7. Pesticide and industrial chemical residues in individual commodities of the dairy products composites of 4 market basket samples, adult Total Diet—October 1977–September 1978

Commodity	Residue	Level,[a] ppm			
Milk	*p,p'*-DDE	T	—	—	T
	α-BHC	—	—	T	T
	Hexachlorobenzene	—	—	—	T
	Dieldrin	—	—	—	T
Evaporated milk	α-BHC	T	—	0.001	0.002
	Heptachlor epoxide	T	—	0.001	T
	Dieldrin	0.002	—	0.001	0.002
	p,p'-Methoxychlor	0.015	—	—	0.005
	p,p'-DDE	—	—	—	0.018
	Oxychlordane	—	—	—	0.001
	Hexachlorobenzene	—	—	—	T
Ice cream	Hexachlorobenzene	0.003	—	0.002	0.001
	Heptachlor epoxide	T	0.001	—	—
	Oxychlordane	T	—	—	—
	Dieldrin	0.002	0.003	—	0.007
	p,p'-DDE	T	—	0.002	0.004
	α-BHC	—	0.001	—	0.002
Cottage cheese	α-BHC	—	T	T	T
	Hexachlorobenzene	—	—	—	T
	p,p'-DDE	—	—	—	T
Processed cheese	α-BHC	0.003	0.003	0.007	0.002
	Lindane	0.002	—	—	T
	Heptachlor epoxide	0.001	0.003	0.003	0.004
	Dieldrin	0.002	0.004	0.005	0.005
	p,p'-Methoxychlor	0.005	T	0.012	—
	Hexachlorobenzene	T	T	0.001	T
	Oxychlordane	—	0.002	0.001	0.002
	p,p'-DDE	—	0.039	0.002	0.002
Natural cheese	Hexachlorobenzene	T	T	0.001	0.002
	α-BHC	0.004	0.006	0.004	0.004
	Oxychlordane	0.001	0.003	0.001	—
	Heptachlor epoxide	T	0.007	T	0.001
	p,p'-DDE	0.002	0.002	0.004	0.006
	Dieldrin	T	0.010	0.003	0.004
	trans-Nonachlor	—	0.001	—	—
	p,p'-Methoxychlor	—	T	—	—
Butter	Hexachlorobenzene	0.003	0.002	0.002	0.004
	α-BHC	0.026	0.010	0.011	0.006
	Oxychlordane	0.006	0.005	0.005	0.007
	Heptachlor epoxide	0.002	0.007	0.005	0.003
	p,p'-DDE	0.004	—	0.004	0.242
	Dieldrin	0.003	0.020	0.011	0.010
	PCB (Aroclor 1254)	—	0.08	—	0.04
	p,p'-Methoxychlor	—	—	0.006	—
	Lindane	—	—	—	0.002
	p,p'-DDT	—	—	—	0.010
Ice milk	Hexachlorobenzene	T	—	—	0.001
	α-BHC	—	—	—	0.001
	p,p'-DDE	—	—	—	0.003
	Dieldrin	—	—	—	0.004
	Heptachlor epoxide	—	—	—	T
	Oxychlordane	—	—	—	T
Buttermilk	None found	—	(not in diet)		
Nonfat dry milk	None found	—	—	—	—
Skim milk	None found	—	—	—	—

Note: T = trace.
[a]Dash indicates none found.

X. Oils, Fats, and Shortening

These composites contained the second highest number of residues reported for any food class and were the source of most of the fungicide residues. Eighty-six fungicide residues, all found generally at low levels, were distributed among 5 compounds: pentachloroanisole, pentachlorobenzene, PCNB, pentachloroaniline, and pentachlorothioanisole. Of the 5 organochlorine pesticides reported, hexachlorobenzene was detected in almost all of the composites, although the majority of such residues were at the trace level. Other organochlorine pesticides reported were dieldrin, α-BHC, *p,p'*-DDE, and toxaphene. Two organophosphorus compounds, fonofos and malathion, were also reported. All 6 metals were reported with zinc and cadmium found at moderate levels in most of the composites; lead and mercury were found in fewer than half of the composites. The more recently identified com-

Table 8. Pesticide and industrial chemical residues in individual commodities of the meat-fish-poultry composites of 4 market basket samples, adult Total Diet—October 1977–September 1978

Commodity	Residue	Level,[a] ppm			
Roast beef	Hexachlorobenzene	T	T	T	—
	α-BHC	T	T	0.002	0.002
	Oxychlordane	T	T	0.001	0.003
	p,p'-DDE	0.018	0.009	—	0.006
	p,p'-TDE	—	0.004	—	—
	p,p'-DDT	—	T	—	—
	Dieldrin	—	0.006	0.011	0.007
	Heptachlor epoxide	—	0.004	0.006	0.004
Hamburger	Hexachlorobenzene	0.001	T	T	0.001
	α-BHC	T	0.001	—	0.002
	Lindane	0.001	—	—	—
	Oxychlordane	0.003	T	—	0.003
	Heptachlor epoxide	0.002	0.002	0.002	0.004
	p,p'-DDE	0.023	0.005	0.017	0.006
	p,p'-TDE	0.010	—	—	—
	p,p'-DDT	0.024	—	—	—
	Dieldrin	0.002	0.004	0.003	0.007
Pork chops	*p,p'*-DDE	T	0.002	0.002	0.001
	Heptachlor epoxide	—	—	T	—
	α-BHC	—	—	—	T
	Hexachlorobenzene	—	—	—	T
	Lindane	—	—	—	0.005
	Oxychlordane	—	—	—	T
Bacon	*p,p'*-DDE	0.004	0.004	0.002	0.002
	p,p'-DDT	0.009	—	0.009	—
	α-Chlordane	0.004	—	—	—
	β-Chlordane	0.005	—	—	—
	Heptachlor epoxide	—	0.003	0.002	T
	Dieldrin	—	0.003	0.002	T
	Oxychlordane	—	0.002	—	T
	trans-Nonachlor	—	0.001	—	—
Chicken	Dieldrin	0.003	T	—	0.001
	p,p'-DDE	0.006	0.002	0.003	0.002
	p,p'-DDT	0.006	—	—	—
	Hexachlorobenzene	—	T	T	—
	Oxychlordane	—	—	—	T
	Endrin	—	—	—	0.001
Fish fillet	α-BHC	—	0.004	—	T
	Hexachlorobenzene	—	0.004	—	T
	Toxaphene	—	0.149	—	—
	p,p'-DDE	—	—	—	0.014
	p,p'-DDT	—	—	—	0.025
	PCB (Aroclor 1254)	—	—	—	0.031
	p,p'-TDE	—	—	—	0.005
	Dieldrin	—	—	—	T
		(Tuna)	(Tuna)	(Tuna)	(Salmon)
Fish, canned	*p,p'*-DDE	0.008	0.003	—	—
	p,p'-TDE	0.008	0.003	—	—
	α-BHC	—	—	—	0.005
	Hexachlorobenzene	—	—	—	0.001
	PCB (Aroclor 1254)	—	—	—	T
	Dieldrin	—	—	—	0.001
Lunchmeat	Oxychlordane	0.001	0.002	T	0.001
	p,p'-DDE	0.011	0.003	0.002	0.003
	p,p'-DDT	0.005	T	—	0.004
	Heptachlor epoxide	—	0.002	0.003	T
	Dieldrin	—	0.003	0.003	0.002
	α-BHC	—	0.001	0.001	0.001
	Hexachlorobenzene	—	T	T	T
	Lindane	—	0.001	T	T
Frankfurters	Hexachlorobenzene	T	0.001	T	T
	α-BHC	T	0.001	0.001	0.002
	Lindane	T	—	—	0.002
	Oxychlordane	0.003	T	—	0.001
	p,p'-DDE	0.020	0.005	0.002	0.018
	Heptachlor epoxide	—	T	0.001	0.001
	Dieldrin	—	0.003	0.002	0.001
	p,p'-TDE	—	—	—	0.003
	p,p'-DDT	—	—	—	0.024
Liver	Hexachlorobenzene	0.001	—	—	T
	Oxychlordane	—	—	—	0.001
	p,p'-DDE	—	—	—	0.001
	Heptachlor epoxide	—	—	—	0.001
	Dieldrin	—	—	—	0.002
	α-BHC	—	—	—	T
Eggs	*p,p'*-DDE	—	—	—	0.002
	Hexachlorobenzene	—	—	—	T
Ham	Oxychlordane	T	—	—	T
	p,p'-DDE	0.002	—	T	0.001
	p,p'-TDE	0.002	—	—	—
	Heptachlor epoxide	—	T	—	—
	p,p'-DDT	—	—	—	0.001
Round steak	*p,p'*-DDE	0.009	0.003	0.002	0.004
	Lindane	0.001	—	—	—
	α-BHC	—	T	T	0.002
	Hexachlorobenzene	—	—	T	—
	Heptachlor epoxide	—	—	0.002	—
	Dieldrin	—	—	0.004	—
	Oxychlordane	—	—	—	T
Veal	Hexachlorobenzene	0.001			T
	α-BHC	0.003	Not	0.001	Not
	Oxychlordane	0.002	in	T	in
	Heptachlor epoxide	T	diet	0.005	diet
	p,p'-DDE	0.003		0.002	
	Dieldrin	0.001			0.004
Lamb	Hexachlorobenzene			T	0.002
	α-BHC	Not	Not	—	0.001
	Oxychlordane	in	in	—	0.003
	p,p'-DDE	diet	diet	—	0.154
	Dieldrin			—	0.003
Shrimp	β-BHC	—	Not	0.001	Not
			in		in
			diet		diet

Note: T = trace.
[a]Dash indicates none found.

pounds, 2-chloroethyl linoleate and 2-chloroethyl palmitate, were both detected in a few of the composites in this food class. 2-Chloroethyl linoleate was found in 4 composites at levels ranging from 0.075 to 0.523 ppm with an average level of 0.0454 ppm for all 20 composites.

XI. Sugar and Adjuncts

Only 4 organochlorine pesticides were found in these composites: α-BHC was found in 13 composites with 5 containing trace levels; lindane was found in 12 composites with 7 containing trace levels; and trace amounts of *p,p'*-DDE and hexachlorobenzene were found in one composite each. One organophosphorus pesticide, malathion, was found in 6 composites at levels ranging from trace to 0.019 ppm. Most of the composites contained zinc, lead, and cadmium residues; arsenic, selenium, and mercury residues were found in one composite each. Three fungicides—pentachloroanisole, pentachlorobenzene, and PCNB—were found at the trace level in one composite each; a fourth fungicide, pentachloroaniline, was found at the 0.001 ppm level in one composite.

XII. Beverages

Only metal residues were reported for the composites in this food class. A total of 35 lead, zinc, and cadmium residues were found at low levels in the 20 beverage composites; arsenic and selenium residues were found in one composite each. This was the only food class in which none of the composites contained mercury. No other residues were found.

Discussion

From the 20 market baskets collected during this reporting period, a total of 1357 residues of 61 compounds were reported for the 240 composites analyzed. Of these residues, 738 (54.4%) were metals, 455 (33.5%) were pesticides, 97 (7.2%) were fungicides, 46 (3.4%) were industrial chemicals, and 21 (1.5%) were herbicides. When these results are compared on a percentage basis with the previous year (19), which included 5 additional market baskets, the current report shows a slight reduction in metals and pesticides and an increase in fungicides and industrial chemicals; the herbicides remained about the same. The same trend can be seen in a numerical com-

TABLE 9. Recovery data for compounds from fortified adult Total Diet samples, October

Compound Added	Type of Food Composites	Spike Level, ppm	Range of Blank Level, ppm	Range of Total Found, ppm[a,b]	No. of Recovery Attempts
Tecnazene	Fatty	0.002	—[c]	0.001 - 0.002 (0.0017)	5
	Nonfatty	0.002	—	0.002 - 0.003 (0.0023)	5
trans-Nonachlor	Fatty	0.005	0 - 0.001	0.004 - 0.007 (0.0051)	3
	Nonfatty	0.005	—	0.005 - 0.008	2
Parathion-methyl	Fatty	0.010	—	0.004 - 0.010 (0.0078)	5
	Nonfatty	0.010	—	0.007 - 0.011 (0.0086)	7
Ronnel	Fatty	0.003	—	0.002	2
	Nonfatty	0.003	—	0.002 - 0.004 (0.0030)	6
Dieldrin	Fatty	0.003	0 - 0.005	0.003 - 0.008	2
		0.03	0 - 0.004	0.021 - 0.033 (0.026)	3
	Nonfatty	0.003	—	0.002 - 0.004 (0.0028)	4
		0.03	—	0.019 - 0.034 (0.026)	5
Endrin	Fatty	0.005	—	0.003 - 0.004	2
	Nonfatty	0.005	—	0.004 - 0.006 (0.0049)	6
Lindane	Fatty	0.002	0 - 0.001	0.001 - 0.004 (0.0022)	3
	Nonfatty	0.002	0 - 0.001 (0.0004)	0.002 - 0.003 (0.0022)	6
Oxychlordane	Fatty	0.004	—	0.003 - 0.005 (0.0037)	3
	Nonfatty	0.004	—	0.002 - 0.005 (0.0036)	6
Ethion	Fatty	0.010	—	0.005 - 0.006 (0.0057)	3
	Nonfatty	0.010	—	0.002 - 0.012 (0.0088)	5
PCNB	Fatty	0.002	—	0.001 - 0.002 (0.0014)	3
	Nonfatty	0.002	—	0.001 - 0.002 (0.0019)	6
p,p'-Methoxychlor	Fatty	0.020	—	0.015 - 0.022 (0.017)	4
	Nonfatty	0.020	—	0.010 - 0.022 (0.0173)	7
Parathion	Fatty	0.004	—	0.002 - 0.003 (0.0027)	4
		0.04	—	0.029 - 0.034 (0.032)	3
	Nonfatty	0.004	—	0.002 - 0.004 (0.0032)	7
		0.04	—	0.017 - 0.043 (0.030)	6
Chlordane	Fatty	0.10	—	0.069 - 0.100 (0.085)	3
	Nonfatty	0.10	—	0.084 - 0.117 (0.104)	6
Captan	Fatty	0.01	—	0 - 0.002 (0.001)	3
	Nonfatty	0.01	—	0.002 - 0.006 (0.0041)	6

1977-September 1978

Compound Added	Type of Food Composites	Spike Level, ppm	Range of Blank Level, ppm	Range of Total Found, ppm[a,b]	No. of Recovery Attempts
Fonofos	Fatty	0.005	—	0.002 - 0.004 (0.0027)	3
	Nonfatty	0.005	—	0.003 - 0.005 (0.0041)	6
Heptachlor epoxide	Fatty	0.02	—	0.014 - 0.021 (0.017)	3
	Nonfatty	0.02	—	0.010 - 0.023 (0.017)	4
4(2,4,5-TB)	Fatty	0.08	—	0.024	1
	Nonfatty	0.08	—	0.025 - 0.056	2
2,4,5-TP	Fatty	0.02	—	0.006	1
	Nonfatty	0.02	—	0.011 - 0.019	2
PCP	Fatty	0.04	—	0.009 - 0.016	2
		0.08	—	0.017 - 0.052 (0.031)	10
	Nonfatty	0.04	0 - 0.003 (0.001)	0.002 - 0.038 (0.022)	19
		0.08	0 - 0.002 (0.005)	0.021 - 0.050 (0.032)	10
2,4-D	Fatty	0.04	—	0.040	1
	Nonfatty	0.04	—	0.023 - 0.049 (0.035)	6
Carbaryl	Nonfatty	0.20	—	0 - 0.28 (0.171)	33
o-Phenylphenol	Nonfatty	0.40	—	0 - 0.64 (0.301)	33
Arsenic	Fatty	0.26	0 - 0.37 (0.094)	0.24 - 0.59 (0.339)	10
	Nonfatty	0.26	0 - 0.03 (0.006)	0.18 - 0.34 (0.265)	28
Cadmium	Fatty	0.20	0 - 0.034 (0.010)	0.174 - 0.318 (0.214)	19
	Nonfatty	0.20	0 - 0.078 (0.021)	0.166 - 0.309 (0.218)	39
Lead	Fatty	0.20	0 - 0.04 (0.014)	0.16 - 0.27 (0.217)	19
	Nonfatty	0.20	0 - 0.29 (0.125)	0.17 - 0.56 (0.258)	40
Mercury	Fatty	0.04	0 - 0.024 (0.005)	0.026 - 0.077 (0.046)	10
		0.06	0 - 0.009 (0.004)	0.044 - 0.070 (0.059)	6
	Nonfatty	0.04	0 - 0.003	0.039 - 0.049 (0.044)	26
		0.06	0 - 0.009 (0.001)	0.058 - 0.069 (0.065)	12
Selenium	Fatty	0.20	0 - 0.18 (0.036)	0.18 - 0.35 (0.226)	10
	Nonfatty	0.20	0 - 0.18 (0.019)	0.15 - 0.43 (0.216)	28
Zinc	Fatty	30	3.3 - 5.8 (4.64)	32.5 - 37.0 (34.81)	14
	Nonfatty	30	0.1 - 11.8 (3.44)	22.2 - 43.2 (32.31)	40

[a] Numbers in parentheses represent average levels.
[b] These values are uncorrected for background.
[c] Dash means none found in blank.

parison of the results from this reporting period with those from the twelfth report (21) in the series, which contained the same number of market basket samples. The number of compounds detected has increased from 47 to 51 and from 51 to 61 through the last 3 reporting periods. This increase can best be explained by improved instrumentation which permits the identification and quantitation of the 2-chloroethyl esters of fatty acids and also the fungicide metabolites of PCNB and tecnazene.

The fungicides accounted for 97 residues. The greatest number of these were PCNB and 3 of its metabolites—pentachloroaniline, pentachloroanisole, and pentachlorothioanisole—all found predominantly in the oil-fat composites. Another fungicide, tecnazene, and 4 of its metabolites—tetrachloroaniline, tetrachloroanisidine, tetrachlorothioanisole, and tetrachlorobenzene—were found mostly in the potato composites. This Market Basket series marks the initial reporting of pentachlorobenzonitrile, an impurity in the manufacture of chlorothalonil; pentachlorobenzonitrile was found in one leafy vegetable composite and in one garden fruit composite—although no chlorothalonil residues were reported.

The increase in the number of industrial chemical compounds was due to the identification of the 2-chloroethyl esters of fatty acids in foods and spices. Pentachlorobenzene, an impurity found in PCNB and one of 10 compounds which accounted for the 46 industrial chemical residues, was reported in 19 oil-fat composites at low levels. The industrial chemical reported at the highest level was 2-chloroethyl linoleate, found at the 0.523 ppm level in one of the 4 oil-fat composites containing this residue. The other 2-chloroethyl esters—the palmitate, laurate, myristate, and caprate—were found mainly in the grain-cereal composites. Of the 4 PCBs reported, one was found in a meat-fish-poultry composite, 2 were found in legume vegetable composites, and the fourth, Aroclor 1254, was found in 5 meat-fish-poultry composites and in one oil-fat composite; Aroclor 1254 was also the PCB found at the highest level, 0.05 ppm, in one of the meat-fish-poultry composites.

The metals accounted for slightly more than half of the residues reported. The highest levels of zinc, arsenic, and mercury were found in the meat-fish-poultry composites. The leafy vegetable composites contained the highest cadmium level, the legume vegetable composites contained the highest lead level, and the grain-cereal composites contained the highest selenium level. The only composites without mercury were the beverages. Arsenic was not found in either the dairy or the leafy vegetable composites, and selenium was totally absent only in the fruit composites. Except for the usually high zinc residues, the metal reported at the highest level was selenium, found at the 0.450 ppm level in a grain-cereal composite.

Throughout this reporting period, the individual items making up the dairy composites and the meat-fish-poultry composites from 4 of the 20 market baskets—one from each region—were analyzed separately for pesticide and industrial chemical residues. The resulting data, which are presented in Tables 7 and 8, serve as an item-by-item comparison between regions and provide a profile of the composites reported for each of these 2 food classes.

A recovery study was also performed in which a few composites from each market basket were fortified with a commonly found residue compound; the fortified composites were analyzed together with the other composites. Data from these recovery studies are presented in Table 9.

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METALS AND OTHER ELEMENTS

Influence of Phosphorus and Calcium on Flame Atomic Absorption Spectrophotometric Determination of Lead in Canned Fish Products

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Lead was determined in the presence of whole multiples of the P/Ca ratio found in Portuguese canned fish by flame atomic absorption spectrophotometry with and without using an ashing aid. Under our experimental conditions, use of the ashing aid eliminates P and Ca interference. Results with real samples, spiked with 1, 2, 3, and 4 ppm lead, are presented and statistically treated.

Current AOAC methods for lead in fish include determination by atomic absorption spectrophotometry (AAS), **25.083**, and dithizonate determination using an "ash-aid" solution, **25.095** (1). In the AAS method, lanthanum is added as a buffer solution at the last stage in certain cases (2, 3).

Phosphorus and calcium are present in canned fish and similar products and produce a background effect that enhances the absorption signal (4). The interference caused by these 2 elements must be removed before proceeding with AAS.

We investigated the use of the ash-aid solution specified in **25.095(c)** (1) to overcome this interference.

Experimental

Apparatus

(a) *Spectrophotometers.*—Varian 1000 without background corrector; Varian 1250 with background corrector.

(b) *Dishes.*—Vitreosil, 7 cm diameter. Boil dishes in 20% nitric acid for 15 min, then rinse in water until neutral. Immerse in 30% nitric acid for minimum of 48 h, and then rinse in water until neutral.

(c) *Furnace.*—Heraeus.

(d) *Glassware.*—Duran 50 volumetric flasks, pipets, watch glasses, rods.

Reagents

(a) *Ash-aid solution (25.095(c)).*—Dissolve 40 g $Al(NO_3)_3 \cdot 9H_2O$ and 20 g $Ca(NO_3)_2 \cdot 4H_2O$ in 100 mL water.

(b) *Lead standard solution.*—25 μg/mL of Pb (as lead nitrate). Prepare by diluting 1000 μg/mL Pb solution (Titrisole No. 9969, E. Merck, Darmstadt, GFR) with water.

(c) *Phosphorus standard solution.*—20 000 μg P/mL. Prepare by diluting Merck H_3PO_4, with water.

(d) *Calcium standard solution.*—20 000 μg Ca/mL. Prepare from Merck $CaCO_3$, Merck HNO_3, and water.

(e) *Water.*—Distilled in Quickfit glass still, or equivalent.

(f) *20% nitric acid.*—Dilute Merck HNO_3 to 20% with water.

(g) *Pure nitric acid.*—Carlo Erba. Dilute to 30% and 20% with water. Use to prepare dishes and glassware for use (*Apparatus* (**b**) and (**d**)).

Determination

Spectrophotometry was carried out using an oxidizing air–acetylene flame at 217 nm, with a 1 nm slit width.

Different series of tests were performed and the Pb content was kept constant for each. The P/Ca ratio was obtained from official tables for Portuguese food (5).

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The formula $A = f(x)$, in which $x = n(P/Ca)$, n = a whole number, and P/Ca = the known tabulated ratio (5), was studied. So that the P/Ca ratio would not vary for each series, the quantity of Pb was kept constant and whole multiples of the P/Ca ratio were used.

The amounts that were measured for use with the prepared Vitreosil dishes for each series of tests are given in Tables 1 and 2. We studied a series in which v = 5.00 and 1.50 mL (representative of 5 and 2 ppm Pb, respectively, in 20 g samples of fish). Trial 6 was the blank solution in each series.

The solutions were evaporated overnight at 85°C in a temperature-controlled steam baths and transferred to a cooled furnace, and then the temperature was slowly increased to 500°C and held overnight. After cooling, 25 mL 20% nitric acid was added to the ashed contents of the dishes. The dishes were covered with prepared watch glasses and kept overnight at 85°C in a temperature-controlled steam bath. The contents of each dish were transferred to separate 100 mL volumetric flasks with water, and diluted to volume later. The time and temperatures used are those normally fixed for determinations of samples of canned food (1).

Results

The results obtained are given in Figure 1. The values of A used to construct the graph represent those levels of A that were obtained after we had subtracted the values obtained with the blank solution.

The results obtained with a spectrophotometer with a background corrector coincided almost exactly with those obtained without using a corrector, in which the background had been corrected by our blank solution.

Canned fish samples of different origins were spiked with different levels of lead (1.00, 2.00, 3.00, and 4.00 ppm). The

Table 1. Amounts measured for each series (without ash-aid)

Trial	25 μg/mL Pb soln (mL)[a]	20 000 μg/mL P soln (mL)	20 000 μg/mL Ca soln (mL)
1 (×)	v	2.50	0.70
2 (2×)	v	5.00	1.40
3 (3×)	v	7.50	2.10
4 (4×)	v	10.00	2.80
5 (5×)	v	12.50	3.50
6 (blank)	v	—	—

[a]v = quantity of Pb solution for each series of determinations.

Table 2. Amounts measured for each series (with ash-aid)

Trial	25 μg/mL Pb soln (mL)[a]	20 000 μg/mL P soln (mL)	20 000 μg/mL Ca soln (mL)	Ash-aid (mL)
1 (×)	v	2.50	0.70	3.00
2 (2×)	v	5.00	1.40	3.00
3 (3×)	v	7.50	2.10	3.00
4 (4×)	v	10.00	2.80	3.00
5 (5×)	v	12.50	3.50	3.00
6 (blank)	v	—	—	3.00

[a]v = quantity of Pb solution for each series of determinations.

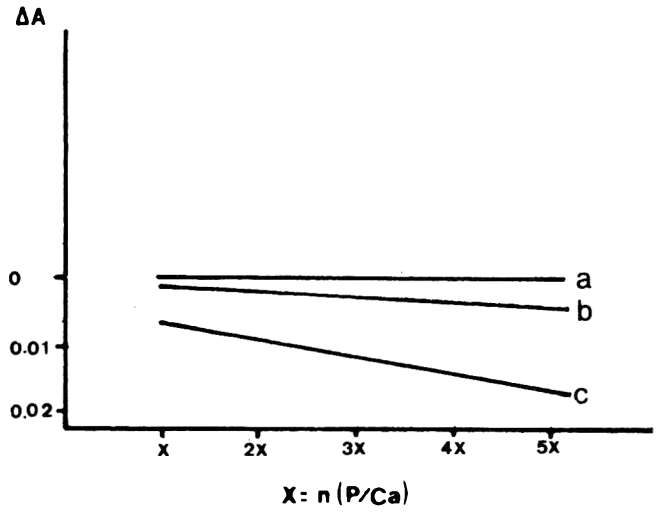

Figure 1. Elimination of P/Ca interference for each series. a, with ash-aid (Table 2); b, without ash-aid in which v = 1.50 mL (Table 1); c, without ash-aid in which v = 5.00 mL (Table 1).

spiked samples and a blank, with ash-aid solution added, were dried overnight at 85°C in a temperature-controlled steam bath and then treated as previously described in *Determination* until a 100 mL solution of nitric acid was obtained. Results are given in Table 3.

Discussion

We observed that using the ash-aid solution, in addition to its primary function as an adjuvant to ashing for 20 g samples of canned fish or similar products, improves the solubility of the ash, reduces calcination time, and has a leveling effect against matrix components.

Figure 1 confirms that the ashing aid is effective in eliminating P/Ca interference when Pb is determined: (a) presents a straight line, which is indicative of no P/Ca interference; (b) and (c) show a definite slope, (c) being more marked (greater quantity of Pb) than (b); both (b) and (c) clearly show the uncompensated interference of the P/Ca ratio.

The degree of the slope of (b) and (c) is directly related to the amount of Pb available for absorption and may be influenced by pyroformations at given temperatures during the ashing process, which may block Pb, either by inclusion or occlusion.

In the following formula, X^{n+} may be Pb^{2+}, Al^{3+}, or CA^{2+}

$$X^{n+} + PO_4^{3-} \longrightarrow X_4(P_2O_7)_n$$

If the concentration of CA^{2+} and Al^{3+} is significant in relation to Pb^{2+}, the reaction will take place without interference from Pb^{2+} because of its competition with Ca^{2+} and Al^{3+}.

Given the amphoteric nature of aluminum compounds, the solubility of ashed products is greater when the ash-aid solution is used, because insolubility only begins to occur when n is ≥ 4.

It is very desirable to eliminate all possible P/Ca interference when lead is determined in canned fish or similar products. Therefore, we suggest using the ash-aid solution not only in AOAC dithizonate determinations, but also in all AAS determinations

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Table 3. Recovery of lead added to canned fish samples

	Δ ppm, 1.00		Δ ppm, 2.00		Δ ppm, 3.00		Δ ppm, 4.00	
Sample	Δ ppm found	Rec., %	Δ ppm found	Rec., %	Δ ppm found	Rec., %	Δ ppm found	Rec., %
A	1.09	109.0	1.90	95.0	3.14	104.7	4.18	104.5
B	0.97	97.0	1.91	95.5	2.85	95.0	3.98	99.5
C	0.94	94.0	2.18	109.0	3.05	101.7	4.06	101.5
D	1.02	102.0	2.01	100.5	2.82	94.0	3.78	96.8
E	1.07	107.0	1.92	96.0	3.21	107.0	3.93	98.3
F	1.08	108.0	1.96	98.0	2.88	96.0	3.87	96.8
G	0.98	98.0	2.13	106.5	3.01	100.3	4.20	105.0
H	0.91	91.0	1.86	93.0	3.05	101.7	4.12	103.0
I	0.95	95.0	1.94	97.0	3.03	101.0	3.94	98.5
J	1.05	105.0	2.03	101.5	2.90	96.7	3.94	98.5
v	0.00416		0.01078		0.01652		0.01869	
s	0.06450		0.10383		0.12851		0.13671	
$s_{m/m} \times 100$	2.03		1.65		1.36		1.08	

PLANT TOXINS

Determination of Cyanogenic Glycoside in Seeds by Molecular Absorption Spectrometry in the Gas Phase

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As a means of evaluating cyanogenic glycoside content, cyanide was determined in apple, cherry, almond, and peach seeds. Sample preparation involved enzymatic hydrolysis and collection of hydrogen cyanide in strong base. For analysis, cyanide was converted to ammonium by addition of excess $KMnO_4$ followed by treatment with H_2SO_4, and the resulting NH_4^+ was determined by the novel gas-phase molecular absorption spectrometric technique. An atomic absorption spectrophotometer, with a flow-through absorption cell replacing the flame, was used in the analysis. Recovery of amygdalin was 98.4 ± 1.2% complete. Cyanogenic glycoside contents determined in seeds (0.93% for apple, 2.05% for cherry, 2.43% for peach, and 0.00% for almond, reported in terms of amygdalin), were confirmed by a standard titration method. The lowest detectable concentration was 0.2 μg NH_4^+/mL, which corresponds to a detection limit of 0.5 mg amygdalin in the seed sample.

Amygdalin and prunasin are cyanogenic glycosides found in seeds of plants of the Rosaceae family, which includes the apple, cherry, almond, and peach. Prunasin, which differs structurally from amygdalin by one less glucose unit, is found in all parts of these plants; amygdalin is found only in the seeds (1). A recent study showed that as seeds mature, the amygdalin content reaches a maximum, while the prunasin content decreases (2). Thus, in the seed, prunasin may be thought of as a precursor of amygdalin formation. Because cyanogenic glycosides are naturally occurring compounds that are potentially toxic, their occurrence in the Rosaceae has been known for a long time, and their reactions have been studied extensively. The hydrolysis of the glycosides under the influence of the enzyme emulsin, also naturally found in seeds of the Rosaceae, yields hydrocyanic acid, benzaldehyde, and glucose. Enzymatic hydrolysis of amygdalin, as well as various acid and base hydrolyses, has been reviewed (3). The quantitation of cyanogenic glycoside in samples is frequently accomplished by measuring one of the products of hydrolysis.

AOAC method **26.115** (4) is the standard method for determining cyanogenic glycosides in beans. In this method, the cyanogenic glycoside is steam-hydrolyzed and the evolved HCN is collected in a solution of sodium hydroxide. The collected cyanide is measured by titration with a standard silver nitrate solution. Instrumental techniques applied to determining amygdalin include spectrophotometry (5), potentiometry (6), liquid chromatography, nuclear magnetic resonance spectrometry, chemical ionization mass spectrometry, and gas chromatography (7).

In the past decade, a novel technique for the determination of certain ionic species in solution has been developed by Syty (8) and coworkers. In this technique, the analyte is converted, in a fast reproducible step, to a volatile molecular species which is determined by measuring its molecular absorbance in the gas phase. The rapid conversion of the test species into the volatile product takes place in a closed vessel when the analyte sample is injected into an aliquot of suitable reagent. The volatile compound is then carried by a stream of inert gas to a flow-through absorption cell that is positioned in place of the burner head in an atomic absorption spectrophotometer. Here, a narrow band of radiation, corresponding to an absorption maximum of the evolved compound, is passed through the cell, and the transient absorbance signal of the compound is measured.

The technique of gas-phase molecular absorption spectrometry was first reported for sulfite evolved as SO_2 (8), and has since been applied to the determination of sulfur dioxide in stack gases (9) and of total sulfur in kerosene (10). It has also been used to determine bromide and iodide evolved as Br_2 and I_2 (11), sulfide evolved as H_2S (12), nitrite evolved as NOCl (13), ionic mercury evolved as Hg^0 (14), and ammonium evolved as NH_3 (15). Ammonium analysis has been used to determine total nitrogen in flour (15). A rapid, specific method for the determination of ammonium, urea, and nitrate nitrogens in fertilizer was another recent application (16).

The determination of cyanide (17) by evolution of ammonia and gas-phase molecular absorption spectrometry is applied in the present paper to the determination of cyanogenic glycoside in seeds of several plants of the Rosaceae family. First, the glycoside in the ground seed sample is enzymatically hydrolyzed, forming HCN gas that is collected in an NaOH solution. The collected cyanide is then oxidized to cyanate with $KMnO_4$ and the cyanate is converted to ammonium by the addition of H_2SO_4 as follows (15):

$$CN^- + 2\ MnO_4^- + 2\ OH^- \rightarrow CNO^- + H_2O + 2\ MnO_4^{-2}$$
$$CNO^- + 2\ H^+ + 2\ H_2O \rightarrow NH_4^+ + H_2CO_3^-$$

Finally, the resulting ammonium solution is evaluated by injecting aliquots into an alkaline solution and measuring the absorbance of the evolved ammonia gas.

Experimental

Apparatus and Reagents

Apparatus for gas-phase molecular absorption spectrometry used in this investigation is illustrated in Figure 1 of reference 14. Cylindrical reaction vessel used to evolve ammonia from sample is 28 cm high and 2 cm in diameter, and has an internal volume of 53 mL. A 25 mL buret for delivering 6 mL aliquots of 10N NaOH is attached to one sidearm of reaction vessel. Ammonium-containing samples are injected into vessel through injection port covered with rubber septum, using B-D 1 mL plastic syringe. Nitrogen serves as carrier gas and flows continuously through apparatus at 0.77 L/min. It enters reaction vessel through glass tube with eyedropper tip that is submerged in sodium hydroxide solution. Carrier gas bubbling through solution ensures rapid mixing of injected ammonium-containing sample with NaOH solution. Released ammonia gas is carried by continuously flowing nitrogen from reaction vessel to 15 cm long flow-through absorption cell. After absorbance signal is recorded, reaction vessel is drained through stopcock at bottom of vessel, and fresh aliquot of NaOH is added to prepare apparatus for injection of next sample.

The flow-through absorption cell is mounted in place of a burner head and flame in the light path of a Perkin-Elmer

Model 460 atomic absorption spectrophotometer. Transient absorbance exhibited by ammonia gas as it passes through absorption cell is recorded on Sargent-Welch Model XKR 10-mV strip chart recorder. Radiation source for gas-phase molecular absorption spectrometry is a deuterium arc lamp which normally functions as background corrector in conventional atomic absorption work. All absorbance measurements are made at 194.0 nm with slit setting of 2.0 nm. Absorbance caused at 194.0 nm by quartz windows of absorption cell and by continuous flow of carrier gas is close to 0.200. This background is corrected at the start by setting spectrophotometer readout to zero before any injections of sample are made.

Commercial, 98.1% pure, amygdalin was obtained from Aldrich Chemical Co. (Milwaukee, WI). Peach, apple, and cherry seeds were obtained from F. W. Schumacher Co., horticulturists (36 Spring Hill Rd, Sandwich, MA 02563), and almonds were purchased packaged under brand name "Golden Harvest Raw Almonds."

Preparation of Seed Samples

If required, shell or hull is removed and seeds are pulverzied by chopping small portions in food blender for a few seconds at high speed. Except for apple seeds, this procedure results in homogeneous samples suitable for analysis. In apple, presence of strands of seed skin together with pulverized seed core results in non-homogeneous mixture. Core and not the skin is expected to contain cyanogenic glycoside, so bulk of skin should be removed to ensure reproducibility of sampling. To accomplish this, ground mixture is sieved by passing it through a 2 mm mesh screen. Separation of skin from core is not quantitative. However, bulk of skin particles are removed, and particles that pass through screen give homogeneous mixture suitable for analysis. All ground seed samples are stored in air-tight Nalgene containers.

Hydrolysis of Cyanogenic Glycoside to Cyanide

Procedure for enzymatic hydrolysis of cyanogenic glycoside and for collection of evolved hydrogen cyanide is derived from published method (3). Accurately weighed portion of powdered seed sample (10 g for apple, 5 g for almond and cherry, and 4 g for peach) is placed in 500 mL round-bottom flask. For samples used in spike recovery analyses, accurately weighed amount of analyzed amygdalin is also placed in flask at this point. An aliquot of water is added (20, 100, 40, and 40 mL for almonds, apples, cherries, and peaches, respectively), and flask is placed in 38°C circulating water bath. Remaining pieces of apparatus are quickly assembled onto round-bottom flask, and each joint and connection is sealed with vacuum grease to make system air-tight. Air enters apparatus through tip of Pasteur pipet that extends into hydrolysis flask through thermometer adapter. Tip of pipet is submerged in seed-water slurry so that passing air causes rapid mixing action. Hydrogen cyanide gas, evolved in hydrolysis of cyanogenic glycoside, is carried by air stream through sidearm, ending in second Pasteur pipet. Tip of this pipet extends into 100 mL volumetric flask containing 20 mL 5N NaOH solution. After apparatus is assembled, air flow is started through apparatus immediately and adjusted to 0.4 L/min for almond and peach samples and 1.16 L/min for apple and cherry. For each sample, HCN gas is collected for 4 h.

Although lengthy collection time recommended in literature (5) for hydrolysis of cyanogenic glycosides was used consistently throughout this investigation, we determined in a separate experiment (see *Results*) that 1.5 h would have been adequate for complete collection. Analysts may wish to adopt steam hydrolysis and distillation procedure (4), which is complete in 2 h and which we used only in connection with standard method.

Conversion of Cyanide to Ammonium

To sodium hydroxide scrubbing solution that contains collected cyanide, 15 mL 0.12M $KMnO_4$ is added and solution is mixed thoroughly by swirling. Next, 10 mL 16N H_2SO_4 is added with swirling. Solution is cooled, then diluted to 100.0 mL with water.

Determination of Ammonium

A 6.0 mL aliquot of 10N NaOH is added from sidearm buret into reaction vessel. Recorder is turned on, and small portion of baseline is traced out while carrier gas flows continuously through NaOH in reaction vessel and through absorption cell. When ammonium-containing sample is injected, absorbance begins to increase within seconds and rapidly reaches maximum. After maximum is passed, absorbance returns to baseline only very gradually. Entire absorbance trace need not, however, be recorded. As soon as peak maximum has been traced, vessel is drained through bottom stopcock and then refilled with fresh aliquot of 10 N NaOH, causing rapid return of pen to baseline, preparatory to next injection of sample. Peak height remains proportional to concentration of ammonium over substantial range of concentrations. Obvious advantage of measuring peak height instead of area under recorded peaks is speed. A typical NH_3 signal requires about 20 s to reach its maximum, but it takes 3–4 min to record entire absorbance trace. In addition, actual measurement of peak areas is more time consuming than measurement of peak heights.

For each sample, standard, and blank, at least 3 injections are recorded. Average peak absorbances are recorded and corrected for the appropriate blanks, then evaluated with aid of calibration curve constructed by using ammonium standards prepared by dissolving reagent grade NH_4Cl in water. Evaluation by method of standard additions is not applicable to present technique because of slight nonlinearity detected in calibration curve in the vicinity of origin. Spiked samples should be tested only to evaluate spike recovery.

Results and Discussion

Recovery Study

Table 1 shows recovery of amygdalin added to almond, apple, cherry and peach seeds. To calculate percent recovery, the cyanogenic glycoside (in terms of amygdalin) determined in unspiked samples was subtracted from the total found in the spiked samples and the difference was divided by the true mass of pure amygdalin in the added spike. The results, averaged over all 4 kinds of seeds, show an average recovery of 98.4 ± 1.2%. The slightly incomplete recovery of amygdalin is attributed to incomplete hydrolysis and collection of HCN, not to the proposed finishing method. To support this assumption, recovery of amygdalin added to almond and peach seeds was also repeatedly evaluated by the standard method (4) of distillation and titration with $AgNO_3$. An average 94.3 ± 1.5% recovery was obtained. Consequently, because the slight curvature in the vicinity of the origin prevented evaluation of seed samples by the method of standard additions, all results given below, which were obtained by the proposed method using a calibration curve, are corrected for incomplete recovery by dividing by 0.984. All results for the standard method are correspondingly corrected by dividing by 0.943.

Table 1. Recovery of amygdalin from seeds by proposed gas-phase absorbance method

Wt sample, g	Pure amygdalin added, g	Rec., %
	Almond	
5.0598	0.297	99.7
5.1010	0.298	96.2
5.1193	0.300	98.0
5.0114	0.299	96.9
5.1020	0.301	96.7
5.0517	0.300	97.4
5.0592	0.300	97.3
Av. rec. ± SD		97.4 ± 1.0
	Apple	
8.0683	0.199	99.4
6.0883	0.199	100.6
6.0682	0.187	102.1
6.0588	0.201	100.6
Av. rec. ± SD		100.7 ± 0.9
	Cherry	
5.0735	0.212	97.9
5.1185	0.217	100.5
5.0782	0.208	96.8
5.0741	0.219	95.8
5.0726	0.210	103.1
Av. rec ± SD		98.8 ± 2.6
	Peach	
2.0611	0.205	98.3
2.0379	0.214	97.1
2.0407	0.240	94.9
Av. rec. ± SD		96.8 ± 1.4

Comparison with Standard Method

The results of repeated analyses of apple, cherry, and peach seeds by the proposed method are compared with those obtained by the standard method in Table 2. No cyanogenic glycoside was detected in almond seeds by either method.

Data in Table 2 show good agreement between the proposed method and the titration method. The greatest difference is for apple seeds, 3%. The reproducibility of the proposed method is good; the greatest variability is represented by 3% relative standard deviation in the results for peach seeds.

Table 2. Determination of cyanogenic glycoside (reported as amygdalin, %) by proposed gas-phase absorbance method and by the standard method

	Proposed method	Standard method	Difference
	Apple		
	0.92	0.96	
	0.93	0.95	
	0.94	0.98	
	0.93	0.96	
	0.93	—	
Av. ± SD	0.93 ± 0.00	0.96 ± 0.01	−3%
	Cherry		
	2.06	2.06	
	2.01	2.01	
	2.08	1.98	
	2.06	2.00	
	2.03	2.00	
Av. ± SD	2.05 ± 0.02	2.01 ± 0.02	+2%
	Peach		
	2.42	2.43	
	2.49	2.44	
	2.32	2.49	
	2.49	2.50	
	2.45	—	
Av. ± SD	2.43 ± 0.05	2.46 ± 0.03	−1%

Calibration Curve and Detection Limits

The linearity of the ammonium calibration curve obtained by the proposed method has been reported (15). In the present work, a definite, though slight, curvature of the calibration curve was detected for ammonium concentrations in the 0.0–8.0 μg/mL range. At concentrations greater than 8.0 μg/mL, the curve was linear. The maximum extent of linearity was not checked in this work, but it did extend beyond 108 μg NH_4^+/mL. To verify the reproducibility of the injection and signal collection steps in the proposed gas-phase absorbance method, 5 consecutive injections of 5.00 μg NH_4^+/mL standard were made. The mean peak height absorbance was 0.0057 with a standard deviation of ± 0.0002 (or 3.5%).

The lowest measurable absorbance signal was 0.0002 under the instrumental conditions used, which represents a concentration of 0.2 μg NH_4^+/mL. In terms of amygdalin, this means that the smallest amount detectable is 0.5 mg if the procedure described above is followed exactly (i.e., the HCN trapping solution is ultimately diluted to 100.0 mL before testing and 1 mL aliquots are injected for analysis).

Selection of Hydrolysis Parameters

The completeness of hydrolysis as a function of time was studied for almond, apple, cherry, and peach seeds. Hydrolysis was monitored by measuring the quantity of HCN that was collected during each successive hour of hydrolysis. Almond samples, which are free of any natural cyanogenic glycosides, were spiked with 0.1132 g pure amygdalin. Other seeds were prepared for analysis as indicated under *Experimental* and were tested unspiked. The results (Table 3) indicate that collection of HCN was almost complete during the first hour of hydrolysis and was probably entirely complete just after the start of the second hour. Consequently, it can be assumed that a 1.5 h hydrolysis period would have been an adequate alternative to the 4 h interval used in collecting the data given in Table 2. However, it would not necessarily be safe to adopt the shorter interval with either spiked samples or with seeds other than the ones tested here because the proportion of cyanogenic glycoside to seed mass would be different and would affect the rate of the enzymatic hydrolysis.

The seed sample size used in the hydrolysis is not a critical variable in the analysis. It was selected to be sufficiently large to produce an adequate absorbance signal that fell well on the linear portion of the calibration curve.

The volume of water used to hydrolyze each seed type was determined by taking the suggested mass of seed and adding enough water to make a nonviscous slurry. This meant adding 20 mL water to almonds, 100 mL to apple, and 40 mL to cherry and peach seeds. Significantly decreasing the volume of water resulted in a viscous slurry and inefficient hydrolysis. Increasing the volume of water had no effect.

The rate of air flow during hydrolysis was chosen mainly to produce adequate mixing (0.40 L/min for almond and peach, and 1.16 L/min for apple and cherry seeds). Greatly lower air

Table 3. Hydrolysis of cyanogenic glycoside in seeds as function of time

Collection interval, min	Av. peak height absorbance			
	Spiked almond	Peach seeds	Cherry seeds	Apple seeds
0–60	0.057	0.060	0.059	0.022
60–120	0.000	0.003	0.001	0.002
120–180	0.000	0.000	0.000	0.000
180–240	—	0.000	0.000	0.000
240–300	—	0.000	0.000	—

flow rates caused imprecise recovery of amygdalin, and greatly higher air flow rates were expected to affect the efficiency of the NaOH trapping solution in absorbing the evolved HCN.

Conclusion

The proposed method for determining cyanogenic glycoside in seeds offers excellent selectivity. To be a possible interferent, a species must be evolved from the seed–water slurry during hydrolysis, must be absorbed by the NaOH trapping solution, must withstand treatment with $KMnO_4$ and H_2SO_4, must yield a volatile product on injection into base, and the product must absorb at 194 nm.

In addition to excellent accuracy, the proposed method offers good reproducibility and low detection limits.

For unspiked samples of apples, cherry, and peach seeds, the 4 h hydrolysis time can be safely decreased to 1.5 h, as the data have indicated. Beyond hydrolysis, about 5 min is required for conversion of CN^- to NH_4^+, and about 15 min for data collection and evaluation using gas-phase molecular absorption spectrometry. The standard titration method requires a similar length of time.

The proposed method represents a good alternative for the determination of cyanogenic glycoside in seeds of plants of the Rosaceae family.

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ALCOHOLIC BEVERAGES

Malt Beverages and Brewing Materials: Gas Chromatographic Determination of Ethanol in Beer

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The American Society of Brewing Chemists collaboratively tested a gas chromatographic method for determination of ethanol in beer. Ten laboratories collaborated in a comparison of the ASBC reference method for alcohol by distillation with the GC method. The latter was shown to be at least as precise as the former, it is more rapid, and it can be used for in-line or process monitoring. The method has been adopted official first action.

The American Society of Brewing Chemists (ASBC) has released a method for the determination of ethanol in beer using gas chromatography to the Associate Referee, who is also liaison between ASBC and AOAC, for recommendation for adoption as official first action. The release of this method is considered timely because interest has been growing in markets around the world for reduced alcohol malt beverages. These reduced alcohol products can be required to meet stringent ethanol content standards set by religious laws or legal restrictions. The suggested gas chromatographic method is specific for ethanol and can be adapted to determinations across a broad range of ethanol concentrations.

ASBC collaboratively tests published methods of interest to the brewing industry and, after clearing the results through the ASBC Technical Committee and interested members in open meetings, issues a standardized protocol and report in the *Journal of the ASBC*. Subsequently, the accepted method is published in the ASBC *Methods of Analysis* handbook. After several years of use in the industry, a method is released to AOAC for official action.

The gas chromatographic method for ethanol was collaboratively tested by ASBC in 1979 (1, 2). Ten collaborating laboratories participated in the study, which compared the ASBC reference method for alcohol by distillation (3) with the recommended gas chromatographic method. This method involves direct injection of an aliquot of beer onto a porous polymer-packed column under isothermal conditions and equipped with a flame ionization detector.

Ethanol in Beer
Gas Chromatographic Method
First Action
ASBC-AOAC Method

Method Performance

Ethanol 4–6% v/v (S_x = 0.052–0.062, S_o = 0.041–0.058)

Principle

n-Propanol internal std is added to sample, and EtOH is detd by GC using flame ionization detection.

Reagents and Apparatus

(a) *n-Propanol.*—Internal std. 5% aq. stock soln. Refrigerate.

(b) *Ethanol std solns.*—3, 4, 5, 6, 7, and 8% aq. EtOH solns. Det. exact % EtOH by pycnometer, **9.013**(a), hydrometer, **9.014**, or refractometer, **9.016**. Alternatively, prep. std solns by quant. diln of concd EtOH soln analyzed by one of above technics. Keep solns refrigerated.

(c) *Gas chromatograph.*—With flame ionization detector and 6 ft × ⅛ in. stainless steel or glass column contg 80–100 mesh Chromosorb 103. He or N carrier gas 20 mL/min; injector temp. 175°; column temp. 185° isothermal (adjust temp. so EtOH elutes in 1 min, *n*-propanol in 1.6 min); detector temp. 250°; chart speed 0.2 in./min; attenuation as required for on-scale peaks.

Calibration

Pipet 5.0 mL EtOH std solns into sep. g-s flasks. Add 5.0 mL internal std soln to each and mix well. Inject 0.2 μL of each soln in duplicate and measure peak hts (integrator may be used). Calc. ratio of EtOH to *n*-propanol peaks and average for each concn. Plot ratio against concn and calc. slope of line (*F*). Repeat analysis of 5% EtOH std soln each day.

Determination

Decarbonate beer by filtering thru S&S 560 or equiv. paper. Pipet 5.0 mL into g-s flask. Add 5.0 mL aq. *n*-propanol internal std soln. Mix thoroly by swirling, and inject 0.2 μL onto GC column, and det. ratio of EtOH to *n*-propanol peaks.

Calculation

Ethanol, %(v/v) = (peak ht ethanol/peak ht *n*-propanol)/*F*

Results and Discussion

Three sample pairs were sent to each of the collaborators, each pair containing approximately 4, 5, and 6% ethanol (Table 1). The study was designed as a combined Youden unit block. Paired *t*-testing between the reference method and the recommended gas chromatographic method showed no consistent statistical differences between methods ($P < 0.5$). Analysis of variance with estimation of error components showed that within-laboratory error compondents were essentially of the same magnitude across each of the 3 sample pairs as well as across both methods. However, *F*-ratio testing of between-laboratory error leads to the selection of the gas chromatographic method as being less prone to operator bias; a significant between-laboratory effect as shown in one of the 3 sample pairs for the GC method as opposed to 2 of the 3 sample pairs for the reference distillation method.

When the data was screened for Youden rank-sum outlying laboratories, Laboratory 4 gave consistently high ethanol readings for the GC method and Laboratory 6 gave consistently low ethanol readings for the RM method, and both qualified as outlying (P = 0.05) laboratories. The variability within-laboratories (repeatability) and variability among laboratories (reproducibility) and their respective coefficients of variation for each method and each ethanol level were computed, and each method over all levels of ethanol concentration (Table 2).

The coefficients of variation of repeatability and of reproducibility for any ethanol level and over all levels are under 2%. The data indicate that the GC method gives repeatability,

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

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Table 1. Collaborative study results for percent alcohol (v/v)

Lab.	Sample A-X		Sample A-Y		Sample B-X		Sample B-Y		Sample C-X		Sample C-Y	
	RM	GC	RM	GC	RM	GC	RM	GC	RM	GC	RM	GC
1	4.07	4.05	3.92	3.94	4.86	5.00	4.86	5.07	6.14	6.27	6.06	6.32
2	4.06	4.16	4.02	4.05	5.00	5.06	5.04	5.16	6.30	6.25	6.16	6.28
3	4.11	4.17	4.07	4.11	5.02	5.16	5.10	5.16	6.33	6.32	6.28	6.39
4	4.04	4.36	3.96	4.05	4.92	5.35[a]	4.93	5.17	6.21	6.62[a]	6.16	6.51
5	4.09	4.14	3.99	4.07	5.01	5.04	5.05	5.11	6.37	6.30	6.33	6.26
6	3.83	4.11	3.87	4.00	4.88	5.03	4.85	5.12	6.02	6.10	6.01	6.20
7	4.08	4.24	4.04	4.10	5.02	5.15	5.06	5.30	6.30	6.32	6.30	6.26
8	4.11	4.01	4.07	4.02	5.01	4.97	5.10	4.95	6.33	6.22	6.29	6.37
9	4.22	4.19	4.10	4.09	4.90	4.95	5.14	5.10	6.38	6.17	6.44	6.19
10	4.00	4.04	3.91	3.97	4.94	4.93	5.00	5.02	6.22	6.26	6.22	6.24
Mean	4.06	4.15	4.00	4.04	4.96	5.03[b]	5.01	5.11	6.26	6.25[b]	6.23	6.28

[a]Outliers by Dixon's test.
[b]Data of collaborators with outliers are deleted from the means.

Table 2. Statistical summary for determination of ethanol in beer

Statistic	Ref. method[a]				GC method[b]			
	4%	5%	6%	All levels	4%	5%	6%	All levels
Mean	4.048	4.998	6.268	5.105	4.081	5.071	6.262	5.138
Repeatability								
SD	0.0614	0.0697	0.0957	0.0584	0.0664	0.0672	0.0510	0.0616
CV, %	1.52	1.39	1.53	1.14	1.63	1.33	0.81	1.20
Reproducibility								
SD	0.0751	0.0820	0.0967	0.0979	0.0805	0.0976	0.0722	0.0802
CV, %	1.86	1.64	1.54	1.92	1.97	1.92	1.15	1.56

[a]Lab. 6 outlier by Youden's test.
[b]Lab. 4 outlier by Youden's test.

reproducibility, and their coefficients of variation comparable to those obtained from the reference method.

A 2-way analysis of variance to compare the mean values of the methods for each ethanol concentration, a 3-way analysis of variance to compare directly the mean value of reference method with that of GC method were performed. The statistical summary is presented in Table 3. Note that all P values are greater than 0.05, which is the critical point for accepting the null hypothesis that the 2 means are not significantly different.

Table 3. Comparison of means between reference method and GC method (Labs 4 and 6 outliers by Youden's test)

Item	4%	5%	6%	All levels
Reference method	4.054	5.007	6.278	5.085
GC method	4.084	5.071	6.276	5.159
P-value	>0.05	>0.05	>0.05	>0.05

The recommended gas chromatographic method has been shown to be at least as precise as the reference method for alcohol by distillation. It also can be performed much more rapidly and can be used for in-line or process monitoring. Perhaps of greater importance, it has been accepted by various overseas agencies as the preferred method for the determination of ethanol in malt beverages subject to regulation by religious law.

Recommendation

It is recommended that the method for determination of ethanol in beer by gas chromatography be adopted official first action.

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TECHNICAL COMMUNICATIONS

Determination of Citrinin in Corn and Barley on Thin Layer Chromatographic Plates Impregnated with Glycolic Acid

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A method is described for the determination of citrinin in corn and barley. The mycotoxin is extracted with a mixture of acetonitrile-10% glycolic acid in water, defatted with isooctane, and transferred to chloroform according to published methods. The mycotoxin is separated by thin layer chromatography (TLC) on plates previously impregnated with 10% glycolic acid solution in ethanol; identity is confirmed by chemical tests. Citrinin is then quantitated by the limit detection method. Recoveries of citrinin from corn and barley samples spiked at levels of 50, 80, 150, 300, 500, and 1000 μg/kg were in the range 91–98%. The minimum detectable concentration is 15–20 μg/kg. Recoveries obtained with and without glycolic acid in the extraction solvent were compared. Sensitivities on TLC plates (limits of detection, μg/spot) impregnated with glycolic acid were compared with those on plates impregnated with oxalic acid.

Citrinin was first isolated from *Penicillium citrinum* Thom (1), but this mycotoxin can be produced and isolated from other species of *Penicillium* (2–5) and from several species of *Aspergillus* (3, 5). Citrinin has been found as a natural contaminant in corn and silage (6); corn, mixed feeds, apples, and pears from Malaysia and Portugal (A. Gimeno & M. Ligia Martins, unpublished); barley and oats in Denmark (7); wheat, rye, barley, and oats in Canada (8); apples in Portugal (9); barley in Spain (10); and peanut pods (11).

The toxicity of citrinin has been studied experimentally in a variety of animal species. Carlton (5) reported on studies by many authors on toxicity of citrinin in the rat, mouse, hamster, guinea pig, rabbit, dog, chicken, and chicken embryo. Nephropathy associated with cereal feed naturally contaminated with citrinin (and ochratoxin A) has been observed in swine in Denmark (7).

Methods are available for determining citrinin in barley (12); in corn, silage, mixed feeds, and feed pellets (6); in grain (13); in corn and barley (14); in raw materials and mixed feeds (15–17); in apples, pears, and their juices and jams (9); in grains and other agricultural products (18); and in animal feedstuffs (19, 20).

The present method, which is more sensitive than the methods previously mentioned, is derived from methods described in refs 9, 15, 16, and 21. If only citrinin is analyzed in corn and barley, 8 samples can be processed in 3–4 h for qualitative analysis and in an additional 1–2 h for quantitative analysis. The method has been used with good results for determining citrinin in wheat and peanuts, and in mixed feeds for poultry and pigs.

METHOD

Apparatus

See ref. 9 and the following:

(a) *Multiple evaporation system.*—Buchi No. RSB/40-5-100 (Buchi Products, Switzerland), or equivalent, adaptable to rotary evaporator and to pear-shape glass flask mentioned in ref. 9.

(b) *TLC plates impregnated with glycolic acid.*—20 × 20 cm glass, unactivated, precoated with 0.25 mm Sil G-25HR (Macherey-Nagel and Co., Duren, GFR, No. 809033) and divided into twenty 1 cm strips by using TLC plate scriber. Impregnate plates with glycolic acid by dipping into 10% glycolic acid solution in ethanol for 1–1.5 min. Let dry 1 h in horizontal position under hood at room temperature. Then heat 20 min at 65°C. Store in plate desiccator. Use plates at room temperature. (Alternatively, let plates dry 2 h under hood at room temperature, and omit heating at 65°C.)

Reagents

(a) *Aluminum chloride solution.*—Dissolve 20 g reagent grade $AlCl_3 \cdot 6H_2O$ in 100 mL ethanol.

(b) *Glycolic acid solution.*—10% w/v in water and 10% w/v in absolute ethanol.

(c) *Mycotoxin standard.*—Citrinin (Sigma No. C1017, Sigma Chemical Co., St. Louis, MO). Store in well sealed aluminum foil-wrapped containers at 4°C.

(d) *Mycotoxin standard solution.*—Citrinin, 30 μg/mL. Prepare with reagent grade $CHCl_3$. Store solution in well sealed aluminum foil-wrapped containers or in amber vials at 4°C. Solution should be at room temperature when used. Check standard as in ref. 5.

(e) *Developing solvents for TLC.*—Prepare fresh with reagent grade chemicals as shown:

(1) Ethyl ether–hexane–ethyl acetate–90% formic acid (70 + 90 + 40 + 2) (9, 16, 17).

(2) Toluene–ethyl acetate–$CHCl_3$–90% formic acid (70 + 50 + 50 + 20) or (80 + 50 + 69 + 6) (16, 17).

(3) Ethyl ether–hexane–ethyl acetate (50 + 100 + 50) (9, 16, 17).

Extraction and Cleanup

Weigh 60 g sample ground to pass 0.8–1 mm mesh sieve and place in 500 mL amber glass-stopper Erlenmeyer flask. Add 180 mL acetonitrile and 20 mL 10% glycolic acid solution in water, stopper (secure stopper with masking tape), and shake vigorously 30 min. Filter extract through 9 cm, MN 640w paper. Collect 60 mL filtrate and place in 150–200 mL amber separatory funnel (PTFE stopper and stopcock). Extract lipids with two 60 mL portions of isooctane (2,2,4-trimethylpentane) according to Stoloff et al. (21). Upper layer of isooctane can be removed by using 50 mL syringe with 8–9 cm needle Teflon tube, 1–2 mm id. (It does not matter if small amount of isooctane remains.) If emulsion delays separation, add 5 drops of 2N HCl as an aide. Add 30 mL water and shake 30 s. Continue as described by Gimeno and Ligia Martins (9).

Thin Layer Chromatography

For spotting and developing TLC plates, see ref. 9, with the following changes: (a) Place 2 spots of 6 μL sample extract solution on TLC plate. (b) Draw transverse line on plate 10–11 cm from imaginary spotting line as solvent stop.

Procedure.—Develop plates A, B, and C with solvents 1, 2, and 3, respectively. Remove plates from tank and let dry

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in dark hood. Force final drying with gentle current of nitrogen or moderately hot air (not more than 35–40°C). No odor of formic acid should be noted.

See ref. 9 for UV visualization of citrinin and chemical tests by NH_3 vapor (22), and spraying with aluminum chloride solution in ethanol (15). R_f values of citrinin with developing solvents 1, 2, and 3 are ca 0.58, 0.69, and 0.50, respectively. Compare sample chromatogram with internal and external standards.

Quantitative Analysis

Use limit of detection method described in ref. 9.

Results and Discussion

Samples of corn and barley, uncontaminated with citrinin and previously ground, were spiked with citrinin standard to obtain artificial contamination levels of 50, 80, 150, 300, 500, and 1000 μg/kg. Each sample was thoroughly mixed. Citrinin was in contact with the sample before extraction for 24 h in the freezer. Each sample was analyzed by 4 methods:

(1) The present method.

(2) Method *1*, but with 4% KCl solution in water (9, 15, 17, 19, 21) added in the extraction solvent, instead of 10% glycolic acid solution in water.

(3) Method *1*, but omitting, after defatting, the step to extract the initial extract with chloroform, as mentioned in ref. 9. Therefore, in Method *3*, after defatting, the acetonitrile is filtered through 9 or 11 cm MN 640w paper filled to the top with anhydrous sodium sulfate. The filtrate is collected in a pear-shape glass flask (9). Then 10 mL $CHCl_3$ is added to the separatory funnel as a rinse. The rinse is passed through the same sodium sulfate and added to the filtrate already collected. The anhydrous sodium sulfate is washed with three 10 mL portions of $CHCl_3$, and the filtrate is added to that already collected. Then the extract is evaporated and redissolved according to ref. 9.

(4) Method *3*, but with 4% KCl solution in water (9, 15, 17, 19, 21) added in the extraction solvent, instead of 10% glycolic acid solution in water.

Table 1 gives the results obtained by the 4 methods. Recoveries when glycolic acid was added in extraction solvent (Methods *1* and *3*) were better than without it, especially for low citrinin levels. Recoveries by Method *3* were slightly better than those for Method *1*. However, by Method 3, there were problems with interferences in some barley samples (old barley and barley with added preservatives). These problems have been solved in the present method (Method *1*). Method *3*, which is shorter than Method *1*, can be used for determining citrinin in corn.

Using the present method, we found citrinin in 2 samples of corn at levels of 10 150 and 12 050 μg/kg. These samples were analyzed by Methods *2*, *3*, and *4*, and results were similar to those for Method *1*.

Samples of corn uncontaminated with citrinin and previously ground were spiked by adding corn naturally contaminated with 10 000 μg citrinin/kg to obtain citrinin levels of 50, 80, 150, and 300 μg/kg. Each sample was thoroughly mixed. Citrinin was in contact with the sample before extraction for 24 h in the freezer. Each sample was analyzed by the 4 methods, with 3 trials. Recovery results were similar to those obtained when citrinin standard was added.

We have not investigated the recovery of citrinin for different times of toxin contact with sample in the freezer and at room temperature before extraction. However, Chalam and Stahr (6) noted that storage of samples with citrinin residues at room conditions results in degradation of the mycotoxin so that estimation is difficult.

Stoloff (23, 24) reported attempts by an investigator to improve citrinin recovery by using oxalic acid and chelating agents, including salts of ethylenediaminetetraacetic acid (EDTA), in the extraction solvent. So far, those attempts have been ineffective, and Stoloff, as AOAC General Referee, recommended continuing the extraction studies (23, 24). We believe that use of glycolic acid may solve citrinin recovery problems especially when citrinin levels are low.

Marti et al. (14) obtained excellent results for citrinin in corn and barley, using the developing solvent chloroform–methanol–hexane (64 + 1 + 35) on TLC plates previously impregnated with 10% oxalic acid solution in methanol. This procedure totally inhibited the tendency of citrinin to streak.

We have studied the use of TLC plates previously impregnated with 10% glycolic acid solution in ethanol and with the developing solvents described here. Results were also excel-

Table 1. Recovery (%) by 4 methods of citrinin added to corn and barley[a]

	Corn, added, μg/kg						Barley, added, μg/kg					
	50	80	150	300	500	1000	50	80	150	300	500	1000
						Method 1						
Mean[b]	91.0	92.0	94.0	95.6	97.0	97.6	92.3	92.0	94.6	96.6	97.0	98.0
SD	1.0	1.0	1.0	1.52	1.0	1.52	0.57	2.0	1.52	0.57	1.0	1.0
CV, %	1.09	1.08	1.06	1.59	1.03	1.56	0.62	2.17	1.61	0.60	1.03	1.02
						Method 2						
Mean	70.0	73.0	81.6	90.0	92.0	96.3	73.0	74.6	80.6	91.6	93.3	95.6
SD	2.0	3.0	1.52	1.0	1.0	1.52	2.0	2.51	2.08	0.57	0.57	1.15
CV, %	2.85	4.10	1.87	1.11	1.08	1.57	2.73	3.37	2.58	0.63	0.62	1.20
						Method 3						
Mean	93.0	93.6	95.0	97.0	98.0	99.6	94.0	94.6	96.0	97.6	98.3	99.3
SD	1.0	0.57	1.0	1.0	1.0	0.57	1.0	0.57	1.0	0.57	0.57	0.57
CV, %	1.07	0.60	1.05	1.03	1.02	0.57	1.06	0.60	1.04	0.58	0.58	0.57
						Method 4						
Mean	77.0	77.0	84.6	92.3	92.6	96.6	76.3	75.6	84.3	92.6	93.3	95.3
SD	1.0	2.0	1.52	0.57	0.57	1.52	3.21	2.30	2.08	1.52	1.52	1.52
CV, %	1.29	2.59	1.80	0.62	0.62	1.57	4.21	3.04	2.46	1.64	1.63	1.60

[a]See *Results and Discussion* for description of methods.
[b]Based on 3 determinations.

lent; citrinin appears as a compact circular spot without tendency to streak.

Table 2 gives the sensitivity on the TLC plate (limit of detection, μg/spot) of citrinin in pure solution and in 6 μL extracts of corn and barley samples, after development and visualization according to the proposed method. The results were obtained for TLC plates impregnated with glycolic acid and with oxalic acid (14). Sensitivities were better with glycolic acid than when oxalic acid was used. We believe that citrinin screened on TLC plates impregnated with glycolic acid develops more compactly than on plates impregnated with oxalic acid. By the method described here, the minimum detectable concentrations (μg/kg) for citrinin were 18–20 in corn and 15–18 in barley.

As a preventive step in analysis of mycotoxins, both natural and artificial light should be avoided during analysis. Care must be taken that diameter of spots, once total volume has been deposited on the plate, does not exceed 2–2.5 mm (dry spots with nitrogen gas). The analysis should be performed as rapidly as possible, especially quantitation by the detection limit method, because quantities of mycotoxin are low and losses can occur on the TLC plate. Chalam and Stahr (6) recommended completing analysis of citrinin in one day. If this is not possible, the sample residue should be stored as a film in the freezer.

Quantitation by the limit of detection method is more accurate than visual comparison with different intensities of standard (25). It is easier for the human eye to determine the least spot of mycotoxin detected in a contaminated sample than to compare the spot with standards of different intensities. Quantitation by serial dilution is time consuming, but with practice can be less labor-intensive.

Table 2. Sensitivity (detection limit, μg/spot) on TLC plates impregnated with glycolic acid or oxalic acid, for citrinin standard solution and citrinin standard added to 6 μL extract of corn and barley[a]

TLC plates	Visualization (see text)	Std soln, μg	Std added to 6 μL product,[b] μg
Impregnated with glycolic acid[c]	$AlCl_3$/UV 366 nm	0.0004	0.0005–0.0006
Impregnated with oxalic acid[d]	$AlCl_3$/UV 366 nm	0.0009	0.0015

[a]Results based on 6 trials.
[b]Sample extract solution of corn and barley.
[c]10% glycolic acid solution in ethanol.
[d]10% oxalic acid solution in methanol.

We believe that the use of glycolic acid in the extraction solvent and use of TLC plates previously impregnated with glycolic acid are important improvements in methods for the determination of citrinin.

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Recommended Modification of Dilution Procedure Used for Bacteriological Examination of Shellfish

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The dilution procedure recommended by the American Public Health Association for use in bacteriological analysis of shellfish samples may cause an underestimation of the number of bacteria in shellfish. Modifications of that procedure to include preparation of the first dilution of the shellfish homogenate on a weight rather than a volume basis, and the inoculation of 1 g portions of the shellfish from a dilution of the homogenate, have been shown to significantly increase the recovery of fecal coliform bacteria from oyster samples. It is recommended that these modifications be adopted by the American Public Health Association.

Results from bacteriological analysis of solid and semisolid foods are usually reported in numbers of bacteria per weight of food even though all steps beyond the initial dilution for homogenization involve volumetric measurements (1–3). This procedure is accurate only if the addition of the food to the dilution water does not significantly alter the density of the diluent during the homogenization procedures. For this reason and for ease in calculation, 50 g food is homogenized with 450 mL diluent, a dilution ratio of 1:10.

In the analysis of shellfish, the American Public Health Association (APHA) procedure (4) directs that the initial dilution be made by homogenizing equal weights of shucked shellfish and dilution water, a dilution ratio of 1:2. It has been our experience that when oysters *(Crassostrea virginica)* are homogenized in this ratio, the resulting mixture may be quite frothy and differ significantly in density from the diluent. The density of the homogenate appears to depend on the physiological condition of the oysters, the post-harvest age of the oysters, and mechanical factors of the blending process including style of blender cup, blending speed, and time. We have observed oyster homogenates to be as much as 40% less dense than the diluent. Therefore, volume measurement of the homogenate can introduce considerable error into data calculations.

The APHA procedure recognizes that, with some species of shellfish, the grinding of equal weights of shellfish and dilution water results in a mixture with a consistency too heavy for pipetting. Their recommendation is to use 1 part shellfish and 3 parts dilution water. However, if a 200 g shellfish sample is used as suggested, the total 800 mL volume cannot be comfortably handled within the 1 L blender jar available in most laboratories.

This paper outlines a suggested modification in the procedure for diluting shellfish samples before cultures for bacteriological analysis. This alteration, depicted in Figure 1, provides for greater accuracy and can be performed using the same equipment and materials specified in the APHA procedure (4).

Experimental

Oysters used in this study were harvested in Mississippi and Louisiana during early 1983 and represented samples of live shellstock, shucked oysters from processing lines, and oysters purchased at the retail level. Two hundred g oyster meat was weighed into a 1 L Waring blender jar followed by the addition of an equal weight of buffered water. A homogenate was formed by blending for 1 min in a Waring Model 700 blender operated at ca 15 000 rpm. Immediately after blending, 2 sets of culture media were inoculated for enumeration of fecal coliforms. Inoculation of one set of media followed the APHA procedures (4). The second set followed the procedure hereafter referred to as the modified procedure, outlined below:

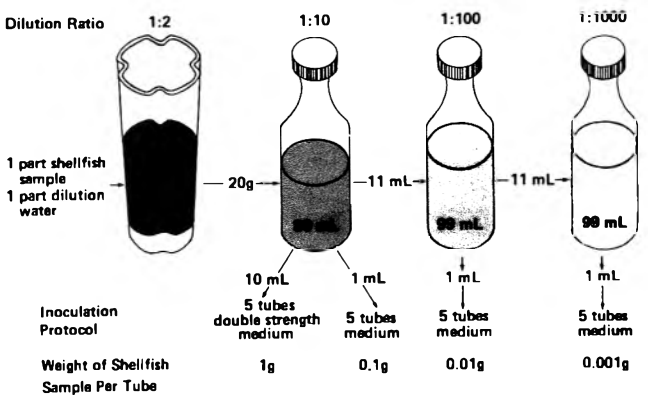

Figure 1. Modified dilution and inoculation procedure for analysis of shellfish.

Procedure

Inoculate:

(a) Each of 5 tubes containing 10 mL double strength medium with 10 mL 1:10 dilution of shellfish sample (equal to 1 g shellfish). Note: The 1:10 dilution should be made by weighing 20 g ground sample into dilution bottle containing 80 mL sterile phosphate-buffered dilution water or 0.5% sterile peptone water.

(b) Each of 5 tubes of single strength medium with 1 mL 1:10 dilution of shellfish sample (equal to 0.1 g shellfish).

(c) Each of 5 tubes of single strength medium with 1 mL 1:100 dilution of shellfish sample (equal to 0.01 g shellfish). Note: The 1:100 dilution may be made by adding 11 mL of the 1:10 dilution prepared in (a) above to 99 mL dilution water.

(d) Each of 5 tubes of single strength medium with 1 mL 1:1000 dilution of shellfish sample (equal to 0.001 g shellfish). Note: The 1:1000 dilution may be made by adding 11 mL of the 1:100 dilution prepared in (c) above to 99 mL dilution water.

The modified procedure differs from the APHA procedure in 3 points: *(1)* preparation of the first dilution of the homogenate on a weight rather than a volume basis; *(2)* inoculation of the first series of tubes of presumptive medium with the dilution prepared on a weight basis rather than inoculation of the homogenate directly into the presumptive medium; and *(3)* use of double strength presumptive medium to receive the 1 g portions of sample.

The weighing technique used in the modified procedure involved pipetting homogenate into a tared dilution bottle containing 80 mL diluent until 20 g homogenate had been added. The volume of homogenate which the 20 g represented was recorded.

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Two technicians were used in this study so that inoculation of both sets of media could begin simultaneously immediately after blending. The period of time required to inoculate the media was approximately the same for both techniques. The technicians alternated inoculation procedures on each oyster sample to reduce technician bias in the results.

Fecal coliforms were enumerated by the most probable number technique using 5 tubes of media at each dilution. Lauryl sulfate tryptose broth was used as the presumptive medium and EC medium for the confirmation of fecal coliforms (4).

Results and Discussion

Results from the analysis of 49 oyster samples revealed that the volume of homogenate required to equal 20 g ranged from 21.5 to 28.0 mL ($\bar{x}$ = 24.7 mL). Error resulting from dilution by volume rather than weight could be expected to range from 7.5 to 40% ($\bar{x}$ = 23.5%).

To determine if the theoretical error resulted in actual difference in fecal coliform enumeration, we first considered the number of positive EC-tubes (5). In 40.8% of the 49 paired analyses, the modified procedure produced more positive EC-tubes than did the APHA procedure, and in 44.9% of the analyses, both procedures gave the same number of positive tubes. A paired t-test (t = 2.640, 48 df) revealed that the number of positive EC-tubes produced by the modified procedure was significantly increased compared with the APHA procedure. Overall, the APHA procedure recorded 89 positive EC-tubes whereas the modified procedure gave 110 positive tubes, or an increase of 23.6%.

Fecal coliform MPN values were computed from the positive EC-tube data and further comparisons were made. The modified procedure gave MPN values which were higher than those obtained by the APHA procedure in 42.9% of the paired analyses. In 26.5% of the paired analyses, both procedures produced equal MPN data. A paired t-test was performed on log-transformed MPN data and indeterminate MPN values ($<$18) were assigned an arbitrary value of 1. This test showed there was a significant difference in the 2 procedures (t = 2.067, 48 df). When the MPN values from each procedure were ranked separately, the median values for the modified and APHA procedures were 40 and 20, respectively.

It should be noted that for shellfish to be considered as satisfactory at the wholesale market levels, the fecal coliform density must not exceed an MPN of 230 per 100 g (6). If all 5 MPN tubes which receive 1 g portions of shellfish are positive and all tubes at other dilutions are negative, the resulting fecal coliform MPN is 230. Thus, it is extremely critical that the correct weight of shellfish meat be implanted in those tubes. It is our experience that it is much easier to achieve this by using a dilution of the homogenate as in the modified procedure than by using the thick, foamy, and often clumpy homogenate suggested in the APHA procedure.

Recommendation

These data indicate that the modified procedure recovers a significantly greater number of fecal coliforms from oyster samples and that this difference is traceable to the fact that the APHA procedure does not account for errors produced when air is introduced into the homogenate during blending. This difficulty can be overcome by adopting the dilution procedure described herein which calls for the initial dilution of the oyster homogenate to be made on a weight rather than a volume basis. There is no reason to believe that other species of shellfish would produce results different from those obtained with oysters. Thus, we recommend that procedure 3.11 on page 45 of the American Public Health Association, *Recommended Procedures for the Examination of Sea Water and Shellfish*, 4th Ed., 1970, be altered to read as stated in *Experimental*.

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Extraction of Pesticides from Environmental Samples by Steam Distillation

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Steam distillation was used to extract several pesticides from samples of soil, plant tissue, and air (by using polyurethane foam filters). The major advantages of steam distillation over most other procedures are the small amounts of organic solvent required (10 mL) and the elimination of extract cleanup. In addition, the procedure is reasonably rapid and requires no special laboratory glassware. Recoveries of 22 pesticides from fortified samples were >75%, except for toxaphene and methoxychlor; recoveries were greater for the more volatile pesticides. Steam distillation extraction recoveries of chlorinated hydrocarbon insecticides from weathered (33 years) soil samples, compared with exhaustive Soxhlet extraction, were similar, except for DDT residues which depended on soil type. DDT residue extraction from a muck by distillation was poor (only 21–60% compared with Soxhlet extraction). The procedure is apparently limited to fairly nonpolar pesticides that are stable in steam or heat.

Several methods (i.e., shake, Soxhlet, partitioning, resin adsorption, and others) exist for extraction of pesticides from environmental samples (soil, plant, animal, water, and air filter). All have merit and each is particularly suited for specific pesticide–sample combinations. No one is universally good for all types of samples. Extraction by distillation is among the oldest methods for separating volatile from nonvolatile organic compounds, but has received only limited use for extracting pesticides from environmental samples (1–9). Specifically, steam distillation has been the preferred method for extracting some thiocarbamate herbicides (1, 4, 9).

The principles of steam distillation have been described (10). The organic compound must have an appreciable vapor pressure of about 1 kPa at 100°C to distill with steam. Steam distillation takes place below the boiling temperature of water and in many cases well below the boiling point of the pesticide.

Because the distillate provides such a clean extract, steam distillation extraction of pesticides from environmental samples might have wider applicability than heretofore appreciated. One disadvantage of most extraction procedures is the necessary extract cleanup, primarily from plant and animal samples.

The distillation procedure described here is applicable to the extraction of several pesticides from plant, soil, polyurethane air filters, and, presumably, water.

METHOD

Apparatus and Reagents

(a) *Flasks.*—1 L round boiling, 24/40 or 45/50 joint necks; and 500 mL Erlenmeyer, 24/40 joint neck.

(b) *Condenser.*—Liebig or equivalent, 20 cm jacket, 24/40 joints both ends.

(c) *Adapter connections.*—105° angle vacuum, 24/40 joints; 75° angle, 24/40 joints; and 24/40 by 45/50 joints.

(d) *Heater.*—Bank of 6, same as for Soxhlet extraction.

(e) *Soxhlet extractor.*—45/50 condenser size and 3.2 id × 7.5 cm fritted glass thimbles.

(f) *Antifoam agent.*—Hodag FD-12 (Hodag Chemical Corp., 7247 N Central Park Ave, Skokie, IL 60076).

(g) *Column material.*—Florisil PR grade (Floridin Co., Central Order Dept, PO Box 187, Berkeley Springs, WV 25411).

(h) *Solvents.*—Distilled in glass.

(i) *Gas chromatograph.*—Electron capture (EC) and photoionization (PI) detectors. Glass columns 4 mm id × 1.8 m long, packed primarily with 3–5% OV-1, OV-17, and OV-101 on Gas-Chrom Q or Chromosorb W (AW, DMCS). Operating conditions for EC and PI, respectively: column temperatures, various; detectors, 300 and 230°C; carrier gases, argon–methane (95 + 5) and argon, usually at 50 and 35 mL/min.

Samples

(a) *Soil.*—See Table 1. Place 100 g 2 mm sieved soil into 1 L boiling flask. Determine moisture content separately.

(b) *Plant.—Zea mays* L. (corn) above soil portion. Chop or cut 40 g fresh plant sample into <1 cu. cm chunks, then macerate in 200–300 mL water, and place contents in 1 L boiling flask.

(c) *Air filter.*—Polyurethane foam (5 cm od × 5 cm long). Place 2 air filters into 45/50 adapter connection.

Steam Distillation Extraction

Fortify with known quantity of internal standard if desired. Add a few grains of sand and bring water in 1 L boiling flask to 750 mL. Add 1 mL antifoam agent if foaming occurs.

Place glass wool loosely in one end of 75° angle adapter connection to prevent carry-over of plant or soil sample if bumping occurs. Assemble extraction apparatus (Figure 1), turn condenser water on, and distill with high heat. Heavy rubber band over joints helps to prevent leaks. Aluminum foil around boiling flask and 75° angle connector aids boiling and reduces heat required.

Table 1. Soil characteristics

Soil type	pH	Organic matter, %	Moisture tension at 33 kPa, %
Galestown sandy loam	6.7	5.2	15.6
Evesboro loamy sand (9)	5.8	2.1	11.5
Chester loam (9)	5.5	2.7	19.9
New Jersey muck[a] (9)	5.5	21.1	39.6
Sassafras sandy loam (9)	5.9	2.5	13.4

[a]Descriptive term, not a soil series.

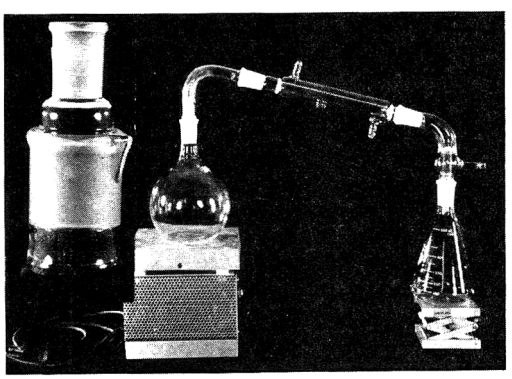

Figure 1. Apparatus for steam distillation extraction of pesticides from samples of soil, plant, and air (polyurethane foam filter).

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Mention of proprietary products does not imply endorsement or approval by the U.S. Department of Agriculture to the exclusion of other suitable products.

Collect 400 mL distillate, about 2.25 h under stated conditions. Rinse condenser and 105° angle vacuum connector with three <1 mL portions of methanol. Glassware from contaminated samples can be decontaminated with an additional 100 mL distilled water. Place distillate in 500 mL separatory funnel. Add 2 mL 1N HCl to reduce emulsions and prevent organic solvent extraction of amines (8).

Add 5 mL organic solvent (CCl_4 for PI detector or 2,2,4-trimethylpentane for EC detector) to separatory funnel. Shake, let separate, and transfer organic solvent to 15 mL pointed test tube. Repeat extraction with additional 5 mL solvent. Bring organic solvent in test tube to 10 mL, adjusting for any water that might be present. Add ca 1 g anhydrous Na_2SO_4 and shake.

Assay by GC and calculate pesticide quantity (when injection quantities of standard and sample extract are equal) by:

$$\text{Pesticides, } \mu g/\text{sample} = (\text{sample peak/std peak}) \times \text{ng in 10 } \mu L \text{ std}$$

Divide μg/sample by soil or plant weight to obtain μg/g.

Soxhlet Extraction

Place 1 cm Florisil, then <50 g 2 mm sieved soil in thimble and cover with glass wool to prevent splashing. Place a few grains of sand, 200 mL hexane, 35 mL methanol, and 15 mL water in 250 mL Soxhlet boiling flask. Assemble Soxhlet apparatus, turn on condenser water, and extract with moderate heat for 12 h.

After cooling, place contents of boiling flask in 500 mL separatory funnel, add 100 mL saturated aqueous NaCl solution, shake, and let separate. Draw off aqueous layer into boiling flask and transfer hexane layer to 100 mL volumetric flask by passing through anhydrous Na_2SO_4. Re-extract aqueous layer with 20 mL hexane and combine with previous extract. Dilute extracts to volume and assay by GC.

If cleanup is necessary, remove 10 mL hexane extract and place on Florisil minicolumn (0.8 g in disposable pipet capped with 0.5 cm Na_2SO_4 activated at 130°C overnight). Rinse with 5 mL hexane and discard eluate. Elute column with 10 mL toluene containing 2% methanol. Assay by GC. Calculate μg/g as above, except multiply result by 10.

Table 2. Recovery of pesticides from fortified Galestown sandy loam soil by steam distillation extraction

Fortification μg	Concn.[a] μg/g	Recd. %	Mean
Benefin			
1.5	0.02–0.02	86 ± 27[b]	
3.75	0.04	99[c]	
7.5	0.08–0.09	79 ± 10	
30	0.34–0.37	79 ± 8	86 ± 29
Butylate			
103	1.08–1.10	97 ± 8	
258	2.01–2.17	92 ± 7	
516	5.26–5.56	95 ± 10	
1031	10.7–11.1	100 ± 4	96 ± 15
***cis*-Chlordane**			
25.1	0.25–0.26	95 ± 10	
125.3	1.25–1.45	91 ± 3	
250.5	2.65–2.67	92 ± 1	93 ± 10
***trans*-Chlordane**			
30.1	0.30–0.32	92 ± 7	
150.5	1.50–1.74	92 ± 3	
300.9	3.18–3.21	89 ± 3	91 ± 8
DCPA			
4.4	0.05–0.05	82 ± 11	
11.0	0.11–0.12	82 ± 8	
22	0.24–0.26	98 ± 11	
88	0.99–1.09	91 ± 7	88 ± 19
***p,p'*-DDT**			
50.0	0.50–0.53	87 ± 14	
250.0	2.49–2.89	74 ± 5	
500.0	5.28–5.33	65 ± 5	75 ± 16
Dieldrin			
20.0	0.21–0.21	108 ± 7	
50.0	0.39–0.53	98 ± 8	
100.0	1.00–1.08	99 ± 3	
501.3	5.30–5.34	92 ± 2	99 ± 11
2,4-D Propylene Glycol Butyl Ether Ester			
20.4	0.21–0.21	90 ± 15	
51.1	0.40–0.43	67 ± 3	
102.2	1.02–1.11	73 ± 7	
204.3	2.13–2.19	96 ± 3	82 ± 17
2,4,5-T Isooctyl Ester			
40.1	0.41–0.44	68 ± 4	
100.3	0.98–1.02	59 ± 1	
200.5	2.09–2.15	94 ± 3	
401	4.26–4.34	76 ± 5	74 ± 7
Endrin			
50.0	0.50–0.53	101 ± 9	
250.0	2.49–2.89	95 ± 4	
500.0	5.28–5.33	90 ± 2	95 ± 10
Heptachlor[d]			
20.0	0.21–0.21	86 ± 25	
50.0	0.39–0.42	89 ± 3	
99.9	1.00–1.08	88 ± 7	
199.8	2.08–2.15	110 ± 14	94 ± 30
Heptachlor Epoxide			
2.01	0.02–0.02	88 ± 4	
10.0	0.10–0.12	84 ± 6	
20.1	0.21–0.21	82 ± 4	85 ± 9
Lindane			
21.8	0.22–0.23	91 ± 8	
50.2	0.39–0.42	83 ± 2	
100.5	1.00–1.09	89 ± 4	
200.9	2.09–2.16	97 ± 3	90 ± 9
PCNB			
2.6	0.02–0.03	86 ± 10	86 ± 10
Toxaphene			
30.36	0.29–0.32	65 ± 15	65 ± 15
Trifluralin			
20.0	0.21–0.21	85 ± 13	
69.7	0.69–0.73	83 ± 8	
100.3	1.00–1.09	90 ± 5	
696	7.36–7.42	95 ± 1	88 ± 16
2,4-D Isooctyl Ester			
52.3	0.54–0.58	99 ± 10	
130.7	1.27–1.33	80 ± 7	
262.0	2.73–2.80	97 ± 2	
522.9	5.55–5.65	100 ± 4	94 ± 13
2,4,5-T Propylene Glycol Butyl Ether Ester			
19.7	0.20–0.21	87 ± 6	
39.4	0.39–0.41	88 ± 17	
98.6	0.98–1.07	88 ± 2	
197.1	2.05–2.12	99 ± 21	91 ± 28

[a] Range of concentrations in oven-dry soil.
[b] Mean and standard deviation of 3 or more replications.
[c] One value.
[d] Measured as heptachlor plus hydroxychlordene, the extraction transformation product.

Results and Discussion

Steam Distillation Extraction of Fortified Samples

In general, the recovery of pesticides from fortified soil was 80–90% (Table 2). Heptachlor was partially (5–40%) transformed to hydroxychlordene (Figure 2) during extraction. Recovery of toxaphene was 65% and recoveries of isooctyl ester of 2,4,5-T and *p,p'*-DDT were 75%.

Recoveries of pesticides from fortified corn were 80–90% for the more volatile pesticides (Table 3) and lower for the less volatile toxaphene and methoxychlor.

Recoveries of pesticides from polyurethane foam air filters, either from trapping of vapors or fortification of the air filters (butylate) were 90–100%, except for dieldrin (Table 4). Dieldrin losses could have been due to inefficient trapping or extraction recovery. The latter was believed the major loss

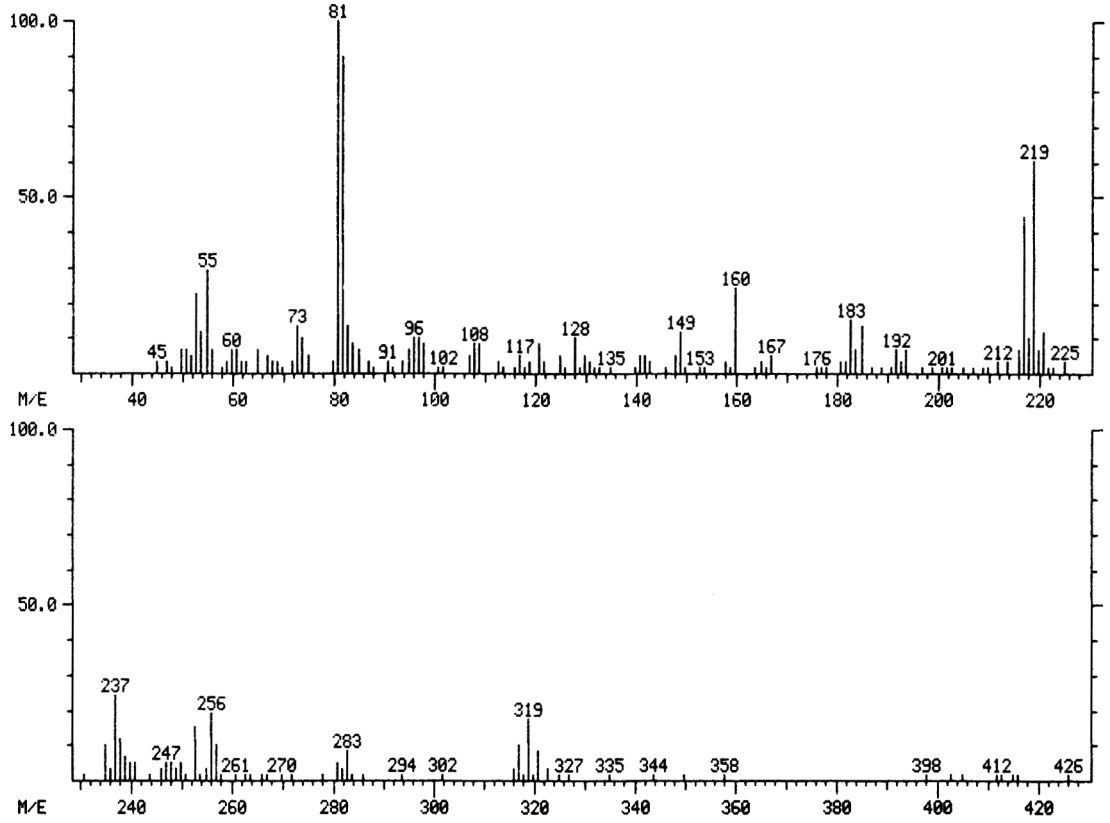

Figure 2. Mass spectrum of heptachlor transformation product (hydroxychlordene) during steam distillation extraction of soil or plant tissue.

Table 3. Recovery of pesticides from fortified cornstalks by steam distillation extraction

Fortification, μg	Concn,[a] μg/g	Recd, %	Mean
Butylate			
103	ca 5	93 ± 21[b]	
258	ca 13	85 ± 34	
576	ca 29	107 ± 17	
1031	ca 52	112 ± 5	99 ± 44
DBCP			
4.0	0.13–0.4	86 ± 6	
40.4	1.83–2.39	74 ± 6	80 ± 8
Dieldrin			
20	ca 1	82 ± 31	
50	ca 2.5	83 ± 13	
100	ca 5	85 ± 6	
200	ca 10	102 ± 6	88 ± 35
EPTC			
30	0.69–0.93	100 ± 3	
150	5.24–5.51	68 ± 7	84 ± 22
Heptachlor[c]			
20.0	ca 1	78 ± 19	
50.0	ca 2.5	78 ± 17	
99.9	ca 5	75 ± 9	
199.8	ca 10	91 ± 23	81 ± 35
Lindane			
20.1	ca 1	92 ± 23	
50.2	ca 2.5	98 ± 15	
100.5	ca 5	90 ± 10	
200.9	ca 10	95 ± 17	94 ± 34
Methoxychlor			
2.22	0.14–0.20	38 ± 6	
11.1	0.77–1.27	37 ± 6	37 ± 9
PCNB			
0.52	0.04–0.07	96 ± 12	
20.9	0.64–0.81	90 ± 4	93 ± 13
Toxaphene			
12.14	0.84–1.22	56 ± 12	
60.7	5.14–5.85	51 ± 6	53 ± 14
Trifluralin			
20.0	ca 1	107 ± 18[b]	
50.1	ca 2.5	87 ± 8	
100.3	ca 5	94 ± 7	
200.5	ca 10	106 ± 9	99 ± 28

[a]Range of concentrations on fresh corn.
[b]Mean and standard deviation of 6 replications for first 5 pesticides and 3 replications for remainder.
[c]Measured as heptachlor plus hydroxychlordene, the extraction transformation product.

Table 4. Air filter trapping (24 h) and extraction efficiency

Pesticide	Percent of applied[a]		
	Air filter	Fiber glass	Total
Butylate	102 ± 34[b]	—	102 ± 34
Dieldrin	15 ± 6	70 ± 6	84 ± 9
Heptachlor[c]	73 ± 20	17 ± 12	91 ± 23
Lindane	78 ± 23	15 ± 9	93 ± 24
Trifluralin	79 ± 12	21 ± 7	100 ± 14

[a]Applied to fiber glass cloth where volatilization occurred and the pesticide vapors were trapped by the air filters (10), except for butylate which was applied directly to the air filter.
[b]Mean and standard error for 6 replications.
[c]Includes 0.9% hydroxychlordene.

in this case, because dieldrin had been efficiently trapped previously. Heptachlor transformation to hydroxychlordene was considerably less (<1%) than for soils or corn. Presumably, because the air filters were in the neck of the boiling flask rather than in the boiling flask, the time of exposure to steam or hot water was reduced.

Steam Distillation vs Soxhlet Extraction

Tables 5 and 6 show steam distillation extraction compared with Soxhlet extraction (one extraction by each method per sample) from field plots treated with chlorinated hydrocarbon insecticides 33 years previously. The results were generally analogous to those in Table 2, if the assumption is made that Soxhlet extraction was comprehensive for weathered samples. The more volatile insecticides or transformation products were extracted nearly as well by steam distillation as by Soxhlet. Two exceptions were aldrin and β-HCH (Table 5) in which distillation extraction was only 78 and 38% as efficient, respectively, as Soxhlet extraction. However, the less volatile insecticides, primarily methoxychlor (Table 5) and DDT residues (residues refer to all compounds given in Table 6), were poorly extracted by steam distillation compared with Soxhlet extraction. A paired *t*-test for the values in Table 5 indicated a significant (95% confidence level) difference between steam distillation and Soxhlet extraction, but only a slightly significant (80%) difference when β-HCH and methoxychlor were excluded from the *t*-test.

Extraction efficiency of DDT residues (Table 6) by steam distillation extraction depended on soil type. Steam extraction of DDT residues from the Chester loam soil was as good or better than the 75% recovery from fortified Galestown sandy loam soil samples (Table 2). However, steam extraction recoveries of DDT residues from the New Jersey muck were poor, only 21 to 60% compared with Soxhlet extraction. Steam extraction of DDT residues from the Sassafras and Evesboro soils was about 50–60% as effective as Soxhlet extraction.

Table 5. Comparative steam distillation and Soxhlet extraction of Evesboro loamy sand samples from plots treated with insecticides in 1949

Plot	Insecticide	Extraction method		(Steam/Soxhlet) × 100, %
		Steam, ppb	Soxhlet, ppb	
5–6–11[a]	aldrin	8.1 ± 2.4	13.3 ± 7.3	61
5–6–16[b]		11.9 ± 0.3	22.1 ± 1.0	54
2–3–16[b]		33.7 ± 8.3	28.3 ± 0.1	119
Mean				78
5–6–11[a]	dieldrin	11 315 ± 605	11 800 ± 585	96
5–6–16[b]		1245 ± 4	1425 ± 45	87
2–3–16[b]		1695 ± 95	1710 ± 15	99
Mean				94
5–6–14	*cis*-chlordane	111	94	118
2–3–19		374	393	95
Mean				107
5–6–14	*trans*-chlordane	190	264	72
2–3–19		404	439	92
Mean				82
5–6–14	heptachlor epoxide	50	37	135
2–3–19		132	131	101
Mean				118
2–3–28	α-HCH	42	36 ± 2	117
5–6–12		9	11 ± 9	82
Mean				100
5–6–27	β-HCH	6780 ± 25	15 940 ± 900	43
2–3–27		5000 ± 95	15 640 ± 130	32
Mean				38
5–6–26	γ-HCH	107 ± 6	119 ± 1	90
2–3–26		60 ± 1	78 ± 6	77
5–6–12		59	50 ± 11	118
2–3–14		122 ± 21	86 ± 14	142
2–3–11		87 ± 3	65 ± 5	134
Mean				112
5–6–18	methoxychlor	42 ± 3	112 ± 7	38
2–3–18		62	311	20
Mean				29
5–6–14	nonachlor	190	264	72
2–3–19		404	439	92
Mean				82
5–6–13	toxaphene	12 945 ± 360	16 915 ± 1350	77

[a]Dielrin-treated plot.
[b]Aldrin-treated plot.

Table 6. Comparative steam distillation and Soxhlet extraction of soil samples from plots treated with DDT or DDD in 1949

Soil[a]	Extraction method: Steam, ppb	Extraction method: Soxhlet, ppb	(Steam/Soxhlet) × 100, %
	4,4′-Dichlorobenzophenone		
Sassafras l	42 ± 3	50 ± 3	84
Chester l	36 ± 0	21 ± 29	171
New Jersey muck	50 ± 4	222 ± 41	23
	o,p′-DDE		
Sassafras l	39 ± 1	32 ± 37	122
Chester l	38 ± 3	39 ± 9	97
New Jersey muck	92 ± 10	154 ± 6	60
	p,p′-DDE		
Sassafras l	2580 ± 185	4930 ± 75	52
Chester l	2905 ± 30	3465 ± 985	84
New Jersey muck	5010 ± 395	11 215 ± 2005	45
	o,p′-DDD		
Evesboro ls[b]	730 ± 75	965 ± 5	48
Evesboro ls[b]	695 ± 3	1515 ± 1	64
	o,p′-DDT		
Sassafras l	1440 ± 110	2345 ± 29	61
Chester l	500 ± 45	485 ± 145	103
New Jersey muck	2650 ± 210	6720 ± 495	39
	p,p′-DDD		
Evesboro ls[b]	1080 ± 55	1680 ± 30	64
Evesboro ls[b]	1385 ± 20	2405 ± 660	58
	p,p′-DDT		
Sassafras l	4635 ± 335	10 960 ± 605	42
Chester l	1615 ± 180	2275 ± 160	71
New Jersey muck	6915 ± 640	33 730 ± 2900	21

[a] l = loam and s = sand.
[b] Treated with DDD; all other plots treated with DDT.

Conclusions

Steam distillation extraction was applicable to extraction of several pesticides from soils, fresh corn stalks, and polyurethane foam used for air filters. Although extraction from water was not tried, it seems evident that the same pesticides could also be steam extracted from water. The small amounts of organic solvent and the elimination of extract cleanup were also distinct advantages of the procedure.

Steam distillation extraction was not as efficient as Soxhlet extraction from weathered soil samples of certain soil types, especially a New Jersey muck. However, steam distillation had the advantage that larger samples, compared with the Soxhlet method, were extracted. Steam distillation was as good or better than Soxhlet for extraction of the more volatile pesticides.

Some pesticides were not extractable by steam distillation. For example, recoveries of *s*-triazine and halogenated phenoxyacetic acid herbicides were <2% of fortification. Presumably, the method is limited to steam- and hot water-stable and nonpolar pesticides with about 1 kPa vapor pressure at 100°C.

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