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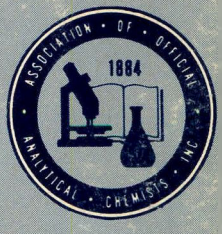

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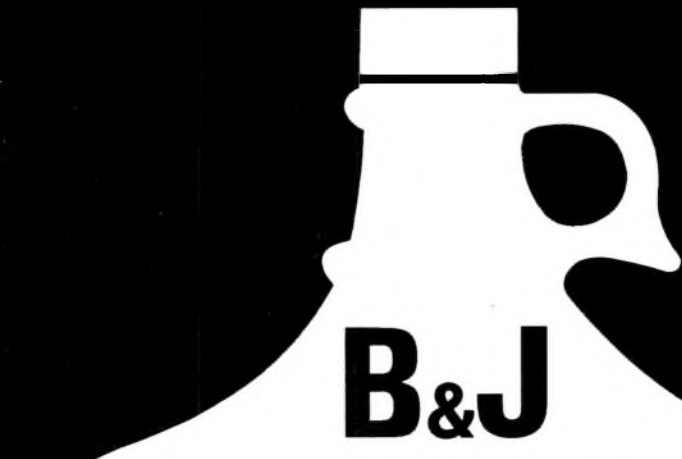

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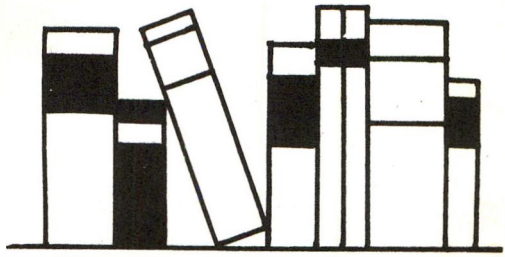

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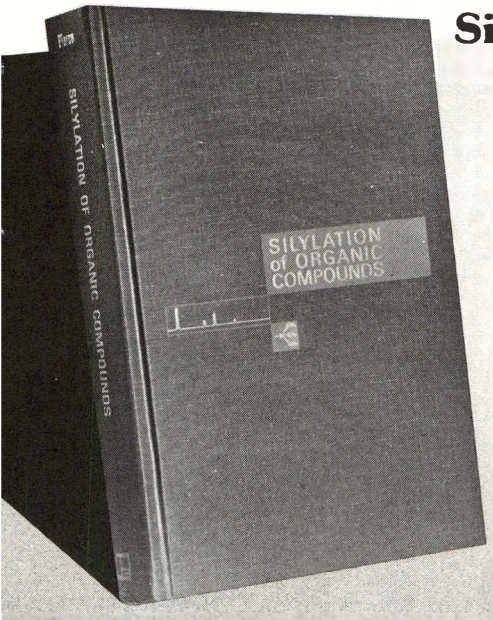

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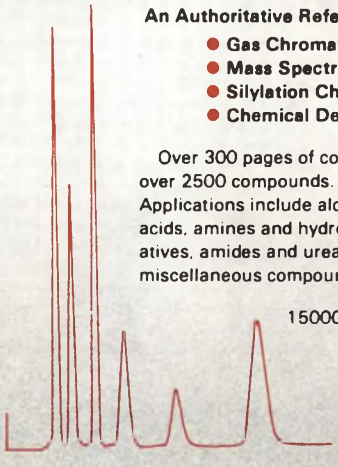

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Basic and Applied Research in the Analytical Sciences
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INSTRUCTIONS TO AUTHORS

Scope of Articles

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Methods, Results and/or Discussion, Acknowledgments, and Recommendations (applicable to reports of General and Associate Referees) should be placed in separate sections under appropriate headings typed in capitals and lower case letters, centered on the page, *not* underscored.

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Symbols and Abbreviations

kg	kilogram(s)
g	gram(s)
mg	milligram(s)
μg	microgram(s)
ng	nanogram(s)
L	liter(s)
mL	milliliter(s)
μL	microliter(s)
m	meter(s)
cm	centimeter(s)
mm	millimeter(s)
μm	micrometer(s) (*not* micron)
nm	nanometer(s) (*not* millimicron)
A	ampere(s)
V	volt(s)
dc	direct current
ft	foot (feet)
in.	inch(es)
cu. in.	cubic inch(es)
gal.	gallon(s)
lb	pound(s)
oz	ounce(s)
ppm	parts per million
ppb	parts per billion
psi	pounds per square inch
sp gr	specific gravity
bp	boiling point
mp	melting point
id	inside diameter
od	outside diameter
h	hour(s)
min	minute(s)
s	second(s)
%	percent
⅋	standard taper
N	normal
M	molar
mM	millimolar

(Note: Spectrophotometric nomenclature should follow the rules contained in *Official Methods of Analysis*, "Definitions of Terms and Explanatory Notes.")

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PRESIDENT'S ADDRESS

From Introspection to New Horizons

JAMES P. MINYARD, JR
Mississippi State Chemical Laboratory, Mississippi State, MS 39762

Much of Nature, and most of our science as analytical chemists, is rhythmic or cyclic in its manifestations. Diurnal cycles, growth and death, and a great deal of analytical chemistry is associated with oscillations—electromagnetic radiation and various spectra of molecules, atoms and ions, chromatographic peaks and valleys, and the growth and death of test organisms in toxicological testing. Previous Presidents have addressed AOAC's "Growing Pains," as examined by Helen Reynolds last year, and the "Breaking Away Process" described by Elwyn Schall concerning AOAC's emergence as a scientific society independent of its surrogate mother, FDA. These are manifestations of the rhythm of growth, change, and mutation of a dynamic organism.

I wish to consider AOAC's present status for a few minutes. We are confronted with unparalleled opportunities for new growth today, as a consequence of some self-examination we have had underway for several years. Many initiatives and opportunities lie on our horizon for expanded services to our members and to the international body of analytical scientists.

I am happy to announce that AOAC is now in a reasonably stable position regarding finances to maintain present programs. Seizing new opportunities for service will require increased fund support, of course. We have a very capable staff under Executive Director MacLean's direction, good fiscal management practices, good office equipment, and a solid base from which we can undertake new programs in an "expanding mode" for AOAC.

Such expansion is exemplified by the recent establishment of three Regional Sections of AOAC, the Pacific Northwest, the Midwest, and the Northeastern Section within the last 2 years. AOAC has conducted, for the past several years, consistently excellent spring and fall meetings. New opportunities for service in the international sphere are beginning to emerge, particularly in the fields of laboratory certification, development and training of laboratory personnel, QA/QC programs for national and international laboratories, and contracting with Federal, Provincial, and State Agencies for methods development and collaborative validation studies. New thrusts have continued to emerge under the leadership of our Board of Directors and capable staff to develop stable funding for AOAC from private and government sustaining members, as well as from membership fees and sales of publications, including the *Official Methods of Analysis*. Our many committees, boards, and ad hoc groups have continued their vigorous activities while making new plans for the future. The future for AOAC appears to be bright.

No President is able to achieve much in his or her year of tenure in that office. That certainly has been true in my own term,

Presented at the 96th Annual International Meeting of AOAC, Oct. 25-28, 1982, Washington, D.C.

during which I have been privileged to serve you and our Association. Nevertheless, I would like to recount a few of the thrusts of your Board of Directors this year, and my focus for that group on behalf of you, our member scientists.

Many new industry members have been appointed to committees, boards, and other working groups serving the interests of our Association. The infusion of their viewpoints and talents into our working groups will probably become one of the most far reaching initiatives that I undertook on AOAC's behalf. This was done as a consequence of our modification of AOAC's By-Laws two years ago, when we realized our Association needed to utilize fully the talents of our industrial scientific colleagues. We have for too many years relegated our industrial members to the status of "second class citizens" in our Association. My appointments to each of the 7 Methods Committees A–G included one or two industry scientists. We added our industrial colleagues to almost every other committee as well. It is my dream that we can soon vote to modify our Association's Constitution and By-Laws to remove all vestiges of discrimination among our members, so that there will be only one class of AOAC membership, namely, "Member." I would hope that all privileges and rights for voting and holding office, including President, would then be available to all qualified member scientists, regardless of affiliation. We can, and *must* in my opinion, broaden our base of membership and make all AOAC scientists peers in name and in fact, affording all members opportunity for participation in the governance of our Association.

Our Long-Range Planning Committee laid before the Board of Directors several years ago many recommendations for improvements in our Association. Most of these have been achieved. Pervasive LRPC themes were (1) that AOAC should undertake a variety of new thrusts connected with areas of membership, (2) that management of collaborative studies should be active rather than passive, and (3) that recommendations to improve our Association's internal management structure should be studied. The LRPC suggested that we could benefit from a professional review of internal staff structure and management. The Board deferred this recommendation for a few years, while we sought to help upgrade our office staffing and management capabilities internally by working closely with Executive Director MacLean and his staff.

Last year your Board felt that an external study of AOAC's niche in the scientific community, and particularly among our biologically oriented colleagues, was appropriate. President Helen Reynolds and the Board implemented this study when we requested that the Federation of American Societies for Experimental Biology, FASEB, explore with their membership and a variety of other biologically oriented organizations what their perceptions of AOAC are.

FASEB scientists have worked for nearly a year, asking representatives of these groups where they felt our particular strengths and weaknesses lie, what they knew of our goals, and how we might better relate to the international biological community, both in our traditional and new non-traditional ways. The FASEB report was received in July 1982. It has been read and reviewed by your Board of Directors. FASEB findings indicate that some members of the biological and other non-chemical scientific community understood AOAC and our modes of operation well, but others are not familiar with AOAC, or have misunderstandings of our function and goals. Most were interested in exploring mutual areas of interest with us. This report reveals that AOAC has a "gold mine" of opportunities for future growth and service in disciplines of toxicology, veterinary analytical methodology, and related sciences. It suggests several new opportunities for service to certain segments of our traditional federal and state agencies who have always been our major constituency.

The FASEB report to the AOAC will be available, at least in its Summary of Recommendations, to all of our membership through publication in the *Journal* (see Executive Director's report, this issue). FASEB staff scientists explored AOAC's opportunities for new relationships and initiatives in toxicology, biological testing, mutagenicity test methods, validations of multiresidue methodology, and many other areas. Many fields of analytical science activities are open for fruitful collaboration and involvement by AOAC, if we can recruit key scientists from the biological community.

The need for a name change of AOAC was even more sharply focused by these FASEB discussions with the biological community.

The participants of our "AOAC Name Change Contest," which was announced by President Reynolds in her address to you last year, submitted many suggestions. The AOAC Staff members selected their favorite of those submitted, and recommended their preference to the Board, according to the judging procedure established by President Reynolds. The Board accepted the Staff choice for information purposes, and have declared Mr. Michael D. Uptmor, Assistant Research Scientist, International Minerals & Chemicals Corporation, Terre Haute, Indiana as the Contest winner for his suggestion of "The Association of the Official Analytical Community." We hope Mike will enjoy his prize, a free trip to the Philadelphia Centennial Spring Meeting in 1984, as promised by President Reynolds last year.

You, as members of AOAC, should discuss this name or others which you may feel are better. The previous Association name change took about 10 years of discussion before consummation, so we have some time to think. Please discuss it with friends, and send your comments to the AOAC office. Maybe we can arrive at a consensus by 1984, our Centennial Year!

More important than a change of name, however, is a needed change in our membership attitudes to persons who are not involved in chemistry as "chemists." We must welcome all scientists active in disciplines requiring analysis, whether they be in the microbiological, macrobiological, or other related sciences. New testing concepts, including RIA assays, monoclonal antibody assays, enzymatic assays, and similar analytical techniques, now appear regularly as Associate Referee topics under our General Referees in Methods Committees E, F, and G. This is a field "white unto the harvest," if we relate with a professional attitude towards scientists and engineers employing analytical techniques different from our traditional concepts of analysis by chemical methodology.

A major thrust in my year as President has been to establish a Subcommittee within the Long-Range Planning Committee to consider the issue of membership in our Association. LRPC had initially recommended that the Board of Directors establish a Membership Committee. I felt that the LRPC members who initiated the whole issue must first wrestle with this matter themselves for a year or so before we organize a new committee. LRPC Chairman Mike Wehr appointed this Subcommittee on Membership and Helen Reynolds, FDA, agreed to serve as Chairman, with Carolyn Andres, FDA, Denver; Fred Baur, industry (retired) and AOAC Consultant; William Cobb, FDA, Rockville; and Al Hoffman, National Research Council of Canada, Ottawa. My charge to this Subcommittee stated that AOAC must examine first who we are, by examining present member structure and goals; then determine what we wish to become as an Association; and finally determine how we may best grow to that goal by modifying our attitudes, establishing internal and external structures, and building relationships with sister associations and societies having goals similar to ours. We need to find who are our members and interested constituency, and what scientific and institutional resources are available from our members.

I have made a concerted effort this year to assemble a complete list of the people who consider themselves members or friends of AOAC. We need to determine names, addresses, phone numbers, and the scientific talents and interests of all members and all affiliated and interested scientists who are not presently members, but who can and will become members if our Association grows in directions of their interests. We solicited this spring, through a Survey Form sent to every reader of *The Referee*, information about each of our members and interested constituents. About 1100 persons have responded from all over the world. I urge each of you to fill out one of these forms, if you have not done so, so we can add your name to our AOAC Scientific Talent and Resources file. Don't duplicate if you have already submitted one, unless data have changed. The information that we have already received will soon be loaded into the AOAC Office's word processors and computer. This information will be available upon request.

A *Directory* listing members' names and addresses has been assembled for all members about whom we have gathered information. AOAC will publish this *Directory* in early 1983, including all of you who have provided information by the end of 1982. It will be provided to every AOAC member as a benefit of membership.

This year, the Committee on Constitution and By-Laws was requested to bring several recommendations to the Board and our

members. These address 3 vital issues connected with AOAC membership and governance.

The first deals with necessary changes in our Constitution and By-Laws to provide for the establishment of Regional Sections of the AOAC.

The Committee on the Constitution was also asked to recommend changes in our By-Laws in *Article IV: Directors; Section I. Board of Directors* to clarify the matter of election of the Secretary/Treasurer to the Board of Directors each year by the Membership. They have recommended a change which includes the statement "No member of the Board of Directors may be elected for more than six (6) consecutive years." For that office alone on the Board, no maximum term of office is established in present By-Laws. All other Directors (of which the Secretary/Treasurer is one) have a 6-year maximum continuous term of office, e.g., as a member elected through the normal succession of 3 years as Director, one year as President-Elect, one as President, and one as Immediate Past-President. Under this By-Law change, all members serving on the Board of Directors would have a maximum term of 6 consecutive years. The Secretary/Treasurer could be reelected by our members for a second term of office, if desired, provided a minimum of one year interruption of service on the Board had occurred. The Board of Directors has approved both of these recommendations of the Committee on the Constitution.

Finally, the Board approved the recommendation from the Membership Subcommittee of LRPC that member dues be increased from $10.00 per year to a level of $25.00 per year, beginning 1983, with annual reviews as appropriate. This is a large percentage increase, but the recommended dues are comparable to other major scientific societies. The Board feels they are appropriate for a prestigious Association like AOAC.

Membership dues at present do not cover even the cost of publishing and mailing *The Referee* to members. Benefits to members will, if this dues increase is ratified, include discounts for preregistration at the Annual Meeting, a free copy of the *AOAC Directory*, receipt of *The Referee* and other AOAC mailings, methodology information, referral and free job advertisements for members in *The Referee*, and "Tips of the Trade" published in *The Referee* as space permits. Information on this dues increase has been published in *The Referee*, September 1982, along with information on the By-Laws Amendments just discussed.

A second major thrust of my term has been to attempt to incorporate good management practices into our Board activities, and into the governance and structure of every Committee. We have tried to assemble Terms of References from every Committee, encouraging the Committees to examine their internal structures, basic functions, membership type and compositions, and to set for themselves long-range and short-term goals. Each Committee is urged to conduct periodic internal reviews of its functions, structures, and goal achievements. The Board of Directors will continue to review those for each Committee, and make or suggest needed changes externally if the Committee is not fruitful or lacks proper internal organization.

It is imperative that every Committee and Board of our Association continue this self-examination and goal-setting to maintain orderly growth and produce results. The effectiveness of each Committee and Board of AOAC must be judged by its reports, recommendations, publications, short courses, or other activities which contribute positively to AOAC goals. We cannot afford unproductive groups in AOAC. Many of our major committees have been at work all year long. Some have not. The time is long past that we can manage our varied activities and interests with one 3-hour Committee meeting per year at our Annual Fall Meeting.

In addition to the Long-Range Planning Committee's Membership Subcommittee activities, LRPC has held several 4–6 hour dialogues with the program planning staff and senior scientists in the Food and Drug Administration, USDA-Agricultural Research Service and USDA-Food Safety and Inspection Service, and EPA Office of Toxic Substances and Office of Pesticide Programs. These discussions have explored opportunities for AOAC to be of service to these Agencies. Such discussions will be regular features of the LRPC activities as long as they are productive. They have already given us many leads on new directions for our Association. LRPC hopes to get AOAC methods development and collaborative studies integrated into the planning cycles for allocation of personnel time in each of the Federal Agencies supporting AOAC as government sustaining

members. This same type of interaction and dialogue will be undertaken with Canadian Provincial Agencies, National Canadian Agencies, and State Agencies as time and resources permit.

William Horwitz, Chairman, Committee on Collaborative Studies, and his Committee have established a strong working set of Subcommittees. They have worked all year to develop a comprehensive document on the AOAC collaborative study process. This will be a major landmark when published, and will serve as a centerpiece in our Centennial Year Celebration in 1984. This Committee has been working with the International Committee on Harmonization of Collaborative Studies, sponsored by IUPAC. Dr. Horwitz and his group, and Harold Egan, recently retired as Her Majesty's Chemist, UK, and now a consultant for AOAC, have put in hundreds of hours to produce a major policy document which will describe how AOAC and other international collaborative studies should be designed, and how statistical analyses of such studies should be conducted.

The International Cooperation Committee is examining our expanding opportunities for service internationally. This committee needs to define more explicitly the role that AOAC might strive to establish in the next decade as an international authority on analytical methodology, how active we should become in each organizational sphere, and through what agencies or intersociety relationships AOAC should continue to provide input into the international scientific and regulatory milieu.

Many of our members are traveling overseas to represent their own Agencies in meetings of WHO, FAO, ISO, CIPAC, *Codex Alimentarius*, and the like. Many of these scientists and administrators can and have told the AOAC story abroad by simply extending their trip a few days and making key contacts with major officials in overseas governmental agencies equivalent to EPA, FDA, USDA, and DOI, and international scientific groups.

For example, this year, Mike Wehr from Oregon visited officials in Japan and the Philippine Islands on behalf of his State Department of Agriculture and AOAC, making many key contacts for our Association. Our AOAC Representative, Margreet Tuinstra, William Horwitz, and Harold Egan have represented us at ISO meetings, Codex, and at other major European functions dealing with food, agricultural products, and other areas of interest to AOAC. Charles Gehrke on our Board visited the Peoples' Republic of China recently as an invited U.S. scientist. He spread the AOAC story to top administrators in the Department of Agriculture of the Peoples' Republic, inviting them to attend our 1984 Centennial Meeting. Other members who may be visiting overseas on behalf of their employers, and who wish to offer their services in this manner, should contact Executive Director MacLean. A small amount of money invested in such contacts can reap great rewards in expanded influence of AOAC in the international arena, and in recruitment of leading world scientists to participate as members our Association and in its activities.

Many new and exciting thrusts are occurring in other Committees such as the Instrument Specification, Automated Analysis, and Data Handling Committee chaired by Jack Plimmer, and the Committee on Laboratory Quality Assurance, chaired by Keith McCully.

Fred Garfield is presently writing a book for AOAC on quality assurance and quality control for analytical laboratories, as a part of his activities as Chairman of the Laboratory Accreditation Committee. This issue of laboratory certification is still very much alive. AOAC receives repeated requests to provide a mechanism for laboratory certification. We are being asked if we can provide primary and secondary reference standards, quality control check sample programs, and similar services for laboratories engaged in areas related to AOAC's traditional interests, as well as in some newer and non-traditional interests.

Opportunities for AOAC growth are present everywhere. Our growth as an Association will be directly related to our willingness to entertain new concepts, provide new techniques for scientific measurements, and involve our most capable colleagues in the business of helping provide accurate, reproducible, and well validated methods in fields of chemistry, biochemistry, biophysics, biological sciences, and toxicology.

It has been a delight to serve as your President this year, despite the work load involved. Thank you for the privilege you have afforded me to grow as a scientist and administrator. I assure you that I shall continue to work for our Association for many years. Thank you for your kind support and your many hours of dedicated service to our splendid organization—AOAC!

WILEY AWARD ADDRESS

Successful Interagency Cooperation – The Diehlstadt Story

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In 1972, I was one of more than 100 people involved in a very complex effort that included at least 10 federal and state agencies. The project was successful beyond every expectation. We have wanted to write and report this success story for years; the Wiley Award and required address provides the opportunity.

Aflatoxin, a carcinogenic compound made by molds on agricultural commodities, was detected by the Food and Drug Administration (FDA) in cornmeal prepared from 1971 white corn grown in the Boothill region of Southeastern Missouri (1). All corn grown in the region was immediately suspected of being contaminated. Very little of the corn was sold; no one wanted it. In the spring of 1972, the Agricultural Stabilization and Conservation Service (ASCS) still had 1.8 million bushels of white and yellow corn under loan. Usually by late spring, most of the corn from the previous year's harvest has been sold by the farmers to repay loans. The ASCS was faced with the unpleasant choice of recalling loans—causing great financial loss to the farmers and perhaps destroying the loan program—or accepting delivery of the corn, paying for it, and owning all of the aflatoxin-contaminated corn in the region. A meeting was called by Robert Hanson of the ASCS, May 24, 1972, at Columbia, MO, to discuss possible solutions to the problem. Robert Hanson will be the only person I mention by name because he was the one who made the most important decisions and took the necessary steps to see the project to its conclusion. The rest of us cooperated. The agencies involved are listed in Table 1.

By June 15, 1972, the ASCS decided, on Hanson's recommendation, to accept delivery of approximately 400,000 bushels of white corn at an elevator in Diehlstadt, MO. The farmers would be paid $1.20 per bushel or

their loans would be credited with that amount. Since it could cost the agency well over $400,000, it was decided to learn as much as possible about sampling and monitoring corn for aflatoxin at the elevator and in laboratories. The Northern Regional Research Center (NRRC) was asked to prepare a plan for sampling and testing for aflatoxin at the Diehlstadt elevator and for research in the laboratory. ASCS personnel were responsible for taking delivery and testing the corn at the elevator. The Missouri State Inspection Service graded truckloads of corn as delivered and separated the bright greenish-yellow fluorescent (BGYF) particles associated with the mold that produces aflatoxin and possibly aflatoxin (2, 3). NRRC trained ASCS personnel in the rapid screening tests to be done at the elevator and conducted research at the laboratory. Agricultural Marketing Service (AMS) personnel installed the primary and secondary sampler, sampled, and studied sampling techniques. The FDA was informed of all plans. Cooperation of the farmers and of the Extension Service was, of course, essential to the project.

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Table 1. Agencies involved in the Diehlstadt project

Agricultural Stabilization and Conservation Service (ASCS) USDA
Missouri State ASCS
Missouri State ASCS Committee
Northern Regional Research Center, ARS, USDA
Grain Division, Agricultural Marketing Service (AMS) USDA
Statistical Staff, AMS
Missouri State Grain Inspection Department
Food and Drug Administration
Farmers and Extension Service

Table 2. Time table for Diehlstadt project, 1972

May 25, ASCS meeting to discuss aflatoxin in southeastern Missouri corn
June 15, ASCS decides to take delivery of white corn at Diehlstadt elevator
June 19, NRRC asked to develop research plans on aflatoxin in white corn
July 24, Final meeting of involved agencies
July 24–26, ASCS technicians trained at NRRC for tests at Diehlstadt
August 3–5, Sampler installed and laboratory prepared at Diehlstadt
August 7–September 21, White corn delivered at Diehlstadt elevator

The final planning session was held in Kansas City, MO, July 24, 1972, even as ASCS technicians and AMS personnel were being trained at NRRC. The time schedule (Table 2) for the project is of interest when one considers how much was accomplished. In less than 2 weeks, continuous primary and secondary samplers were installed at the Diehlstadt elevator—a job that was considered impossible by more than one expert. An efficient young AMS employee and Mr. Hanson were responsible for this feat. A weighing building on the premises was converted to a laboratory—rather primitive, to be sure, but usable. The building had to be wired for electricity, water was brought in with a garden hose, an oven hood was installed, and laboratory stools and benches were made from scraps of wood used in the remodeling.

On August 7, 1972, the first deliveries were accepted. All deliveries were controlled by telephone calls. The first farmers to be called were obviously the most patient and the best-natured. The elevator had 5 vertical bins and 2 horizontal storage bins that could accommodate almost any combination of aflatoxin-contaminated corn, aflatoxin-free corn, and U.S. Sample Grade corn. As truckloads of corn were delivered at the "office-laboratory" (former weighing house), they were probed 15 times to obtain a 10 lb sample. The probe samples were used for grading and rapid screening tests at the elevator to segregate aflatoxin-contaminated corn from good corn and corn of the poorest grade—U.S. Sample Grade. One of the first steps at the elevator was to inspect corn lots for BGYF particles under longwave ultraviolet (365 nm) black lights (2, 3). If BGYF was present, corn then was tested for aflatoxin by the rapid minicolumn method in the laboratory set up in the weighing house (4). The 50 g, whole-kernel sample for assay was selected to contain fluorescing particles, so our results indicated more aflatoxin than actually was present. The flow sheet (Figure 1) shows the distribution of the corn at the elevator based on results of black light or BGYF and minicolumn tests on the probe samples of the truckloads of corn. These tests were used at the elevator to separate the 250 to 400 bushel truckloads of corn into aflatoxin-contaminated corn, "clear" or toxin-free corn, and U.S. Sample Grade corn. Probe sampling, grading, and screening tests took about 30–40 min.

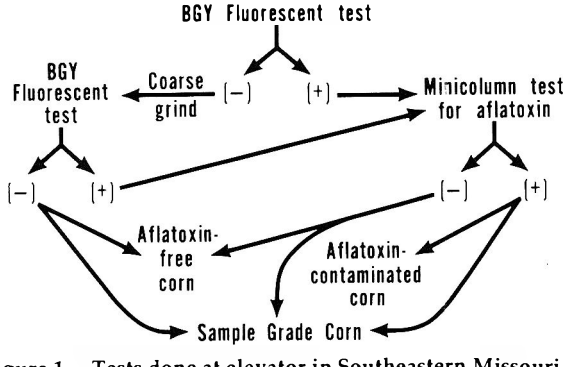

Figure 1. Tests done at elevator in Southeastern Missouri.

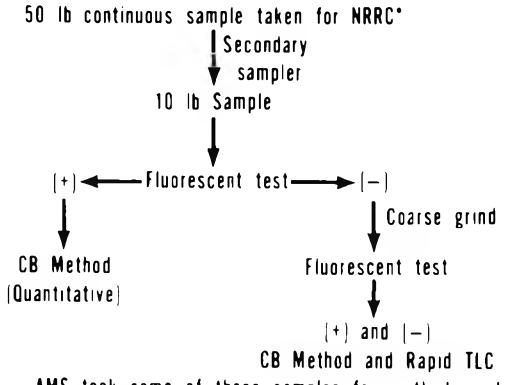

Figure 2. Analyses and testing at NRRC.

Because of the unloading facilities at the elevator, trucks were backed up waiting to be unloaded rather than to have the corn tested for aflatoxin.

Ten pound samples were shipped to NRRC in Peoria, IL, to check the results of the BGYF test and to determine quantitatively the aflatoxin levels by the CB method, which since has been approved by the Association of Official Analytical Chemists (5) and American Association of Cereal Chemists (6) for corn (Figure 2). Small portions of whole kernels were taken from samples for mold profile studies. The whole-kernel samples were examined for BGYF under ultraviolet light (365 nm) and then cracked for a second examination. The cracked corn was ground to pass a No. 20 sieve and then blended in a twin-shell blender to obtain representative 50 g subsamples for analysis.

Quantitative determinations were made at NRRC to determine the actual incidences and levels of aflatoxin in the 1971 white corn under loan in Southeastern Missouri (Table 3). There was only a 13% incidence of aflatoxin in levels of 20 or more ppb (FDA guideline), which was not nearly as high as was expected when the project was started. Determinations also were made on the reliability of rapid screening tests done at the elevator (Table 4). We wanted to know the exact aflatoxin levels in the aflatoxin-free or clear corn, in the Sample Grade corn, and in the contaminated corn; the levels were 1 ppb, 17 ppb, and 28 ppb, respectively. About one-third of the corn placed in the bin for contaminated corn had toxin levels below the FDA guidelines. The quantity of good corn classified as contaminated can be explained by the method of obtaining the analytical sample at the elevator. The rapid screening tests at the elevator were definitely effective in segregating aflatoxin-contaminated corn from good corn. The aflatoxin-free corn was sold immediately after quantitative determinations were completed. U.S. Sample Grade corn and corn placed in the bin for contaminated corn were sampled continuously as 2000 bushels were unloaded. Ten pound samples were ground, blended, and analyzed by an improved minicolumn procedure (7). These results were confirmed later at NRRC (8). All lots that tested below 20 ppb were sold, with FDA approval, for animal feed.

The 6 scientific studies completed at NRRC on the 1971 white corn under loan are listed in Table 5. There is no doubt that the Diehlstadt project provided a unique opportunity to study the application of rapid screening tests to detect aflatoxin in corn at an elevator. Indeed, the results of the project proved that corn could be monitored for mycotoxins at the earliest buying point in the market system. Of practical importance, many farmers who faced financial disaster were saved, although they did not make money on the situation. Much to their surprise, the ASCS did not lose money at Diehlstadt (Table 6). In fact, they

Table 3. Aflatoxin incidence in white corn samples under loan at Diehlstadt, 1971

Grade U.S. No.	Number assayed	Negative samples	Aflatoxin, ppb		
			Below 20	20–100	>100
None	1			1	
1	371	280	61	27	3
2	477	354	82	39	2
3	107	73	22	9	3
4	72	32	16	15	9
5	80	38	20	19	3
SG	175	112	28	26	9
Total	1283	889	229	136	29

Table 4. Aflatoxin levels in truckloads of 1971 white corn stored in Southeastern Missouri—by storage bin [a]

Aflatoxin, ppb	U.S. Sample Grade [b]	Aflatoxin-free [c]	Aflatoxin-contaminated [c]
None	112	686	91
<20	28	79	122
20–29	7	10	28
30–100	19	7	65
>100	9	0	20
Total	175	782	326
Average aflatoxin level, ppb	17	1	28

[a] As determined by AOAC-AACC method at NRRC.
[b] All Sample Grade corn was stored in one bin because of poor quality, regardless of aflatoxin content.
[c] As determined by rapid screening tests with BGYF fluorescence and minicolumn.

Table 5. Studies on aflatoxin in white corn under loan, 1971

I. Incidence and level (9)
II. Effectiveness of rapid tests in segregating contaminated corn (10)
III. Association with bright greenish-yellow fluorescence (11)
IV. Mold flora (12)
V. Aflatoxin prediction from weight percent BGYF (13)
VI. Confirmation of rapid screening tests for aflatoxin done at corn elevator (8)

Table 6. Fate of about 400,000 bushels of white corn delivered at Diehlstadt elevator [a]

1. Clean corn (1 ppb aflatoxin), 200,000+ bushels sold at $2.70 per bushel
2. U.S. Sample Grade (17 ppb aflatoxin, ~70,000 bushels sold at discount)
3. Contaminated corn (28 ppb aflatoxin) ~110,000 bushels
   a. 11,000 bushels (>20 ppb) to NRRC for research
   b. 14,000 bushels (<20 ppb) sold at $2.58–$3.25 per bushel
   c. >80,000 bushels (34 ppb) destroyed

[a] Delivery brought $1.20 per bushel.

may have made a little. When it was decided to accept delivery of the white corn on a one-time basis, the agency was prepared to spend about $400,000 to solve the acute problem in the Boothill region in return for the research that could be done. Table 6 summarizes the ASCS sales of white corn after delivery. This is not an accountant's report that can be audited, because the exact number of bushels is not available. After all expenses were paid for the sampling and tests at Diehlstadt, a half million dollars had been taken in for the corn sold.

Persons in industry commented that the project was one of the most successful examples of cooperative effort they had seen. Why the success? At least 10 state and federal agencies were involved. More than 100 persons of different backgrounds and expertise worked on the project. Opinions have come from a number of people that I shall quote without giving credit to the individuals voicing them. "There was very little administrative meddling." "The project was completed before the administrators knew it was being done." However, everyone was kept fully informed. "Once decisions were made, persons implementing them in the field were given latitude to do so." "Some persons in higher positions were so sure such a complicated scheme would not work that they kept their distance until the project was successful." No matter why the effort was successful, it was fun and exciting to be a part of it.

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PESTICIDE RESIDUES

Gas Chromatographic Determination of Fumigant Residues in Stored Grains, Using Isooctane Partitioning and Dual Column Packings

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A gas chromatographic (GC) procedure for determining fumigants in grains was developed. Fumigants were leached from grain samples with the official AOAC method using acetone-water (5 + 1). They were then partitioned from the leachate with isooctane, yielding a dry, stable extract that was analyzed by GC. Fortified sample recoveries ranged from 90 to 100%. Two GC columns were used, 20% OV-101 and 20% OV-225/20% OV-17 (2 + 1). These columns gave dissimilar retention profiles and baseline resolution for the 7 fumigants investigated: chloroform, ethylene dichloride, carbon tetrachloride, trichloroethylene, chloropicrin, ethylene dibromide, and tetrachloroethylene. Further tests showed that grain samples could be screened for fumigant residues by direct injection of the acetone-water leachates obtained using the AOAC method.

The official AOAC method (1) for the determination of fumigants in grain is based on the work of Heuser and Scudamore (2). Fumigants are extracted/leached from grain by soaking for 48 h in acetone-water (5 + 1), a portion of which is then dried by salting out with NaCl and $CaCl_2$ before gas chromatographic (GC) assay.

The accuracy of the AOAC method was later evaluated by Clower (3), using a 3.6 m OV-17 column in a gas chromatograph equipped with a linearized ^{63}Ni electron capture (EC) detector. The OV-17 column resolved multiresidue fumigants better than previously used columns, and the ^{63}Ni EC detector lowered the detection limits. Clower observed high (105–115%) recoveries, however, and concluded that they were caused by the water removal (salting out) step.

The current AOAC method has proven useful for fumigant determination because the initial leaching step works well for whole-kernel grains. However, recoveries are consistently high. Recoveries obtained in this laboratory using the AOAC method ranged from 125 to 150%. Fumigants also deteriorate in acetone solution. Furthermore, GC columns that could simultaneously resolve commonly used fumigants have not been available. Compounds such as carbon tetrachloride (CCl_4), chloroform ($CHCl_3$), ethylene dichloride (EDC), and trichloroethylene (TCE) chromatographed unresolved or in tightly grouped patterns on recommended column packings. Even the OV-17 column could not separate chloropicrin (CP) and tetrachloroethylene (PCE). To correct these problems, a procedure was devised to obtain fumigant recoveries of about 100% and to inhibit fumigant deterioration in solution. Column packings were found that can satisfactorily chromatograph fumigants that are generally used on stored grains.

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Experimental

Sampling

Nine kg (20 lb) grain samples were collected from grain cars and storage bins in the midwestern United States as part of the Food and Drug Administration surveillance program for pesticides and industrial chemicals (4). Samples were kept frozen (−14°C) from the time of collection until analysis.

Reagents

(a) *Solvents.*—Acetone (Fisher), hexane, isooctane, and deionized water, all pesticide quality. Check each solvent separately with GC.

(b) *Saturated NaCl solution.*—Certified ACS, in deionized water.

(c) *Anhydrous Na_2SO_4.*—Certified ACS, condition 12 h at 750°C in muffle furnace.

(d) *Fumigant standards.*—CCl_4, $CHCl_3$, EDC, TCE, CP, PCE, and ethylene dibromide (EDB), all pesticide quality when available; otherwise, ACS or reagent grade.

Standard Solutions

Fumigants are irritants and are toxic. Prepare all standards in hood.

Stock solutions.—Prepare stock solutions by adding specified amount of fumigant to 90 mL isooctane in 100 mL volumetric flask, using well rinsed microliter syringe (3, 5) or pipet (see Table 1). Swirl flask during addition to aid dispersion

Table 1. Dilutions for preparing stock standard solutions

Fumigant	bp, °C	d^{20}	Amt, μL, dild to 100 mL	Stock concn, mg/mL	Quantn limit, ng[a]
$CHCl_3$	62	1.483	25	0.371	0.9
EDC	83	1.257	2[b]	25.1	50.0
CCl_4	77	1.594	200[c]	0.064	0.2
TCE	87	1.465	25	0.381	0.8
CP	112	1.657	35	0.580	1.8
EDB	132	2.179	45	0.981	2.1
PCE	121	1.623	13	0.211	0.6

[a] Based on 10% FSD.
[b] mL.
[c] Then 2 mL, each diluted to 100 mL.

of fumigant. Dilute to volume with isooctane and mix. Stock solutions stored at ≤14°C can be used for 1 year.

Fortifying solution.—For recovery determinations, prepare mixed spiking solution by diluting stock solutions 1:10 with hexane. Add 15 μL of this solution to 50 g grain samples after they have been immersed in 150 mL acetone-water (5 + 1).

Working solutions.—Prepare one of the following mixed working standards by bringing stock solutions to room temperature and diluting 1000-fold in one of the working solvents. Six μL of either working solution will give 30–50% full-scale deflection (FSD) for each fumigant at stated instrumental conditions. *Rapid screening.*—Using well rinsed 100 μL syringe, add 50 μL of each stock solution to 40 mL acetone-water (5 + 1) in 50 mL volumetric flask. Dilute to volume with same solvent and mix. Working standards prepared in acetone will deteriorate. Prepare these working solutions weekly and complete all GC as soon as possible when screening method is used. *Isooctane partitioning.*—Prepare working solution as described above, using acetone-isooctane (3 + 97) in place of acetone-water (5 + 1). Working standards prepared in acetone-isooctane solution can be used for 1 month.

Apparatus

(a) *Glassware.*—250 mL screw-cap Erlenmeyer flasks, 50 and 100 mL glass-stoppered volumetric flasks, 10 mL repeater pipet, and 5 mL septum-cap vials.

(b) *Gas chromatograph.*—Tracor 560, equipped with constant current ^{63}Ni EC detector and 1.8 × 4 mm id glass columns and operated at following conditions: injection port 150°C; column 75–85°C; detector 350°C; carrier gas (CH_4/Ar) flow rate 30 mL/min for OV-101 packing, 45 mL/min for OV-225/OV-17 packing; $CHCl_3$ retention time (RT) 3.5–4 min on both columns from point of injection.

(c) *Recorder.*—Leeds & Northrup 610; 1 mV full scale, chart speed 12.7 mm/min.

(d) *Column packings.*—(1) 20% OV-101 on 80–100 mesh Chromosorb W(HP); (2) 20% OV-225/20% OV-17 (2 + 1) on 80–100 mesh Chromosorb W(HP). Prepare separate batches of 20% OV-101, 20% OV-225, and 20% OV-17 (6). Condition each at 240°C for 48 h. Fumigant elution sequence for 20% OV-101 packing is shown in Figure 1. Sequence for 2 + 1 mixed packing is shown in Figure 2. If similar pattern is not found for mixed packing, then mixture should be adjusted as follows:

Adding small amounts of 20% OV-225 to mixture changes elution pattern by moving CCl_4 peak towards solvent front and away from $CHCl_3$ peak. Adding 20% OV-17 to mixture reverses 2-peak pattern by moving CCl_4 towards $CHCl_3$ peak. Adjustment of mixture, which assures

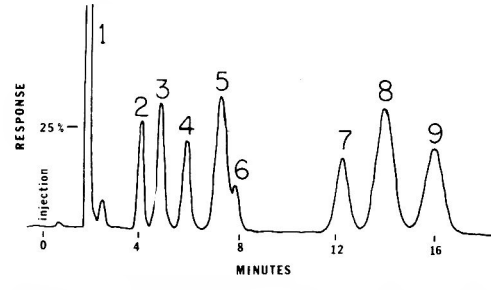

Figure 1. Fumigant GC elution pattern on 20% OV-101 packing. 1, acetone front followed by small unknown peak; 2, 2.2 ng $CHCl_3$; 3, 151 ng EDC; 4, 0.38 ng CCl_4; 5, 2.3 ng TCE; 6, isooctane; 7, 3.5 ng CP; 8, 5.9 ng EDB; 9, 1.3 ng PCE.

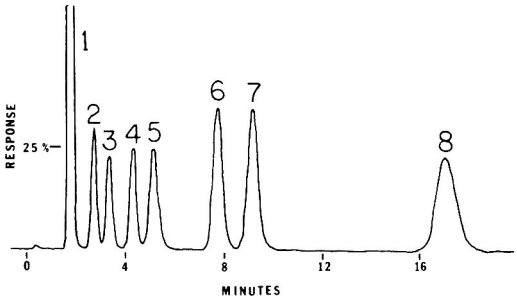

Figure 2. Fumigant GC elution pattern on 20% OV-225/20% OV-17 (2 + 1) mixed packing. 1, acetone-isooctane (3 + 97) front; 2, 0.38 ng CCl_4; 3, 2.2 ng $CHCl_3$; 4, 2.3 ng TCE; 5, 151 ng EDC; 6, 1.3 ng TCE; 7, 3.5 ng CP; 8, 5.9 ng EDB.

precise duplication between preparations, is optimal when CCl_4 and $CHCl_3$ peaks are cleanly resolved as in Figure 2. Add phase increments of 0.5–1 g/7 g total mixture if additional adjustment should be necessary. This column will yield marked RT changes from 20% OV-101 column (Table 2).

Determination

Possible loss of fumigant residues should be minimized during sample preparation and weighing. Rapidly weigh 50 g frozen whole grain into 150 mL acetone–water (5 + 1) in 250 mL screw-cap Erlenmeyer flask; secure cap. Let sample extract/leach 48–72 h in dark at room temperature with occasional swirling. Towards end of extraction period, let solids settle until supernate becomes clear. Filtering is rarely needed.

For rapid screening analysis, remove small portion of acetone–water leachate from Erlenmeyer flask, place in vial, and directly chromatograph 6 μL (2 mg) sample. Compare to reagent blank and working standard prepared with 5 + 1 solution.

To carry out isooctane partitioning step, transfer 25 mL aliquot of leachate to 125 mL separatory funnel. Add ca 25 mL saturated NaCl solution and ca 60 mL deionized water. Add 10 mL isooctane; add another 10 mL isooctane to second separatory funnel. Shake first funnel vigorously 1 min, let layers separate, and drain aqueous portion into second separatory funnel. Shake second funnel 1 min, let layers separate, and discard aqueous portion. Combine isooctane portions (20 mL), add ca 1 g anhydrous Na_2SO_4, and mix. Chromatograph 6 μL sample, equivalent to 2 mg, and compare to reagent blank and working standard.

Table 2. Fumigant retention time relative to $CHCl_3$ at 75°C on OV-101 and mixed packing

Fumigant	OV-101	Mixed
$CHCl_3$	1.00	1.00
EDC	1.17	1.44
CCl_4	1.39	0.84
TCE	1.66	1.24
CP	2.63	2.39
EDB	2.96	4.25
PCE	3.38	2.05

Results and Discussion

Salting Out Method.—When acetone is salted out from the 5 + 1 extracting solution of the AOAC method, the fumigants remain with the acetone portion, which is 83.3% (v/v) of the total solution. The reduction of solution volume caused by the water loss is compensated for in this procedure by substituting in the calculations a 125 mL factor for the 150 mL 5 + 1 solution actually used. In practice, however, the volume percentage of acetone recovered from the AOAC procedure is closer to 75%, which indicates that some of it is lost with the water. Fumigant recoveries also are increased following the salting out steps (3). Since the 5 + 1 solution is prepared by adding water to a specific volume of acetone, these recovery trends suggest that acetone is selectively lost while the fumigants are concentrated to a point beyond the proportional loss of water. These trends also occurred in fortified reagent blanks which were free of any sample effects. It was concluded that the salting out procedure, especially the final $CaCl_2$ drying step, was substantially affecting the accuracy of the AOAC method.

Rapid Screening.—Heuser and Scudamore (7) verified that 15–20% water in acetone was needed to efficiently leach fumigants from whole-kernel grain. The wet leachate generally required dehydration before GC injection because moisture will sometimes overload EC detectors. $CaCl_2$ was employed because it was one of 79 possible salting out agents found by Matkovitch and Christian (8) that satisfactorily separated acetone and water. However, the $CaCl_2$ salting out step adversely affected fumigant recoveries, so the possibility of direct injections of the wet leachates was investigated.

Fumigant standards were prepared in both 5 + 1 and straight acetone (dry) solutions. Six μL

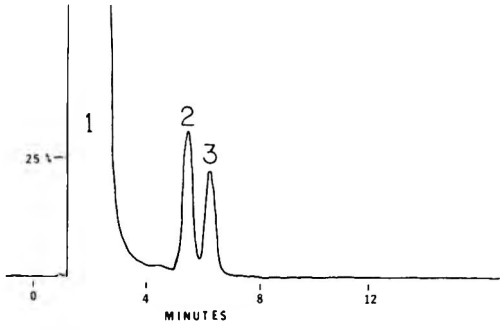

Figure 3. Screening chromatogram of oat sample (2 mg) on OV-101 column. 1, acetone-water (5 + 1) front; 2, acetone impurity; 3, 1.7 ppm CCl_4.

injections were made, and their respective chromatograms were compared. Two differences were noted. First, the solvent fronts of the wet solutions were 4–6 mm broader than their dry counterparts. Also, all fumigant retentions were 4–6 mm longer in wet solution. Second, the fumigant peaks nearest the solvent front responded the most (30%) in wet solution. This difference of peak height magnitude between wet and dry solutions decreased as the RTs increased, so that the later-eluting fumigants responded about the same in both solutions. It appeared that the wet acetone formed an azeotrope upon vaporization and eluted at the solvent front. No deleterious effects were observed, i.e., replicate injections demonstrated good response and repeatability, with coefficients of variation of <5%. A typical screening chromatogram is shown in Figure 3. In this instance, the oat sample was leached with an acetone solution that contained an interference peak.

Partitioning Step.—Isooctane is highly immiscible with water and is almost completely recovered from the partitioning step. When fortified samples were partitioned with one 10 mL portion of isooctane, CCl_4 and EDB were recovered in the 80–90% range, while the other fumigants were recovered in the 90–100% range. Using two 10 mL portions of isooctane brought all recoveries into the 90–100% range.

Table 3. Fumigants (ppm) detected in grains by screening and partitioning techniques

Grain	Fumigant	Screened	Partitioned
Wheat	CCl_4	0.6	0.6
Wheat	CCl_4	7.1	6.9
	EDB	20.8	20.7
Wheat	CCl_4	3.5	3.7
	EDB	4.5	4.7
Milo	CCl_4	8.7	8.6
	EDC	39.0	33.0
	EDB	12.0	11.0
Oats	CCl_4	1.7	1.8
Corn	CCl_4	3.0	3.1

Two factors become apparent in this step. First, the test fumigants are not lost into the headspace gas during vigorous agitation at room temperature. Second, these fumigants will partition into another solvent, in this case into a nonchlorinated, fairly stable solvent such as isooctane. Other solvents such as hexane or petroleum ether can also partition fumigants. Rains and Holder (9) used multiple portions of hexane to directly extract EDB from biscuits and flour. Hexane, however, will chromatograph in the elution regions of other known fumigants. In addition, several brands of pesticide grade hexane and petroleum ether contain interferences. The boiling points of hexane (69°C) and petroleum ether (35–75°C) also indicate that isooctane (99°C) is the least volatile of the 3 solvents at ambient temperatures. Furthermore, hexane and petroleum ether are not recovered from aqueous media as well as isooctane,

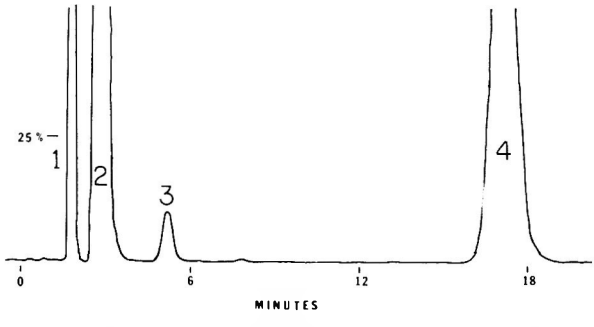

Figure 4. Typical chromatogram of partitioned grain sample (2 mg milo) on mixed column. 1, acetone-isooctane (3 + 97) front; 2, 9 ppm CCl_4 (off scale); 3, 33 ppm EDC; 4, 11 ppm EDB (off scale).

Table 4. Recoveries (%) of fumigants from 2 wheat samples, using screening and partitioning techniques

Fumigant	Fort. level, ppm	Sample 1		Sample 2	
		Screened	Proc.[a]	Partitioned	Proc.[a]
$CHCl_3$	1.11	105	95	96	105
EDC	75.3	—	—	99	95
TCE	1.14	99	96	98	99
EDB	2.94	102	99	99	100
PCE	0.633	108	101	97	94

[a] Procedural standard.

undergoing 10–20% volume losses. Thus, isooctane is best suited for this step.

Table 3 shows the fumigant residues detected in several 50 g grain samples, using both the screening and partitioning techniques. The chromatogram of the milo sample seen in Figure 4 demonstrates that EDC is not as responsive to EC detection as CCl_4 or EDB.

Recovery Studies.—Recovery studies were conducted on both techniques. Relatively clean grain samples were fortified with fumigants. CP was omitted because it deteriorates rapidly in acetone solutions. Procedural standards were included to check the fortification technique for each fumigant used. Table 4 shows the recoveries obtained when the screening method was applied to one wheat sample and the partitioning method to another. Table 5 is a comparison of the recoveries obtained when both techniques were applied to the same oat and corn samples. Recoveries from the screened samples were slightly higher than those from the partitioned samples, but overall recoveries were about 100%. These satisfactory recoveries, together with the acceptable duplications from both techniques and the agreement of results for incurred fumigant residues, signify that both techniques are accurate.

Fumigant Persistence in Grains.—Accumulating evidence has shown that fumigants in solution, or permeated through whole-kernel grain, do not readily escape into the environment. Heuser and Scudamore (7) found that none of the fumigants in their study penetrated the headspace above the 5 + 1 extracting solution after 4 h. They also found EDB residues in empty grain bins 3 months after its application. In McMahon's studies (10), the persistence of EDB in grains led to disproportionately high EDB recoveries.

This persistence can be illustrated further by a wheat sample that had sat loosely sealed in a fruit jar in this laboratory for $2\frac{1}{2}$ years and still contained most of the CCl_4 originally determined, 0.6 ppm. Subsequent to that observation, 4 grain samples were tested for their ability to retain fumigants. Each sample was divided into 2 portions, the first of which was analyzed directly by the partitioning method. The second portion was heated in a 125°C oven for 4 h before analysis by the same method. The results in Table 6 suggest a delayed evaporation or breaking down of incurred fumigants. EDB appears to be more heat labile in the grain matrix than CCl_4 or EDC.

Attempts also were made to hasten the initial extraction step by using a blender, but these failed. For example, a 50 g milo sample was blended to a pulp in (and under the surface of) acetone–water (5 + 1), using a blender equipped with a Polytron head. Blending time was 3 min. The resultant amounts of fumigants were less than those from the same milo sample soaked for 72 h (Table 7). The accompanying spiked sam-

Table 5. Recoveries (%) of fumigants from oat and corn samples, using screening and partitioning techniques

Fumigant	Fort. level, ppm	Oats		Corn	
		Screened	Partitioned	Screened	Partitioned
$CHCl_3$	1.11	103	95	102	93
EDC	75.3	105	94	105	92
CCl_4	0.192	—	—	104	100
TCE	1.14	100	95	99	93
EDB	2.94	105	102	104	96
PCE	0.633	100	96	99	93

Table 6. Fumigants detected (ppm) in 50 g grain samples before and after heating at 125°C

Grain	Fumigant	Before heating	After heating	% Remaining
Wheat	CCl_4	3.6	1.0	28
	EDB	4.6	0.2	4
Milo	CCl_4	8.7	3.4	39
	EDC	36.0	25.0	69
	EDB	12.0	1.5	12
Oats	CCl_4	1.8	0.9	50
Corn	CCl_4	3.1	1.0	32

Table 7. Fumigants (ppm) found in blended or soaked milo sample

				Blended rec., %	
Fumigant	Blended	Soaked	% Diff.	Milo	Proc.[a]
$CHCl_3$	—	—	—	96	99
EDC	19	116	84	—	91
CCl_4	10	19	47	—	94
TCE	—	—	—	100	97
EDB	20	39	49	—	96
PCE	—	—	—	99	97

[a] Procedural standard.

ples, also blended 3 min, showed no losses, indicating that the fumigants added were neither absorbed by the milo nor evaporated during blending. Similar results have been found with other grain samples. These results confirm that the efficient extraction of fumigants from whole-kernel grains is a leaching process.

Summary

Data have been presented showing that the salting out procedure for separating water from the initial leaching solution of the AOAC method yields high recoveries and can be omitted. The wet leachates from that method can be directly screened with GC if the assay is completed as soon as possible. Moreover, test fumigants in solution do not escape into the headspace gas at ambient temperatures. Therefore, the partitioning of fumigants with another solvent such as isooctane is a practical means of obtaining dry, stable conditions for the GC determination of fumigants. The 2 column packings provide suitable resolution and replication for the quantitation of fumigants in grains and grain products.

Acknowledgments

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Applicability of a Carbamate Insecticide Multiresidue Method for Determining Additional Types of Pesticides in Fruits and Vegetables

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Several fruits and vegetables were fortified at a low (0.02-0.5 ppm) and at a high (0.1-5 ppm) level with pesticides and with a synergist, and recoveries were determined. Analyses were performed by using 3 steps of a multiresidue method for determining *N*-methylcarbamates in crops: methanol extraction followed by removal of plant co-extractives by solvent partitioning and chromatography with a charcoal-silanized Celite column. Eleven compounds were determined by using a high performance liquid chromatograph equipped with a reverse phase column and a fluorescence detector. Twelve additional compounds were determined by using a gas-liquid chromatograph equipped with a nonpolar packed column and an electron capture or flame photometric detector. Recoveries of 10 pesticides (azinphos ethyl, azinphos methyl, azinphos methyl oxygen analog, carbaryl, carbofuran, naphthalene acetamide, naphthalene acetic acid methyl ester, napropamide, phosalone, and phosalone oxygen analog) and the synergist piperonyl butoxide, which were determined by high performance liquid chromatography, averaged 100% (range 86-117) at the low fortification level and 102% (range 93-115) at the high fortification level. Quantitative recovery of naphthalene acetamide through the method required that an additional portion of eluting solution be passed through the charcoal column. Recoveries of 7 additional pesticides (dimethoate, malathion, methyl parathion, mevinphos, parathion, phorate oxygen analog, and pronamide), which were determined by gas-liquid chromatography (GLC), averaged 108% (range 100-120) at the low fortification level and 107% (range 99-122) at the high fortification level. DDT, diazinon, dieldrin, phorate, and pirimiphos ethyl, which were determined by GLC, were not quantitatively recovered.

Pesticide residues in crops can be determined by single or multiresidue analytical methods. When the pesticide application history is not known, or when a crop has been treated with more than one pesticide, the use of a multiresidue method, which selectively determines each residue, is generally preferred because of reduced analysis time and costs. Such multiresidue analytical approaches have been successfully applied to the determination of volatile organohalogen, organophosphorus, and organonitrogen pesticides in foods (1, 2) by using gas-liquid chromatography (GLC) to separate the individual residues for detection by selective and sensitive detectors. This multiresidue concept was also used in a method for determining *N*-methylcarbamate insecticides (3). That method used high performance liquid chromatography (HPLC) to separate the individual residues for detection by a selective and sensitive post-column fluorometric labeling technique.

The purpose of the work reported here was to investigate the applicability of the *N*-methylcarbamate method (3) to additional chemical types of pesticides with the use of HPLC or GLC determinative techniques. A number of pesticides which are not readily determined by GLC exhibit natural fluorescence (4-7). Recently, single residue methods were developed for the determination of the fluorescent pesticides carbaryl (8), naphthalene acetic acid (9), thiabendazole (10), and warfarin (11), using a high performance liquid chromatograph with a fluorescence detector. Of interest was whether fluorescent pesticides could be determined by a multiresidue method—in this case, by the carbamate method. Thus, 7 naturally fluorescent pesticides (carbaryl, carbofuran, naphthalene acetamide, naphthalene acetic acid methyl ester, napropamide, phosalone, and phosalone oxygen analog) and the fluorescent synergist piperonyl butoxide were selected for study. Although azinphos ethyl, azinphos methyl, and azinphos methyl oxygen analog do not fluoresce, their hydrolysis product, anthranilic acid, does; therefore, the 3 azinphos compounds were also included in the study.

Twelve additional (or nonfluorescent) representative pesticides which can be determined by multiresidue methods using GLC (1, 2) were selected for study on the basis of their range of polarity: DDT, diazinon, dieldrin, dimethoate, malathion, methyl parathion, mevinphos, parathion, phorate, phorate oxygen analog, pirimiphos ethyl, and pronamide. Study of these compounds was undertaken to provide information on the capability and limitations of the

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carbamate method (3) to recover polar and nonpolar pesticides determined by GLC.

The crops used in the study were broccoli, green beans, lettuce, pears, peaches, and strawberries.

METHOD

In the carbamate insecticide method (3), methanol and a mechanical ultrasonic homogenizer are used to extract the field-incurred residues. Water-soluble plant co-extractives and nonpolar plant lipid materials are removed from pesticide residues by liquid-liquid partitioning. Additional crop co-extractives (e.g., carotenes, chlorophylls) are removed by using a charcoal-silanized Celite column.

The basic carbamate method (3) was used in the study with the following additions/changes:

Reagents (Changes in Ref. 3)

(a) *Solvents.*—Acetone, acetonitrile, methanol, methylene chloride, petroleum ether, and toluene. Distilled-in-glass grade (Burdick & Jackson Laboratories, Inc., Muskegon, MI 49442).

(d) *Sodium hydroxide solution.*—0.2N. Pipet 27 mL clear supernate NaOH in water (1 + 1) into 100 mL volumetric flask. Dilute to volume with water and mix (5N NaOH). Pipet 40 mL 5N NaOH into 1 L volumetric flask. Dilute to 1 L with degassed ultrapure water, and mix well but gently to minimize re-incorporation of air into solution.

Reagents (f) and (g) are omitted.

HPLC Operating Parameters (Change in Ref. 3)

Adjust mobile phase flow rate to 1.50 ± 0.02 mL/min at 50% acetonitrile in water. Equilibrate system at 12% acetonitrile in water for 10 min, inject sample, and begin 30 min linear gradient to 70% acetonitrile in water, then 100% acetonitrile for additional 5 min. For determination of azinphos pesticides, adjust post-column flow rate of 0.2N NaOH to 0.50 ± 0.02 mL/min. Operate column oven at 35°C. Operate hydrolysis chamber at 100°C only for the determination of azinphos pesticides; otherwise, maintain chamber at ambient temperature. Set fluorescence detector excitation and emission wavelengths at 278 and 306 nm, respectively. Set detector photomultiplier gain at low and time constant at 1 s. Adjust sensitivity so that 50 ng carbofuran produces 50% full scale response on printer plotter (recorder) at attenuation of 5. For determination of fluorescent compounds, use excitation and emission wavelengths in Table 1.

GLC Apparatus and Parameters (Additions to Ref. 3)

The apparatus and parameters given in Refs. 1 and 2 were used with the following changes in Ref. 1, sections **29.008** and **29.040.**

29.008

(b) *Column.*—Glass, 1.85 m × 4 mm id packed with 5% (w/w) OV-101 on 80–100 mesh Chromosorb W HP. Use 60 mL argon-methane (95 + 5)/min as carrier gas for ^{63}Ni electron capture

Table 1. HPLC and fluorescence characteristics of 11 pesticides/metabolites

Compound	Maximum E_x[a]	Maximum E_m[a]	Wavelengths used with HPLC E_x	Wavelengths used with HPLC E_m	Retention time rel. to carbofuran	Amt producing 50% FSD[b] response, ng
Azinphos ethyl[c]	320	400	320	400	1.46	5
Azinphos methyl[c]	320	400	320	400	1.29	5
Azinphos methyl oxygen analog[c]	320	400	320	400	0.81	15
Carbaryl	286	338	288	330	1.06	3
Carbofuran	278	306	288	330	1.0[d]	90
Naphthalene acetic acid methyl ester	286	338	288	330	1.44	4
Naphthalene acetamide	286	337	288	330	0.77	3
Napropamide	296	342	288	330	1.36	4
Phosalone	287	320	288	330	1.70	90
Phosalone oxygen analog	288	318	288	330	1.30	90
Piperonyl butoxide	295	330	288	330	1.74	5

[a] E_x = nm excitation; E_m = nm emission.
[b] FSD = full scale recorder deflection.
[c] Fluorescence of anthranilic acid measured after post-column degradation of parent.
[d] Carbofuran elutes in ca 20 min.

Table 2. GLC characteristics of pesticides/metabolites

Compound	Retention time rel. to chlorpyrifos	Amt producing 50% FSD[a] response, ng	
		ECD[b]	FPD-P[c]
DDT	3.09	4	
Diazinon	0.51		0.7
Dieldrin	1.87	1.5	
Dimethoate	0.41		1
Malathion	0.89		1.5
Methyl parathion	0.69		1
Mevinphos	0.13		1
Parathion	0.98	5	1
Phorate	0.36		0.5
Phorate oxygen analog	0.66		0.5
Pirimiphos ethyl	1.14		1.5
Pronamide	0.51	1.5	

[a] FSD = full scale recorder deflection.
[b] Electron capture detector.
[c] Flame photometric detector operated in the phosphorus mode.

detector and nitrogen as carrier gas for flame photometric detector.

(c) *Electron capture detector (ECD).*—Constant current variable frequency ^{63}Ni electron capture detector.

29.040

(i) *Flame photometric detector operation in phosphorus mode (FPD-P).*—Adjust gas flows to 100 mL hydrogen/min and 120 mL air/min. Operate at sensitivity that produces 50% full scale deflection (FSD) for 1.5 ng chlorpyrifos.

Determination (Changes in Ref. 3)

When gas-liquid chromatograph with ECD is used, dilute 1 mL methanol sample solution to 5 mL with methanol and inject 5 μL onto column.

When gas-liquid chromatograph with FPD-P detector is used, evaporate 1 mL methanol sample solution just to dryness, using vacuum rotary evaporator. Immediately dissolve residue in 5 mL acetone. Inject 5 μL onto GLC column.

Results and Discussion

Recovery studies were conducted to determine the applicability of the *N*-methylcarbamate insecticide multiresidue method to the determination of the 22 pesticides and the synergist. Fruits and vegetables were fortified with the compounds at low (0.02–0.5 ppm) and high (0.1–5 ppm) levels by the addition of 15 mL of a methanol fortification solution to 150 g crop in a glass homogenizer jar. A 285 mL portion of methanol was added, and the sample was homogenized immediately. A crop control and duplicate fortification analyses were performed. Each set of 5 samples (1 control, 2 low-level, and 2 high-level fortifications) was subjected to the extraction and co-extractive removal steps within one 8 h working day. Subsequently, HPLC or GLC was used to determine the pesticides after elution from the charcoal-silanized Celite column with 125 mL toluene–acetonitrile (1 + 3). Also, an additional 100 mL toluene–acetonitrile column eluate and the petroleum ether layer from the partitioning step were monitored for the pesticides.

Seven naturally fluorescent pesticides (carbaryl, carbofuran, naphthalene acetamide, naphthalene acetic acid methyl ester, napropamide, phosalone, and phosalone oxygen analog) and the synergist piperonyl butoxide were determined by HPLC with the fluorescence detector. Azinphos ethyl, azinphos methyl, and azinphos methyl oxygen analog were determined after post-column in-line hydrolysis to fluorescent anthranilic acid. Table 1 contains HPLC and fluorescence characteristics of these 11 compounds.

DDT, dieldrin, and pronamide were determined by using a gas-liquid chromatograph equipped with an ECD. Diazinon, dimethoate, malathion, methyl parathion, mevinphos, phorate, phorate oxygen analog, and pirimiphos ethyl were determined by using a gas-liquid chromatograph equipped with an FPD-P. Parathion was determined by GLC, using either the ECD or the FPD-P. GLC retention and response data are given in Table 2.

HPLC Recovery Data

Tables 3 and 4 show the recovery data for the compounds determined by HPLC. At the low fortification level (0.02–0.5 ppm), the overall average recovery of azinphos ethyl, azinphos methyl, azinphos methyl oxygen analog, carbaryl, carbofuran, naphthalene acetic acid methyl ester, naphthalene acetamide, napropamide, phosalone, phosalone oxygen analog, and piperonyl butoxide was 100%; individual recoveries ranged from 86 to 117%. The overall average recovery of these pesticides at the high fortification level (0.1–5 ppm) was 102%; individual recoveries ranged from 93 to 115%. Except for naphthalene acetamide, 90+% of the quantity of each pesticide added to the crop was recovered in the first toluene–acetonitrile fraction. Approximately 80% of the naphthalene acetamide eluted in the first fraction from the charcoal–silanized Celite column. The re-

Table 3. Recovery (%) of pesticides and metabolites from crops at low fortification levels through method with determination by HPLC

Pesticide/metabolite	Added, ppm	Crop 1[a]		Crop 2[a]		Av.
		Pears		Green beans		
Carbaryl	0.02	103	105	105	108	105
Carbofuran	0.2	102	103	102	102	102
Naphthalene acetic acid methyl ester	0.05	97	96	97	101	98
Naphthalene acetamide	0.02	96	100	99	96	98
Napropamide	0.02	92	89	98	98	94
Phosalone	0.5	95	92	113	117	104
Phosalone oxygen analog	0.5	—[b]	—[b]	108	116	112
Piperonyl butoxide	0.05	86	88	90	92	89
		Peaches		Broccoli		
Azinphos ethyl	0.05	101	103	105	103	103
Azinphos methyl	0.05	102	103	105	102	103
Azinphos methyl oxygen analog	0.05	104	104	108	103	105
Phosalone	0.5	94	93	89	93	92
Phosalone oxygen analog	0.5	95	95	—[b]	—[b]	95

[a] Duplicate determinations.
[b] Interference precluded quantitating recovery.

maining naphthalene acetamide was found in the subsequent toluene–acetonitrile fraction. Approximately 4% of the piperonyl butoxide and 1% of the naphthalene acetic acid methyl ester were found in the petroleum ether layer after partitioning. The other fortified pesticides were not found in the petroleum ether layer with a detection limit of 0.4% (approximately 5% FSD response) for each respective pesticide.

Quantitative recovery is only one aspect to be considered in evaluating the applicability of the method to these pesticides. The other aspect is selectivity. Selectivity is a function of the chromatographic system and associated detector to preferentially monitor the individual compounds of interest and exclude detection of other materials. Selectivity is also a function of the ability of the crop co-extractive removal procedures (i.e., cleanup) to eliminate components which would be detected with the determinative procedure. Numerous extraneous fluorescent peaks were observed at the 288 nm excitation and 330 nm emission wavelengths used for the naphthalene pesticides carbofuran and phos-

Table 4. Recovery (%) of pesticides and metabolites from crops at high fortification levels through method with determination by HPLC

Pesticide/metabolite	Added, ppm	Crop 1[a]		Crop 2[a]		Av.
		Pears		Green beans		
Carbaryl	1	100	103	104	94	100
Carbofuran	1	106	102	104	99	103
Naphthalene acetic acid methyl ester	1	95	92	97	95	95
Naphthalene acetamide	0.1	98	101	100	93	98
Napropamide	0.5	99	98	99	97	98
Phosalone	5	104	105	99	111	105
Phosalone oxygen analog	5	103	106	115	103	107
Piperonyl butoxide	1	100	97	97	93	97
		Peaches		Broccoli		
Azinphos ethyl	1	106	105	104	104	105
Azinphos methyl	1	106	106	105	104	105
Azinphos methyl oxygen analog	1	108	109	108	105	108
Phosalone	5	106	105	107	105	106
Phosalone oxygen analog	5	106	104	—[b]	—[b]	105

[a] Duplicate determinations.
[b] Interference precluded quantitating recovery.

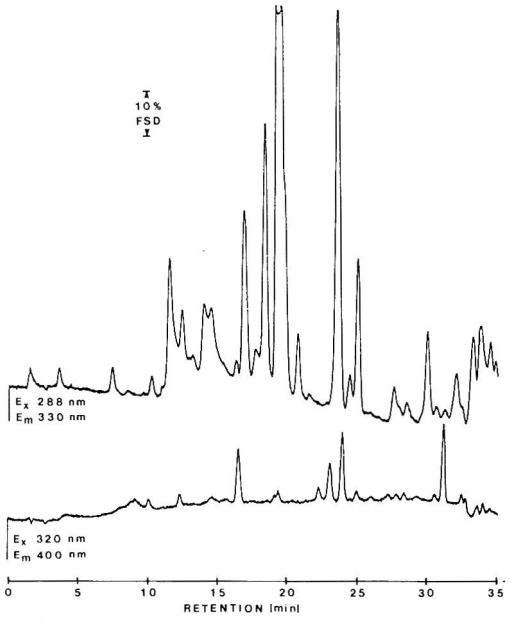

Figure 1. HPLC chromatogram of broccoli control showing effect of different fluorescence excitation and emission wavelengths.

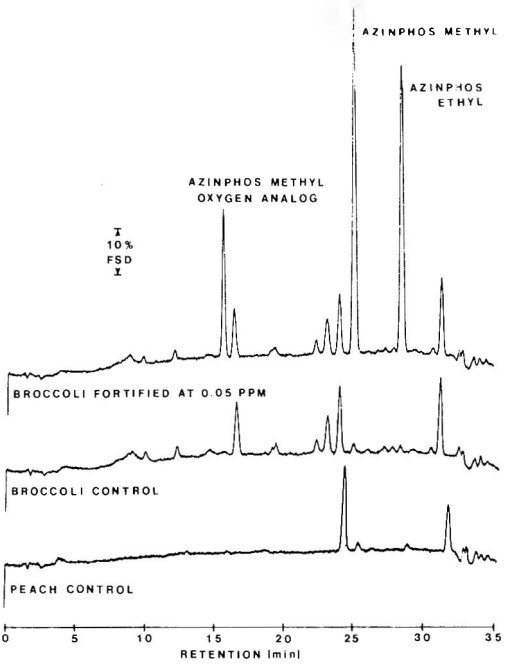

Figure 2. HPLC chromatograms obtained by using fluorescence detector at 320 nm E_x and 400 nm E_m and post-column hydrolysis.

alone. An example is shown in Figure 1. The extraneous responses, which are not present in the reagent blank, may be due to fluorescent plant co-extractives. Of the fluorescent pesticides studied, only phosalone oxygen analog could not be quantitated because of an interfering peak. Although additional extraneous peaks were observed (Figure 1), none prevented the quantitation of the other fluorescent pesticides.

The selectivity for the detection of azinphos compounds is greatly increased over that of the naphthalene pesticides with the change in excitation wavelength to 320 nm and emission wavelength to 400 nm and the addition of base to the column eluent. An example of the increased selectivity is shown in Figure 1. Most of the extraneous peaks observed in the broccoli control sample at 288 nm excitation and 330 nm emission are absent at 320 nm excitation and 400 nm emission. A chromatogram of the broccoli sample fortified with azinphos compounds (Figure 2) shows that the small extraneous peaks present in the broccoli control sample do not interfere in the quantitation of azinphos residues.

GLC Recovery Data

Tables 5 and 6 show the recovery data for the compounds determined by GLC. The crops were fortified with DDT, diazinon, dieldrin, dimethoate, malathion, methyl parathion, mevinphos, parathion, phorate, phorate oxygen analog, pirimiphos ethyl, and pronamide at 0.05 and 1 ppm. The overall average recovery of the organophosphorus insecticides dimethoate, malathion, methyl parathion, mevinphos, parathion, and phorate oxygen analog at the 0.05 ppm fortification level was 108%; individual recoveries of these compounds ranged from 100 to 120%. The overall average recovery of these pesticides at the 1 ppm fortification level was 107% and ranged from 99 to 122%. The high recovery bias was due in part to partial evaporation of the 5 mL acetone added to the 500 mL round-bottom flask to dissolve pesticide residues for the GLC analyses. Approximately 5% of the acetone evaporates while it is pipetted onto the surface of the round-bottom flask and swirled in the flask for 20 s to dissolve residues.

Pronamide, an organochlorine herbicide, was quantitatively recovered through the method. Recoveries averaged 106% at the 0.05 ppm fortification level and 105% at the 1 ppm level.

The nonpolar organohalogen insecticides DDT and dieldrin were not quantitatively recovered through the method (Tables 5 and 6). Less than 70% of these fortified residues was found in the

Table 5. Recovery (%) of pesticides and metabolites from crops at 0.05 ppm fortification level through method with determination by GLC

Pesticide/metabolite	Crop 1[a]		Crop 2[a]		Av.
Electron capture	Pears		Green beans		
DDT	56	62	69	66	63
Dieldrin	68	70	71	64	68
Parathion	—[b]	—[b]	100	100	100
Pronamide	105	105	105	107	106
Flame photometric	Strawberries		Lettuce		
Diazinon	74	83	81	83	80
Dimethoate	118	115	118	114	116
Malathion	112	109	112	109	110
Methyl parathion	112	108	111	108	110
Mevinphos	—[c]	—[c]	106	100	103
Parathion	103	120	105	106	108
Phorate	46	38	46	46	44
Phorate oxygen analog	106	107	105	106	106
Pirimiphos ethyl	69	84	68	77	74

[a] Duplicate determinations.
[b] Parathion in control sample (0.02 ppm).
[c] Mevinphos in control sample (0.28 ppm).

toluene–acetonitrile eluate from the charcoal–silanized Celite column. The remainder of these residues was found in the petroleum ether layer after partitioning. Similarly, approximately 20% of diazinon and pirimiphos ethyl was found in the petroleum ether layer. Thus, it appears that the relatively nonpolar pesticides are not quantitatively recovered through the method. Although phorate oxygen analog was quantitatively recovered, phorate recovery values were approximately 50%. About 15% of the fortified phorate was found in the petroleum ether layer. No phorate was found in the second acetonitrile–toluene eluate from the charcoal–silanized Celite column.

The lettuce and strawberry crop controls injected onto the gas-liquid chromatograph equipped with a FPD-P were free of extraneous

Table 6. Recovery (%) of pesticides and metabolites from crops at 1 ppm fortification level through method with determination by GLC

Pesticide/metabolite	Crop 1[a]		Crop 2[a]		Av.
Electron capture	Pears		Green beans		
DDT	57	47	45	45	48
Dieldrin	76	68	62	57	64
Parathion	103[b]	101[b]	108	110	106
Pronamide	105	102	111	103	105
Flame photometric	Strawberries		Lettuce		
Diazinon	85	88	85	85	86
Dimethoate	107	117	99	106	107
Malathion	106	122	103	114	111
Methyl parathion	104	116	105	107	108
Mevinphos	—[c]	—[c]	105	103	104
Parathion	102	115	106	107	108
Phorate	50	41	50	57	50
Phorate oxygen analog	100	110	102	104	104
Pirimiphos ethyl	80	87	71	69	77

[a] Duplicate determinations.
[b] Recoveries uncorrected for parathion present in control sample (0.02 ppm).
[c] Mevinphos in control sample (0.28 ppm).

peaks except for 0.28 ppm mevinphos found in the strawberry control sample. The green bean and pear crop control samples injected onto the gas-liquid chromatograph equipped with an ECD were relatively free of extraneous peaks, the largest being 5% FSD.

Conclusion

The basic multiresidue method originally developed for the determination of the *N*-methylcarbamate insecticides in fruits and vegetables was found to be applicable to several fluorescent pesticides, organophosphorus insecticides, a synergist, and a herbicide. However, the method does not appear to quantitatively recover the relatively nonpolar pesticides. The HPLC chromatograms obtained for the determination of the azinphos compounds and the GLC chromatograms obtained with FPD-P and ECD were relatively free of extraneous peaks, indicating adequate removal of any interfering crop coextractives. However, in the HPLC determination of those pesticides detected with the fluorescence detector set at 288 nm excitation and 330 nm emission wavelengths, chromatograms of the crop controls were found to contain extraneous peaks. One of those peaks interfered with the quantitation of phosalone oxygen analog.

Acknowledgments

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Nitro Musk Fragrances as Potential Contaminants in Pesticide Residue Analysis

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Synthetic nitro musks, fragrance ingredients of soaps, lotions, and other products commonly used in the home and laboratory, are potential interferences in analyses of foods for pesticide residues. These fragrances are recovered by extraction-cleanup procedures of the AOAC multiresidue method for fatty and nonfatty foods and have electron capture gas-liquid chromatographic properties similar to the organochlorine residues for which the method has official status. This report presents the analytical evidence that led to the identification of musk xylol (1-[1,1-dimethylethyl]-3,5-dimethyl-2,4,6-trinitrobenzene) as a contaminant in a sample of fish; mass spectra and data on the behavior in the AOAC multiresidue method of musk xylol, musk ambrette (1-[1,1-dimethylethyl]-2-methoxy-4-methyl-3,5-dinitrobenzene), musk tibetene (1-[1,1-dimethylethyl]-3,4,5-trimethyl-2,6-dinitrobenzene), and musk ketone (1-[4-(1,1-dimethylethyl)-2,6-dimethyl-3,5-dinitrophenyl]ethanone); and information on some sources of these chemicals in the laboratory environment.

Synthetic nitro musks are widely used as fragrances in soaps, detergents, lotions, and other scented products (1, 2). The artificial musks whose chemical structures are shown in Figure 1 are recovered by the AOAC multiresidue method for organochlorine and organophosphorus pesticides (3) and give electron capture (EC) gas-liquid chromatographic (GLC) responses in the retention range of the organochlorine pesticides and polychlorinated biphenyls (PCBs). Thus, these fragrances could interfere with the determination of the organochlorine residues for which the AOAC method has official status.

Our interest in nitro musks began with an investigation to identify a contaminant in a homogenized sample of catfish received from the Food and Drug Administration (FDA) District laboratory in Minneapolis, MN. The Minneapolis District laboratory had used the AOAC multiresidue method to analyze 3 samples of fish from a lake in Illinois and had detected an electron-capturing unknown in the ethyl ether–petroleum ether (6 + 94) Florisil column eluate of one sample. Under an ongoing FDA program for the identification of unrecognized industrial chemical contaminants in foods, the Minneapolis District laboratory sent a portion of the sample containing the unidentified contaminant and related analytical data to our laboratory for further study. Re-analysis of the sample and interpretation of GLC data obtained with EC, nitrogen/phosphorus (NP), mass spectrometric (MS), and other selective detectors led to the tentative identification of the unknown as 1-(1,1-dimethylethyl)-3,5-dimethyl-2,4,6-trinitrobenzene (musk xylol, Figure 1B). GLC/MS comparison of the contaminant with an authentic sample of musk xylol confirmed this identification.

Musk xylol has not previously been reported as a contaminant in foods or the environment. Because this chemical is a common ingredient of toiletries (1, 2) and was found in only one of 3 fish samples caught in the same lake, we suspected that the sample could have become contaminated outside the aquatic environment. Our investigation did not include analysis of personal care products used by those involved in the collection or original analysis of the sample. However, qualitative EC/GLC or GLC/MS analyses of a sample of soap and 3 samples of hand lotions used in our laboratory showed the presence of musk xylol in each product. Musk ambrette (1-[1,1-dimethylethyl]-2-methoxy-4-methyl-3,5-dinitrobenzene, Figure 1A) and musk ketone (1-[4-(1,1-dimethylethyl)-2,6-dimethyl-3,5-dinitrophenyl]ethanone, Figure 1D) were also found in 2 of the 3 hand lotions examined. In addition, EC and NP/GLC chromatograms derived from several previously analyzed fish samples indicated the presence of one or more of these substances. For these reasons, the fragrances identified in the hand lotions and the structurally similar musk tibetene (1-[1,1-dimethylethyl]-3,4,5-trimethyl-2,6-dinitrobenzene, Figure 1C) were studied to determine their behavior in the AOAC pesticide multiresidue method; all 4 compounds were quantitatively recovered.

Contamination by products scented with nitro musks is only one route through which these compounds occur in foods. Musk ambrette has been used as an artificial flavor in the United

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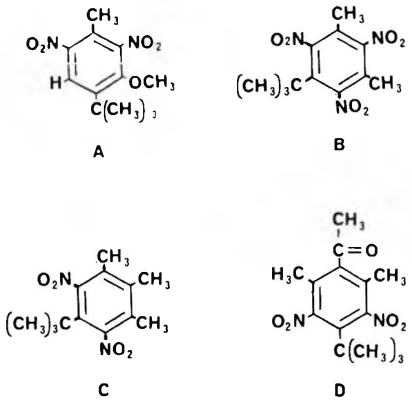

Figure 1. Chemical structures of A, musk ambrette; B, musk xylol; C, musk tibetene; D, musk ketone.

States (4). Musk xylol and musk ketone, although not approved as food additives in the United States, are approved for use as artificial flavoring substances by the Council of Europe (5). By analogy with other industrial chemicals that have solubility properties and other chemical/physical characteristics which allow them to be recovered by the AOAC multiresidue method, it appears that nitro musks have the potential to reach the aquatic environment and to ultimately contaminate foods via this route. The purpose of this report is to help the residue analyst to recognize these compounds in foods and to caution those involved with sample handling that nitro musks are ingredients of many products commonly used in the laboratory.

Experimental

Reagents

(a) *General reagents.*—See sec. 29.002 (3).

(b) *Pesticides.*—Aldrin, azobenzene, heptachlor epoxide, malathion, parathion, and trifluralin were used as GLC reference standards. All were obtained from the U.S. Environmental Protection Agency, Health Effects Research Laboratory, Research Triangle Park, NC 27711.

(c) *Nitro musks.*—Musk ambrette, musk ketone, musk tibetene, and musk xylol were supplied by the FDA Division of Cosmetics Technology, Washington, DC 20204. These chemicals are commercially available from ICN Life Sciences Group (K&K), 121 Express St, Plainview, NY 11803.

Apparatus

(a) *General apparatus and glassware.*—See sec. 29.005.

(b) *Gas chromatograph with constant current EC detector.*—Hewlett-Packard 5730A gas chromatograph with Hewlett-Packard 18713A EC detector and the following glass columns: (*1*) 1.8 m × 4 mm id, packed with 5% OV-101 on 80–100 mesh Chromosorb W (HP); (*2*) 1.8 m × 4 mm id, packed with 1 + 1 mixture of 5% OV-101 on 80–100 mesh Chromosorb W (HP) and 7.5% OV-210 on 80–100 mesh Chromosorb W (HP); (*3*) 1.4 m × 2 mm id, packed with 2% OV-101 on 100–120 mesh Chromosorb W (HP). Operating conditions: temperatures (°C)—column 200, inlet 250, detector 300; 5% methane/95% argon carrier, 60 mL/min (columns 1 and 2); nitrogen carrier, 30 mL/min (column 3); recorder span, 1 mV. The attenuator was set to give ca 50% full scale deflection (FSD) for 1 ng heptachlor epoxide in all work except determinations of ratios of EC to NP response for reference compounds and the unidentified contaminant. For EC to NP response ratio measurements, the EC attenuator was adjusted so that the same amount of the chemical used to determine NP response gave an EC response of 10–90% FSD.

(c) *Gas chromatograph with NP detector.*—Hewlett-Packard 5730A gas chromatograph with Hewlett-Packard 18789A NP detector and 1.4 m × 2 mm id glass column packed with 2% OV-101 on 100–120 mesh Chromosorb W (HP). Operating conditions: temperatures (°C)—column 200, inlet 250, detector 250; nitrogen carrier, 30 mL/min; air, 50 mL/min; hydrogen, 1 mL/min; recorder span, 1 mV; electrometer sensitivity (max., range 1), 5×10^{-12} A full scale at attenuation 1; detector voltage, adjusted to ca 15 V to give 30–50% FSD for 1 ng malathion at attenuation 32. At these conditions, 0.5 ng azobenzene gave >30% FSD and 1 μg octadecane gave <5% FSD at attenuation 32. For sample and reference compound analyses, attenuation settings of 8, 16, 32, and 64 were used as required to give 10–90% FSD for the compounds of interest.

(d) *Combined gas chromatograph-mass spectrometer-data system.*—Varian 1700 gas chromatograph/Finnigan 1015 quadrupole mass spectrometer/Finnigan 6000 data system. The gas chromatograph was directly coupled to the mass spectrometer through a 15 m × 0.2 mm id SE-52 wall-coated open tubular (WCOT) capillary column. Splitless injections (6) were made at the following operating conditions: temperatures (°C)—injector 250, column 50 for injection, then raised directly to 200; helium carrier, 1 mL/min; mass spectrometer pressure, 2×10^{-5} torr; filament emission, 500 μA; preamplifier, 10^{-7} A/V; 70 electron volt primary ionizing voltage in

electron impact (EI) mode; scanned mass range, m/z 39–450; data acquisition under computer control.

Analysis of Fish

Fish were prepared for analysis by the AOAC general method for organochlorine and organophosphorus pesticides (3) as described in edible portion guide, sec. 141.12c, of the FDA Pesticide Analytical Manual, Volume I (PAM I) (7). Ground fillets of ocean perch were used as the substrate for recovery studies. Samples of thoroughly ground and mixed edible tissue were extracted by using the official fatty food extraction procedure for fish, sec. **29.012(e)** and/or the nonfatty food extraction procedure for high moisture products containing less than 5% sugar, sec. **29.011(a)**(*1*). The latter procedure, which uses acetonitrile as the extractant and is normally applied to fruits and vegetables, was modified for application to fish as described in PAM I (7), sec. 211.13(f)(2), i.e., by reducing the sample size so that the total amount of fat did not exceed 2 g. Residues in extracts obtained by the modified nonfatty food extraction procedure were partitioned into petroleum ether, sec. **29.011(e)**, and cleaned up by Florisil column chromatography, sec. **29.015**, before GLC analysis. Extracts obtained by the fatty food extraction procedure were cleaned up for GLC analysis by acetonitrile partitioning, sec. **29.014**, and Florisil column chromatography, sec. **29.015**. The Florisil cleanup procedure was used without modification in recovery studies; for other analyses of fish, the procedure was modified to elute the Florisil column with 250 mL petroleum ether before the usual ethyl ether-petroleum ether (6 + 94) and (15 + 85) eluates were collected. This modification was used to remove potential contaminants, such as PCBs, which could interfere with the GLC determination of nitro musks.

Results and Discussion

Figure 2 shows EC chromatograms of (A) a mixture of musk ambrette, musk xylol, musk tibetene, and musk ketone, and (B) the ethyl ether–petroleum ether (6 + 94) Florisil eluate of the catfish sample containing the unidentified contaminant. GLC analysis of the unknown with element-selective detectors indicated the presence of nitrogen and the absence of chlorine, bromine, iodine, phosphorus, and sulfur; i.e., the NP detector gave a positive response at the retention time of the electron-capturing contaminant, but neither the halogen-specific microcoulometric detector nor the phosphorus/sulfur-selective flame photometric detector gave any response.

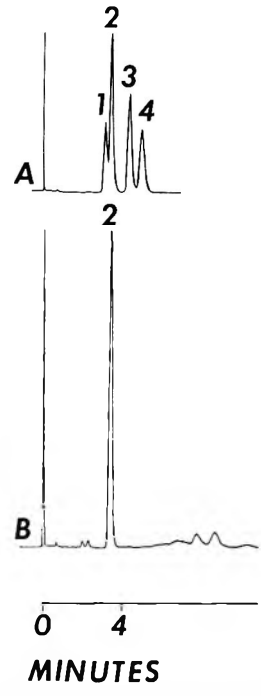

Figure 2. EC/GLC chromatograms of nitro musks: A = mixture of 1, musk ambrette (0.62 ng); 2, musk xylol (1.24 ng); 3, musk tibetene (0.93 ng); and 4, musk ketone (0.68 ng); B = ethyl ether-petroleum ether (6 + 94) Florisil eluate of catfish sample (7.46 mg sample equivalent injected). 5% OV-101 column at GLC parameters in *Apparatus* (b).

The background-subtracted mass spectrum of the unknown, which was essentially identical to that of musk xylol (Figure 3), did not provide sufficient information for us to deduce the structure from first principles. No similar spectra were found in available compilations of mass spectral data (8, 9). We were initially uncertain as to whether the apparent molecular ion at m/z 297 was the molecular ion or even whether this relatively low intensity ion (relative intensity enhanced by a factor of 50 in mass spectrum of musk xylol, Figure 3) was produced by the contaminant of interest. However, the m/z 297 ion profile peaked at the GLC retention time of the electron-capturing substance and no evidence was apparent for higher mass ions similarly associated with this substance. In addition, the odd mass number of the apparent molecular ion was consistent with the presence of nitrogen, either singly or in odd number. Chemical ionization/MS analysis confirmed that the ion observed at m/z 297 in the EI spectrum

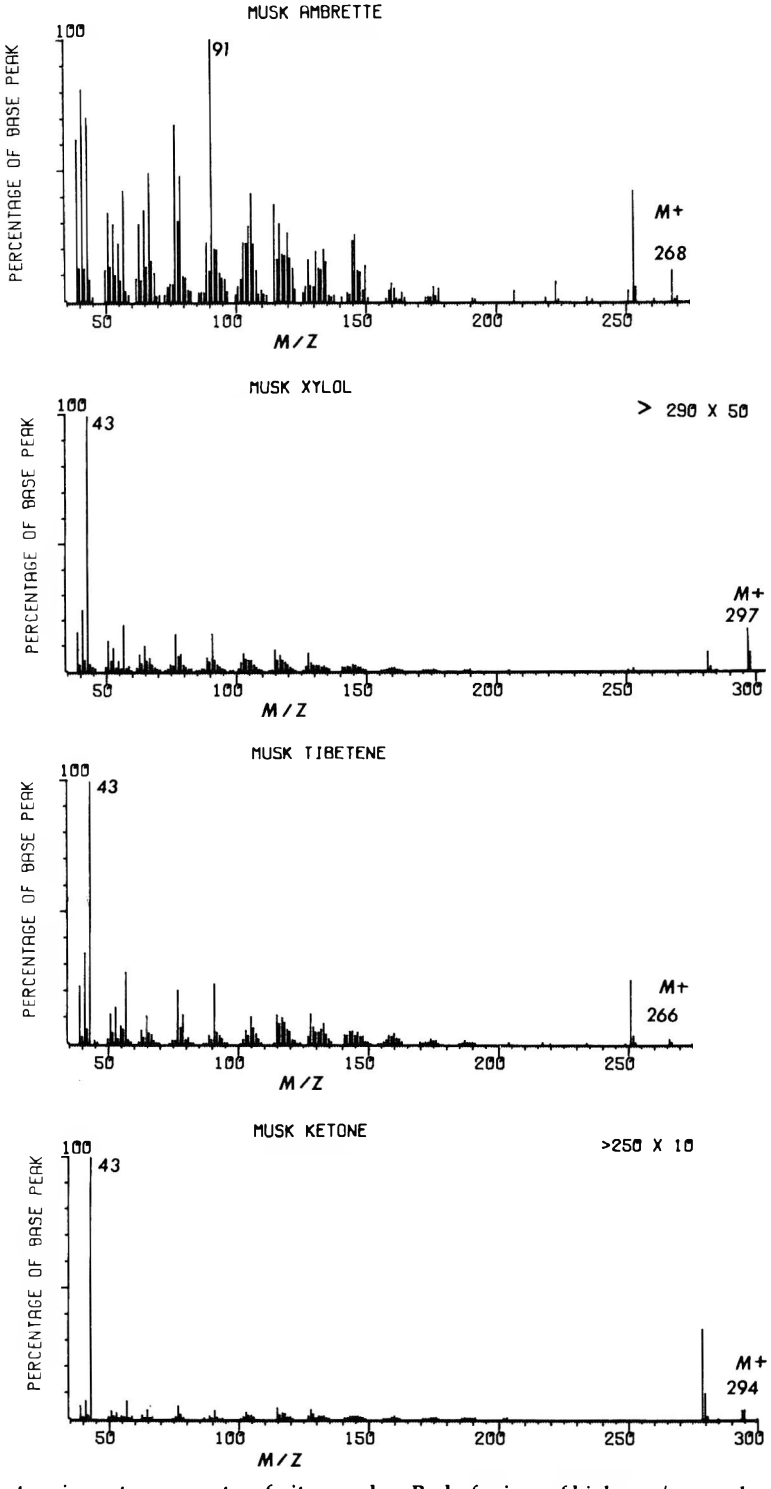

Figure 3. Electron impact mass spectra of nitro musks. Peaks for ions of higher m/z are enhanced as indicated in spectra of musk xylol and musk ketone.

was the molecular ion and indicated that the unknown contained one or more nitro groups.

The key to the identification of the contaminant was its very strong EC response for a nonhalogenated organonitrogen compound as shown by an exceptionally high ratio of EC to NP response for such a compound. This ratio was determined for the unknown by dividing its EC response (% FSD × EC attenuation factor) by its NP response (% FSD × NP attenuation factor) when equal amounts of the ethyl ether–petroleum ether (6 + 94) eluate of the catfish sample were analyzed on 2% OV-101 GLC columns at the operating conditions described under *Apparatus* (b,c). Ratios of EC to NP response were similarly determined for several electron-capturing reference compounds that contain nitrogen and/or phosphorus: azobenzene 3.3, malathion 20, parathion 38, trifluralin 150, unknown 350. The EC to NP response ratio for the unknown was much greater than that for any of the selected reference compounds and was more than twice that of trifluralin, a herbicide with very high electron affinity resulting from the presence of a trifluoromethyl group and 2 nitro groups in its molecular structure.

Considering all the available data, we suspected that the unknown contained 3 nitro groups and had a molecular formula (e.g., $C_{12}H_{15}N_3O_6$) that could yield a parent ion at m/z 297. A search of the chemical literature indicated that musk xylol was a good possibility. GLC analyses of a musk xylol reference standard on OV-101 and mixed OV-101 + OV-210 columns produced peaks at the identical retention times of the contaminant in the catfish. In analyses by EC/GLC and NP/GLC with OV-101 columns, the standard musk xylol gave the same ratio of EC to NP response as the contaminant. The identification of the contaminant as musk xylol was verified by GLC/MS comparison with the reference standard. As determined by EC/GLC, the concentration was 0.32 ppm.

After we learned that musk xylol was one of several nitro musks used as fragrances in soaps, lotions, perfumes, creams, etc. (1, 2), qualitative analyses revealed that hand lotions and soap in use in our laboratory contained several of these compounds. Figure 4 shows EC/GLC chromatograms of petroleum ether extracts of 3 hand lotions and a sample of soap. The presence of musk xylol in the soap and in one of the lotions was confirmed by GLC/MS. In addition to musk xylol, musk ambrette and musk ketone were tentatively identified in the other 2 hand lotions by retention times on 2 GLC columns. These

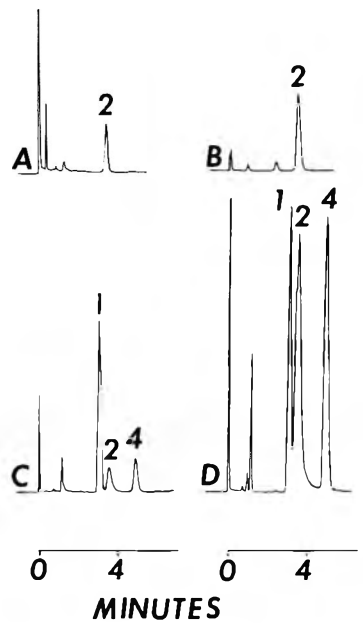

Figure 4. EC/GLC chromatograms of extracts of A, hand lotion; B, hand soap; C and D, other hand lotions used in our laboratory. 5% OV-101 column at GLC parameters in *Apparatus* (b). 1, musk ambrette; 2, musk xylol; 4, musk ketone.

fragrances and musk tibetene were also found in analyses of several other scented products used in the authors' homes, e.g., perfume, rug deodorant, and toilet tissue.

Because nitro musks were present in products used in our laboratory, their analytical properties were studied. EC/GLC retention time and response data for the fragrances (Table 1) were obtained by using OV-101 and mixed OV-101 + OV-210 columns at conditions normally used for pesticide residue analysis to allow direct comparison with the data reported for a large number of pesticides and industrial chemicals in PAM I, Tables 331A and 331B (7). The nitro musks eluted from each GLC column in the same retention range as many organochlorine pesticides and industrial chemicals (e.g., chlordane, PCBs, etc.), and their EC responses approximated that for an equal amount of heptachlor epoxide. Recoveries of the nitro musks by the AOAC pesticide multiresidue method (3) were determined from fortified samples of ground fillets of ocean perch. As shown in Table 2, quantitative recoveries were obtained with both the fatty food and nonfatty food extraction/cleanup procedures of the method. Musk xylol and musk tibetene

Table 1. GLC retention times and EC response data for nitro musks [a]

Compound	R_A [b]		Amount for 50% FSD, ng [c]
	OV-101	OV-101 + OV-210	
Musk ambrette	0.58	1.02	1.3
Musk xylol	0.64	1.01	1.3
Musk tibetene	0.80	1.21	1.4
Musk ketone	0.91	1.72	1.5

[a] Columns: 5% OV-101; 1 + 1 mixture of 5% OV-101 and 7.5% OV-210. GLC parameters given in *Apparatus* (**b**).
[b] Retention time relative to aldrin. *p,p'*-DDT, a secondary retention time standard, had R_A values of 3.04 (OV-101) and 3.23 (OV-101 + OV-210) at the stated GLC parameters.
[c] Nanograms needed to give same peak height (% FSD) as 1 ng heptachlor epoxide when 1 ng heptachlor epoxide gives ca 50% FSD. Data are for OV-101 column.

were recovered in the ethyl ether–petroleum ether (6 + 94) Florisil column eluate; musk ambrette and musk ketone were split between the ethyl ether-petroleum ether (6 + 94) and (15 + 85) eluates.

EI mass spectra are presented in Figure 3. Musk ambrette gives a complex spectrum with a base peak at m/z 91 and many other peaks, including that for the molecular ion, M^+, with intensities greater than 10% of the base peak. The spectra of musk xylol, musk tibetene, and musk ketone greatly resemble each other in general appearance; each exhibits a base peak at m/z 43, an M^+ peak that is very low in intensity relative to the base peak (i.e., ≤1%), and a very similar pattern of peaks for ions below m/z 200 (the most abundant of which are also abundant in the spectrum of musk ambrette). For all 4 compounds, the $[M - 15]^+$ ion produces the most prominent peak in the higher mass region of the spectrum. In general, the mass spectra of these fragrances provide little of the type of information needed for molecular structure determinations in the absence of reference spectra.

A review of EC and NP chromatograms of samples previously analyzed in our laboratory revealed that a few fish samples caught in highly industrialized areas gave peaks at or near the retention times of nitro musks. In later analyses of reserved portions of these samples, several nitro musks were tentatively identified at low levels (<0.2 ppm) by retention times on 2 GLC columns using both EC and NP detectors. Attempts to confirm or deny these findings by GLC/MS (EI) were unsuccessful; the presence of interfering substances in these fish and the lack of abundant peaks above m/z 200 in the spectra of musk xylol, musk tibetene, and musk ketone made it difficult to obtain meaningful background-subtracted spectra for the residues indicated by the EC and NP/GLC analyses.

In summary, musk fragrances, ingredients of products commonly used in the home and laboratory, have been detected in several fish samples. The source of these contaminants has not been determined; it is possible that they were added during sample handling rather than through environmental contamination. Regardless of the mode of entry of nitro musks into foods, the data presented should prove useful to

Table 2. Recovery of nitro musks from fish by AOAC pesticide multiresidue extraction/cleanup procedures for fatty and nonfatty foods

Compound	Added, ppm	Rec., %		Florisil eluate [c] contg compd
		FF [a]	NFF [b]	
Musk ambrette	0.24	93, 93	103, 108	1, 2
Musk xylol	0.48	89, 79	105, 106	1
Musk tibetene	0.36	98, 83	106, 98	1
Musk ketone	0.26	98, 83	83, 115	1, 2

[a] Fatty food extraction/cleanup, secs **29.012(e)**, **29.014**, **29.015**.
[b] Nonfatty food extraction/cleanup, secs **29.011(a)**(*1*), **29.011(e)**, **29.015**. Extraction modified for fish as in PAM I (7), sec. 211.13(f)(2).
[c] 1 = ethyl ether–petroleum ether (6 + 94); 2 = ethyl ether–petroleum ether (15 + 85).

the analyst if these compounds are encountered in residue analyses.

Acknowledgments

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High Resolution Gas Chromatographic Analysis of Nonpolar Chlorinated Hydrocarbons in Human Milk

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A gas chromatographic method is described for the analysis of human milk to determine polychlorinated biphenyls (PCBs) as 72 congeners plus *p,p'*-DDE, mirex, hexachlorobenzene, and octachlorostyrene. The detection limit for individual compounds is about 0.05 ng/g when 30 g milk is analyzed. Total PCBs can be estimated with a detection limit of 1-5 ng/mL milk. Analytical precision is better than ±10% for all compounds at 20-50 ng/mL whole milk.

Two extensive studies on chlorinated hydrocarbons in human milk have recently been published. The U.S. National Study (1) used methodology which closely followed the EPA *Manual of Analytical Methods* (2), involving glass wool-mediated extraction of fats from the milk, acetonitrile–hexane partitioning, Florisil column and silica gel column cleanup, and packed column gas chromatography with electron capture detection (3, 4). The second major study was confined to residents of Michigan (5) and was carried out as an adjunct to a series of analyses for polybrominated biphenyls; hence, the methodology was primarily designed for the brominated compounds. These surveys yielded an excellent picture of the extent of contamination by the insecticides. However, 2 questions remain which are of particular importance in New York State: Which polychlorinated biphenyl (PCB) congeners are present, and what quantity of mirex is present?

Milk samples contain many fewer PCB congeners than did the commercial mixtures that originally contaminated the environment. Some congeners in milk have been identified in Japan (6) and Sweden (7), but the methods used are unsuitable for extensive surveys. Present EPA methodology is also inadequate for discriminating and quantitating PCB congeners accurately (8). In Holland, a method for analyzing cow milk on a PCB-congener basis has recently been implemented (9), but only 29 congeners are quantitated.

We have developed an analytical system based on Apiezon L-coated glass capillary gas chromatography (GC) with electron capture detection, which separates 72 PCB congeners from a mixture of Aroclors 1221, 1016, 1254, and 1260. In addition, this system separates hexachlorobenzene, octachlorostyrene, *p,p'*-DDE, and mirex from all of the PCB congeners (10). The high resolution of the chromatography allows a much abbreviated cleanup, an advantage already appreciated by several authors (3, 9, 11). Because the limiting factor determining sensitivity in previous methods has been reagent blank signal (4), reduction of the number of cleanup steps and the amount of solvent required for cleanup improves sensitivity, as does the sharp peak shape achieved by glass-capillary chromatography.

Mirex has not been unequivocally identified in human milk from the United States (1), but because it is an important pollutant of Lake Ontario (12) it is of special concern in this state. The detection limit in previous work was 30 ng/g whole milk (1, 3). Our goal was to produce a method that is routinely capable of detecting 0.1 ng mirex/g (13), so that suspect samples could be subjected to further cleanup on activated Florisil (8, 10, 13–15) and examined by GC–mass spectrometry.

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Experimental

Apparatus

(a) *Gas chromatograph.*—Hewlett-Packard 5840A with extended memory, ASCII interface board, and Hewlett-Packard 5880 capillary injector. (The original 5840 injector is not suitable because it is susceptible to irreversible contamination by fatty residues from samples.) A 30 m (0.3 mm id) borosilicate glass capillary column coated with Apiezon L, HCl (gas) etched 3 h at 375°C, silanized with hexamethyl-disilizane, and coated by passing a solution of Apiezon L (2%) in isooctane (one-half the column volume) through it at 2 cm/s under nitrogen pressure, using 2 half-turn plugs of mercury to form an even coating. A second buffer column is connected to the end of the capillary to ensure even coating speed (16; H. R. Buser, personal communication).

(b) *Glassware.*—Standard equipment: Soxhlet apparatus; 6-ball Snyder columns; Kuderna-

Danish evaporators with 12 mL ground-glass graduated centrifuge cone receivers; 30 × 1 cm chromatographic columns with fritted glass ends and 100 mL reservoirs. Calibrate Kuderna-Danish receivers by pipeting 2.00 mL water from a grade A pipet and scratching a mark with a diamond point if existing mark is inaccurate.

(c) *Tissumizer.*—With W-18 head (Tekmar Co., Cincinnati, OH).

(d) *Milk collection jars.*—Sputum jars (125 mL) with metal screw caps, and fitted with Teflon cap liners, cut from Teflon sheets. Each jar and cap has a number engraved on it.

Reagents and Media

(a) *Hexane.*—Pesticide pure grade (AOAC solvent purity test, Ref. 15)

(b) *Sodium sulfate.*—Anhydrous, granular, Soxhlet-extracted overnight with hexane.

(c) *Florisil column.*—Packed by pouring 10 g Florisil (Fisher Scientific Co., Fairlawn NJ, F-100 60–100 mesh) into column, tapping column vertically, and adding layer of sodium sulfate (ca 1 cm) to the top. Activate Florisil 8 h at 450°C, cool in desiccator, deactivate by adding water (2%, v/w) dropwise while shaking, and then shake 0.5 h.

(d) *Glass wool.*—Soxhlet-extracted overnight with hexane.

(e) *PCB reference standards.*—See Ref. 10.

Procedure

Extraction.—Weigh collection (sputum) jars and number them indelibly before sending to the collector. Weigh full jar on receipt from collector, and determine weight of milk. Freeze-dry contents and weigh powder. Place 2.0 g (when available) on a weighing paper; pour powder onto plug of glass wool in Soxhlet extractor, and place second plug on top to retain powder. Extract 15 h at 6 cycles/h. Evaporate extract to <2 mL in Kuderna-Danish apparatus. When extract has cooled to room temperature, adjust volume to 2.0 mL with hexane.

Fat determination.—Measure two 50 μL volumes of extract into tared aluminum cups. Evaporate hexane by placing cups (contained in small stainless steel rack that will accommodate 24 cups) on hot plate at ca 100°C. Reweigh cups to nearest 10 μg on balance such as Cahn Electrobalance (Ventron Instrument Corp., Paramount, CA). Heat cups again, and weigh to constant weight. Take mean of pairs. If weights differ >10% of mean weight, repeat determination.

Cleanup.—Quantitatively transfer remaining 1.9 mL extract to top of 10 g 2% deactivated Florisil column (1 cm id) overlaid with 1 cm hexane-washed granular anhydrous sodium sulfate. Elute column with hexane. All PCB, mirex, photomirex, *p,p'*- and *o,p'*-DDE, hexachlorobenzene, and octachlorostyrene emerge in first 40 mL eluate (14). These are the prevalent chlorinated hydrocarbon pollutants of the New York State region (1, 13, 14). Other common electron-capturing pesticides remain on the column, particularly dieldrin, endrin, *p,p'*- and *o,p'*-DDT and -DDD, hexachlorocyclohexane isomers, (lindane, etc.), and chlordane isomers. These more polar compounds can be collected for further analysis by eluting column with dichloromethane or ethyl ether–hexane (1 + 9) (40 mL).

Analysis.—Evaporate cleaned up extract and adjust to 1.00 mL with hexane in graduated centrifuge cone, which serves as Kuderna-Danish receiver. Transfer solution to 1.5 mL Hewlett-Packard crimp-sealed vial. Make 2 μL automatic injections with following typical conditions:

purge valve switch: 0.5 min
initial temperature: 70°C
hold time: 1 min
program rate: 10°/min for 6 min then 3°/min to 230°C
final time: 10 min, depending on column characteristics
injector temp: 225°C
detector temp: 300°C
flow rate: 20–30 cm/s

Adjust above conditions to achieve the more difficult separations of the following compounds, found in samples from this region, from PCB congeners: (*i*) hexachlorobenzene from 2,2'4'-trichlorobiphenyl and 2,2',5'-trichlorobiphenyl, (*ii*) octachlorostyrene from the first pentachlorobiphenyl of Aroclor 1254 (substitution pattern unknown), (*iii*) *p,p'*-DDE from 2,5,2',3',4'-pentachlorobiphenyl, and (*iv*) mirex from 2,3,6,2',3',4',5'-heptachlorobiphenyl and 3,4,2',3',4',5'-hexachlorobiphenyl.

A typical chromatogram of the mixture of PCB, hexachlorobenzene (HCB), *p,p'*-DDE, octachlorostyrene, and mirex is shown in Figure 1. PCB structures and weights were determined as described previously (10). Calibrate the chromatograph microprocessor with appropriate weights, and transmit results via ASCII interface to cassette of Texas Instruments Silent 700 data terminal or other data storage device.

With PDP 11/45 or other computer, apply dilution factors and accession information to data accumulated during automated GC run, and

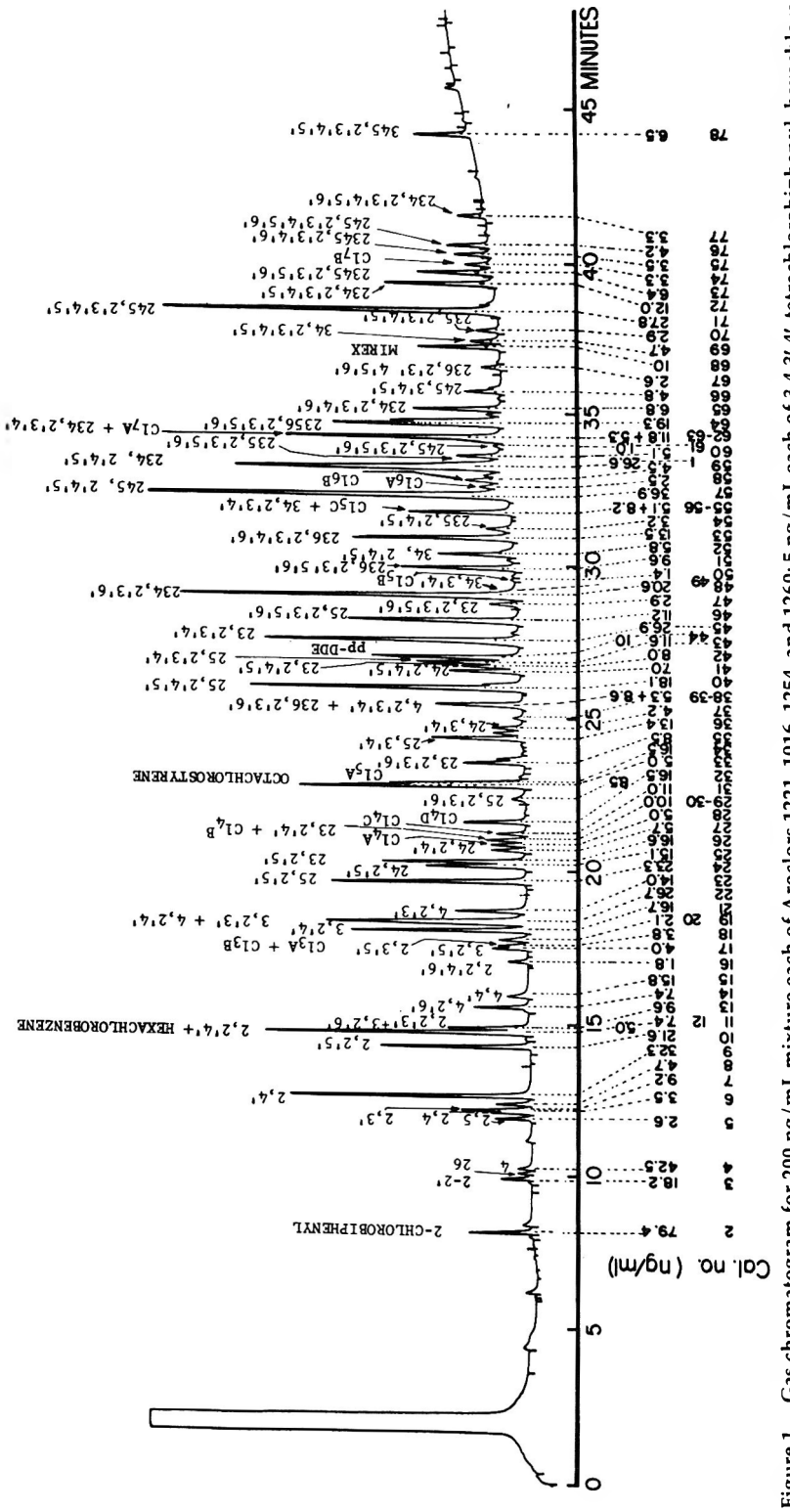

Figure 1. Gas chromatogram for 200 ng/mL mixture each of Aroclors 1221, 1016, 1254, and 1260; 5 ng/mL each of 3,4,3',4'-tetrachlorobiphenyl, hexachlorobenzene, and octachlorostyrene; and 10 ng/mL of *p,p'*-DDE and mirex in hexane. The proposed structure, estimated mass represented by each peak, and our calibration numbers are shown. Column used was 29.1 m × 0.29 mm id borosilicate glass. Chromatographic conditions as given in text.

Table 1. Computer Generated Report.

EXPERIMENTAL SAMPLE HEXANE BLANK SAMPLE RAN 03/16/81

DILUTION FACTOR= 1.0 GLC VIAL # 11

PCB SUBSTITUTION PATTERN	AROCLOR MIX	NG/G	PCB SUBSTITUTION PATTERN	AROCLOR MIX	NG/G
2	21	12.	CL5B	54,60	0.00
2,2'	21	0.00	236,2'3'5'6'	54,60	0.00
4	21,16	0.00	34,2'4'5'	54,60	0.18
2,5	21,16	0.80E-02	236,2'3'4'6'	54,60	0.00
2,4	21,16	0.00	235,2'4'5'	54,60	0.00
2,3'	21,16	0.00	CL5C	54,60	0.00
2,4	21,16	0.00	34,2'3'4'	54,60	0.00
2,4'	21,16	0.58	245,2'4'5'	54,60	0.10
2,2'5'	16	0.00	CL6A + PHOTOMIREX	54,60	0.00
2,2'4'	16	0.00	CL6B	54,60	0.83
2,2'3'+3,2'6'	16	0.16	234,2'4'5'	54,60	0.07
4,2'6'	16	0.40E-02	235,2'3'5'6'	54,60	0.92
4,4'	21,16	0.00	245,2'3'5'6'	60	8.9
2,2'4'6'	16	0.00	CL7A	54,60	0.22
3,2'5'	16	0.00	234,2'3'4'	54	11.
2,3'5'	16	0.00	2356,2'3'5'6'	54,60	0.60E-01
CL3A	16	0.00	234,2'3'4'6'	54,60	0.19
CL3B	16	0.00	245,3'4'5'	54,60	0.00
3,2'4'	16	0.53E-01	236,2'3'4'5'6'	54,60	0.00
3,2'3'+4,2'4'	16	0.22E-01	MIREX	NA	0.00
4,2'3'	16	0.00	34,2'3'4'5'	54,60	0.00
25,2'5'	16,54	0.00	235,2'3'4'5'	54,60	0.00
24,2'5'	16,54	0.00	245,2'3'4'5'	54,60	0.00
23,2'5'	16,54	0.00	234,2'3'4'5'	54,60	0.00
24,2'4'	16,54	0.57	2345,2'3'5'6'	60	0.00
CL4A	16,54	0.00	CL7B	60	0.0
23,2'3'	16,54	0.00	2345,2'3'4'6'	60	0.00
CL4C	16,54	0.86E-01	245,2'3'4'5'6'	60	0.00
CL4D	16,54	0.00	234,2'3'4'5'6'	60	1.5
CL5A	54,60	0.00	345,2'3'4'5'	60	0.16
23,2'3'6'	54,60	0.41	HEXACHLOROBENZENE	NA	0.90E-02
25,3'4'	54,60	0.00	OCTACHLOROSTYRENE	NA	0.66E-01
24,3'4'	54,60	0.79	34,3'4'	48	0.22
4,2'3'4'	54,60	0.19E-01	CL4B		0.00
236,2'3'6'	54,60	0.41			
25,2'4'5'	54,60	0.70E-02			
24,2'4'5'	54,60	0.00			
23,2'4'5'	54,60	0.00			
25,2'3'4'	54,60	8.0			
DDE	NA	0.00			
23,2'3'4'	54,60	0.13			
25,2'3'5'6'	54,60	0.27			
23,2'3'5'6'	54,60	0.80			
234,2'3'6'	54,60	0.23			

TOTAL PCB 56. EDIT 46.6
TRUE TOTAL 9.4ng/g
TOTAL HEXACHLOROBENZENE <1
TOTAL OCTACHLOROSTYRENE <1
TOTAL MIREX <1
TOTAL DDE <1

format results for report (e.g., Table 1). Report in Table 1 is designed to display data at 2 levels of understanding: as congener-by-congener analysis, with indication of Aroclor mixtures which could have given rise to the residue, and as total PCBs, obtained by summing all congener concentrations. The report summarizes the non-PCB analyses after the PCB total to gain an overall impression of contamination in the sample.

Edit report, and check chromatograms of hexane blanks in particular (Figure 2 and Table 1). Thus far, peaks have been automatically identified as PCB congeners by the integrator, which operates by first attempting to identify a peak designated during calibration as reference peak. It chooses a large peak within a 5% retention-time window of the designated retention time. Retention times of all other calibrated peaks are then adjusted in proportion to differ-

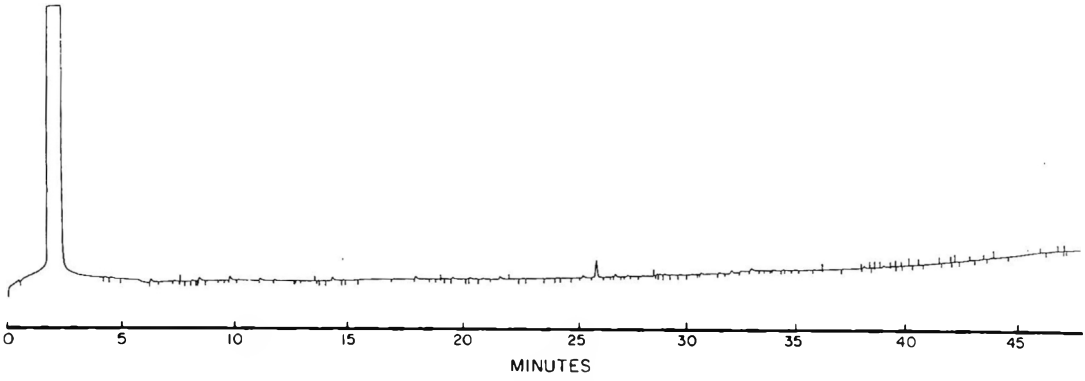

Figure 2. Chromatogram of hexane blank equivalent to processing 30 g milk.

ences in retention times observed between calibrated reference peak retention time and its known retention time.

In baseline studies, no peak or the wrong peak may be identified as the reference, in which case all resultant expected retention times will be incorrect. Wrong identification is rarely a problem with samples, because a peak that occurs in all biological samples is chosen as a reference (2,3,4,2′,4′,5′-hexachlorobiphenyl), but it may be a problem with blanks. In the example illustrated in Figure 2, a peak with the retention time of the reference peak was identified, and the process of editing the report could begin.

If a peak occurs within a 2% retention-time window of a calibrated congener (e.g., the peak at 8.18 min corresponding to 2-chlorobiphenyl), it will be reported; many which do not fall inside the 2% windows are eliminated from the analysis automatically (e.g., the peaks at 9.71 and 26.06 min). Edit out any remaining peak that is clearly not due to PCB congeners, e.g., a small signal resulting from solvent impurities or a baseline shift. (At 38.21 min, a peak corresponding to Cl_7B has been integrated. Reference to the chromatogram shows this to be spurious, being caused by an unexplained baseline shift.) Edit out highly improbable values, such as one for the presence of 2-chlorobiphenyl when the other components of Aroclor 1221 are absent. Eliminate any integrated peak that is distorted or has a shape uncharacteristic of a PCB congener (in Figure 2 there are peaks at 34.11 min: 234,2′3′-4′-hexachlorobiphenyl, 33.69 min: 245,2′3′5′-6′-heptachlorobiphenyl, and at 26.78 min: 25,2′3′4′-pentachlorobiphenyl). No other major contributors to the total background PCB sum can be eliminated; a total of 46.6 ng/g of spurious signal is edited from the report.

Subtract the edited peaks to get total PCB signal from hexane reagent blank. In Figure 2 this is 9.4 ng/mL, which corresponds to a concentration of 0.31 ng/g for 30 g milk and represents the detection limit for total PCBs for a 30 g sample. Run a blank after every fifth milk sample; subsequent analyses are acceptable so long as hexane blank total after editing is <50 ng/mL (i.e. < about 2 ppb total PCB in whole milk).

Milk chromatograms normally require only minor editing for extraordinary irregularities, such as negative peaks (see 32 min in Figure 3), and then only if area reported is obviously very different from reported areas of similar-sized peaks in chromatogram.

For proper statistical analysis of the large number of data points across a large number of samples, reformat data in report to be readable by a packaged statistical program, such as the BMP79 Statistical Package (17).

Quality control.—This type of complex analysis calls for an innovative quality control strategy. Before starting an automatic run, analyze 3 types of standards: (i) a hexane blank; (ii) a mixture of all the compounds to be determined, in this case, Aroclors 1221, 1016, 1254, and 1260, each at 200 ng/mL, plus 3,4,3′,4′-tetrachlorobiphenyl (a congener unique to Aroclors 1242 and 1248), hexachlorobenzene, and octachlorostyrene, each at 5 ng/mL, plus *p,p′*-DDE and mirex at 10 ng/mL; and (iii) a standard solution resembling samples being analyzed, in this case, a hexane solution of a few separate PCB congeners and insecticides (a quasi-milk mixture) as a final check of integrator operation on a chromatogram exhibiting isolated peaks with a hexane background in between.

Compute data for the 3 chromatograms, and plot on the quality control chart the total PCBs

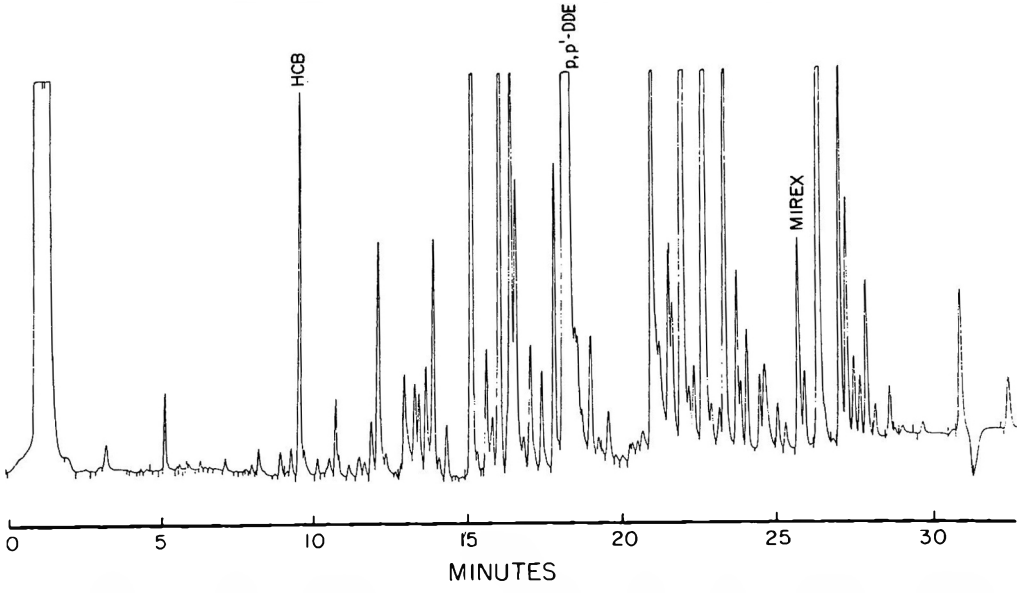

Figure 3. Gas chromatogram of typical human milk on typical 30 m × 0.3 mm id Apiezon L-coated glass capillary column. (Not same column used in Figure 1 and 2. Dilution factor 0.03.)

in the hexane blank, total PCBs in the complex mixture, and individual insecticide and PCB congeners in the quasi-milk mixture. Analyze these control solutions after every sixth milk sample, and plot results after run is complete (Figure 4). If quality control is lost as defined by the requirements of the particular analytical situation, repeat GC analysis. Spiked milk samples are not practical for quality control because human milk is not readily available.

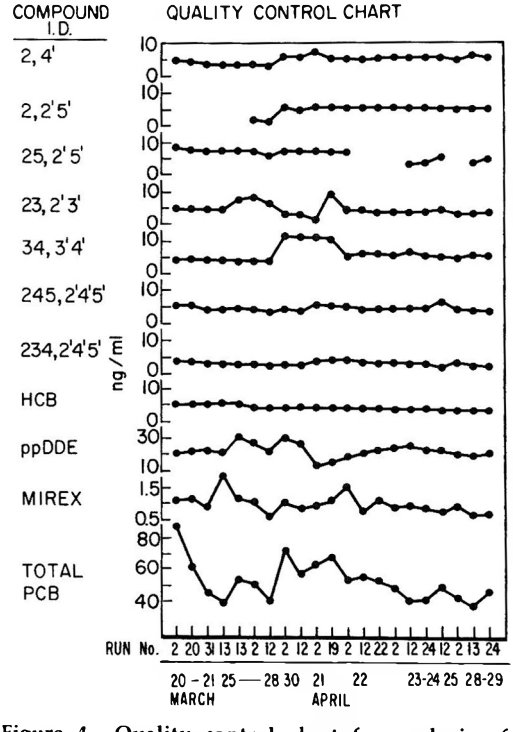

Figure 4. Quality control chart for analysis of quasi-milk mixture.

Spiking.—To determine efficiency of analytical recovery, use realistically fortified cow's milk. To spike 100 g cow's milk, add 100 μL of a 100 μg/mL solution of PCBs in acetone very slowly from a 100 μL syringe, while homogenizing milk with Tissumizer. This method will produce a spike that is as intimately bound to milk constituents as possible.

Results and Discussion

Recovery Efficiency

In 3 separate experiments, the recovery of major PCB congeners from cow's milk spiked with Aroclors 1221, 1016, 1254, and 1260 (Table 2) showed that the extraction method gives repeatable results for the same congeners in different PCB mixtures. 2,5,2',5'-Tetrachlorobiphenyl in Aroclor 1016 had a background peak interferent, which accounts for an apparent recovery greater than 100%. There is an apparent loss of the more volatile mono- and dichlorobiphenyls, probably during freeze-drying. In our opinion, this drawback is compensated by the method's simplicity and by the low detection

Table 2. Recovery of major compounds of Aroclors in 3 experiments

Aroclor	Congener	% Recovery 1	2	3
1221	2	33	33	26
	4	29	29	29
	2,2′	50	50	57
	2,5	43	29	29
	2,4	51	49	51
1016	2,4′	35	37	35
	2,2′5′	52	52	50
	4,2′,6′	71	71	71
	3,2′,4′	83	88	85
	2,5,2′,5′	107	112	107
1254	2,5,2′,5′	83	83	83
	2,5,2′,3′,6′	73	74	74
	2,3,2′,3′,6′	73	73	73
	2,5,3′,4′	96	93	96
	2,5,2′,4′,5′	72	71	71
	2,3,2′,3′,4′	70	68	70
	2,5,2′,3′,5′,6′	71	71	71
	2,3,6,2′,3′,5′,6′	73	68	73
	2,3,6,2′,3′,2′,6′	72	72	72
	2,4,5,2′,4′,5′	65	66	66
	2,3,4,2′,4′,5′	68	68	65
	2,3,4,2′,3′,4′	65	71	77
1260	2,5,2′,3′,6′	77	80	80
	2,5,2′,4′,5′	70	81	76
	2,3,2′,3′,4′	81	89	89
	2,5,2′,3′,5′,6′	64	75	71
	2,3,6,2′,3′,5′,6′	72	80	76
	2,4,5,2′,4′,5′	66	77	69
	2,3,4,2′,4′,5′	66	73	69
	2,3,4,2′,3′,4′	66	74	65
	2,3,5,6,2′,3′,5′,6′	64	71	67
	2,4,5,2′,3′,4′,5′	61	67	62
	2,3,4,2′,3′,4′,5′	68	74	68

limits attainable because of the small quantity of reagents and solvents required.

Precision

The overall precision of the method may be affected by variance introduced during sample workup and during GC analysis. The procedure with the least precision is the Soxhlet extraction, which must be optimized by assuring at least 6 siphon cycles/h and by locating the milk powder not more than 1 cm from the bottom of the extraction chamber. The GC integrator precision may be affected both by large shifts in baseline (due to late-eluting materials from samples) and by poor identification of peaks (due to retention time shifts). Either problem can be minimized by observations made while editing the report.

Overall precision for each of 32 major congeners is better than 5% RSD (Table 2). Precision for total PCBs is somewhat worse; it was similar to that predicted by summing the variances of the individual measurements (10). Table 3 shows the statistics for 10 replicate analyses of a standard mixture. Table 4 shows the statistics produced for several samples of milk from 2 mothers.

Table 3. Mean and standard deviation (ng/mL) for 10 replicate determinations of selected congeners of a standard mixture (see Figure 1)

Congener[a]	$\bar{X}$	±SD
2,2′,5′-TCB	20	0.5
2,5,2′,5′-TCB	21	0.3
2,5,2′,4′,5′-PCB	17	0.5
3,4,2′,4′,5′-PCB	5	0.3
3,4,2′,3′,4′-PCB	7.9	0.7
2,4,5,2′,4′,5′-HCB	34	4.0
2,3,4,2′,4′,5′-HCB	24	1.0
Total PCBs	755	15

[a] TCB, trichlorobiphenyl and tetrachlorobiphenyl; PCB, pentachlorobiphenyl; HCB, hexachlorobiphenyl.

Detection Limit

A chromatogram of a solvent and reagent blank equivalent to processing 30 g whole milk (Figure 2) and the report produced from this reagent blank via the ASCII interface of the chromatograph (Table 1) illustrate 2 problems associated with detection limit determination when a microprocessor integrator is used for area measurements.

The first and simpler problem is defining a detection limit for an individual compound which gives a Gaussian peak, well separated from other peaks in the chromatogram. If the rate of change of signal at the commencement of the peak becomes less than the chosen slope sensitivity or maximum slope sensitivity (0.01), the peak will suddenly become undetectable. This sensitivity will change for the same peak in different chromatograms. In a crowded chromatogram, the peak may be easier to define because slope changes caused by neighboring peaks

Table 4. Mean and standard deviation (ng/g adjusted to 3% fat) for determination of selected PCBs and total PCBs in human milk

Congener[a]	Mother A (n = 3) $\bar{X}$	SD	Mother B (n = 7) $\bar{X}$	SD
3,4,2′,3′,4′-PCB	1.2	0.23	0.45	0.22
2,4,5,2′,4′,5′-HCB	4.5	0.56	1.06	0.45
2,3,4,2′,4′,5′-HCB	2.8	0.29	0.70	0.21
Total PCBs	36	1.1	16	3.3
Fat (%)	2.0	0.6	4.4	2.9

[a] PCB, pentachlorobiphenyl; HCB, hexachlorobiphenyl.

may facilitate definition of the beginning and end of the zone. However, when the peak is poorly separated from one or two neighbors and the relative size of the neighbors changes, the peak may disappear into a neighbor or may incorporate the neighbor. For example, in Figure 1, octachlorostyrene could interact with the adjacent pentachlorobiphenyl with unknown structure (Cl_5A).

Where a particular compound such as mirex is of great concern, conditions are adjusted and, if necessary, columns are recoated to achieve a separation at least as good as that shown in Figure 1. Mirex detectability at 10 ng/mL would be impaired if the concentration of the 3,4,2',3',-4',5'-hexachlorobiphenyl were increased to 5 times that illustrated. As it is, in the presence of 200 ng Aroclor 1260/mL the detection limit is well below 1.0 ng/mL.

The second problem is to define the detection limits of total PCBs. In conventional analysis, where a simple analog signal is being measured with a device such as a chart recorder, the detection limit is defined as some multiple of the background noise (18). Here, where total PCBs is the sum of a large number of measurements, such a definition is not practicable. Following previous practice (4, 19), we have defined it empirically as the apparent total PCB value produced by a reagent blank brought through the analytical process. It depends, of course, on the weight of the milk processed, here assumed to be 30 g wet weight. Results are rejected if a preceding blank run, after editing, exceeds some pre-ordained maximum, in this work 50 ng/mL of hexane, corresponding to about 2 ng/g wet weight of a whole-milk sample weighing about 30 g.

Conclusion

This method allows analysis in milk for 78 chlorinated hydrocarbons at high sensitivity and accuracy in less than 1 h of GC time, excluding sample preparation. With a time-share computer, the large volume of data can be analyzed statistically, so that congener-by-congener analysis can be handled in a realistic time.

Acknowledgments

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MYCOTOXINS

International Mycotoxin Check Sample Survey Program. Part III. Report on Performance of Participating Laboratories for Determining Ochratoxin A in Animal Feed

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A sample of animal feed made from barley, naturally contaminated at about 1000 μg ochratoxin A/kg, was analyzed by 44 laboratories in 24 countries. The AOAC method for determining ochratoxin A in barley was used by 27 (61%) of the participating laboratories. Results from laboratories using this method were grouped around 2 maxima: one below 100 μg/kg and a second near 940 μg/kg, the median concentration for results reported by all 44 participating laboratories. Results from laboratories using methods other than the AOAC method were more widely distributed with no apparent maximum near 1000 μg/kg.

As the number of mycotoxins found to contaminate food increases, more laboratories around the world are undertaking or expanding existing programs for their analysis. These laboratories can be greatly assisted by periodic control or comparison of their results with those of other laboratories doing similar analyses. Such a program, organized on an on-going basis by the International Agency for Research on Cancer (1, 2), exists for those laboratories involved in the analysis for aflatoxins. More than 70 laboratories participating in that program requested that a similar program to assure analytical quality be organized for laboratories which analyze foodstuffs for ochratoxin A. Consumption of this mycotoxin, which has been reported to contaminate a number of cereal grains (barley, wheat, maize) (3), has been associated with cancer in several species of animals (4–6). In view of this interest, the International Mycotoxin Check Sample program has been expanded to include the analysis of foodstuffs contaminated with this mycotoxin.

Methods

In the current study, 120 g portions of a bulk sample of commercially prepared, naturally contaminated animal feed made from barley were distributed to 69 laboratories. Before dispatch by airmail to these laboratories, samples were ground, thoroughly mixed, and checked for homogeneity. Four samples chosen at random showed ochratoxin A concentrations of 1140, 1110, 1090, and 1130 μg/kg; mean 1120; SD 20; CV 2%. Participants were requested to analyze each sample twice, either in duplicate using the same method or by using 2 different methods.

A standard sample of ochratoxin A was provided to participants if requested, but they were instructed to use it only after having verified its concentration after dilution. Standards furnished by IARC were used in about 85% of the analyses.

Exclusion of Outlying Results

Outlying results were excluded, according to the Dixon test (7), before statistical analysis using techniques developed by the Association of Official Analytical Chemists (AOAC). Results from 2 laboratories, 7080 and 12 300 μg/kg, were excluded in this way.

To avoid giving undue weight in the statistical analysis to results from laboratories reporting duplicate results for a given method as opposed to those from laboratories reporting a single result per method, the second reported result of duplicates from a single method has been excluded from distribution curves and the statistical analyses which follow. The option of using the mean of duplicate results rather than only the first reported value was discarded because it is not statistically sound to directly compare single values with mean values. Both results had to be retained, however, for laboratories that submitted duplicate results from 2 different methods.

Results

Results were received from 44 laboratories in 24 countries. The official AOAC method for

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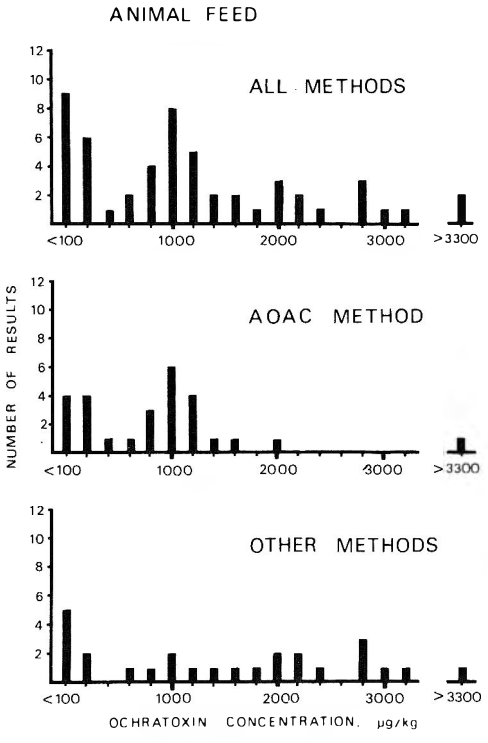

Figure 1. Frequency distribution by method of analysis for results of determination of ochratoxin A in animal feed made from barley. Single bars at right of each graph represent out-lying values not considered in statistical analysis (Tables 1 and 2).

determining ochratoxin A in barley (8–10), used by 27 laboratories, was the only method used by enough participants to justify separate statistical analysis. Results from other methods, which include thin layer chromatography (11–15) and high performance liquid chromatography (16, 17), were grouped for statistical analysis. Results from unpublished methods were also included in this group.

Figure 1 shows frequency distributions of these results. Results were rounded to the nearest 200 µg/kg and thus each bar corresponds to a group of results falling within a small range of concentrations. For example, in the graph for all methods, the bar of height 8 corresponding to a concentration of 1000 µg/kg indicates that 8 results lie within the range 900–1100 µg/kg. In addition, the first bar on the left of each distribution represents those laboratories reporting less than 100 µg/kg, and the bar on the far right represents results which were excluded as outliers (> 3300 µg/kg).

Two-thirds of the participating laboratories analyzed the sample in duplicate using a single method. For this subgroup of laboratories, Table 1 gives the repeatability (within-laboratory precision) and reproducibility (total or within plus between-laboratory precision), expressed as percent coefficient of variation.

Table 1. Within-laboratory (repeatability) and within- plus between-laboratories (reproducibility) precision, as coefficient of variation (CV), for subgroup of laboratories reporting duplicate results from a single method

Statistic	AOAC method	Other methods	All methods
Within-laboratory CV, %	8.9	8.0	8.7
Total CV, %	65.5	82.1	84.2

The overall statistical analysis of the results is given in Table 2. The median, mean, standard deviation, percent coefficient of variation, and number of results involved have been calculated for each of the 2 method subgroups, as well as for the group including all methods.

Discussion

It should be noted and emphasized that this report does not refer to a collaborative study and that participants in the program were allowed to and often did make modifications to published methods. In addition, the quality of results grouped in a given category of methods can and does vary widely, because the laboratories involved range from those just beginning aflatoxin analysis to those who have carried out such analyses routinely for many years. Statistical analyses reported in this paper should therefore always be interpreted as comparisons between *laboratories* using different methods and not as comparisons between the methods themselves.

Two maxima are observed in the distribution of reported results: one at less than 100 µg/kg and a second near 1000 µg/kg. For such a dis-

Table 2. Statistical analysis of results for determination of ochratoxin A in animal feed made from barley, by method of chemical analysis

Method	Concn of ochratoxin A, µg/kg: Median	Mean	SD	CV, %	*N*
AOAC method	890	770	530	69	26
Other methods	1300	1400	1100	79	25
All methods	940	1100	910	84	51

tribution, the actual ochratoxin concentration is probably better represented by the median value rather than by the mean. In fact, the median of the results from laboratories using the AOAC method falls near the maximum in the distribution at 1000 μg/kg. Significantly, no maximum is observed near 1000 μg/kg in the distribution of results from laboratories not using the AOAC method.

Laboratories involved in mycotoxin analysis are encouraged to participate in future check sample surveys. Interested laboratories should contact the authors.

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EGGS AND EGG PRODUCTS

Gas-Liquid Chromatographic Determination of Trace Amounts of Nitrite in Egg, Egg White, and Egg Yolk

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A simple, sensitive, and practical method is described for determination of nitrite in egg, egg white, and egg yolk. Egg is deproteinized by adding a mixture of ammonium thiocyanate, mercuric chloride, and zinc acetate, and centrifuged. Nitrite in the supernate is converted to tetrazolophthalazine by reaction with hydralazine in acidic solution and then determined by gas-liquid chromatography with an electron-capture detector (GLC-ECD) and a column of OV-225 on Chromosorb W(HP). Nitrite concentrations from 5 to 50 ng/mL are calculated from peak height; the detection limit is 3 ng/mL extract. Recoveries from eggs, egg whites, and egg yolks ranged from 91.7 to 98.0%. The mean nitrite concentration in 50 egg samples was 0.04 ppm (0.01–0.11 ppm) with a detection limit of 4 ng nitrite/g.

The toxicity of nitrite toward humans is due primarily to the possibility that it can cause methaemoglobinanemia in infants and can form carcinogenic nitrosamines under certain conditions; therefore, its determination at trace levels is of great interest. Nitrite in food is commonly determined by a colorimetric method based on the formation of an azo dye produced by coupling with Griess reagent (1–3) or diazotization of sulfanilic acid and subsequent coupling with *N*-(1-naphthyl)ethylenediamine (4, 5). However, all of these colorimetric methods are limited by the fact that occasionally turbid samples, such as biological, and slightly colored samples can affect the color of the azo dye and, consequently, the accuracy of the nitrite determination. Recently, gas-liquid chromatography (GLC) has been used for this purpose (6–17). Several groups have studied oxidation of nitrite to nitrate and nitration of benzene (or a benzene derivative) in the presence of sulfuric acid as catalyst (7–9, 11, 12). However, this technique is nonspecific for nitrite and requires vigorous derivatization conditions. The method that involved formation of triazole derivatives by reaction with *o*-phenylenediamine was specific for nitrite, but was not suitable for trace nitrite analysis (6, 10, 13). A GLC method for nitrite reported recently by Funazo et al. (17) was not suitable for routine food analysis because of interference by nitrate.

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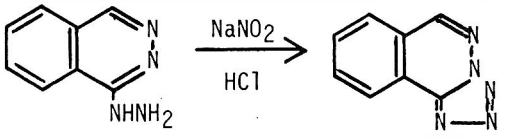
We found that tetrazolophthalazine (18) can be prepared quantitatively by a reaction with nitrite and hydralazine and extracted with several organic solvents over a wide pH range (14–16). This specific reaction for nitrite and the



favorable GLC properties permits quantitation by GLC with electron capture detection (19).

Because the spectrophotometric methods were not suitable for determination of nitrite in egg samples, which give turbid extract solutions, we studied the combination of hydralazine reaction and GLC. Nitrite was extracted from deproteinized egg with aqueous solution, reacted, and analyzed by GLC-ECD without further cleanup.

METHOD

Apparatus

(a) *Gas chromatograph.*—Shimadzu GC-4BM equipped with ^{63}Ni electron capture detector. Operating conditions: temperatures (°C)—column 210, detector 300, injector 300; nitrogen flow rate ca 50 mL/min.

(b) *GLC column.*—Glass, 100 cm × 2 mm id, packed with 3% OV-225 on 80–100 mesh Chromosorb W(HP).

(c) *Gas chromatograph–mass spectrometer (GLC-MS).*—Shimadzu LKB-9000.

(d) *High-speed blender.*—Nihon Seiki model equipped with stainless cup (Nihon Seiki Co., Ltd, Tokyo, Japan).

Reagents

(Double-distilled water, the last distillation carried out in glass, was used throughout.)

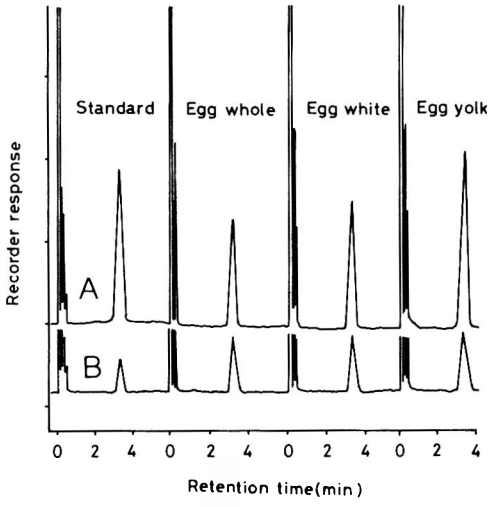


Figure 1. Gas chromatograms of A, xylene extracts of standard reaction mixture, to which nitrite was added at 0.05 ppm to various egg samples; and B, reagent blanks. Sample size 1 μL.

(a) *Hydralazine solution.*—Dissolve 1.0 g hydralazine (Tokyo Kasei Kogyo Co., Ltd) in 100 mL water.

(b) *Deproteinizing solution A.*—Dissolve 90 g ammonium thiocyanate and 80 g mercuric chloride in 1 L water.

(c) *Deproteinizing solution B.*—Dissolve 125 g zinc acetate in 500 mL water.

(d) *Standard sodium nitrite solution.*—(1) *Stock solution.*—0.1 mg NO_2/mL. Dissolve 0.15 g sodium nitrite, previously dried 1 h at 110°C, in enough water to make 1 L solution. (2) *Working solution.*—0.1 μg NO_2/mL. Transfer 1.0 mL stock solution to 1 L volumetric flask, dilute to volume with water, and mix.

(e) *Solvents.*—Distilled-in-glass xylene and other solvents.

Sample Extraction

Place egg sample in stainless cup and homogenize in blender to form slurry. Weigh 5 g homogenate into 10 mL glass-stopper test tube (11.5 × 1.5 cm id). Add 1 mL each of deproteinizing solutions A and B, followed by 3 mL water; shake vigorously 2–3 min. Let stand 30 min at room temperature, and then centrifuge 10 min at 1400 × g.

Derivative Formation

Transfer 2 mL extract to test tube. Add 0.5 mL hydralazine solution and 1 mL 2M HCl (if necessary, add 1 mL 5% mercuric chloride solution to counteract sulfide interference). React in 70°C water bath with occasional shaking for 20 min and then cool to room temperature. Add 2 g sodium chloride and 2 mL xylene, and shake vigorously 1 min. Centrifuge contents 10 min at 1400 × g. Pipet xylene phase into pyrex test tube (10 × 0.9 cm id) and dry with small amount of anhydrous sodium sulfate.

Gas-Liquid Chromatography

Inject 1 μL derivative solution into gas chromatograph with electron capture detector. Determine nitrite by comparing with standard curve prepared as follows: Prepare 2 mL solutions of 5, 15, 25, 35, and 50 ng nitrite/mL by pipetting working standard solution into test tube, adding 0.5 mL hydralazine solution, and proceeding as described above. Plot peak height (cm) against μg NO_2.

Results and Discussion

The tetrazolophthalazine product of the reaction of nitrite with hydralazine was extracted with organic solvent and analyzed by GLC-ECD (14–16, 19). Typical chromatograms are shown in Figure 1. Sharp symmetrical peaks allow peak heights (cm) to be used for calculations. Response was linear between 5 and 50 ng nitrite and the detection limit was 3 ng nitrite/mL extract. The relative standard deviation for 5 determinations was 3.4% for 5 or 10 ng nitrite and 4.6% for 50 ng, and the reproducibility was considered satisfactory.

To obtain good accuracy and sensitivity for determination of nitrite, various deproteinizing reagents reported by other investigators (11, 12,

Table 1. Effect of various deproteinizing reagents on recovery of added nitrite in whole egg [a]

Deproteinizing reagent	Condition of egg ext	Rec., %
1. $K_4Fe(CN)_6$(12%) $Zn(CH_3COO)_2$(22%)	turbid, colorless	58.1
2. $K_4Fe(CN)_6$(6%) $Zn(CH_3COO)_2$(12%)	turbid, yellow	54.8
3. $ZnSO_4$(12%) 1N NaOH	turbid, yellow	3.2
4. NH_4SCN(9%) plus $HgCl_2$(8%) $Zn(CH_3COO)_2$(25%)	transparent, colorless	97.2
5. $HgCl_2$(5%) Na_2CO_3(0.5M)	turbid, yellow	29.0
6. $Ba(OH)_2$(16%) $ZnSO_4$(12%)	transparent, light yellow	90.3
7. $K_2Al_2(SO_4)_4$(Sat.)	turbid, yellow	6.4

[a] 25 ng nitrite and 1 mL deproteinizing reagent were added to 5 g whole egg sample, which was processed by described procedure.

Table 2. Recovery of added nitrite from whole egg, egg white, and egg yolk

Sample	Added, μg/g	No. of anal.	Rec., %[a]
Whites	0.01	5	91.7 ± 4.2
	0.02	5	95.1 ± 2.9
	0.05	5	96.9 ± 3.4
	0.10	4	97.8 ± 2.5
Yolks	0.01	6	92.8 ± 5.0
	0.02	6	94.7 ± 4.9
	0.05	5	97.1 ± 3.2
	0.10	4	96.4 ± 2.9
Whole	0.01	6	92.3 ± 3.7
	0.02	6	95.7 ± 2.4
	0.05	5	98.0 ± 2.0
	0.10	4	96.3 ± 2.8

[a] ± Standard deviation.

Table 3. Nitrite levels in whole egg

Concn range, ppm	No. of eggs
≤0.01 ~ <0.02	3
≤0.02 ~ <0.03	13
≤0.03 ~ <0.04	10
≤0.04 ~ <0.05	7
≤0.05 ~ <0.06	9
≤0.06 ~ <0.07	3
≤0.07 ~ <0.08	1
≤0.08 ~ <0.09	2
≤0.09 ~ <0.10	1
≤0.10 ~	1
$\bar{x}$ = 0.04 ppm (n = 50)	

20, 21) were tried with 25 ng nitrite in 5 g whole egg. All combinations gave turbid and/or colored extracts, except ammonium thiocyanate and mercuric chloride with zinc acetate, as shown in Table 1. Furthermore, the influence of various deproteinizing reagents on the formation of tetrazolophthalazine was studied, and the recovery yield of nitrite in whole egg was higher with the above-mentioned combination than with other deproteinizing reagents (Table 1). An adequate volume of deproteinizing reagent was 1 mL each of ammonium thiocyanate + mercuric chloride and zinc acetate. Because 20 min is the minimum time for obtaining a transparent extract from egg, 30 min was used in this method.

Benzene, toluene, xylene, ethyl acetate, butyl acetate, and methyl isobutyl ketone were tried as extraction solvents for tetrazolophthalazine. Extraction yields for ethyl acetate and butyl acetate were lower than those for benzene, toluene, and xylene, and extraction with methyl isobutyl ketone prevented the determination of the tetrazolophthalazine peak because of overlapping interfering peaks. Xylene was preferred because it did not co-extract interferences from egg and did not form an emulsion with water.

To investigate the effect of various possible interfering ions in egg, fluoride (0.1 mg), chloride (1.0 mg), bromide (0.1 mg), iodide (0.1 mg), carbonate (1.0 mg), nitrate (1.0 mg), sulfate (0.1 mg), sulfide (0.1 mg), sodium (1.0 mg), zinc (1.0 mg) and ammonium (1.0 mg) were mixed with 25 ng nitrite under the same reaction conditions described. These ions, except for sulfide, did not interfere with the formation of tetrazolophthalazine (nitrite recoveries ranged from 94.8 to 103.7% with an average recovery of 100.2%). On the other hand, addition of 0.1 mg sulfide gave recoveries of nitrite ranging from 24.8 to 33.4%. However, this interference can be prevented by addition of mercuric chloride; then recoveries averaged 92.5%.

The method described has been tested for analysis of whole egg, egg white, and egg yolk. No samples showed any interference with the determination of nitrite (Figure 1). To check validity of the proposed method, recoveries of nitrite added to 5 g samples were determined, as shown in Table 2. The detection limit was 4 ng nitrite/g egg sample. Fifty egg samples were collected from the market and analyzed by the proposed method. Results given in Table 3 show a mean concentration of 0.04 ppm (ranging from 0.01 to 0.11 ppm). Furthermore, GLC–mass spectrometry showed the tetrazolophthalazine peak to be free from interfering substances.

The proposed method facilitates the determination of nitrite in egg, without interferences. The GLC method is simple and sensitive, and the entire procedure can be completed within 80 min.

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DRUGS

High Pressure Liquid Chromatographic Assay for Prednisone in Bulk Drug Substances and Tablets

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A high pressure liquid chromatographic (HPLC) method was developed for the assay of prednisone in bulk drug substances and tablets. The sample was dissolved in water-methanol and an aliquot was analyzed by using HPLC. The average recovery of prednisone added to a prednisone tablet composite was 99.5% with a coefficient of variation of 1.07%. Prednisone was determined in 46 tablets (1-50 mg prednisone/tablet) formulated by 22 manufacturers, using the HPLC method and the USP blue tetrazolium assay. The results show that the HPLC method is more specific and faster than the USP method.

A review of information furnished by formulators of prednisone tablets showed that prednisone drug substance used in the United States in 1979 was manufactured by 4 suppliers, 1 domestic and 3 foreign. Approximately 22 manufacturers of prednisone tablets and a larger number of distributors were in operation that year.

The blue tetrazolium reaction is widely used for the determination of corticosteroids, and it is the official USP XX method (1) of analysis for prednisone drug substance and tablets. The official compendium employs the slightly modified procedure of Mader and Buck (2) for the quantitation of prednisone. Blue tetrazolium oxidizes the α-ketol moiety of the C_{17} side chain of corticosteroids in strongly alkaline solution and is quantitatively reduced to a highly colored formazan whose concentration is measured spectrophotometrically. The reaction is subject to interference from reducing materials (3) as well as substances that change the pH of the reaction solution (4).

Several investigators have used high pressure liquid chromatography (HPLC) to determine prednisone in corticosteroid mixtures (5-9) and degradation products from certain steroids (10-12). This paper reports the development of an HPLC method for determining prednisone in bulk drug substances and tablets. The multicomponent mobile solvent system was optimized by using a procedure similar to that reported by previous workers (13, 14). Forty-six brands of prednisone were analyzed by this HPLC method and by the USP blue tetrazolium method, and the results were compared.

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Experimental

Apparatus

(a) *High pressure liquid chromatograph.*—Model 204 (Waters Associates, Milford, MA 01757), equipped with Model M-6000A 6000 psi pump, Model U6K loop injector, Model WISP 710A and B automatic injector, Model 440 ultraviolet (UV) light detector with sensitivity range from 0.005 to 2.0 absorbance units full scale (AUFS) at 254 nm, and Model 730 recording integrator.

(b) *Reverse phase HPLC columns.*—4.6 mm × 25 cm 5 μm C_{18} octadecylsilane (Zorbax ODS. E.I. duPont de Nemours & Co., Wilmington, DE 19898; Ultrasphere, Altex Scientific, Berkeley, CA 94710), 8 mm × 10 cm 10 μm C_{18} octadecylsilane (RCM-100, Waters Associates), 4.6 mm × 25 cm 5 μm trimethylsilane (Zorbax TMS, E.I. duPont de Nemours), 4.6 mm × 25 cm 5 μm C_8 octylsilane (Zorbax C-8, E.I. duPont de Nemours).

(c) *Recording spectrophotometer.*—Cary 15 (Varian Instruments, Palo Alto, CA 94303).

(d) *Filters.*—Type LS, 5 μm pore size (Millipore Corp., Bedford, MA 01730).

Reagents

(a) *Acetonitrile, methanol, and tetrahydrofuran (THF).*—HPLC or distilled-in-glass grade. Peroxides were removed from THF by passing it through aluminum oxide column containing 10 g aluminum oxide/100 mL THF (15).

(b) *Mobile solvent.*—Mix 250 mL peroxide free THF with 62 mL methanol and dilute to 1 L with water. Pass through 5 μm filter before use.

(c) *Standards.*—1,4-Pregnadiene - 3,11 - dione-17-carboxylic acid (synthesized by M. Maienthal, Food and Drug Administration (FDA), Washington, DC); tetramethyl ammonium hydroxide and acetanilide (Eastman Organic Chemicals, Rochester, NY 14650); 1,4-androstadiene-

3,11,17-trione (Research Plus Steroids, Denville, NJ 07834); blue tetrazolium (Dajac Laboratories, Philadelphia, PA 19124); prednisone, prednisolone, hydrocortisone, cortisone acetate, hydrocortisone acetate, prednisolone acetate, and prednisone acetate (USP Reference Standards).

(**d**) *Internal standard solution.*—Dissolve 22 mg acetanilide in 100 mL methanol and dilute to 200 mL with water.

(**e**) *Prednisone standard.*—Dissolve dried, accurately weighed amount of USP Reference Standard prednisone in methanol-water (1 + 1) to yield ca 0.2 mg/mL solution. Sonicate 1 min to dissolve prednisone, if necessary. Transfer 5 mL to 50 mL volmetric flask, add 5 mL internal standard, and dilute to volume with methanol-water (1 + 1). Prepare dilute solution fresh daily.

Sample Preparation

Weigh and finely powder ≥20 prednisone tablets. Transfer accurately weighed portion of powder, equivalent to ca 20 mg prednisone, to 100 mL volumetric flask. Add 5 mL water, swirl, and sonicate 1 min to dissolve powder; then add 50 mL methanol and repeat sonication 1 min. Dilute to volume with water and mix. Transfer 5.0 mL to 50 mL volumetric flask, add 5.0 mL internal standard solution, and dilute to volume with methanol-water (1 + 1). Filter through 5 μm filter in syringe filter holder attached to glass syringe before HPLC.

Content Uniformity

Place 1 tablet in volumetric flask of size that yields ca 0.2 mg/mL prednisone concentration. Proceed as directed under *Sample Preparation*, starting with "Add 5 mL water . . .," except for 1 mg tablets, dilute directly to 50 mL after adding 5 mL internal standard solution.

System Suitability Test

Let chromatographic system equilibrate with mobile solvent ca 30 min; then inject 10 μL aliquots of dilute standard. Retention time (RT) for prednisone should be ca 8 min. Coefficient of variation (CV) of 5 replicate injections of standards should be ≤2%. Resolution factor should be ≥3 for prednisone and internal standard.

HPLC Determination

Let chromatographic system equilibrate by passing mobile solvent through column ca 30 min. Chromatograph equal volumes (typically 10 μL) of samples and standards, using mobile solvent flow rate of 1 mL/min and detection at 254 nm. Use average of duplicate injections of each determination for calculation.

Blue Tetrazolium Determination

The USP XX (1) Assay for Steroids (spectrophotometric) and Single-Steroid Assay (thin layer chromatographic (TLC)) procedures were used.

Method Development

Linearity.—Prednisone.—The peak areas per μg prednisone determined by HPLC were linear over a concentration range of 10 ng–4 μg/injection. The average peak area per μg prednisone injected was 5935 with a CV of 1.5% for 8 different concentrations, each injected in duplicate. The proposed HPLC procedure specifies a concentration of 20 μg prednisone/mL or 0.2 μg prednisone/10 μL injected.

Acetanilide.—The peak areas per μg acetanilide determined by HPLC were linear over a concentration range of 5 ng–4.2 μg/injection. The average area per μg injection was 16 807 with a CV of 1.2% for 13 different concentrations, each injected in duplicate. The proposed HPLC procedure specifies a concentration of 11 μg/mL or 0.11 μg acetanilide/10 μL injected as the internal reference standard.

Comparison of internal vs external standard.—Six portions of prednisone composite, equivalent to one average tablet weight, were quantitated by use of the external standard. For 15 analyses, an average CV of 0.47%, with a range from 0.21 to 1.40%, was obtained. Different aliquots of the same samples, analyzed similarly and calculated by use of the internal standard, gave an average CV of 0.81% with a range of 0.18–1.73%. Thus, quantitation of prednisone by the proposed method using either internal or external standard methods gave satisfactory results.

Recovery of USP prednisone from sample composite.—Six portions of sample composite, equivalent to one average tablet weight, were accurately weighed into a volumetric flask and diluted with methanol-water (1 + 1) to yield a concentration of 0.2 mg/mL. Five mL aliquots of this solution were added to a 50 mL volumetric flask and to a 100 mL volumetric flask (which contained 5 mL standard solution containing ca 1 mg accurately weighed USP prednisone). These samples were diluted to volume, mixed, filtered, and then analyzed by HPLC. The average recovery of USP prednisone added to prednisone tablet composites in 33 studies was 99.5% with a range from 97.2 to 103.5% and a CV of 1.07%.

Degradation of prednisone in solution.—Replicate

HPLC analyses of mixed USP prednisone and acetanilide standard in methanol–water (1 + 1) carried out periodically during a 2.5 month period showed that the ratio of prednisone to acetanilide decreased with time. The initial ratio of 1.023 gradually declined to 0.883 (13.7%) over 67 days, an average decrease of 0.2% per day. Similar studies conducted over 12 and 13 day periods gave the same daily rate of change. Comparison of this mixture after 67 days with new standards showed that 87.3% prednisone and 100.0% acetanilide remained. No new peaks were observed on the HPLC chromatogram.

Effect of variation in flow rate on mobile solvent.—A study of the effect of varying flow rates of mobile solvent on prednisone and internal standard using radial compression systems with an 8 mm × 10 cm 10 μm particle size C_{18} reverse phase column was carried out. Variation of flow rate from 0.4 to 3.0 mL/min resulted in a corresponding change from 550 to 2200 psi in the pumping pressure.

A van Deemter plot of height equivalent per theoretical plate vs flow rate indicates that the greatest efficiency of this system for prednisone analysis occurs at a flow rate of 0.6–0.8 mL/min; very little loss in efficiency occurs by operating at 1 mL/min, the flow rate specified in the proposed procedure. Likewise, the efficiency of the system for acetanilide is also satisfactory at 1 mL/min.

Effect of variation of mobile solvent components.—When 25% THF was used in combination with a wide variation of concentration (3.25–25%) of either acetonitrile or methanol in water, adequate separation of internal standard, acetanilide, and prednisone was achieved.

The effect of THF concentration (15–45%) in water on the retention of acetanilide and prednisone was studied. No separation was observed in 45% THF in water (RT 3.5 min for both compounds); 15% THF in water gave an RT of 14 min for acetanilide and 38 min for prednisone with a resolution of 24 and a selectivity factor of 3.3. Decreases in THF concentration from 45 to 15% increase retention of acetanilide by a factor of 4, while that of prednisone is increased by a factor of 11, i.e., the retention of prednisone is about 2.75 times that of acetanilide. This large difference in sensitivity of the 2 compounds to THF concentration results in great versatility in mobile solvent.

The data listed in Table 1 were obtained from the study of the effect of varying the methanol concentration from 0 to 25% on the RTs and resolution of prednisone relative to acetanilide and acid and ketone degradation products of prednisone. The data show a wide range of resolution and RTs for the 4 components, all of which provide adequate separation and quantitation conditions for prednisone.

Varying the THF concentration as little as 1% caused significant changes in RTs of the internal standard, prednisone, and the ketone degradation product of prednisone as well as the resolution between all 4 components. The changes in RTs shown in Table 1 are not the same for each component. For example, as the THF increases from 20 to 25%, the RT for acetanilide decreases by 31%, for prednisone by 54%, and for the ketone derivative by 61%. Table 1 also shows that with a 5% increase in THF concentration, the RT for acetanilide decreases by 32%, that for prednisone by 54%, and that for the ketone by 77%. Separations are adequate for quantitation in most cases shown in Table 1.

RT changes for selected compounds with various mobile solvents.—Table 2 reports the variation of RTs and relative RTs of common related steroids and degradation products of prednisone for 4 mobile solvents. This study shows that common related steroids and degradation products of prednisone at expected levels of <1.0% are separated from the internal standard and prednisone by the proposed procedure (see Figure 1). At this level, they are identifiable and quantitatable if desired.

Limit of sensitivity for prednisone by HPLC.—The lower limit of sensitivity of the proposed system for prednisone was determined using 0.005 AUFS and 10 μL injections of prednisone. The signal-to-noise ratio produced by 1 ng prednisone is 3:1 while that for 0.4 ng sample is 1:1. The lowest practical limit of detectability of prednisone using the conditions described in the proposed procedure is ca 1 ng.

USP prednisone recovery through USP Single-Steroid Assay.—Ten determinations (using Analtech Uniplate, silica gel GF) of USP prednisone using the USP XX Single-Steroid Assay (1) averaged 98.1% recovery with a CV of 2.4% and a range of 93.3–100.9%. A second series of 8 determinations (using Merck silica gel 60, F-254) was made with an average recovery of 97.8%, a CV of 1.4%, and a range of 95.6–99.6%.

Results and Discussion

The purity of bulk drug prednisone samples from 4 manufacturers was checked by the proposed HPLC method (Table 3). USP prednisone was used as the reference standard. The average bulk drug prednisone purity was 99.5%, with a

Table 1. Effect of solvent variation on prednisone, internal standard, and degradation products

% [a]		Retention time, min				Resolution		
Methanol	THF	Acid	Int. std	Prednisone	Ketone	Acid vs prednisone	Int. std vs prednisone	Ketone vs prednisone
—	20	2.93	9.83	18.36	22.46	22.4	12.0	4.54
5	20	4.16	8.66	14.50	17.56	12.6	9.3	4.00
10	20	3.53	7.56	11.13	13.56	13.1	6.0	3.38
15	20	3.40	6.46	8.70	10.50	11.1	3.8	2.85
20	20	2.83	5.50	6.86	8.26	5.79	2.6	2.43
25	20	2.73	4.93	5.76	6.86	5.71	1.7	1.82
15	20	2.40	6.62	9.02	10.82	9.38	4.03	2.79
15	21	2.50	6.23	8.06	9.70	8.73	3.11	3.02
15	22	2.56	6.23	7.76	9.26	7.96	2.62	2.26
15	23	2.76	5.93	7.23	8.63	7.79	2.50	2.84
15	24	2.50	5.46	6.53	7.80	6.60	1.83	2.17
15	25	2.43	5.06	5.86	7.00	5.83	1.09	1.83
12.5	20	2.36	7.06	9.76	11.73	12.08	5.09	3.50
12.5	21	2.43	6.70	9.00	10.83	12.18	4.57	3.32
12.5	22	2.40	6.06	7.86	9.43	9.27	3.52	2.59
12.5	23	2.40	6.03	7.66	9.20	9.23	2.89	2.82
12.5	24	2.43	5.70	6.96	8.36	7.84	2.49	2.33
12.5	25	2.33	5.33	6.36	7.63	6.83	2.04	2.27

[a] All solvents diluted to volume with water.

Table 2. Comparison of retention times (RT) of selected solutes using various mobile solvents

Solute	A[a] RT	A[a] Rel. RT	B[b] RT	B[b] Rel. RT	C[c] RT	C[c] Rel. RT	D[d] RT	D[d] Rel. RT
C_{17} acid	3.28	0.42	3.00	0.47	3.65	0.36	3.15	0.43
Acetanilide	5.98	0.76	5.26	0.82	6.71	0.66	5.62	0.76
Prednisone	7.86	1.00	6.40	1.00	10.15	1.00	7.40	1.00
Cortisone	8.66	1.10	6.93	1.08	11.22	1.11	N.D.[e]	N.D.
C_{17} ketone	9.14	1.16	7.46	1.17	11.65	1.15	8.50	1.15
Prednisolone	9.17	1.17	7.46	1.17	13.01	1.28	9.30	1.26
Hydrocortisone	9.97	1.27	8.03	1.25	13.95	1.37	9.96	1.35
Prednisone acetate	25.06	3.12	16.20	2.53	35.53	3.54	23.54	2.99
Prednisolone acetate	26.41	3.28	16.96	2.65	41.20	3.95	24.60	3.12
Cortisone acetate	27.42	3.41	17.50	2.73	43.80	4.20	24.94	3.16
Hydrocortisone acetate	29.24	3.64	18.90	2.95	41.20	3.95	26.60	3.38

[a] 25% THF; 6¼% methanol in water.
[b] 25% THF; 12½% methanol in water.
[c] 20% THF; 10% methanol in water.
[d] 20% THF; 25% methanol in water.
[e] Not determined.

range of 98.6–101.2%, and the average difference between duplicate injections was 0.13%, with a range from 0.00 to 0.30%.

Of the 11 impurities detected by HPLC (Table 4), 4 were common to all drug substances. The HPLC impurity profile of bulk drug substance prednisone is essentially the same as that obtained by TLC. The amount of UV-absorbing impurities present, approximated on the assumption that they have the same absorptivity value as prednisone, ranged from 0.09 to 0.64% in USP prednisone. Analysis of bulk drug substance and USP prednisone during this study revealed only prednisolone and prednisone acetate as possible related steroids.

Ten portions of composite (from samples supplied by 30 different firms), each portion equivalent to one average tablet weight, were extracted and analyzed by the USP XX Total Steroid Assay (1) and the HPLC procedure. One extraction more than is required by the USP XX was made; this extract was carried separately through the procedure to check for completeness of extraction. Table 5 reports the results of 46 analyses of these samples. The difference between mean values for the blue tetrazolium and HPLC analyses of about 10 portions for each of

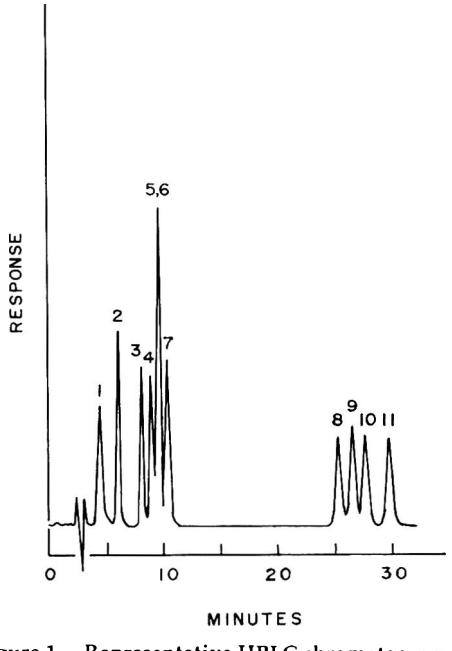

Figure 1. Representative HPLC chromatogram: 1, 1,4-pregnadiene-3,11-dione-17-carboxylic acid; 2, acetanilide; 3, prednisone; 4, cortisone; 5, hydrocortisone; 6, 1,4-androstadiene-3,11,17-trione; 7, prednisolone; 8, prednisone acetate; 9, prednisolone acetate; 10, cortisone acetate; 11, hydrocortisone acetate.

Table 3. Purity (%) of bulk substance prednisone determined by using HPLC[a]

Vol. Injected, μL	Concn	Manufacturer 1	2	3	4
10	4 mg/2 mL	101.2	100.6	100.6	98.9
20	1 mg/50 mL	99.4	99.6	99.4	98.9
20	10 mg/50 mL	99.1	98.9	99.0	98.6
	Av.	99.9	99.7	99.7	98.8

[a] USP prednisone used as external reference standard and calculated as if 100% pure.

Table 4. Retention times[a] relative to prednisone of impurities in bulk drug prednisone determined by using HPLC

		Manufacturer			
Impurity	USP[b]	1	2	3	4
1	0.36 (0.02)	0.37 (0.02)	0.38 (0.03)	0.37 (0.03)	0.39 (0.03)
2	0.52 (T)[c]		0.54 (0.01)	0.53 (0.01)	0.53 (0.01)
3	0.59 (T)	0.60 (0.01)	0.59 (0.04)	0.58 (0.04)	0.59 (0.04)
4			0.65 (T)	0.64 (T)	0.65 (T)
5	0.72 (0.03)	0.74 (0.02)	0.75 (0.3)	0.74 (0.3)	0.73 (0.3)
6	0.84 (0.04)	0.86 (0.02)		0.85 (T)	0.85 (T)
7			0.93 (0.08)	0.92 (T)	0.93 (T)
8	1.16[d] (T)	1.22[d] (0.2)			1.27 (T)
9	1.38 (T)	1.38 (T)	1.38 (0.05)	1.38 (T)	
10	1.77 (T)	1.77 (T)	1.77 (0.05)	1.77 (0.03)	1.78 (0.05)
11		3.11[e] (0.03)	3.18[e] (0.08)	3.18[e] (0.06)	3.21[e] (0.1)
	(0.99)	(0.48)	(0.64)	(0.47)	(0.53)

[a] % in parentheses.
[b] Percent calculated using prednisone as reference standard.
[c] T = trace, <0.01%.
[d] May be prednisolone.
[e] May be prednisolone acetate

these same samples for content uniformity shows an average CV value which was 0.5% higher for blue tetrazolium than for HPLC. This positive bias is reasonable since the blue tetrazolium reaction has been shown to be nonspecific, and many of the excipients used by United States manufacturers of corticosteroid formulations react with blue tetrazolium to give falsely high results (3, 16). In this study, the average variation is small because most interfering substances had been removed by chloroform extraction before analysis.

Four samples of 5, 10, 20, and 50 mg tablets were extracted incompletely by the USP XX procedure. Three tablet sizes were made by the same formulator. The amount of prednisone unextracted ranged from 0.1% in 5 mg tablets to 3% in 50 mg tablets. The average CV for all determinations, summarized in Table 5, was 0.90% for the blue tetrazolium Total Steroid Assay and 1.40% for the proposed HPLC procedure.

Table 6 reports the results of USP Single-Steroid Assay in duplicate for 46 different formulation dosage forms. All samples were within USP limits; the difference between duplicates after elimination of 4 outliers showed an average value of 1.4%, with a range from 0 to 4.45%.

The results reported for prednisone recovery and those in Table 6 were obtained by a person who had no prior experience with the USP Single-Steroid Assay. A total of 39 USP prednisone recoveries by the USP XX Single-Steroid Assay were run by this analyst before performing the test determinations.

Thin layer chromatograms used in the Single-Steroid Assay showed 2–5 extraneous bands. The prednisone band was removed from one developed chromatogram, extracted, and restreaked, and the thin layer chromatogram was developed two more times. In each case, the same bands appeared when the chromatogram was viewed under shortwave UV light, indicating that prednisone degradation occurred on the plate during development. No effort was made to identify the components of the extraneous bands. Prewashing the TLC plates with absolute methanol or 1% citric acid in methanol reduced but did not eliminate those extraneous bands.

Table 7 compares the HPLC analysis of prednisone in whole tablet and composite samples. The average CV for analysis of whole tablets was 2.10%, with a range from 0.14 to 7.56%. Comparable values for composites made from the same samples had an average CV of 0.81% and a range from 0.19 to 4.16%. Table 7 also shows that the average CV for whole tablet analysis is greater than that for the composite, which is due to tablet weight variation (CV 1.68%). The amount of declared drug was 1% higher for whole tablet than for composite analysis. This positive bias, 53 of 75 values greater for whole tablet analysis than for composite analysis, may be due to selective losses of prednisone on mortar and pestle surfaces during composite preparation (Elaine Bunch, FDA, Seattle, WA, personal communication, 1981). Analyses of whole tablets or sample weights equivalent to an average tablet weight of composite by the HPLC method gave comparable results.

Of the corticosteroid alcohols studied, pred-

Table 5. Comparison of prednisone content of tablet composites determined by using blue tetrazolium (BT) assay and HPLC [a]

BT		HPLC	
% Declared	CV	% Declared	CV
100.4[b]	0.74	99.1[b]	0.59
100.7	0.73	100.9	3.2
97.6	1.95	97.8	0.61
94.9	0.95	94.1	0.88
96.6	0.63	96.3	1.97
97.6	0.83	99.1	2.49
98.5	1.07	104.1	1.74
95.8	0.75	94.8	0.84
100.9	0.77	100.7	2.12
92.3	1.21	90.8	0.52
94.6	1.43	97.2	3.39
101.9	0.55	101.6	1.39
98.7	1.61	98.3	1.52
98.2	0.61	95.6	0.61
95.6	0.90	98.5	2.62
93.9	0.67	91.7	0.66
96.9	0.93	95.5	1.2
90.6	0.72	89.8	2.39
98.4	0.91	97.4	0.80
97.9	0.84	95.8	0.37
98.0	0.58	96.3	0.73
98.0[c]	0.94	97.9[c]	2.05
98.6	0.86	102.3	1.09
100.2	0.94	101.2	1.04
97.5	0.71	95.6	1.1
97.5[c]	0.71	95.6[c]	1.1
93.4	1.60	91.8	2.61
101.5	1.18	99.2	0.69
97.4	1.41	95.5	0.75
100.3	0.56	99.7	0.75
95.9	0.91	95.5	4.19
96.9[b]	0.69	95.5[b]	0.29
102.2	0.84	99.9	4.64
95.5	0.62	94.1	0.94
92.1	2.24	95.5	1.95
101.8	0.27	101.9	0.76
98.3	0.95	96.1	1.15
96.5	0.63	93.4	1.87
93.4	0.57	92.6	1.26
101.2	0.42	96.6	1.04
98.6	0.77	98.6	0.81
98.9	0.73	99.0	0.48
99.9	1.17	96.6	0.69
100.6	0.44	101.4	1.2
97.0	0.97	95.2	1.16
98.1	0.52	100.6	0.73
Av.	0.90		1.40

[a] Ten replicates unless otherwise noted.
[b] Nine replicates.
[c] Eight replicates.

Table 6. Assay of prednisone samples by USP XX procedure (duplicate analyses)

Manuf.	Tablet size, mg	% of Declared		Av., %
1	20	100.2	102.3	101.2
2	5	102.0	102.6	102.3
3	5	99.3	101.6	100.4
4	5	95.4	95.6	95.5
5	20	95.4	94.5	95.0
6	5	97.3	99.4	98.4
6	20	100.0	99.7	99.8
6	10	95.7	95.7	95.7
6	5	94.3	97.9	96.1
7	5	95.6	98.9	97.2
8	5	96.9	97.0	97.0
9	5	97.1	95.7	96.4
10	5	105.9	99.2	102.6[a]
		104.5	100.7	102.6
11	20	103.6	103.7	103.6
12	5	97.5	97.7	97.6
13	5[b]	97.6	99.6	93.6
14	5	102.5	100.0	101.3
14	2.5	96.1	95.4	95.7
15	2.5	90.7	100.7	95.7[a]
16	5	87.6	92.9	90.3[a]
17	5	99.9	99.9	99.9
18	50	103.5	105.1	104.2
18	20	103.3	103.4	103.4
18	10	100.2	99.2	99.7
19	5	101.6	102.2	101.9
20	5[b]	99.5	96.7	98.1
21	20	104.4	101.6	103.0
21	1	96.3	97.8	92.0
22	5	96.8	95.5	96.1
23	5	103.8	104.9	104.4
23	1	96.6	101.0	98.8
24	5	102.5	106.3	104.4
25	2.5	98.0	100.3	99.2
25	1	99.1	98.8	99.0
26	20	103.7	102.9	103.3
26	5	96.3	94.8	95.6
27	20[b]	100.3	100.3	100.3
27	2.5	98.0	91.5	94.8[a]
28	50	101.1	103.7	102.4
28	20	101.2	100.7	101.0
28	10	99.3	98.8	99.1
28	5	99.8	100.3	100.0
28	2.5	203.0	99.7	100.8
29	5	95.7	94.6	95.1
30	20	101.7	102.2	102.0
30	5	98.7	95.4	97.0

[a] Emulsion problems during extraction.
[b] Outlier (greater than 4X average deviation from the mean) not included in results.

nisone eluted first from reverse phase HPLC columns. An internal standard should elute before prednisone to avoid potential interference from other corticosteroids. Of 76 different compounds evaluated, only acetanilide had an R_f value sufficiently less than prednisone to give good resolution plus the other qualities necessary to be a suitable internal reference standard. Acetanilide is stable in the solution used in the proposed method for at least 2 months. Furthermore, it does not interfere with known decomposition derivatives of prednisone, related steroids, or excipients contained in prednisone tablet formulations and obeys Beer's law over the range suggested by the proposed procedure (Figure 1). No degradation products of predni-

Table 7. Comparison of prednisone content of whole tablet with composite determined by using HPLC

Manuf.	Tablet: Size, mg	Tablet: Wt variation CV	Tablet: % of Declared[a]	Analysis CV	% of Declared[b]	CV
1	20	1.37	102.8	2.49	102.9	0.68
1	20[c]	1.76	98.2	1.27	98.0	0.50
2	5	1.81	101.8	0.79	99.8	0.87
2	5[c]	1.63	99.6	2.17	97.6	0.69
3	5	2.57	94.2	0.27	95.4	0.27
3	5[c]	2.49	98.1	2.61	96.0	0.41
4	5	0.36	95.9	2.04	93.2	1.68
4	5[c]	1.82	97.0	0.99	95.1	1.17
5	5	1.71	98.1	1.59	97.6	0.65
5	20	1.43	96.2	1.41	93.6	0.66
5	20[c]	1.29	102.6	3.45	95.0	0.60
6	5	2.10	93.9	1.22	94.3	1.22
6	10	1.34	96.3	1.05	94.6	0.41
6	10[c]	0.52	96.7	0.59	95.9	0.56
6	20	0.95	101.6	1.25	99.1	0.74
6	20[c]	1.10	99.0	1.23	95.3	1.09
7	5	0.82	94.8	1.05	93.7	1.02
7	5[c]	1.12	97.0	1.55	94.5	0.54
9	5	0.69	94.9	1.62	94.3	0.70
10	5	2.58	97.0	2.69	97.8	0.51
10	5[c]	3.32	97.7	4.03	97.5	0.67
11	20	2.44	107.7	1.45	106.2	1.12
12	5	2.00	98.8	2.07	98.0	0.88
12	5[c]	3.50	98.0	4.00	99.0	0.70
13	5	1.34	96.1	1.47	95.7	0.45
14	2.5	3.84	95.4	4.39	90.9	0.55
14	2.5[c]	3.86	95.8	3.52	94.0	0.42
14	5	2.09	97.6	2.58	98.0	1.32
14	5[c]	1.80	96.6	.355	97.9	0.31
15	2.5	3.59	96.2	5.97	90.8	0.72
16	5	0.94	97.9	1.33	97.2	1.60
16	5[c]	0.43	99.6	1.00	97.9	0.69
17	5	1.06	95.6	1.56	93.9	0.61
18	10	1.02	95.6	1.29	95.5	0.28
18	10[c]	1.33	96.3	1.73	96.6	0.29
18	20	1.19	109.8	0.99	108.1	1.04
18	50	1.26	107.3	1.47	104.8	0.40
18	50[c]	1.15	104.1	1.45	104.7	0.50
18	50[c]	1.08	105.2	0.65	103.7	0.82
19	5	1.58	97.3	2.01	97.1	0.31
20	5	2.28	96.9	2.65	96.8	0.59
20	5[c]	2.67	98.2	3.26	97.0	0.86
21	1	0.93	91.6	2.64	94.7	0.66
21	20	0.55	101.0	0.92	100.3	1.35
22	5	2.25	95.6	6.48	96.4	0.65
22	5[c]	2.18	95.2	7.56	94.3	2.81
22	5[c]	2.18	98.4	6.77	94.3	0.84
23	1	3.44	97.9	2.53	96.7	1.21
23	1[c]	3.14	98.0	2.08	96.5	1.50
23	1[c]	1.97	97.4	2.94	97.4	0.25
23	5	2.29	100.3	1.19	100.5	0.80
24	5	1.60	101.9	1.54	98.8	1.13
25	1	1.88	94.1	2.04	93.7	0.84
25	1[c]	0.51	96.7	2.64	94.8	0.19
25	2.5	0.73	96.5	1.63	97.0	0.82
25	2.5[c]	0.94	98.1	0.48	95.6	0.56
26	5	2.00	93.9	2.36	92.9	0.58
26	20	2.11	100.0	3.31	99.0	1.72
26	20[c]	0.80	103.8	2.44	102.0	0.82
27	2.5	2.10	97.0	2.28	96.1	0.84
27	20	0.51	101.4	0.46	100.2	1.72
27	20[c]	0.28	98.2	0.83	99.7	0.23
28	2.5	0.59	96.8	0.45	96.2	0.68
28	5	1.54	96.8	1.97	96.7	0.35
28	10	0.42	96.6	0.88	97.8	0.35
28	10[c]	0.62	96.7	0.63	97.4	0.65

Table 7. (cont'd)

Manuf.	Tablet Size, mg	Wt variation CV	% of Declared[a]	Analysis CV	% of Declared[b]	CV
28	20	0.52	91.8	0.75	92.0	0.56
28	200[c]	0.72	102.4	0.54	100.4	0.62
28	50	0.78	99.8	0.14	99.0	0.34
29	5	4.80	96.4	3.71	96.0	4.16
29	5[c]	3.72	99.6	5.28	96.0	1.05
30	5	0.84	96.4	1.63	94.6	1.14
30	5[c]	1.68	97.0	2.10	01.9	0.23
30	5[c]	1.52	94.9	1.00	93.8	0.46
30	20	2.97	101.8	3.06	100.3	0.52
	Av.	1.68		2.10		0.81

[a] Average of 6.
[b] Sample size equivalent to average tablet weight.
[c] Duplicate or triplicate; see next higher results.

sone were detected in commercial tablets analyzed by the HPLC procedure.

The USP blue tetrazolium assay of prednisone gives acceptable results, but it is time consuming and requires a TLC specialist to obtain reproducible results. The lack of specificity of the blue tetrazolium reaction usually results in a positive bias. Degradation of prednisone on the TLC plates during chromatogram development leads to low recoveries. Decomposition of both standard and sample prednisone on the TLC plate must be uniform to yield good results in the USP Single-Steroid Assay. The amount of degradation of prednisone can vary from one side of the plate to the other, which may result in varying positive or negative recoveries depending on whether the standard or sample was decomposed more.

Mass spectrometry of bulk drug substance prednisone and TLC on 20% sulfuric acid in ethanol sprayed plates showed no gross contamination of bulk drug substance prednisone by non-UV absorbing isomeric substances, precursors, or intermediates.

The proposed HPLC assay is specific and determines only prednisone content. Related steroids, precursors, intermediates, and degradation products of prednisone are separated during the chromatography.

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Spectrofluorometric Determination of Pindolol and Its Dosage Form

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A new direct, simple, and sensitive spectrofluorometric method is described for the assay of pindolol and its tablets at room temperature, using ethanol (96% v/v) as solvent. The drug exhibits intrinsic fluorescence. Working wavelengths are 263 nm for excitation and 305 nm for emission. The calibration curve is linear in the range 1.0×10^{-7} to 1.5×10^{-6}M pindolol.

Pindolol, 1-(indol-4-yloxy)-3-(isopropylamino)-2-propanol (CAS 13523-86-9), is a relatively potent beta-blocker adrenergic agent that is prescribed for treatment of angina pectoris, arterial hypertension, and cardiac arrhythmias. The indole ring structure of pindolol suggests the possibility of intrinsic fluorescence activity. Few analytical procedures have been reported for quantitative assay of pindolol in biological fluids and pharmaceutical formulations. Published procedures include a nonaqueous titrimetric method (1) using standard acetous perchloric acid, gas chromatographic techniques (2, 3), and an indirect fluorometric assay (4) for pindolol in plasma and urine. The latter author did record the pindolol fluorescence directly, but found greater sensitivity by using the derivative. In the direct fluorometric procedure, a fluorophore is formed as a result of a condensation reaction between pindolol and orthophthalaldehyde. The fluorophore is intensely fluorescent, but it must be stabilized by the addition of ascorbic acid. The present paper describes a direct and simple fluorometric method for determining pindolol.

Experimental

Apparatus and Reagents

(a) *Fluorescence spectrophotometer.*—Perkin Elmer Model MPF-44 B, equipped with an X-Y recorder Model 056, was used for measuring fluorescence intensity and fluorescence spectra. A 10 × 10 mm quartz cell was used throughout the experiments. The excitation source was a xenon arc lamp.

(b) *Ethanol.*—96% v/v (Riedel-De Haen, Seezle, Hannover, GFR). Use without pretreatment.

Preparation of Standard Curve

Pindolol standard solutions.—Authentic pindolol powder was donated by Sandoz Ltd, Basle, Switzerland. Accurately weigh ca 20 mg authentic pindolol and quantitatively transfer to 100 mL volumetric flask. Dissolve in ethanol and dilute to volume.

To establish the calibration curve, prepare by serial dilution a series of 6 standard solutions whose concentrations range from 1.0×10^{-7} to 1.5×10^{-6}M.

Preparation of Samples

Accurately weigh 20 pindolol tablets (Visken®, purchased from local market) and compute average weight per tablet. Pulverize tablets, accurately weigh amount of powdered tablets equivalent to 20 mg pindolol, and quantitatively transfer to 100 mL volumetric flask. Add ca 80 mL ethanol and shake the flask ca 15 min. Dilute to volume with ethanol, and let contents settle. With rubber teat, pipet aliquot from supernatant layer and, by serial dilution, prepare solution with pindolol concentration ca 1.0×10^{-6}M.

Procedure

Set recorder to zero, excitation monochromator to 263 nm, and emission monochromator to 305 nm. Using ethanol as blank, record fluorescence intensity of each standard solution, starting with most dilute solution. Plot peak heights (measured in millimeters) against concentration.

Under the same conditions, measure fluorescence intensity of pindolol in unknown sample, and determine concentration from linear part of calibration curve.

Results and Discussion

Fluorescence Spectra

The excitation and emission spectra are shown in Figure 1. The intrinsic fluorescence of pindolol exhibits its excitation maximum at 263 nm and emission maximum at 305 nm. All values for fluorescence spectra are uncorrected for

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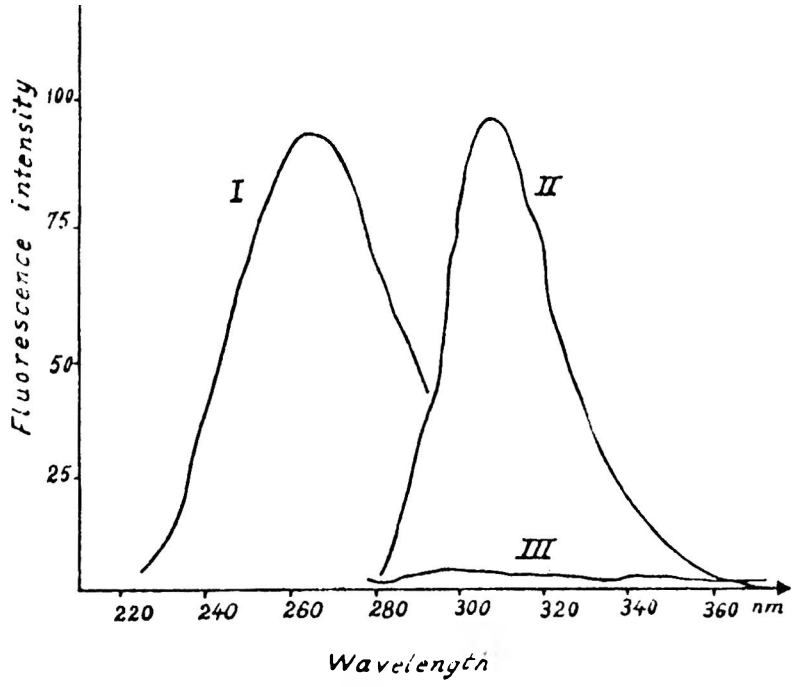

Figure 1. Fluorescence spectra of pindolol in ethanol: I, excitation spectrum; II, emission spectrum; III, emission spectrum for ethanol.

emission characteristics of the response of the spectrofluorometer.

Preliminary studies of alcohol solutions of pindolol showed that the inner filter effect was practically zero under the working conditions. Although the ethanol used as solvent was not spectral grade, there was negligible interference as shown in Figure 1. However, interference of ethanol was serious when the concentration of pindolol was less than 1.0×10^{-7}M. To study the interference of excipients in tablets, the ultraviolet absorption spectrum and the excitation and emission spectra for the alcohol extract of powdered tablets were run. All spectra were identical to those of authentic pindolol.

Calibration Curve and Precision

The calibration curve shows a linear relationship between the fluorescence intensity and pindolol concentration in the range 1.0×10^{-7}M.

To test the precision of the procedure 6 replicate determinations were made on a sample of 1.0×10^{-6}M pindolol concentration over a period of 1 week. The mean fluorescence intensity peak was 104 mm and the standard deviation was 1.8.

Recoveries and Spiking Experiment

The percent recoveries for the assay of authentic pindolol and its tablets are shown in

Table 1. Spectrofluorometric determination of pindolol

Sample	Amt taken, mg	Amt authentic added, mg	Rec., %[a]
Authentic pindolol	20[b]	—	99.1 ± 1.2
Pindolol tablets (Visken)	20[c]	—	98.7 ± 0.8
		5	98.9 ± 1.0

[a] Mean of 6 runs and standard deviation.

[b] 20 mg pindolol was accurately weighed and used to prepare standard solutions of 1.0×10^{-7} to 1.5×10^{-6}M concentration.

[c] An amount of powdered tablets containing 20 mg pindolol was accurately weighed and treated as described in text.

Table 1. These results, in addition to those of spiking experiments, demonstrate good precision and accuracy.

The spectrofluorometric method presented in this work is direct, simple, sensitive, and rapid for the assay of pindolol and its pharmaceutical formulations.

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VETERINARY TOXICOLOGY

Diagnosis of Ethylene Glycol (Antifreeze) Intoxication in Dogs by Determination of Glycolic Acid in Serum and Urine with High Pressure Liquid Chromatography and Gas Chromatography-Mass Spectrometry

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A relatively fast and sensitive high pressure liquid chromatographic (HPLC) and gas chromatographic-mass spectrometric (GC/MS) method was developed for determination of glycolic acid, one of the major metabolites of ethylene glycol, in extracts of canine urine and serum. The procedure involves extraction of glycolic acid with methyl ethyl ketone and derivatization with *O-p*-nitrobenzyl-*N,N'*-diisopropylisourea (PNBDI) in ethyl acetate solution. Recovery was greater than 94% from spiked samples. Ethylene glycol and commercial antifreeze were administered to experimental dogs at different dosage levels to reproduce the naturally occurring toxicosis associated with the consumption of commercial antifreeze. Glycolic acid was determined in either the urine or serum or both from these dogs by HPLC and GC/MS. 1,3-Butanediol, a competitive inhibitor of ethylene glycol biotransformation, was administered to one dog concurrently with antifreeze. In that experiment, it was effective in decreasing glycolic acid formation and prevented acute metabolic acidosis, kidney damage, and death.

Ethylene glycol (EG) intoxication is a common problem in dogs because of its palatability and wide use in commercial antifreeze preparations. Ingestion of as little as 4-5 mL/kg body weight can be fatal (1). Clinical diagnosis is difficult; nonspecific signs may include central nervous system depression, acidosis, vomiting, polyuria, ataxia, and renal failure (2, 3). Current analytical methods using colorimetry (4, 5), thin layer chromatography (6), and gas chromatography (7-9) to detect EG or its metabolites in urine or serum are frequently unreliable because of low recovery, low sensitivity, tailing peaks, or inconsistent results.

EG per se is relatively innocuous; it is the metabolic intermediates, particularly glycolic acid, that cause the severe metabolic acidosis and other complications associated with EG toxicosis (10, 11). Diagnosis of antifreeze intoxication by detection of EG is unreliable due to the rapid clearance of this compound by urinary excretion and metabolic degradation (10, 12, 13). Glycolic acid is the sole metabolite that accumulates in sufficient concentration with an adequate half-life for reliable chemical detection throughout the course of the intoxication (11). The primary purpose of this investigation was to develop specific, highly sensitive, reproducible HPLC and GC/MS methods for diagnosis of EG intoxication by detecting glycolic acid in urine and serum samples. Chemical derivatization of glycolic acid was necessary to facilitate these objectives.

A preliminary investigation was designed to evaluate the effectiveness of 1,3-butanediol, a competitive inhibitor of alcohol dehydrogenase (14), in the treatment of antifreeze intoxication by comparing serum glycolic acid concentrations in a treated vs a nontreated intoxicated dog.

METHOD

Reagents and Apparatus

(a) *Solvents.*—Reagent grade ethyl acetate, methyl acetate, and methyl ethyl ketone (MCB, Norwood, OH); HPLC grade isooctane (Burdick & Jackson Laboratories, Inc., Muskegon, MI).

(b) *Chemicals.*—*O-p*-Nitrobenzyl-*N,N'*-diisopropylisourea (PNBDI) (Regis Chemical Co., Morton Grove, IL); ethylene glycol (Fisher Scientific Co., Fair Lawn, NJ); commercial antifreeze (95% ethylene glycol, Prestone II, Union Carbide Corp., Danbury, CT); glycolic acid (Sigma Chemical Co., St. Louis, MO); 1,3-butanediol (Aldrich Chemical Co., Inc., Milwaukee, WI); and chlorpromazine hydrochloride (Thorazine, Smith Kline Corp., Philadelphia, PA).

(c) *High pressure liquid chromatograph.*—Model ALC/GPC 204 (Waters Associates, Milford, MA) equipped with dual wavelength absorbance detector and dual pen recorder.

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(d) *Gas chromatograph-mass spectrometer.*—Model 5992 B (Hewlett-Packard, Palo Alto, CA) equipped with 8 in. flexible disk data system (master and slave drive). Electron impact ionization with electron energy of 70 eV.

(e) *Columns.*—4.6 mm id × 250 mm Supelcosil LC-Si (5 μm silica, Supelco, Inc., Bellefonte, PA) for all HPLC separations. Six ft × 6 mm od, 2 mm id glass column packed with 3% OV-1 on 100–120 mesh Chromosorb W-HP for all GC/MS separations.

(f) *Derivatization tubes.*—5 mL acylation tubes (Regis Chemical Co.).

(g) *Serum and urine chemical analysis.*—Automated serum analyzer (SMA 12/60, Technicon Instrument Corp., Tarrytown, NY); urinalysis reagent strips (Multistix, Ames Co., Elkhart, IN); and refractometer (American Optical, Buffalo, NY).

Sample Acquisition

Urine and serum samples for blank and spiked determinations were collected from normal, healthy dogs. Specimens were spiked with aqueous solutions of glycolic acid.

Extraction

Urine.—Dilute 20 mL sample to 100 mL with water and filter through Whatman No. 4 paper in Buchner funnel. Add 25–30 g NaCl and adjust to pH 2 with 8–10 drops of concentrated HCl. Extract acid solution 3 times with equal volumes of methyl ethyl ketone, pool organic fractions, and evaporate just to dryness with gentle air stream and little or no heat in fume hood. Dissolve residue in 3 mL ethyl acetate and transfer to 5 mL volume acylation vial for derivatization.

Serum.—Pipet 2 mL serum into 50 mL centrifuge tube. Dilute each sample to 2 times its volume with water and add 1 g NaCl. Acidify to pH 2 with 2–3 drops of 4N HCl and extract with 15 mL methyl ethyl ketone. Centrifuge sample (2000 × g for 10 min) and transfer organic phase to 15 × 150 mm test tube. Evaporate to dryness in warm water bath, using gentle air stream, and dissolve residue in 3 mL ethyl acetate. Transfer solution to 5 mL volume derivatization vial.

Derivatization

Add 20 mg PNBDI to ethyl acetate solution and seal acylation vial with Teflon-lined cap. Incubate vial 2 h in 80°C oven. Cool sample before chromatographic analysis.

Prepare standard curve from solutions of glycolic acid in ethyl acetate (5 and 0.5 mg/mL). Dilute aliquots of these standard solutions to 3 mL with ethyl acetate, and derivatize as above.

Chromatography

Assay the samples by HPLC using solvent system of 16% methyl acetate in isooctane. Operating conditions: temperature ambient; flow rate 3 mL/min; chart speed 0.5 in./min; sensitivity 0.01–0.5 absorbance unit full scale (AUFS); wavelength monitoring 254 and 280 nm; solution volume 3.0 mL; injection volume 3 μL. Measure peak heights for quantitation.

Confirm results with GC/MS if sample is positive for glycolic acid derivative after HPLC analysis. Reduce volume to 0.5–1.0 mL and inject 3 μL. Use selected ion monitoring program (SIM) to scan for ion masses of 120, 136, and 211 m/z (molecular ion). GC operating conditions: injection port 250°C; flow rate 20 mL/min with zero grade helium carrier gas; initial oven temperature 180°C, programmable at 10°/min to 260°C; and solvent delay 1.0 min. Dwell times for SIM program are 1000, 200, and 1000 ms for ions 120, 136, and 211, respectively. Scan speed for scanning mode is 380 amu/s. Peak area may be measured by using a reconstruct and integrate program.

Animal Model

Six dogs of both sexes and varying weights (5–20 kg) and ages (½–10 years) were used in these experiments. The dogs were administered by stomach tube 6 or 12 mL/kg of either reagent grade EG or commercial antifreeze. These doses were mixed with equal volumes of water before administration. Dogs were restrained in metabolism cages with feed and water ad libitum.

Periodic urine and blood samples were collected. One of the dogs (B) was treated intravenously with 6 mL/kg of 20% 1,3-butanediol in 0.85% NaCl solution at time zero. This treatment was repeated every 4–8 h for 48 h. Two dogs (A and B) were treated with chlorpromazine (1 mg/kg iv) just before ethylene glycol administration to prevent emesis. The dogs were observed for clinical signs and were necropsied after death for observation of gross and histopathological lesions.

Results

Derivatization conditions for the formation of *p*-nitrobenzylglycolate (PNBG, $C_9H_9O_5N$, MW 211) were defined through kinetic experiments. Assays of standard glycolic acid solutions at various time intervals during incubation estab-

Table 1. Recovery of glycolic acid, as determined by HPLC

Sample	Amt glycolic acid, mg	Replicates	Recovery, %
Water	0.5	5	98 ± 1.0
Urine	1.0	6	96 ± 2.0
Serum	1.0	5	94 ± 2.7

lished that 2 h at 80°C was sufficient for complete reaction with PNBDI. Twenty mg PNBDI was necessary for complete reaction with up to 6 mg glycolic acid.

The HPLC standard curve of peak heights of PNBG vs mg glycolic acid was linear (r = 0.9975) through the operational range of 0.01–6.0 mg. Retention time in the HPLC solvent system was 14 min and the ratio of peak heights (254 nm/280 nm) was 2.4. Approximately 1–2 ng glycolic acid could be detected as PNBG at maximum sensitivity. Retention time in the GC/MS system was 3.5 min with somewhat less sensitivity (i.e., 10–12 ng).

It was necessary to extract glycolic acid from aqueous solution (water, urine, serum) into organic solvent for the derivatization reaction. Glycolic acid partitioned completely into methyl ethyl ketone below pH 2, if the diluted aqueous solution was saturated with NaCl. Partition coefficients were unfavorable using ethyl acetate, ether, and chloroform.

Spiking and recovery experiments with water, urine, and serum were used to optimize the extraction techniques (Table 1). Dilution and filtration of urine samples and dilution of serum samples were necessary to obtain maximum recovery of glycolic acid.

Of the 6 dogs (A–F) dosed with reagent grade EG or commercial antifreeze (Table 2), three (A,C,E) died acutely within 48 h, becoming depressed and ataxic within a few hours of dosing. These animals exhibited polyuria, vomiting, dehydration, dyspnea, coma, and death. Dog F displayed early central nervous system and pulmonary signs but improved considerably until the third day when death ensued from terminal uremia. The kidney tubules from this dog contained the typical birefringent calcium

Table 2. Dosage levels, survival time, and serum analyses from experimentally poisoned dogs

Dog	Dose (oral)	Survival time, h	Time (h) afer EG dosage	Inorganic phosphorus, mg/100 mL	BUN, mg/100 mL	Creatinine, mg/100 mL
Normal range	—	—	—	3.5–5.5	5–25	0.8–1.5
A	12 mL/kg of antifreeze	16	1	5.0	14	1.1
			16	11.3	46	2.7
B	12 mL/kg of antifreeze + 1,3-butanediol	>168	1	6.4	4	0.9
			24	4.1	6	0.9
			48	4.0	15	1.1
			96	4.5	17	1.1
C	6 mL/kg of antifreeze	48	6	5.7	12	0.9
			12	6.0	14	1.0
			18	6.2	42	1.8
			24	7.2	55	2.4
			30	7.3	58	2.8
			36	8.1	83	4.2
			42	9.2	107	4.9
			48	10.5	136	6.1
D	12 mL/kg of antifreeze	>168	6	5.5	21	1.1
			12	5.9	32	1.4
			18	6.0	23	1.4
			24	7.3	42	2.5
			30	7.1	61	2.6
			36	7.0	80	3.2
			42	8.0	88	3.6
			48	8.2	95	4.5
			54	8.2	103	4.8
			60	8.2	112	5.5
			72	10.0	124	6.8
			96	9.5	139	8.0
E	6 mL/kg of EG	30	14	5.2	25	1.5
			30	8.7	60	3.2
F	12 mL/kg of EG	72	14	3.8	11	1.0
			30	9.1	48	4.4
			72	12.5	160	13.0

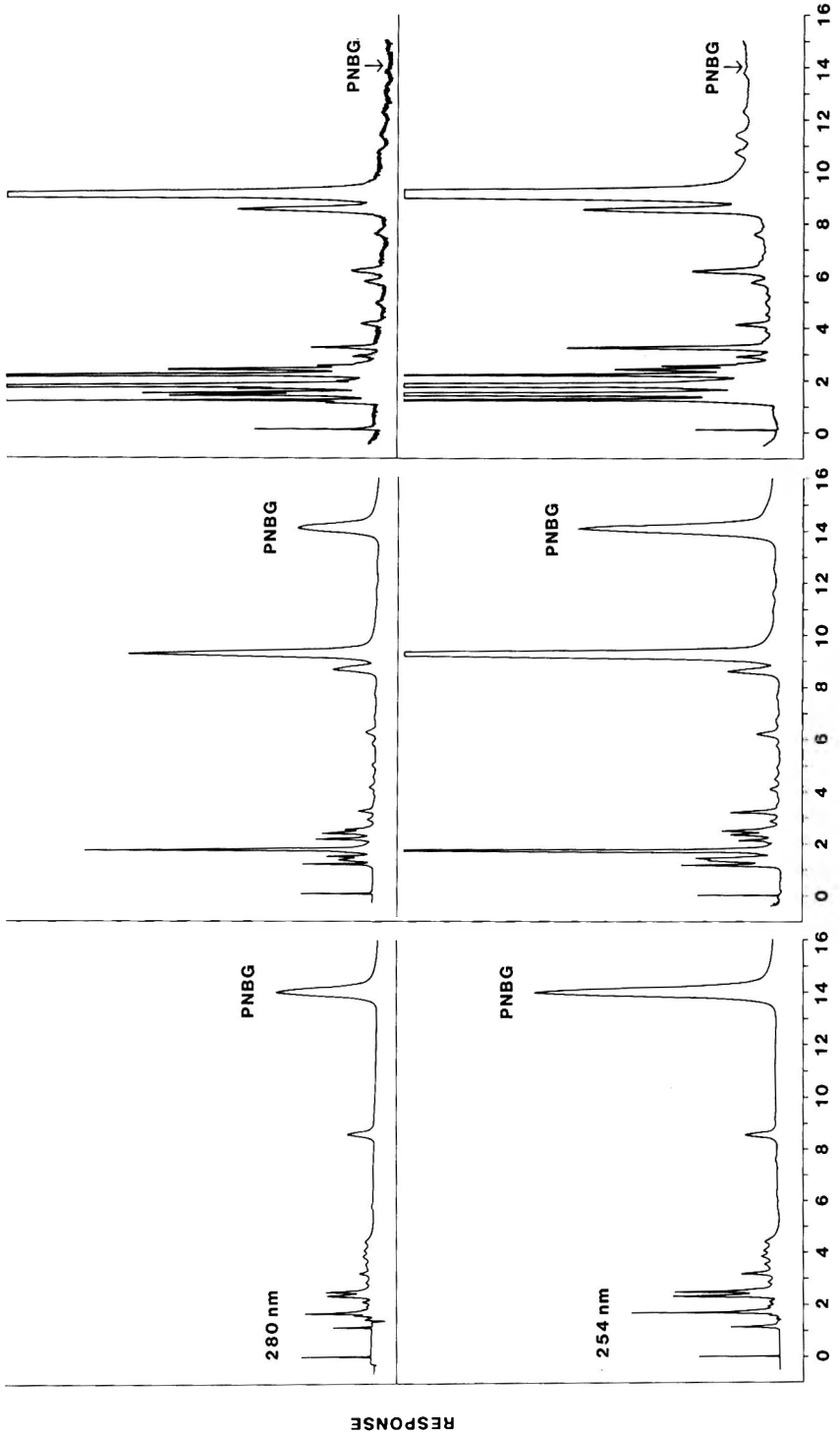

Figure 1. Typical chromatograms for HPLC analysis of glycolic acid as PNBG:
(left) 1 mg standard glycolic acid, 3 mL volume, 3 μL injection, 0.2 AUFS; (middle) dog B serum extract 3 h after EG administration, 3 mL volume, 3 μL injection, 0.2 AUFS; (right) control serum sample, 3 mL volume, 3 μL injection, 0.02 AUFS.

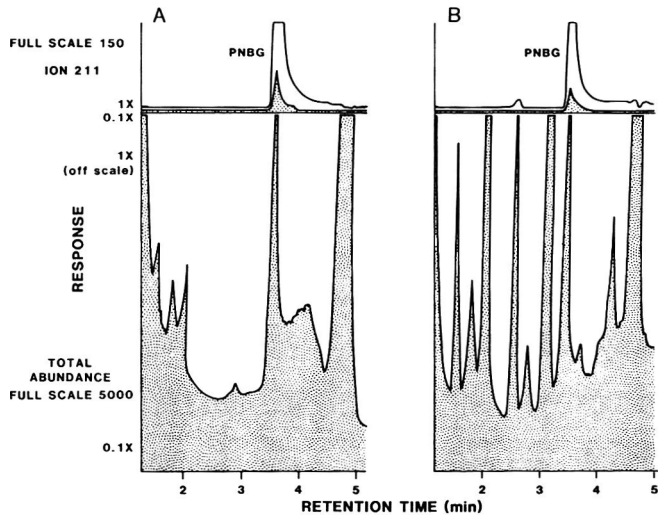

Figure 2. Typical GC/MS total ion program chromatograms for analysis of glycolic acid as PNBG: (A) 1 mg standard glycolic acid, 3 mL volume, 3 μL injection; (B) extract of serum, case 1, 0.8 mL volume, 1 μL injection.

oxalate crystals associated with EG intoxication (1). Dog D displayed early signs of poisoning but survived, possibly as a result of vomition soon after dosage. Dog B (1,3-butanediol treatment) briefly displayed mild signs of ataxia and was subsequently normal.

Serum analyses of dogs A, C, D, E, and F indicated renal dysfunction as evidenced by increasing concentrations of inorganic phosphorus, BUN, and creatinine throughout the course of the poisoning syndrome while values for dog B were normal (Table 2). Renal damage was pronounced by 18–24 h in the affected dogs. Results of urinalyses were characterized by low specific gravity (1.003–1.010) and occasional hematuria, proteinuria, and glycosuria.

Typical HPLC chromatograms of standard glycolic acid assayed as PNBG and extracts of blank serum and serum from a poisoned dog are shown in Figure 1. Chromatographic interferences in the region of interest in no instance prevented detection and measurement of PNBG. Blanks were run for urine and serum samples at higher sensitivity and no peaks were observed with the same retention time or peak height ratio as the derivative.

GC/MS total ion chromatograms of a standard solution of PNBG and of an extract of serum from an affected animal are shown in Figure 2. The upper chromatogram represents ion monitoring of the molecular ion of PNBG, 211 m/z. The mass spectrum values of PNBG are shown in Table 3. Total ion chromatograms of these serum and urine extracts have numerous peaks, some of which co-chromatograph with PNBG.

The SIM program was more sensitive in routinely analyzing samples (ions monitored were 211, 136, 120 m/z). This program provided unambiguous confirmation as the chromatograms demonstrate in Figure 3. The separate monitoring of the 211 ion indicated its presence in the PNBG standard solution and in extracts of serum and urine from poisoned dogs but not in control serum and urine.

Results in urine assays for glycolic acid are shown in Figure 4. Dog D received the larger dose, but some ethylene glycol was lost due to

Table 3. Mass spectrum of PNBG

Mass, m/z	Abundance, %
42	7
50	7
51	12
52	5
62	5
63	12
64	5
76	5
77	9
78	30
79	5
89	35
90	19
106	26
107	7
120	5
136	100
137	15
152	5
153	14
211	7

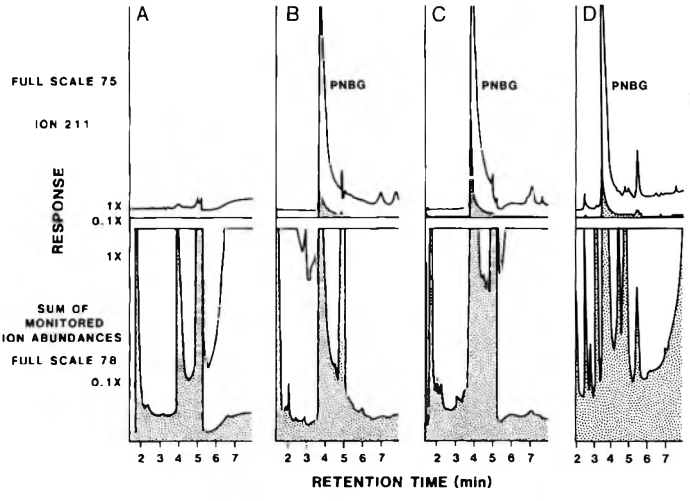

Figure 3. Typical GC/MS selected ion program chromatograms for analysis of glycolic acid as PNBG: (A) control serum, 1.0 mL volume, 3 μL injection; (B) 1 mg standard glycolic acid, 3 mL volume, 3 μL injection; (C) dog B serum extract 3 h after EG administration, 1.0 mL volume, 3 μL injection; (D) extract of serum, case 1, 0.8 mL volume, 1 μL injection.

vomition. Urine excretion of glycolic acid was copious until 30–40 h after ingestion of antifreeze. The increased excretion rate after 18 h may be related to renal dysfunction.

Figure 5 shows serum glycolic acid concentrations in treated dogs vs time after antifreeze ingestion. Serum concentrations were lower for dog D (vomition loss) than for dogs A and C, as expected. Dog B received the 1,3-butanediol treatment, and serum glycolic acid concentrations remained much lower than in the other dogs. Glycolic acid can be detected as early as 3 h or as late as 60 h after ethylene glycol ingestion. No analytical data are included for dogs E and F because these animals were part of an initial pilot study.

Several terminal cases of suspected antifreeze poisoning were submitted to this laboratory. These dogs were showing the initial symptoms of EG intoxication, and the results of serum and urine analyses are shown in Table 4. Corroboration by histopathology showing calcium oxalate crystals in kidney tubules was made in 4 of the 5 incidents. One case was apparently too acute for crystal deposition to occur.

Discussion

This method is specific for glycolic acid and has good sensitivity and recovery. In addition to having enhanced UV absorbance for HPLC detection, the glycolic acid derivative, PNBG, is more chemically suitable (i.e., greater column affinity, stable molecular ion) for GC/MS analysis than the parent compound.

These data suggest that maximum serum glycolic acid concentrations of approximately 30 mg/100 mL and greater may be fatal, while dogs with lower maximum serum concentration might survive.

Glycolic acid was detectable in sera (the preferred sample) of poisoned dogs within hours and these concentrations remained elevated for days, thus establishing this protocol as a viable means for diagnosing antifreeze poisoning in the early stages and improving the potential for therapy.

GC/MS was used only for rapid confirmation for the presence of PNBG and quantitation was not done routinely. SIM can be used for quantitating glycolic acid in lieu of HPLC if desired.

The advantage of the SIM mode for monitoring ion 211 m/z over single ion detection in the scanning mode is increased sensitivity. The peaks at and around the retention time of PNBG are apparently decomposition products of PNBDI or side reaction products of this compound with normal constituents from serum or urine, and

Table 4. Glycolic acid concentrations (mg/100 mL body fluid) in antifreeze toxicity cases, quantitated by HPLC

Case	Sample	Glycolic acid
1	urine	16
	serum	46
2	serum	20
3	urine	15
4	urine	8
5	urine	1.4

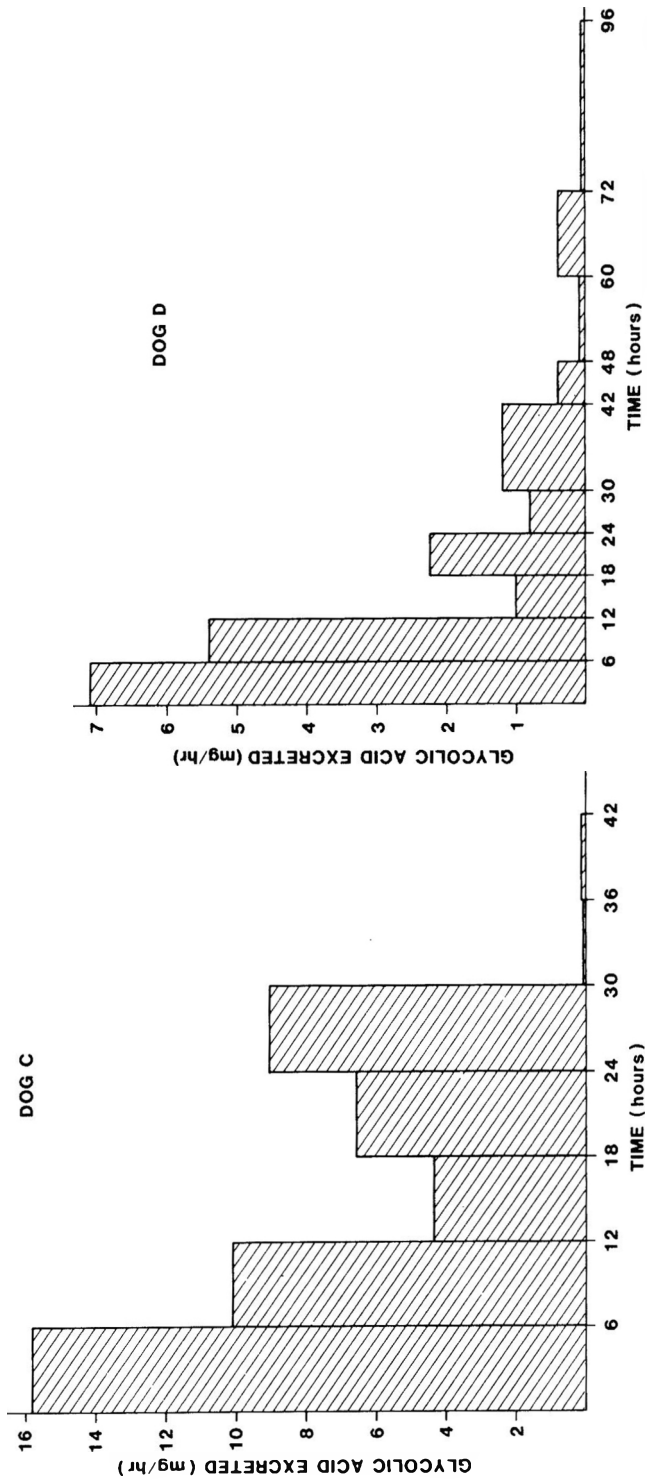

Figure 4. Glycolic acid excretion in urine as determined by HPLC analysis for dogs dosed with ethylene glycol: dog C dosed with 6 mL/kg of antifreeze; dog D dosed with 12 mL/kg of antifreeze.

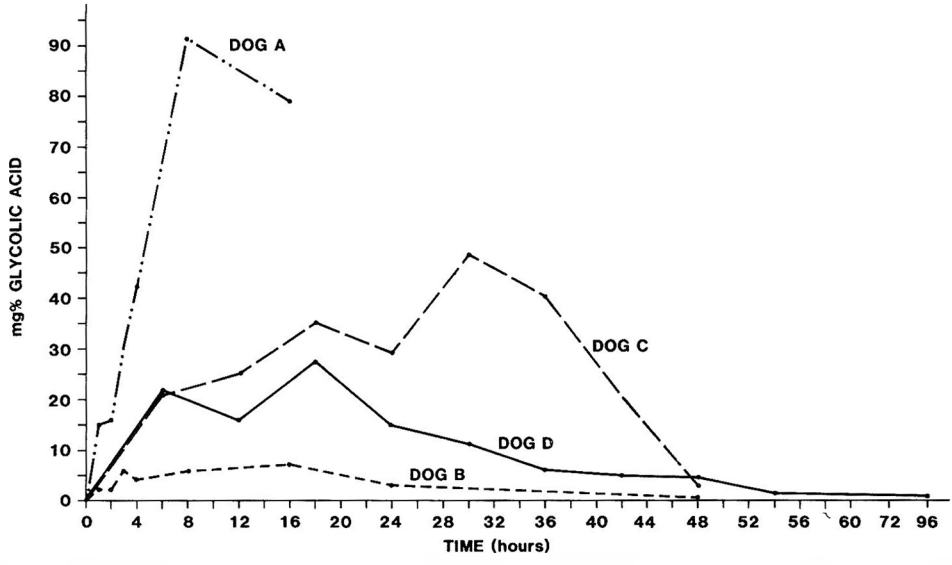

Figure 5. Glycolic acid concentrations in serum as determined by HPLC analysis for dogs dosed with ethylene glycol:

dog A dosed with 12 mL/kg of antifreeze; dog B dosed with 12 mL/kg of antifreeze + 1,3-butanediol treatment; dog C dosed with 6 mL/kg of antifreeze; dog D dosed with 12 mL/kg of antifreeze.

have mass spectra similar to PNBG (except for ion 211 m/z). Hence ion 211 m/z is the most useful for confirming the presence of PNBG; the other ions, 120 and 136 m/z, are rather arbitrary choices and their presence does not provide confirmatory evidence for the presence of PNBG. As a result, the ratio of peak abundances 120/136/211 varies with the composition of the extract; however, no questionable 211 m/z peak was ever observed in blanks, and detection of this molecular ion peak at the proper retention times provides unambiguous confirmation for the presence of PNBG.

1,3-Butanediol was effective in reducing ethylene glycol biotransformation to glycolic acid in the preliminary trial involving one dog. This animal showed no signs of renal damage as evidenced by serum analysis throughout the course of the experiment. These promising results indicate that the full therapeutic potential of this compound should be investigated.

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DRUGS IN FEEDS

High Pressure Liquid Chromatographic Determination of Monensin in Feed Premixes

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A high pressure liquid chromatographic (HPLC) method has been developed to determine monensin in feed premixes. The method is simple and rapid. Monensin is extracted with methanol-water and determined in the extracting solution by HPLC. Average recovery for monensin from a 13.2% premix sample was 103% (coefficient of variation (CV), 2.6%) by HPLC and compares with the value of 100% (CV, 3.4%) obtained by the turbidimetric bioassay method.

Monensin, which is produced by the organism *Streptomyces cinnamonensis*, is used to control coccidiosis in poultry (1) and to promote growth in cattle (2). Monensin ($C_{36}H_{62}O_{11}\cdot H_2O$) has a molecular weight of 688 and exhibits no UV-vis absorption. The compound can be determined in feed premixes by using a cylinder plate method (3), a turbidimetric method (4), or a colorimetric method (5). None of these methods separates monensin from other minor factors. This could lead to inaccurate assay values, depending on the composition of the sample. The bio-autographic method (6, 7) does separate monensin from other minor factors but that procedure is time consuming and is not quantitative.

The object of this study was to develop a faster, more specific assay that is both accurate and precise. Because monensin is nonvolatile and does not exhibit UV-vis absorption, a high pressure liquid chromatographic (HPLC) system was used as a basis of the assay. The refractive index detector responds to many organic compounds but lacks specificity and sensitivity. However, when this detector is coupled with HPLC separation, monensin can be successfully determined in premixes.

Experimental

Reagents and Apparatus

(a) *Solvents.*—Reagent grade methanol; glass-distilled water.

(b) *Monensin reference standard.*—Obtained from Lilly Research Laboratories, Indianapolis, IN.

(c) *High pressure liquid chromatograph.*—Waters Model 6000A pump; Waters Model R401 or DuPont refractive index detector; reverse phase DuPont Zorbax C-8 column, 4 mm id × 30 cm; Micromeretics Model 725 autoinjector; Fisher Recordall Series 5000.

(d) *Standard solutions.*—Weigh 200 mg monensin reference standard into 20 mL volumetric flask. Dissolve monensin in methanol, and dilute to volume with methanol. Use this solution to prepare 2.0 mg/mL standard solution in methanol–water (90 + 10, v/v).

Sample Preparation

Weigh 5 g premix sample into 100 mL volumetric flask. Add 75 mL methanol–water (90 + 10, v/v) and extract monensin by letting mixture stand 16 h (overnight). Periodically swirl flask to ensure complete extraction. Dilute to volume with methanol–water. Dilute an aliquot of this solution with methanol–water to concentration of about 2.0 mg/mL before injection into HPLC system.

Chromatography

Quantitate premix sample solutions by comparing detector responses vs 2.0 mg/mL monensin reference standard solution. Operating conditions: temperature, ambient; mobile phase, methanol–water (90 + 10, v/v); injection volume, 40 μL; retention volume of monensin, 12–14 mL. Compare peak heights or areas of samples and standards for quantitation.

Results and Discussion

Premix samples can be readily assayed for monensin simply by extracting the sample with methanol–water (90 + 10, v/v), making appropriate dilutions, and injecting the sample into the HPLC system. Similar extractions were performed on the inert premix component; no potentially interfering peaks were observed. Typical chromatograms for standards and samples are shown in Figure 1. The HPLC system

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Figure 1. Typical chromatograms for monensin standards and samples: (a) peak response equivalent to 80 μg monensin standard injected onto the column; (b) control premix sample; (c) premix sample containing 13.2% monensin.

Table 1. Assay reproducibility for 13.2% monensin premix sample

	Monensin, %			
Sample	Day 1	Day 2	Day 3	Day 4
1	13.4	13.7	13.0	13.9
2	13.7	13.9	13.2	13.4
3	13.7	13.9	13.0	13.9
Av.	13.6	13.8	13.1	13.7
SD	0.17	0.12	0.13	0.29
CV, %	1.3	0.8	0.9	2.1

Overall av. 13.6 ± 0.35% (CV = 2.6%)

was linear over the range of 40–160 μg monensin injected onto the column. Normally, 40 μL of sample and standard solutions at a concentration of 2 mg/mL are injected. Interference studies were conducted by determining the response of other coccidiostats under the HPLC conditions set for monensin. The compounds narasin, lasalocid, salinomycin, and arprinocid had different retention times under the HPLC conditions used for monensin.

A representative 13.2% premix sample was assayed in triplicate for monensin on 4 different days over a period of 2 weeks. The data shown in Table 1 indicate good day-to-day reproducibility with an overall average for 12 samples of 13.6 ± 0.35% (coefficient of variation (CV), 2.6%). The same sample was assayed in quadruplicate by turbidimetry and gave an average of 13.2 ± 0.44% (CV, 3.4%).

Several premix samples containing different concentrations of monensin were assayed by HPLC and by the routinely used turbidimetric method (4) using an Autoturb® system (Table 2). The Autoturb system (8) automates pipetting of samples, dilution with inoculated broth, and measurement of turbidity. These samples were assayed by one laboratory using turbidimetry, and by another laboratory using HPLC with no special precautions taken. Some differences between 2 methods were observed as shown in Table 2. The HPLC method assays only the monensin whereas the microbiological (turbidimetric) method assays for the total biological activity, including minor factors which have activity against the test organism. Small variations in the minor factor content may cause the

Table 2. Comparative results for HPLC and turbidimetric assay (%) of monensin premix samples

	HPLC			Turbid.		
Sample	n^a	Replicates or range	Mean	n	Replicates or range	Mean
1	2	14.3, 14.5	14.4	2	12.6, 13.7	13.2
2	2	12.3, 13.0	12.7	2	12.6, 13.2	12.9
3	2	13.0, 13.4	13.2	4	12.8–15.2	14.2
4	2	14.5, 14.5	14.5	3	11.2–12.4	11.9
5	2	13.6, 14.4	14.0	3	11.5–12.9	12.0
6	2	8.8, 8.8	8.8	4	8.6– 9.3	9.1
7	2	9.5, 9.7	9.6	3	8.8–10.1	9.2
8	2	9.7, 10.1	9.9	3	9.0–10.6	9.7
9	2	10.1, 10.4	10.3	3	8.6– 9.9	9.4
10	2	12.8, 13.4	13.1	4	12.6–13.9	13.6
11	2	10.4, 10.4	10.4	5	9.9–11.2	10.5
12	2	10.6, 10.8	10.7	3	9.9–10.6	10.4
13	4	16.9–17.8	17.5	2	18.3, 18.6	18.5
14	2	20.0, 20.3	20.2	2	19.9, 20.1	20.0

[a] n = number of assays conducted on sample.

2 methods to yield slightly different results. The premix samples shown in Table 2 are experimental samples made at different concentrations with different matrices. Comparative data indicate good agreement between the 2 methods with a correlation coefficient of 0.95, using linear regression analysis.

The HPLC method is simple, does not require a growth period common to biological methods, and results in greater specificity by separating monensin from its minor factors.

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Rapid Gas-Liquid Chromatographic Method for Determination of Sulfathiazole in Swine Feed

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A gas-liquid chromatographic (GLC) method for determining residues of sulfathiazole (STZ) in swine feed has been developed. Feed is extracted first with acetone and then with ammonia-acetone. STZ is isolated from other feed extractives on a Sephadex LH-20 column with methanol-toluene. The sulfa residues are methylated with diazomethane, and the eluate is evaporated to dryness. A solution containing an internal standard of methyl sulfasymazine is used to dilute the sample before injection onto an OV-25 GLC column. The precision of the method was determined by assaying 10 sets of feed spiked at 0.5, 1, 2, and 5 ppm STZ. The mean recoveries and coefficients of variation were 90.2 (5.90), 89.5 (4.67), 87.4 (5.62), and 87.7% (4.29), respectively. The critical steps of the method, including the stability of STZ, were also determined.

Various colorimetric (1, 2), high pressure liquid chromatographic (3, 4), and gas-liquid chromatographic (GLC) (5, 6) methods to detect and quantitate sulfonamide residues in animal tissues lacked specificity and sensitivity, and were time consuming. We reported earlier (7) a GLC method that uses a rapid Sephadex LH-20 column cleanup step for determining sulfamethazine (SMZ), which is the most widely used sulfa drug, in swine feed. The next most widely used sulfa drug is sulfathiazole (STZ). This paper reports the adaptation of the SMZ procedure to determine STZ.

METHOD

Reagents

See ref. 7, Reagents a-c, e-h plus

Standards.—STZ reference standards (USP). *Stock solution.*—100 μg/mL methanol. *Working solution.*—1.0 μg/mL methanol.

Apparatus

See ref. 7.

Preparation and Evaluation of Sephadex Column

See ref. 7, paragraphs 1 and 2 under this heading.

Plot volume of eluate vs concentration of each fraction. If STZ first appears in fifth fraction, use 40 mL forerun volume. If STZ is in sixth fraction, but is less than 5% of total, use 50 mL forerun. More than 95% of STZ should be collected before fourteenth fraction. If amount of STZ is found in fractions outside these limits, adjust ratio of methanol in eluting solvent to achieve proper elution profile. Additional methanol will decrease retention volume of sulfonamides.

Regenerate column only if background of chromatogram appears to increase. To regenerate Sephadex column, pass four 15 mL portions of methanol through column followed by four 15 mL portions of methanol-toluene (3 + 7). Let each portion enter bed before adding next. Let equilibrate at least 1 h before use.

Extraction

Weigh 50.0 g well mixed feed previously ground to pass 2 mm screen into 250 mL centrifuge bottle. Add 100 mL acetone and vigorously shake 10 min on mechanical shaker. Centrifuge at 2000 rpm for 10 min or until supernate is clear. Decant into 250 mL graduated cylinder with stopper. Add 100 mL 2% NH_4OH-acetone and vigorously shake 1 min. Centrifuge as before and combine extracts. Dilute to 200 mL with acetone and mix. Ignore any cloudiness of extract solution. Stopper and store in refrigerator.

Cleanup

Pipet 8.0 mL clear supernate into 15 mL centrifuge tube. Evaporate *just* to ca 0.5 mL at 40°C with nitrogen. Add 2 mL methanol-toluene (3 + 7) eluting solvent. Caution: Discontinue evaporation before first indication of dryness; do not allow sample to stand in eluting solvent. Proceed to Sephadex column as soon as possible.

Drain solvent in column to level of Sephadex bed and place 50 mL graduated cylinder under column. Transfer residue to column with five 2 mL portions of methanol-toluene (3 + 7). Let each addition enter bed before adding next portion, using care not to disturb column bed.

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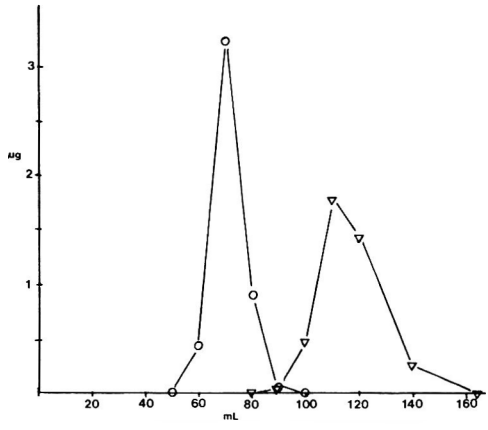

Figure 1. Elution profile of SMZ (○) and STZ (▽) from Sephadex column with 25% methanol-toluene.

Add solvent to maintain 1-20 cm liquid above bed. Collect first 40 mL forerun eluate or modify as necessary (see *Preparation and Evaluation of Sephadex Column*). Wash tip of column with solvent and discard wash. Collect next 90 mL eluate in 100 mL pear-shaped flask. Wash tip with solvent.

Derivatization

Add 0.5 mL diazomethane to eluate, mix, and stopper. Let stand 10 min and carefully rotary-evaporate at 60–70°C to dryness. Cool flask and add 5.0 mL diluting solvent with internal standard (Istd). Stopper and mix.

Pipet 2.0 mL working STZ standard into 100 mL pear-shaped flask. Add 70 mL methanol-toluene (3 + 7), derivatize as above, evaporate, and dilute to 10 mL with diluting solvent with Istd. Inject 3 μL into gas chromatograph and measure peak heights (PH).

Bracket samples with standard STZ injections.

ppm STZ = [PH STZ (sample)/PH STZ (std)] × [PH Istd (std)/PH Istd (sample)] × concn STZ std (μg/mL, final column) × (5.0 mL/8.0 mL) × (200 mL/50.0 g)

Thin Layer Chromatographic (TLC) Identification of STZ

See ref. 7, but use 8.0 mL sample extract.

Results and Discussion

The fortification of feed samples with STZ was carried out in the same manner as described in the method for SMZ (7). A stock standard feed mixture containing 1000 ppm STZ was prepared and then assayed by using a colorimetric method (2). The results of 10 assays (970.1, 971.9, 965.4, 960.6, 955.9, 972.5, 965.4, 967.7, 969.6, 970.1 ppm, mean 966.92, standard deviation 5.27, coefficient of variation (CV) 0.545%) show that the mixture was uniform. As was described for SMZ (7), the stock standard was diluted with ground feed and 50 g portions were spiked with STZ at the 0.5, 1, 2, and 5 ppm levels. All subsequent validation tests were performed with these 4 spike levels.

STZ has an elution profile from a Sephadex LH-20 column that is quite different from that of SMZ. In fact, the 2 sulfa drugs can be almost completely separated with an eluting solvent of methanol-toluene (1 + 3) (see Figure 1).

The problem of low recoveries of SMZ reported earlier (7), which occurred when the evaporation of the extract was continued after the acetone was removed, also applies to STZ. STZ is lost to an even greater extent. It was necessary to evaporate sample extracts to about 0.5 mL rather than risk losing STZ by evaporating all of the acetone. STZ is less stable than SMZ in the extract, even when refrigerated. Except for the sample extract, STZ appears stable in the other solutions used in the method. There was no detectable loss of STZ when it was mixed with feed and stored for up to 6 months in a dry state (Table 1).

The STZ elution volume from the Sephadex column is much larger than for SMZ. Therefore, an attempt was made to decrease the retention volume by increasing the percent methanol in the eluting solvent. Figure 2 shows that STZ emerges earlier with more methanol; however,

Table 1. Stability of STZ under various conditions

Matrix	Period tested	Comment
STZ standard in methanol	6 months	actinic glass at room temperature
STZ mixed with feed	5 months	glass jars at room temperature
STZ in ammoniated acetone	2 days	refrigerated at 4°C
STZ in 30% methanol–toluene eluting solvent[a]	3 days	after Sephadex cleanup; room temperature
Methylated STZ + sulfasymazine (Istd)	1 week	room temperature
Methylated STZ during rotary evaporation of eluting solvent[a]	1 h	tested up to 70°C

[a] In the presence of feed material.

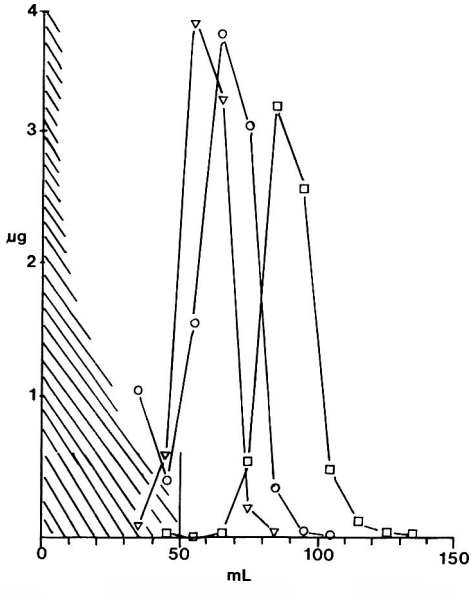

Figure 2. Elution profile of STZ from Sephadex column with 50% (▽), 40% (○), and 30% (□) methanol-toluene in sample matrix. Shaded area represents significant interferences from feed extract.

in a feed matrix a substantial amount of interference occurs at the higher proportion of methanol. Thirty percent methanol–toluene produced the cleanest solution with a reasonable retention volume.

The recovery of STZ was lower when the compound was extracted with 2% NH_4OH–acetone, as required in the SMZ method, than with acetone alone. On the other hand, the recovery of the sulfa drugs from the Sephadex column was improved when the sample extract was treated with ammonium hydroxide. For these reasons the samples were first extracted with acetone and then extracted again with an NH_4OH–acetone solution. Recoveries did not improve when multiple acetone extractions were used rather than a second extraction with ammoniated acetone.

The precision of the proposed STZ method is shown in Table 2. The mean recoveries ranged from 87.4 to 90.2%, with a CV range from 4.29 to 5.90%. These results were slightly lower than those obtained with the SMZ method, in which the mean recovery ranged from 93.4 to 96.0% and the CV was 1.43–4.38%. The lower recoveries and the higher CVs are attributable to STZ adsorption onto feed solids which are not extracted with acetone. Additional loss of STZ is due to adsorption that occurs in the evaporation step described earlier.

The recoveries from spiked commercial feeds agree with the recoveries from the precision test with the exception of the last feed in Table 3. These recoveries may be lower than the other values because of the relatively large amounts of alfalfa in the sample. Losses of STZ seem to occur in the extraction as well as in the evaporation step described earlier. Recoveries of STZ were in the same range as those of the other feeds when the sample was spiked after extraction.

GLC of methyl STZ in this method uses the same conditions as those described in the SMZ method. The treatment of the sulfasymazine Istd is also the same; however, the retention time of STZ is less than that of the Istd. Figure 3 shows a typical chromatogram of a 0.5 ppm spiked feed.

Once the GLC conditions are established and

Table 2. Precision of proposed GLC method for determining STZ

	Spike level, ppm							
	0.484		0.968		1.936		4.840	
Assay No.	Rec.	%	Rec.	%	Rec.	%	Rec.	%
1	0.465	96.1	0.938	96.9	1.893	97.8	4.145	85.6
2	0.388	80.2	0.812	83.9	1.587	82.0	3.957	81.8
3	0.431	89.0	0.810	83.7	1.608	83.1	4.213	87.0
4	0.475	98.1	0.869	89.8	1.700	87.8	4.280	88.4
5	0.419	86.6	0.901	93.1	1.761	91.0	4.526	93.5
6	0.415	85.7	0.860	88.8	1.691	87.3	4.380	90.5
7	0.451	93.2	0.846	87.4	1.692	87.4	4.303	88.9
8	0.427	88.2	0.876	90.5	1.614	83.4	3.967	82.0
9	0.488	92.6	0.849	87.7	1.761	91.0	4.310	89.0
10	0.446	92.1	0.904	93.4	1.607	83.0	4.392	90.7
Mean	0.437	90.2	0.867	89.5	1.691	87.4	4.247	87.7
SD	0.0258		0.0405		0.0951		0.1822	
CV, %	5.90		4.67		5.62		4.29	

Table 3. Assay results for STZ in 4 different types of spiked commercial swine feeds by proposed GLC method

	Spike level, ppm							
	0.484		0.968		1.936		4.840	
Feed	Rec.	%	Rec.	%	Rec.	%	Rec.	%
Co-op Hog Finisher-14 Complete (A)	0.570	117.8	0.854	88.2	1.600	82.6	3.910	80.8
Co-op Sow Lactation	0.442	91.3	0.947	97.8	1.700	87.8	4.294	88.7
Purina Complete Sow Chow w/o	0.530	109.5	0.867	89.6	1.685	87.0	3.950	81.6
Co-op Swine[a] Builder (A)	0.359	74.2	0.698	72.1	1.358	70.1	3.414	70.5

[a] Sample contained large amounts of alfalfa.

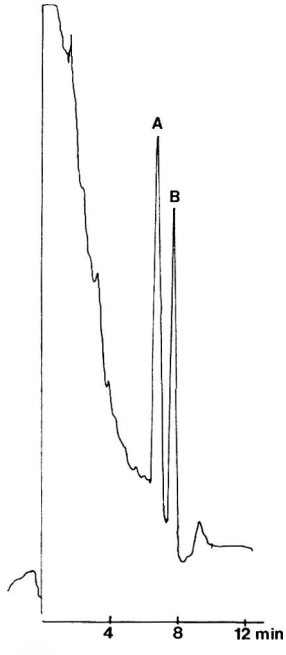

Figure 3. Typical chromatogram of A, STZ, and B, Istd, from feed sample spiked at 0.5 ppm.

the evaluation of the Sephadex column is completed, the assay of feed samples is quite rapid. One analyst can assay 12 samples a day. Samples can continue to be analyzed from day to day without having to regenerate the Sephadex column. The Sephadex column gave reproducible elution profiles after 50 samples had passed through it (7).

This method, as developed for STZ residues in swine feed by using GLC, is sensitive, rapid, and accurate.

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VITAMINS AND OTHER NUTRIENTS

Evaluation of Urea-Acid System as Medium of Extraction for the B-Group Vitamins. Part II. Simplified Semi-Automated Chemical Analysis for Niacin and Niacinamide in Cereal Products

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Efficiency of a urea-acid system was evaluated as a medium of extraction for niacin and niacinamide from cereals and other selected food products. Samples were treated with 1N HCl in the presence of urea and autoclaved 30 min at 15 psi. Extracts were diluted to a known volume, filtered, and analyzed. The analytical results obtained for niacin were compared with values obtained by AOAC method 43.044. In general, the urea-acid extraction system was simple and straightforward with an efficiency comparable to the AOAC method. The analytical manifold designed uses fewer modules, gives net absorbance readings from sample and blank, and is capable of analyzing 50 samples per hour.

Niacin (pyridine-3-carboxylic acid) occurs mainly in the form of its amide (niacinamide), and in tissues is directly linked through the pyridine nitrogen to a ribose residue to form a part of both co-enzyme I and co-enzyme II. The isolation of dinicotinylornithine from the excreta of chicks (1) and the presence of niacin-containing micromolecules in wheat (2) suggest that niacin may also be combined through an amide linkage to other structures. Some materials, for example, the seed coat of corn and other cereal grains, contain a bound form of niacin (3, 4) which is not biologically available to the organism. (The bound form of niacin in corn has been implicated in the pellagragenic effect of this cereal grain.)

All chemical and microbiological procedures for the determination of niacin and niacinamide in a wide variety of food products require the liberation of these compounds from their bound forms. For this reason, samples under study are hydrolyzed with either an acid or an alkaline reagent. The present AOAC method (5) suggests hydrolysis of non-cereal foods and feeds with dilute sulfuric acid and cereal samples with calcium hydroxide to liberate niacin and niacinamide, respectively. During acid or base hydrolysis, the liberated niacinamide is hydrolyzed to niacin. Vaughn and Robbins (6) reported that the usual hydrolytic conversion of heterocyclic carboxamides to the corresponding acids is particularly difficult because the acids are prone to decarboxylation. Leete (7) observed that niacin when heated with calcium oxide gives pyridine. Base hydrolysis of amides is frequently an inefficient process (8). Like aromatic acids, niacin, in the presence of either base or acid increases the formation of a positive charge on the ring nitrogen which then exerts attraction on the electrons of the carboxyl group, drawing them toward the ring and favoring the release of carbon dioxide. With the loss of CO_2, a carbanion is generated which then is stabilized by the positive center on the pyridine ring to yield pyridine.

Considering the competition between the acidic hydrogen of the pyridine carboxyl group and the hydrogen of the acidic solvent (HCl or H_2SO_4) for the nitrogen of the pyridine ring, the rate of decarboxylation of niacin in acidic medium under certain experimental conditions should decrease compared with basic medium. Barton-Wright (9) reported difficulty in the assay of niacin in cereal and cereal products by the chemical method, because of the presence of interfering substances.

In previous studies (10), we reported that urea in the presence of dilute HCl might be used as a common extraction medium for B-group vitamins. In this paper, we describe the evaluation of the urea-acid system as a medium of extraction, and a simplified semi-automated chemical procedure based on Konig's reaction (11) for determination of niacin and niacinamide in cereals and other selected groups of food products.

METHOD

Apparatus

Automated equipment: AutoAnalyzer®II (Technicon Instruments Corp., Tarrytown, NY

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10591) systems including sampler IV with cam 50/h (6:1), proportioning pump III, colorimeter with 50 mm flowcell and 470 nm filters, voltage stabilizer, strip chart recorder, and all AutoAnalyzer II glassware.

Reagents

Use analytical grade reagents and solvents and distilled water throughout analytical procedure.

(a) *Phosphate buffer (pH 7.0) stock solution.*—Dissolve 162.0 g disodium hydrogen phosphate and 50.0 g potassium dihydrogen phosphate in 800 mL hot water, and cool. Dilute to 1 L with water and mix.

(b) *Phosphate buffer working solution.*—Dilute 100 mL phosphate buffer (pH 7.0) stock solution to 1 L with water. Add 1.0 mL Brij-35 (Atlas Chemical Co.) and mix.

(c) *Cyanogen bromide (CNBr) solution, 10%.*—Weigh 100.0 g CNBr and dissolve in ca 700 mL warm water (ca 40°C). Shake until contents dissolve, and dilute to 1 L with water. *Caution:* Perform weighing and reagent preparation steps under hood. Do not let cyanogen bromide or solution come in contact with skin. CNBr is very toxic. Store reagent in brown bottle in refrigerator.

(d) *Sulfanilic acid solution, 10%.*—Add 100.0 g sulfanilic acid in beaker containing 500 mL water. Add ca 50 mL concentrated NH_4OH solution dropwise with shaking to dissolve sulfanilic acid. Adjust pH to 7 with 5N HCl. Dilute to 1 L with water.

(e) *Hydrochloric acid solution, 1N.*—Dissolve 82.5 mL concentrated HCl in ca 800 mL water. Dilute to 1 L with water.

(f) *Ethanol solution, 25% (v/v).*—Dilute 250 mL ethanol to 1 L with water.

(g) *Niacin stock standard solution.*—(A) 1000 μg/mL: Weigh 100.0 mg niacin reference standard (previously dried and stored in dark in desiccator over P_2O_5), and transfer to 100 mL volumetric flask. Add ca 80 mL aqueous ethanol (25% v/v) solution and shake to dissolve. Dilute to 100 mL with aqueous ethanol (25% v/v) solution. Store reagent in brown bottle in refrigerator. Standard is stable 2 weeks when kept in refrigerator.

(h) *Intermediate niacin stock standard solution.*—(B) 100 μg/mL: Pipet 10 mL niacin stock standard solution (A) into 100 mL volumetric flask and dilute to volume with water. Store standard in brown bottle in refrigerator. Prepare fresh weekly.

(i) *Working niacin standards.*—0.5, 1.0, 2.0, 3.0, 4.0, 5.0 μg/mL: Pipet 0.5, 2, 3, 4, and 5 mL aliquots of intermediate niacin stock standard (B) solution, respectively, to six 250 mL Erlenmeyer flasks containing 2.0 g urea. Add 25 mL 1N HCl and dilute with water to ca 75 mL. Digest these standards along with samples. Transfer contents and washings into 100 mL volumetric flask and dilute to volume with water.

(j) *Urea.*—ACS certified (Fisher Scientific Co.).

Sample Preparation

Accurately weigh 2.50 g sample (or equivalent amount to contain niacin in working standards range) and transfer to 250 mL Erlenmeyer flask. Add 2.0 g urea and 75 mL 1N HCl. Swirl flask to dissolve urea and to wet sample particles. Autoclave mixture 30 min at 15 psi. Cool to room temperature and adjust pH of extracts to 6.30–6.50 with 15% NaOH solution (in cases of overshoot, use 1.0N HCl to effect desired pH range). Transfer contents and washings to 100 mL volumetric flask and dilute to volume with water. Take aliquot of diluted sample and centrifuge 10 min. Transfer clear sample onto sampler IV for analysis.

Analytical Procedure

Flow diagram for AutoAnalyzer II continuous flow analytical system and appropriate flow rates of various pump tubings are given in Figure 1.

Sample segmented with air is passed through membrane of 24 in. dialyzer. Phosphate buffer (pH 7.0) segmented with air is introduced into lower part of dialyzer. Dialysates are pumped through AO glass fitting, and portion of stream leaving AO glass fitting is divided into 2 substreams. One substream is mixed with CNBr and sulfanilic acid, respectively, and then allowed to enter sample inlet of colorimeter. Second substream is treated with sulfanilic acid in absence of CNBr and then allowed to enter reference inlet of colorimeter. Net percentage transmission at 470 nm is recorded.

To ensure proper synchronization of system (sample and reference reach their respective flowcell simultaneously), dilute colored solution (e.g., red ink) is aspirated through manifold. Stream which reaches flowcell early is corrected by adding reagent tubing. Five sets of niacin standard (0.5–5 μg/mL) solution should be run through system to determine standard curve. Linear response is observed over this range of niacin concentrations. Manifold design is capable of performing analysis at 50 samples per hour.

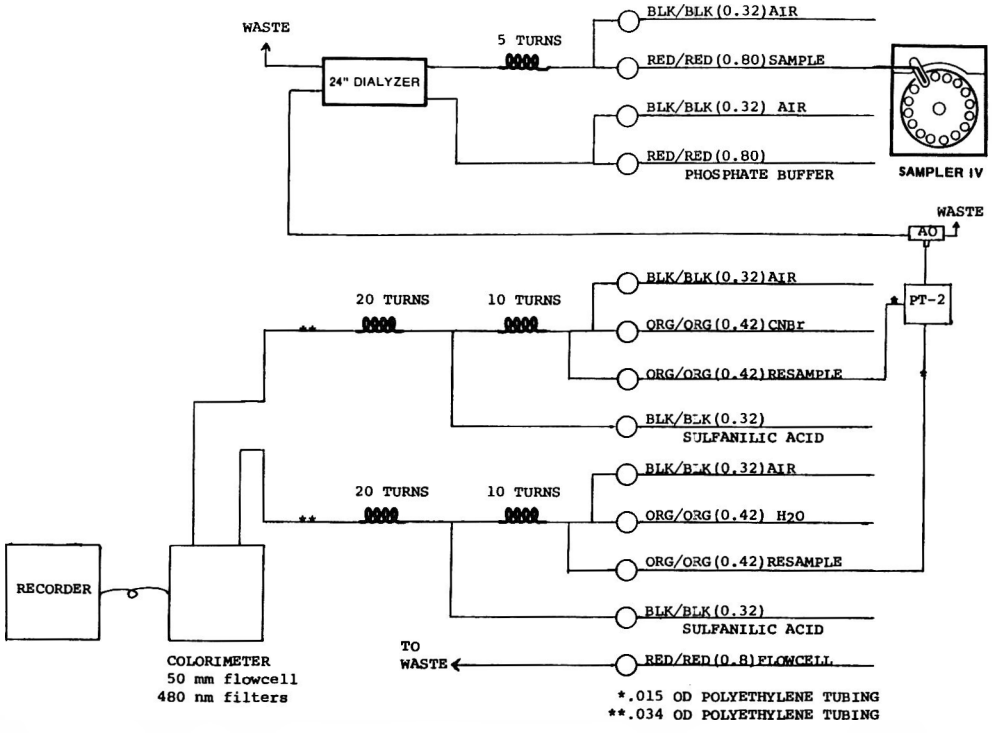


Figure 1. Schematic flow diagram for semi-automated analysis of niacin in food products (figures in parentheses signify flow rates in mL/min).

To start system, water containing a few drops of Brij-35 is pumped through all reagent lines ca 10 min. Reagent lines are placed in their respective reagent solutions with sample line in water, and system is equilibrated 15 min. Standards are run for calibration procedure. Reliability of the system is checked by introducing a known standard between every 20 samples on the sampler tray.

Operating Notes

Since all methods used for sample hydrolysis are likely to cause decomposition of substances other than niacin and niacinamide, it is important to run a blank for every sample assayed. To obtain on-line blank correction, a blanking channel has been added to the present manifold.

Fat and oil as well as sample extracts from fruits and vegetables are likely to contain interfering substances and, therefore, they should be removed from the sample extracts. Fat can be partially removed by filtering a cooled (below 5°C) extract through filter paper (S&S No. 588). Oil and other interfering substances can be removed by extracting the sample extracts with either chloroform or isobutanol.

Multiple working standards are used only to establish linearity of the system. For day-to-day operation, the 5 μg/mL standard niacin solution with standard calibration set to read 60 chart units is recommended for the routine check on the performance of the system.

The stream leaving the colorimeter as waste should be allowed to drip into an Erlenmeyer flask containing a mixture of 2N NaOH and 5% sodium hypochlorite solutions.

It is advisable to set up the entire system under a hood.

Alternative ranges may be obtained by using the standard calibration control on the colorimeter.

The Technicon block digestor can also be used for sample digestion in lieu of autoclaving.

At the end of the day, the system should be shut down by pumping water containing Brij-35 through all reagent lines ca 20–25 min.

Results and Discussion

Several different food samples were extracted with 1N HCl in the presence of 2.0 g urea and analyzed for niacin by using the manifold shown in Figure 1. Measurement of niacin by the present automated system incorporated the

Table 1. Comparison of niacin results obtained by AOAC and automated procedures from cereal and related food products

Sample	AOAC, mg/100 g[a]	Auto., mg/100 g[a]	Diff. AOAC − Auto.	% Bias Auto.	t
Wheat germ	5.1	5.3	−0.2	−3.9	−0.5
Oat Flakes	15.3	14.0	1.3	9.3	3.4**
All Bran	21.3	21.8	0.0	0.0	0.0
Instant Corn Gritt	9.6	9.5	0.1	1.1	0.3
Gold Medal enriched flour	6.1	5.8	0.3	5.2	0.8
Enriched bread	4.1	4.0	0.1	2.5	0.3
Enriched rice	6.8	6.4	0.4	6.3	1.1
Rice Chex	24	23.5	0.5	2.1	1.3
Egg ring	8.3	9.5	−1.2	−12.6	−3.6**
Enriched flour	4.5	4.3	0.2	4.7	0.2
Proprietary cereal products					
(a)	16.5	17.5	−0.9	−5.1	2.4**
(b)	15.9	18.5	−2.6	−14.1	−6.8**
(c)	16.5	17.0	−0.5	02.9	−1.3
(d)	15.3	16.5	−1.2	−7.3	−3.2**

[a] Av. of duplicate analyses.
** Statistically significant at $\alpha = 0.05$ level.

chemical principles of Konig's reaction (11). Gross (12) and Egberg et al. (13) have also reported semi-automated procedures based on Konig's reactions for determination of niacin and niacinamide in food products.

The former author described a single channel manifold designed to obtain the sample and the blank readings separately. The latter authors split the sample on-line and then determined the sample and blank readings by using 2 separate but identical manifold designs. While the procedure described by Gross (12) reqires re-analysis to obtain sample and blank readings, those described by Egberg et al. (13) need extra automated modules.

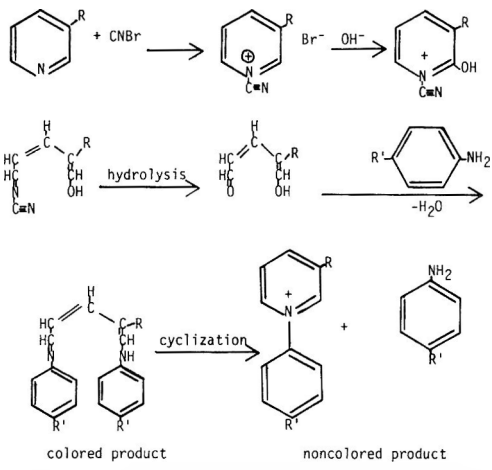
In the present method, the sample extracts were partially purified with the dialyzer, and the dialysate was divided and resampled. One resample stream was treated with CNBr and sulfanilic acid, respectively, and the mixture was then allowed to enter the sample inlet of the colorimeter. The other resample stream was mixed with sulfanilic acid and water and passed through the reference inlet of the colorimeter. A single-pen recorder traced the output signal on a continuous strip chart to produce a single peak for each sample.

The precision of the method was established as the variability for repeated analysis of niacin standard during the day of analysis and from day-to-day operation. The relative standard deviation for duplicate analysis of a 3 μg/mL niacin standard was less than 1%.

Analyzing the various concentrations of niacin standards, even though not at steady-state and the baseline not returning completely to its original starting position, showed no carryover. The accuracy of the method was determined by comparing the automated results with the manual AOAC values. Both analyses were performed on the same samples. The results, which are in agreement within the experimental limits (Table 1), indicated that the urea-acid system was an acceptable extraction medium for niacin and niacinamide from several groups of food and food products.

Literature references (14, 15) indicate that the reaction of niacin and niacinamide with cyanogen bromide and sulfanilic acid (Konig's reaction) to produce color depends on the pH, reagent concentrations, and time. The color intensity is optimum within 2 min and then starts fading rapidly. It is therefore necessary to read color intensities at the time of maximum color development from both sample and standard to get reproducible results. In the automated procedure, sample and standard are treated under identical conditions, so the need for pH correction and color measurement at optimum intensity is eliminated. In addition, the automated procedure protects analysts from the toxic fumes of cyanogen bromide.

In Scheme 1, we propose mechanisms by which the color products are formed and fade in the Konig reactions. Niacinamide reacts to yield the same color as niacin but of lesser intensity



Scheme 1. Possible reaction mechanism for color formation in Konig's reaction (R = $CONH_2$ group; R' = $-SO_3H$ group).

(16, 17); therefore, an efficient extraction medium is necessary to liberate and quantitatively convert niacinamide to niacin to obtain correct analytical results. We observed that the urea–acid system hydrolyzes niacinamide to niacin completely during sample preparation.

Finholt and Higuchi (18) reported that, in strongly acidic solution, the first step in the hydrolysis of niacinamide is the reaction between hydrogen ions and the protonated form of the amide. The reported studies (18, 19) on the acid-catalyzed hydrolysis of amides, in which water attacks the *N*-protonated conjugate acid in the rate-determining step, suggest that the hydrolysis of niacinamide to niacin occurs through a reactive intermediate.

Because the above hydrolytic reactions involve 2 charged species, a positive primary salt effect is expected to increase the rate of hydrolysis of niacinamide in acidic environment. (In alkaline medium, niacinamide will exist in the noncharge form and therefore no primary salt effect will occur.) Mono- and di-HPO_4 ions were reported (18) to exert the strongest effect on the rate of hydrolysis of niacinamide in acidic solution (such phosphate ions are likely to be generated by the degradation of co-enzymes I and II, respectively, during the sample preparation).

Johnson (20) claimed that standard niacinamide solution was completely hydrolyzed (100%) to niacin when heated with 0.6N H_2SO_4 in an autoclave at 15 psi for 1 h. Urea activates dilute HCl or dilute H_2SO_4 to effect a rapid liberation and hydrolysis of niacinamide to niacin from a wide variety of foods and food products.

Because of the strong hydrolytic action of the urea-acid system, the samples containing high levels of carbohydrate and starch produce colored extracts due to rapid degradation of these molecules into smaller units with reactive carbonyl groups. Such samples may require a smaller amount of urea (e.g., 1 g), weak HCl (0.05N), and shorter period (15–20 min) for sample digestion. Some fruits and vegetables may contain free niacin (old meat samples may also contain free niacin due to enzymatic hydrolysis of niacinamide) and, therefore, these samples should be extracted with 0.5 g urea in 1N HCl. Urea has been reported to prevent interaction of protein and carbohydrates (21) and therefore might minimize the formation of interfering substances.

Samples (a total of 45) selected from sandwich breads, hot dog rolls, and hamburger rolls which were expected to contain a minimum of 3.5 μg/mL of niacin, when analyzed by the present method, gave an average value of 4.08 μg/mL of niacin. Several samples from both meat and fruit products were analyzed and compared (Table 2) with the manual AOAC values. The automated procedure gave an average of 1.6 mg/373.24 g higher values (<12.5% at 13 mg/373.24 g) compared with the AOAC method for meat samples. For fruit samples (apples), no difference between the AOAC and automated methods was detected. Recovery studies on

Table 2. Comparison of niacin results (mg/373.24 g) obtained by AOAC and automated methods from meat and fruit products

Sample	AOAC Av.[a]	Auto. Av.[a]	Diff. AOAC − Auto.	% Bias Auto.	SD[b]	*t*
Bologna	12.12	13.72	−1.60	−11.7	0.34	−10.6**
Frankfurter	12.36	13.98	−1.62	−11.6	0.41	−8.9**
Apples	0.94	1.08	−0.15	−13.9	0.16	−2.0

[a] 5 different samples analyzed in duplicate.
[b] Obtained by pooling paired duplicates from AOAC and AAII assays.
** Statistically significant at $\alpha = 0.05$ level.

Table 3. Recovery of niacin added (μg/mL) to extracts from bread and flour samples [a]

Sample	Amt added	Amt expected	Amt found	Rec. %	Loss/amt added
Bread	0	0	5.17		
	0.5	5.67	5.66	99.8	0.01 (2%)
	1.0	6.17	6.15	99.7	0.02 (2%)
Flour	0	0	5.94		
	0.5	6.44	6.42	99.7	0.02 (4%)
	1.0	6.94	6.90	99.4	0.04 (4%)

[a] Analytical results represent average of duplicate analyses.

bread and flour samples spiked at 2 levels of niacin (0.5 and 1.0 μg/mL) are shown in Table 3. The analytical data obtained from both the manual AOAC and automated procedures were statistically analyzed. The error estimate (0.38) obtained from pooling the bologna and frankfurter error estimates in Table 2 was conservatively applied to the data in Table 1, in calculating the *t*-statistics with respect to 10 degrees of freedom.

The results of statistical analysis may be summarized as follows: (a) Seven of 15 different foods showed statistically detectable differences between the automated and AOAC methods (Tables 1 and 2), which is more than chance would predict. (b) In 6 of the detectable differences, the AOAC method showed an average negative (−12%) bias, relative to the automated method, which is more than chance would predict (Tables 1 and 2). (c) Oat flakes (Table 1) showed a positive (9.3%) bias relative to the automated method.

Results obtained for niacin and niacinamide from other groups of food products not reported here, as well as other B-group vitamins using a urea–acid extraction system, will be reported in the future.

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SEAFOOD TOXINS

High Pressure Liquid Chromatographic Determination of Toxins Associated with Paralytic Shellfish Poisoning

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A high pressure liquid chromatographic procedure is described for assay of toxins associated with paralytic shellfish poisoning (PSP). The method is applicable to saxitoxin, neosaxitoxin, gonyautoxins I through IV, and their sulfocarbamoyl derivatives. Toxins are separated on a bonded phase cyano column and detected by fluorescence following alkaline oxidation (NH_4^+ and periodic acid). The utility of the HPLC procedure for research and monitoring is discussed.

Toxic shellfish causing the disorder commonly referred to as paralytic shellfish poisoning (PSP) occur along both the east and west coasts of North America in the summer months. Shellfish become toxic after feeding on marine dinoflagellates of the genus *Gonyaulax* (identical to *Protogonyaulax*, found in some of the more recent literature). Dinoflagellates contain potent neurotoxins (see Figure 1) which accumulate in tissues of shellfish. The unpredictable pattern of this toxicity necessitates constant monitoring of shellfish from beaches in affected areas. The primary analytical method now used for shellfish monitoring is the mouse bioassay as described in *Official Methods of Analysis* (AOAC) (1). Several drawbacks to this bioassay include precision of only ±20%, interference from sodium chloride, and the need to maintain a supply of mice of the proper size (2). In addition, with the recent report of sulfocarbamoyl toxin derivatives (3, 4) present in cultures of *Gonyaulax*, it becomes increasingly important to develop methods that can distinguish the various toxins.

Several other assay techniques have been developed. These include an immunological assay (5), colorimetric assays (6, 7), and a fluorometric assay (8, 9). Of these, the fluorometric assay holds the greatest promise for routine measurement of shellfish toxicity. This assay is based on alkaline oxidation of the shellfish toxins to fluorescent derivatives and has proven to be extremely sensitive and specific. A major drawback of the fluorometric procedure is the very low response to several of the toxins, namely neosaxitoxin and gonyautoxins I and IV. These nonfluorescing toxins can constitute a major portion of the toxin content of shellfish (10), thereby creating an underestimation of total toxicity by this method. Buckley et al. (11) incorporated this method into a continuous flow liquid chromatography system but the procedure does not separate the various toxins to allow for individual quantitation.

In this paper we report the development of a high pressure liquid chromatographic (HPLC) procedure for quantitation of PSP toxins. This method is similar to the toxin analyzer reported by Buckley et al. (11) with several modifications which allow separation and detection of the shellfish toxins illustrated in Figure 1.

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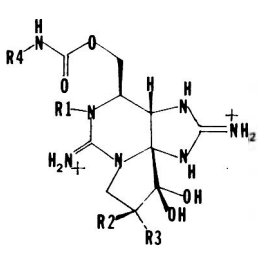
Experimental

HPLC System

Figure 2 illustrates the flow diagram for the HPLC system. Equipment includes a high pressure pump (LDC Minipump Model 2396-89), an injection valve (Rheodyne Inc. Model 7120), and a reagent pump (Technicon AutoAnalyzer pump I). The reagent pump is equipped with standard Technicon pump tubing providing flows of 0.32, 0.42, and 0.23 mL/min for the base,



R1	R2	R3	R4	
H	H	H	H	STX
H	H	H	SO_3^-	B1
H	H	OSO_3^-	H	GTX-II
H	H	OSO_3^-	SO_3^-	C1
H	OSO_3^-	H	H	GTX-III
H	OSO_3^-	H	SO_3^-	C2
OH	H	H	H	NEO
OH	H	H	SO_3^-	B2
OH	H	OSO_3^-	H	GTX-I
OH	OSO_3^-	H	H	GTX-IV

Figure 1. Toxins associated with paralytic shellfish poisoning: STX = saxitoxin, GTX = gonyautoxin, NEO = neosaxitoxin, and B1, B2, C1, C2-*N*-sulfocarbamoyl derivatives.

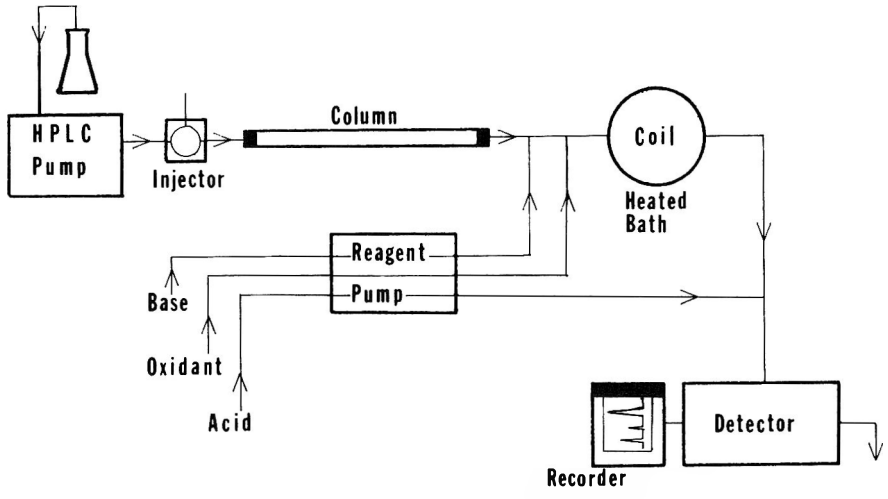


Figure 2. Flow diagram for HPLC system.

oxidant, and acid, respectively. The Perkin Elmer MPF-2A fluorescence spectrophotometer is equipped with a flow cell fabricated from 3 mm id glass tubing (40 μL illuminated volume). Excitation and emission wavelengths of 340 and 388 nm, respectively, are used. Slit widths and detector sensitivity are adjusted to achieve maximum response for shellfish toxins. The HPLC column is a bonded phase cyano column based on 7.5 μm Nucleosil (Macherey-Nagel and Co., Duren, GFR). The packing is prepared by reacting 5 g with 13 g cyanopropyldimethylchlorosilane (Petrarch Systems, Inc., Levittown, PA) and 6.3 g pyridine in 40 mL toluene under reflux for 16 h. The reaction mixture is filtered and washed with methanol, methanol–water (50 + 50), methanol, ether, and toluene in that order. The packing material is then returned to the reaction flask and the reaction and washing procedures are repeated by substituting hexamethyldisilazane (HMDS) for the cyanopropyldimethylchlorosilane reagent. The prepared packing material is then slurry-packed into a 4.0 mm id × 250 mm stainless steel column, using ethyl ether.

The reaction manifold system is comprised of a Teflon capillary tube (0.56 mm id × 4.9 m) as a reaction coil and low volume tees fabricated from plexiglass and $^1/_{16}$ in. stainless steel tubing. The reaction coil has an internal volume of ca 1.2 mL and a 40 s reaction time.

Mobile Phase

The 1M ammonium phosphate buffer (pH 7.0) is prepared from ammonium hydroxide and phosphoric acid, and ionic strength is standardized by measuring ammonium concentration (distillation and titrimetry). Mobile phases for HPLC are prepared by mixing methanol (Mallinckrodt Inc., Nanograde), water, and 1M ammonium phosphate buffer to achieve the desired ionic strength (as NH_4^+) and run at a flow rate of 0.9 mL/min.

Toxin Standards

Toxins are purified from *Gonyaulax* spp. by column chromatographic procedures (3, 4). Purity is checked by TLC on silica gel using pyridine–ethyl acetate–acetic acid–water (15 + 5 + 3 + 4) (3). Pure toxins are diluted in 0.03M acetic acid for use in standardizing the HPLC assay.

Oxidation Reagents

Aqueous solutions of periodic acid (J.T. Baker & Co.), ammonium hydroxide, and acetic acid are prepared and pumped through the lines marked oxidant, base, and acid, respectively (see Figure 2). The concentration of these reagents is determined by the total flow rate through the reaction manifold and the dilution each reagent undergoes as it enters the manifold (see discussion). Response is optimum when using reagents of the following concentrations: 0.065M periodic acid, 2M ammonium hydroxide, and 6.0 M acetic acid.

Sample Preparation

Shellfish samples, obtained from contaminated beaches in Puget Sound, are extracted according to AOAC procedures (1). Before injection into the HPLC system, protein is precipi-

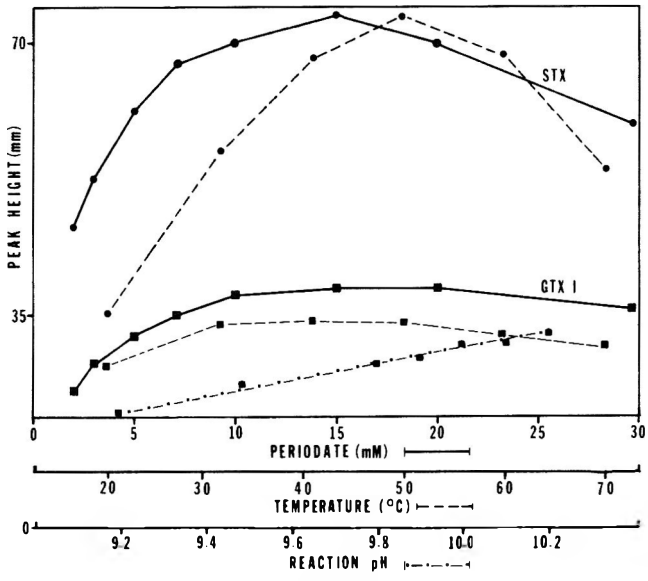


Figure 3. Effect of periodate concentration in reaction coil and reaction coil temperature on response to saxitoxin (STX) and gonyautoxin I (GTX I), and effect of reaction medium pH on response to saxitoxin.

tated by adjusting the pH to 4.0–4.5 with NH_4OH. The sample is then centrifuged and filtered through a 0.45 μm filter to remove particulates.

Dinoflagellate (*Gonyaulax catenella*) cells are extracted in a manner similar to that above except 0.05M acetic acid is substituted for 0.1N HCl, and extracts are not heated. This produces an extract containing predominantly sulfocarbamoyl toxins. To produce an extract in which the sulfocarbamoyl toxins have been hydrolyzed, 0.15N HCl is used and the extract is heated 5 min at 100°C. Both dinoflagellate and shellfish extracts are diluted with water or methanol to reduce the ionic strength to less than 0.03N (as HCl) before injection into the HPLC system.

Results and Discussion

Bates and Rapoport have described the oxidation of shellfish toxins by using hydrogen peroxide (8). The pressures required to retard O_2 bubble formation when using H_2O_2 are incompatible with the Technicon pump and reaction manifold described here. We found that fluorescent derivatives can also be formed using periodate as the oxidant. The fluorescence intensity of neosaxitoxin and gonyautoxins I and IV is less than that for the other toxins, necessitating a separation step such as HPLC. We investigated the parameters which lead to efficient separation and detection of saxitoxin, neosaxitoxin, gonyautoxins I–IV, and the *N*-sulfocarbamoyl derivatives (3, 4, 12).

Post-Column Reaction

To achieve optimum sensitivity, the post-column reaction system must operate efficiently and reliably. In our initial investigations we found that hydrogen peroxide, benzoyl peroxide, or periodate could be used to form fluorescent derivatives. The fluorescence produced for neosaxitoxin and gonyautoxins I and IV was slightly higher with periodate so this reagent was chosen for use in the HPLC system. The parameters which affect fluorescence intensity were investigated. These include periodate concentration, temperature, pH of the reaction medium, and final pH. Figure 3 illustrates the effect of reaction coil periodate concentration on saxitoxin and gonyautoxin I response. The periodate concentration is determined by the concentration of the reagent pumped into the manifold and its subsequent dilution. As can be seen, little change in fluorescence intensity is observed between 10 and 25 mM periodate. Using flow rates of 0.75, 0.32, and 0.42 mL/min for the mobile phase, base, and periodate, respectively, this corresponds to a periodic acid *reagent* concentration between 35 and 90 mM. Small changes in periodic acid or flow rates will not greatly affect fluorescence.

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To keep post-column volume to a minimum, a relatively short reaction coil is used. This coil provides a reaction time of only 40 s, so heating was necessary. Fluorescence intensity was greatly dependent on reaction temperature (see Figure 3); 40–50°C was optimum for both toxins investigated. Toxins were not converted to their fluorescent derivatives at lower temperatures and fluorescence decreased at higher temperatures, indicating possible over-oxidation.

Fluorescence is also affected by pH of the reaction medium (Figure 3). Intensity increases approximately 50% between pH 9.2 and 10.2. Reaction medium pH is controlled by the strength of the base pumped into the reaction manifold, dilution that occurs, and buffering capacity of the mobile phase. While the greatest intensity occurs at higher pH, it is impractical to increase the pH above 10.0 (approximately 2.5N reagent) because subsequent neutralization with acetic acid can lead to the formation of a precipitate. Values above 9.8 were adequate; only a small increase in sensitivity is achieved above this level.

Addition of acetic acid to the effluent immediately after the reaction coil decreases pH and shifts the maximum emission to 390 nm. This increases fluorescence intensity about 10-fold over that at alkaline pH. Fluorescence intensity differs little between pH 5.0 (50% acetic acid reagent) and pH 5.9 (20% acetic acid reagent).

Toxin Separation

Accurate determination of total toxin content by using this method depends on efficient separation. It is especially important that the poorly fluorescing toxins, neosaxitoxin and gonyautoxins I and IV, are well resolved from other toxins. In our initial HPLC studies, several columns were tested. In the reverse phase mode (C_{18}) using an ion-pairing reagent (hexane sulfonic acid), all 4 gonyautoxins co-eluted, as did neosaxitoxin and saxitoxin at a somewhat longer retention time. An amino phase column was also tested extensively. Although this column separated all 6 toxins efficiently, elution times and orders changed after a short period, indicating degradation of packing material. This was especially prevalent following analysis of a number of shellfish extracts. It is presumed that materials in the extracts adsorbed or reacted with the packing material, altering its chromatographic properties.

The bonded phase cyano column has proven to be the most useful for toxin separations. Figure 4 illustrates the separation of saxitoxin,

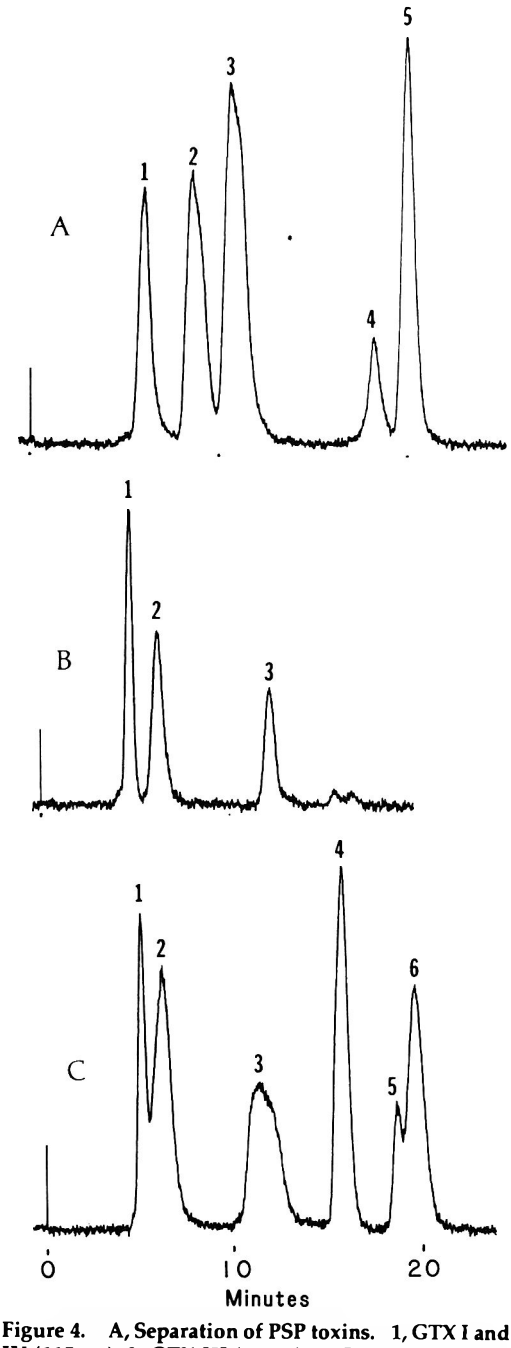

Figure 4. A, Separation of PSP toxins. 1, GTX I and IV (115 ng); 2, GTX III (11 ng); 3, GTX II (16 ng); 4, NEO (132 ng); and 5, STX (15 ng). Mobile phase 20% MeOH with 0.002M Amm. PO_4, switch to 0.008M at 10 min. B, Separation of sulfocarbamoyl derivatives. 1, C1 and C2 (10 ng); 2, B2 (50 ng); 3, B1 (10 ng). Mobile phase 20% MeOH with 0.001M Amm. PO_4, switch to 0.008M at 10 min. C, Separation of PSP toxins and their sulfocarbamoyl derivatives. 1, C1 and C2; 2, GTX I, IV, and B2; 3, GTX III and B1; 4, GTX II; 5, NEO; and 6, STX. Conditions as in B.

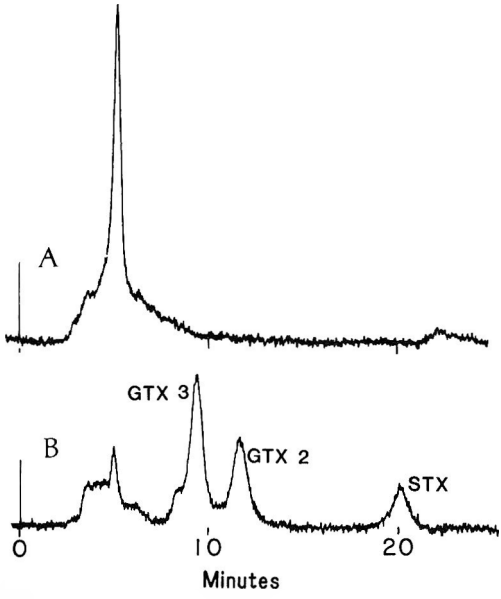

Figure 5. A, Extract of nontoxic mussels. B, extract of toxic mussels (*Mytilis edulis*) showing presence of GTX II, III, and STX. Conditions as in Figure 3A.

neosaxitoxin, gonyautoxins I–IV, and 4 of their sulfocarbamoyl derivatives. Although gonyautoxins I and IV co-elute, as do C-1 and C-2, the other toxins are sufficiently well separated to allow individual determination. Figure 4C shows that several of the sulfocarbamoyl derivatives are not well resolved from other toxins. This is not a problem because the derivatives can be easily hydrolyzed (3) to their parent toxins before analysis. Under the mobile phase conditions used for separation of the toxins, the sulfocarbamoyl derivatives elute much faster. A

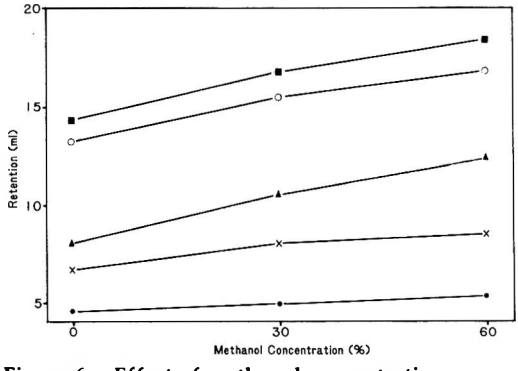

Figure 6. Effect of methanol concentration on retention of PSP toxins. Ammonium phosphate concentration: 0.002M, switch to 0.008M at 10 min. GTX I ●–●; GTX II ▲–▲; GTX III ×–×; NEO ○–○; STX ■–■

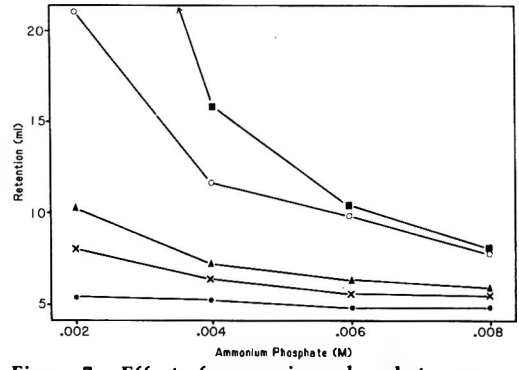

Figure 7. Effect of ammonium phosphate concentration on retention of PSP toxins. Mobile phase is 20% MeOH, 80% water. GTX I ●–●; GTX II ▲–▲; GTX III ×–×; NEO ○–○; STX ■–■

weaker solvent is necessary to separate these compounds. Chromatograms of toxic and nontoxic shellfish extracts prepared according to AOAC procedures are shown in Figure 5. Note the absence of interfering fluorescent material in the area where the toxins elute. The peak eluting near the solvent front generally does not interfere with quantitation of the toxins, although it would interfere with detection of sulfocarbamoyl derivatives.

The most important parameter affecting toxin retention on polar bonded phase columns is ionic strength of the mobile phase. At very low ionic strength, toxins do not elute, while at very high ionic strength, toxins are not retained. The methanol–water ratio is less important in these separations (see Figure 6). Figure 7 illustrates the effect of ammonium phosphate concentration on retention of shellfish toxins. Complete separation of all toxins in a reasonable amount of time is difficult under isocratic conditions; therefore, a step gradient is used. A mobile phase containing 0.002M ammonium phosphate elutes the gonyautoxins and then is switched at 10 min to 0.008M to elute neosaxitoxin and saxitoxin.

Since sodium or potassium phosphate has limited solubility in mobile phases containing methanol, it is necessary to use ammonium phosphate to control ionic strength and pH. Another consideration is the limited solubility of sodium or potassium periodate. Ammonium periodate is much more soluble and elimination of any sodium or potassium compounds is necessary to prevent precipitation of periodates in the reaction manifold.

Mobile phase pH has a dramatic effect on toxin retention. Lowering the pH by even 0.5 unit

greatly reduces the retention of all toxins. In addition, the lifetime of cyano columns for toxin separations is greatly reduced at lower pH values. It is important to maintain the mobile phase at pH 7.0 to preserve column usefulness.

After several weeks of use, retention characteristics of cyano columns tend to deteriorate. This may be due either to adsorption of contaminants onto the packing or to hydrolysis of the bonded phase, thus exposing silica. Deterioration is especially prevalent after analysis of crude shellfish extracts and is evident by misshaped or tailing peaks. In situ deactivation with HMDS can return the column to its original characteristics. A solution of HMDS–pyridine–toluene (2 + 1 + 4) is pumped into the column and reacted overnight. Before and after this treatment, the column is rinsed thoroughly with dry toluene to completely remove any methanol which could react preferentially with HMDS.

The HPLC method described here is useful for determining relative levels of various toxins in both shellfish extracts and cultures of *Gonyaulax*. Overall, this procedure is superior to other chemical assay techniques available for PSP toxins. The reaction manifold and detection system are very reliable; few problems have been experienced after one year of use. The greatest problems have been column stability and sample compatibility. The amino columns that were tested showed erratic behavior and had a useful life of less than one month under continuous operation. High ionic strengths (0.03–0.1M) were necessary to elute the toxins from amino columns; this may have contributed to their rapid deterioration. The cyano columns proved to be useful alternatives although they too suffer from instability problems. Absolute retention times for the toxins tend to lengthen with column use, but this does not present a great problem because relative retention times between various toxins remain unchanged. HMDS treatment greatly improves the performance characteristics of the column when retention times lengthen and peak shape deteriorates.

Ionic strength plays an important part in toxin retention; therefore, injection of samples with high dissolved solids content can affect retention times of the toxins. It is necessary to minimize the ionic strength of shellfish or dinoflagellate extracts to achieve accurate retention times.

The HPLC technique may have its greatest utility in PSP research. With recent reports of toxin derivatives of relatively low toxicity (3, 4, 12), it becomes important to understand the relationship between toxins present in dinoflagellates and those in shellfish. HPLC is ideally suited to this type of research because it provides a rapid, accurate toxin profile with minimum sample preparation. Procedures used previously for this type of determination involve column chromatography in conjunction with TLC and mouse bioassays (3, 11). These lengthy procedures present a greater risk of toxin loss and interconversion (i.e., equilibrium of epimeric pairs or hydrolysis of sulfocarbamoyl derivatives).

Another area in which this procedure may prove useful is monitoring shellfish for total toxicity. As noted, the mouse bioassay now used for shellfish monitoring has several major drawbacks and the availability of an alternative analytical technique would be most useful. The HPLC technique is generally more sensitive than the mouse bioassay. The limits of detection of saxitoxin and gonyautoxins II and III are approximately 0.5 ng; the limits for neosaxitoxin and gonyautoxins I and IV are approximately 25 and 8 ng, respectively. The lower sensitivity to N-1 hydroxy toxins (Neo, GTX I and IV) may limit the utility of this technique for monitoring shellfish toxicity. Often though, if these poorly fluorescing toxins are present, they are accompanied by even larger concentrations of saxitoxin or gonyautoxins II and III. We are currently assessing the usefulness of the HPLC procedure for monitoring shellfish toxicity.

Note added in proof: Commercially available cyano columns also produce adequate toxin separations, although slight adjustments in mobile phase ionic strength may be necessary to obtain proper retention of the toxins.

Acknowledgments

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OILS AND FATS

High Performance Liquid Chromatographic Determination of Malondialdehyde in Vegetable Oils

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A simple high performance liquid chromatographic (HPLC) method has been developed for determining malondialdehyde (MDA) in vegetable oils. MDA was reacted with dansyl hydrazine in an acidic medium, and the product, 1-dansyl-pyrazole, was determined by HPLC, using a Zorbax sil column with mixed mobile phase of *n*-hexane-methylene chloride. MDA can be determined as 1-dansyl-pyrazole by fluorometric detection at a level of 0.01 ppm in vegetable oils.

It is well known that autocatalytic auto-oxidation of edible oils proceeds by the mechanism of free radical chain reaction resulting in the accumulation of hydroperoxides and a variety of secondary oxidation products.

In Japan, peroxidative lipids in vegetable oils have been quantitatively analyzed by peroxide value (POV) (1, 2), and by the thiobarbituric acid (TBA) test (3). The modified TBA test has also been applied to the determination of peroxidative lipids in blood and tissues (4, 5). The chromophore was reported (6) to be derived from the reaction of malondialdehyde (MDA), a peroxidative product of polyunsaturated lipids, with TBA. But because the TBA test is based on the colorimetric determination of pigment at 530 nm, the method is unable to avoid the interference of other compounds which also react with TBA.

Although there are reports of measurement of so-called MDA by the TBA test (4, 5, 7, 8), no information is available on the specific measurement of MDA in oils, blood, and tissues.

Recently, the fluorescent reagent dansyl hydrazine (9) has been used for quantitative analysis of carbonyl compounds, e.g., keto steroids (10–12) and reducing sugars (13).

This paper describes a new HPLC method for measuring free MDA in vegetable oils, after conversion to dansyl-pyrazole.

METHOD

Materials and Reagents

(a) *Malondialdehyde bis(dimethylacetal).*—Nakarai Chemicals Ltd, Kyoto, Japan.

(b) *Dansyl hydrazine (1-dimethylaminonaphthalene-5-sulfonyl hydrazine).*—Tokyo Kasei Co. Ltd, Tokyo, Japan.

(c) *Solvents.*—*n*-Hexane, ethanol, and methylene chloride. Spectroscopic grade (Nakarai Chemicals Ltd).

(d) *Other reagents.*—Guaranteed reagent grade (Nakarai Chemicals Ltd).

(e) *Dansyl hydrazine solution.*—Dissolve 100 mg dansyl hydrazine in 100 mL ethanol.

(f) *Pyruvic acid solution.*—Dissolve 1 g pyruvic acid in 100 mL ethanol.

(g) *Samples.*—Fresh vegetable oils (corn, olive, peanut, rapeseed, sesame, soybean), methyl oleate, methyl linoleate, and methyl linolenate (Nakarai Chemicals Ltd). Vegetable oils were used fresh, and portions were also placed in glass flasks fitted with glass tube condenser and heated 20, 50, and 100 h in a 100°C oil bath. Auto-oxidized fatty acid methyl esters were prepared by transferring 5 mL samples into 20 mL glass-stopper tubes and incubating the tubes 20 h in the dark, at 37°C.

(h) *MDA standard solution.*—Add 228 mg malondialdehyde-bis(dimethylacetal) to 50 mL 0.1N HCl and let stand at 40°C with occasional shaking until miscible. Cool to room temperature, and dilute solution to 100 mL with 0.1N HCl (1000 ppm MDA). This stock solution is reasonably stable for one week in refrigerator. Prepare working solutions of 0.1–1.0 ppm MDA by diluting stock solution with 0.1N HCl. Prepare fresh.

(i) *Synthesis of 1-dansyl-pyrazole standard.*—To 164 mg (1 mM) malondialdehyde-bis(dimethylacetal), add solution of 265 mg (1 mM) dansyl hydrazine in 20 mL 5% HCl–50% ethanol and heat the mixture 10 min in an 80°C water bath.

To the residue obtained by concentrating the reaction mixture, add 50 mL 5% NaOH, and then extract with two 20 mL portions of methylene chloride. Elute methylene chloride extracts on silica gel (Merck, Kiesel gel 60 PF_{254}) column

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with *n*-hexane–ethyl acetate (9 + 1). Recrystallize the second-eluting yellow compound from aqueous methanol to give 1-dansyl-pyrazole, $C_{15}H_{15}N_3O_2S$ (mass spectrum m/z 301 (M^+, 100%), 237 ($M^+ - 64$, 68.8%), 208 ($M^+ - 93$, 79.9%), 170 ($M^+ - 131$, 81.6%); mp 113–113.5°C; infrared (IR) spectrum, γ max. ($CHCl_3$) cm^{-1}: 1618, 1591, 1579, and 1523 (arom C=C and C=N); NMR spectrum ($CDCl_3$, ppm): δ 2.83 (6H, s, $-N(CH_3)_2$); 6.36 (1H, d.d., J = 1.6, 2.2, pyrazole =CH—*CH*=CH—); ca 7.0–7.7 (4H, nm, arom—H and pyrazole N=*CH*—CH=); ca 8.1–8.7 (4H, m, arom—H and pyrazole —N—*CH*=CH—)).

Apparatus

(**a**) *Shaker.*—TS-type (Irie Shokai Co. Ltd, Tokyo, Japan).

(**b**) *Liquid chromatograph.*—LC-2, with fluorescence monitor RF-530 and Zorbax sil (5 μm) column, 2.1 mm id × 25 cm (Shimadzu Seisakusho Ltd, Kyoto, Japan).

Procedure

(*1*) *Preparation of sample solution.*—Weigh 1 g sample into 10 mL volumetric flask. Dilute to volume with ethanol, and shake mixture 5 min. Let stand 10 min to obtain clear supernate.

(*2*) *Reaction and extraction of 1-dansyl-pyrazole.*—Transfer 1 mL sample solution into 20 mL glass-stopper test tube, add 1 mL 0.1N HCl and 0.1 mL dansyl hydrazine solution, and heat mixture 10 min in 80°C water bath. Let reaction mixture cool to room temperature, and add 0.1 mL pyruvic acid solution. Let mixture stand 15 min at room temperature for reaction of excess dansyl hydrazine. Then add 2 mL 0.1N NaOH, 5 mL water, and 1 mL methylene chloride. Shake solution 2 min, then let stand several minutes.

(*3*) *Chromatography of 1-dansyl-pyrazole.*—Remove upper layer by pipetting, and inject 1 μL lower methylene chloride layer into HPLC system by using microsyringe.

One μL reference solution (1 ppm as MDA) injected into HPLC system has retention time of 311 ± 1 s. HPLC conditions: solvent system, *n*-hexane–methylene chloride (4 + 1); flow rate 1.0 mL/min; column at room temperature. Fluorescence detector parameters: excitation wavelength, 365 nm; emission wavelength, 500 nm; sensitivity, high; range, ×2; recorder chart speed, 2.5 mm/min.

If sample peak measurement is not within points on standard curve, dilute sample quantitatively with methylene chloride or concentrate to dryness at room temperature under stream of nitrogen and redissolve in small volume of methylene chloride as required for re-injection.

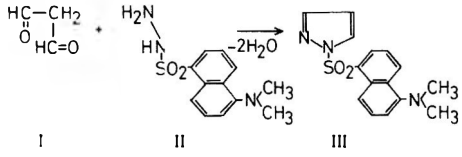

Figure 1. Reaction of malondialdehyde(I) with dansyl hydrazine(II) to give reaction product(III).

(*4*) *Preparation of standard curve.*—Transfer 1 mL of each working solution (0.2, 0.4, 0.6, 0.8, and 1.0 ppm MDA) into separate 20 mL glass-stopper test tubes, and treat as described above (procedure (*2*) and (*3*)). Set HPLC conditions and detector parameters as described in procedure (*3*). If working solutions of 0.01–0.1 ppm MDA were used, set detector parameters as follows: sensitivity, high; range ×1. Prepare MDA calibration curve daily for plotting different amounts of MDA (pg) against observed peak area.

(*5*) *Measurement of peroxide value* (*1, 2*).—Use 1 g sample for the confirmatory official method. Calculate peroxide value in sample, expressed as meq/kg.

(*6*) *Measurement of thiobarbituric acid value* (*TBA value*) (*3*).—Weigh 0.3–0.5 g sample into 20 mL glass-stopper test tube, add 10 mL TBA solution (dissolve 0.7 g TBA in 60 mL water in 200 mL volumetric flask by swirling flask under hot water, and dilute to volume with acetic acid), and mix thoroughly. Then heat mixture 30 min in boiling water bath. Cool to room temperature and measure absorbance of solution at 530 nm. Calculate TBA value of sample, expressed as ppm MDA, with aid of calibration curve for MDA.

Results and Discussion

Structure Elucidation of Reaction Product

The structure of the reaction product of MDA with dansyl hydrazine was confirmed as 1-dansyl-pyrazole by IR, mass, and NMR spectra, and from the report of Knorr (14), which described the formation of pyrazoles from reaction of 1,3-diketones with phenyl hydrazine. The suggested reaction mechanism is shown in Figure 1.

Optimal Assay Conditions for Fluorometric Detector

From the fluorescence spectrum of 1-dansyl-pyrazole in methylene chloride, excitation and

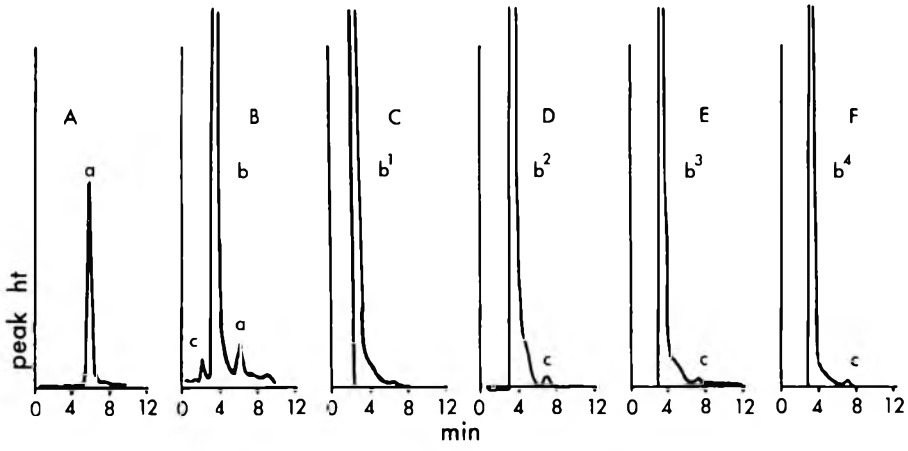

Figure 2. Chromatograms of reaction products of (A) standard MDA (0.4 ppm), and (B) MDA from soybean oil (heated 50 h) with dansyl hydrazine; and reaction products of (C) acetylacetone, (D) glutaraldehyde, (E) acrolein, and (F) *n*-propionaldehyde with dansyl hydrazine. HPLC conditions as described in text for 1 ppm MDA.

a, 1-dansyl-pyrazole; b, unknown compounds; b^1, 1-dansyl-3,5-dimethyl-pyrazole; b^2, dansyl hydrazone of glutaraldehyde; b^3, dansyl hydrazone of acrolein; b^4, dansyl hydrazone of *n*-propionaldehyde; c, unknown compounds.

emission wavelengths were selected as 365 and 500 nm, respectively, for HPLC determination of MDA as 1-dansyl-pyrazole.

Effects of Other Aldehydes and Ketones on HPLC

Dansyl-pyrazole was completely separated from other dansyl hydrazones of *n*-propionaldehyde, acrolein, and glutaraldehyde, and 1-dansyl-3,5-dimethylpyrazole, which was prepared from reaction of acetylacetone with dansyl hydrazine by HPLC (Figure 2). Therefore, this procedure is useful as a confirmatory, quantitative method.

Calibration Curve of MDA as 1-Dansyl-Pyrazole

MDA gave a linear response from 100 to 1000 pg (detector parameters: sensitivity, high; range, ×2) and from 10 to 100 pg (detector parameters: sensitivity, high; range, ×1). The limit of detection of MDA as 1-dansyl-pyrazole for quantitative HPLC is 10 pg (signal/noise = 4).

Recovery Test

For recovery by solvent extraction, 1 mL of 4.18 ppm 1-dansyl-pyrazole ethanol solution (1 ppm as MDA) is used as a sample solution. Results are shown in Table 1. A single extraction with 1 mL

Table 1. Extraction of 1-dansyl-pyrazole from reaction mixture with various organic solvents [a]

Solvent (1 mL)[b]	Peak height, mm[c]	Rec., %
Standard (1-dansyl-pyrazole soln[d])	180.8 ± 0.3	—
n-Hexane	79.8 ± 1.6	44.1
Benzene	152.0 ± 1.0	84.1
Methylene chloride	181.2 ± 0.1	100.2
Chloroform	156.3 ± 5.1	86.4

[a] HPLC conditions as described in text for 1 ppm MDA.
[b] 4.18 ppm 1-dansyl-pyrazole ethanol solution was used as sample solution in the reaction procedure.
[c] Average ± standard deviation, $n = 3$.
[d] 4.18 ppm 1-dansyl-pyrazole CH_2Cl_2 solution.

Table 2. Effect of reaction temperature on formation of 1-dansyl-pyrazole in the reaction procedure [a]

Reaction temp., °C[b]	Peak height, mm[c]	1-Dansyl-pyrazole yield, %
Standard (1-dansyl-pyrazole soln[d])	180.8 ± 0.3	—
40	30.0 ± 2.7	16.6
60	159.5 ± 0.5	88.2
80	166.4 ± 0.3	92.0
100	145.4 ± 3.6	80.4

[a] HPLC conditions as described in text for 1 ppm MDA.
[b] 1 ppm MDA standard solution was used as sample solution in the reaction procedure.
[c] Average ± standard deviation, ($n = 3$).
[d] 4.18 ppm 1-dansyl-pyrazole in CH_2Cl_2 solution.

Table 3. Effect of HCl concentration on reaction of MDA (0.5 ppm) with dansyl hydrazine [a]

HCl concn (N)	Peak height, mm[b]	1-Dansyl-pyrazole yield, %
none	0	0
0.1	83.4 ± 0.6	91.9
0.2	58.1 ± 1.1	64.0
0.5	25.5 ± 1.6	28.1
1.0	8.5 ± 4.5	9.4
Standard (1-dansyl-pyrazole soln[c])	90.7 ± 0.3	—

[a] HPLC conditions as described in text for 1 ppm MDA.
[b] Average ± standard deviation (n = 3).
[c] 2.09 ppm 1-dansyl-pyrazole CH_2Cl_2 solution.

methylene chloride is sufficient to extract almost all of the 1-dansyl-pyrazole (recovery ratio 100.2%) from the reaction mixture. The other solvents are not suitable for extraction of 1-dansyl-pyrazole.

Multiple extraction with these solvents was also examined. Recoveries for three 1 mL extractions were 98.9% (methylene chloride), 95.8% (chloroform) 92.4% (benzene), and 54.7% (n-hexane). Both single and multiple extractions with methylene chloride have been adapted. In the present study, a single extraction with methylene chloride was selected because this extraction does not require use of a separatory funnel and evaporator.

The formation of 1-dansyl-pyrazole was studied at several reaction temperatures. The best yield was 92% after 10 min at 80°C (Table 2).

Table 3 shows that the optimal concentration of HCl is 0.1N for the formation of 1-dansyl-pyrazole. Recoveries of MDA from rapeseed, soybean, corn, and peanut oils at 2 fortification levels are shown in Table 4. Recoveries from rapeseed oil and soybean oil were satisfactory, but recoveries from corn oil and peanut oil were as low as 71.0–79.5%.

Measurement of POV and Determination of MDA in Vegetable Oils

POV (1, 2) was measured and levels of MDA were determined by the TBA test (3) and by the present method in fresh and heated vegetable oils and auto-oxidized fatty acid methyl esters (Table 5).

For fresh corn oil and sesame oil heated 20 h, MDA found by the present method was greater than for the TBA test. In all other cases, MDA found by the TBA test was 2–20 times MDA amounts found by the present method. Furthermore, in the case of auto-oxidized methyl linolenate, the TBA value was 147.03 ppm as MDA, but only 2.63 ppm MDA was found by the dansyl hydrazine-HPLC method. These results do not show a correlation between the TBA value and MDA found by the present method.

The merits of the dansyl hydrazine-HPLC method can be summarized as follows: The method is not affected by carbonyl compounds such as n-propionaldehyde, acrolein, glutaraldehyde, and acetylacetone. A reproducible and rapid determination of MDA with dansyl hydrazine is obtained by the HPLC-fluorometric detector system. The 1-dansyl-pyrazole formed from MDA can be detected at a level of 0.01 ppm in vegetable oils.

Table 4. Recoveries of MDA from heated oils of rapeseed, soybean, corn, and peanut [a]

Oil (heated 20 h)	MDA added, μg	Peak height,[b] mm	Found, μg	Rec., %
Rapeseed	0	11.7 ± 0.09	0.82	—
	2.0	39.5 ± 0.5	2.78	98.0
	4.0	67.0 ± 0.5	4.71	97.3
Soybean	0	4.0 ± 0.03	0.28	—
	2.0	31.5 ± 0.25	2.21	96.5
	4.0	59.9 ± 0.4	4.21	98.3
Corn	0	3.2 ± 0.04	0.23	—
	2.0	25.2 ± 0.1	1.77	77.0
	4.0	45.7 ± 2.9	3.21	74.5
Peanut	0	5.2 ± 0.09	0.33	—
	2.0	23.5 ± 0.5	1.92	79.5
	4.0	38.9 ± 0.2	3.17	71.0

[a] HPLC conditions as described in text for 1 ppm MDA.
[b] Average ± standard deviation (n = 3).

Table 5. POV measurement and determination of MDA levels in vegetable oils and fatty acid methyl esters by the TBA test and by the dansyl hydrazine–HPLC method

Sample	Time heated, h	POV, meq/kg	MDA, ppm	
			TBA test	HPLC
		Oils		
Soybean	0	2.43	0	ND[a]
	20	11.17	0.86	0.28
	50	31.40	4.31	0.79
	100	37.92	5.05	0.57
Corn	0	4.12	0.11	0.23
	20	48.00	0.70	0.23
	50	92.19	6.09	0.35
	100	52.16	6.75	0.28
Rapeseed	0	3.60	1.36	0.24
	20	17.09	5.00	0.82
	50	28.84	6.39	1.93
	100	89.79	51.43	2.35
Olive	0	5.35	0.03	ND
	20	8.83	0.70	0.27
	50	21.61	1.05	0.33
	100	8.56	1.26	0.28
Peanut	0	3.11	0.03	ND
	20	20.43	0.80	0.33
	50	37.90	1.10	0.35
	100	27.50	1.95	0.38
Sesame	0	2.98	0.03	ND
	20	20.02	0.16	0.27
	50	35.75	1.15	0.29
	100	14.92	1.43	0.47
		Auto-oxidized Fatty Acid Methyl Esters		
Me oleate	20	18.50	2.58	0.44
Me linoleate	20	62.50	5.36	0.75
Me linolenate	20	799.30	147.03	2.63

[a] ND = <0.01 ppm.

However, the present method only measures free MDA in vegetable oils. The MDA-induced method for hydroperoxidized fatty acids and glycerides is under investigation.

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PLANT TOXINS

Simple Colorimetric Method for Determination of Episulfides, Using 4-(*p*-Nitrobenzyl)-Pyridine

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Episulfides were determined by a simple colorimetric assay using 4-(*p*-nitrobenzyl)-pyridine, which has been used previously for the detection and analysis of epoxides, organophosphates, and other alkylating agents. With episulfides, absorbance was directly proportional to concentration up to an absorbance of at least 1.0. No interference was observed with a variety of non-episulfide sulfur-containing substances. The colorimetric method is useful in monitoring 1-cyanoepithioalkane formation from the degradation of glucosinolates containing terminal double bonds.

The degradation of glucosinolates containing terminal double bonds to form episulfide-containing compounds, known as 1-cyanoepithioalkanes, has been studied in our laboratory (1, 2). These compounds have been determined routinely by gas chromatography; however, a simple and rapid colorimetric assay for episulfide-containing compounds, in the presence of sulfur or other sulfur-containing compounds, would facilitate current work on the purification of protein factors implicated in 1-cyanoepithioalkane formation from glucosinolates. Such an assay should also prove valuable whenever other episulfide-containing compounds are encountered. In this paper, we report the detection and analysis of episulfides by using 4-(*p*-nitrobenzyl)-pyridine (NBP). This reagent has been used previously for the analysis of epoxides (3).

Experimental

Apparatus and Reagents

(a) *Spectrophotometers.*—Beckman DK-2A ratio recording spectrophotometer for determination of λ_{max} and Gilford 240 for routine analysis at fixed wavelength.

(b) *NBP reagent.*—5% (w/v) 4-(*p*-nitrobenzyl)-pyridine in acetone.

(c) *Other reagents.*—0.1N potassium hydrogen phthalate; 1.0M K_2CO_3; 50% (v/v) aqueous acetone.

Sample Preparation

Racemic 1-cyano-2,3-epithiopropane and diasteriomeric (2*S*)-1-cyano-2-hydroxy-3,4-epithiobutanes were prepared enzymatically from sinigrin and epiprogoitrin, respectively, by the procedure of Petroski and Tookey (2). Styrene episulfide, *N*-(2,3-epithiopropyl)-phthalimide, 2,3-epithiopropyl-*p*-methoxyphenyl ether, 1,2-epithiocyclo-hexane, and 1,2-epithiododecane were synthesized from the corresponding epoxides by the procedure of Lüthy and Benn (4), and their structures were confirmed by spectroscopic methods. All other sample compounds were obtained commercially.

Procedure

Add potassium hydrogen phthalate (1.0 mL, 0.1N) and NBP reagent (1.0 mL), with mixing, to sample consisting of a solution of the episulfide (1–100 μmoles depending on ϵ) in 2 mL acetone. Heat sample in a 50–60°C water bath until evaporation of the acetone is nearly complete (ca 10 min); then increase temperature of water bath to 100°C and heat sample an additional 45 min. Remove sample from water bath, let cool to below 50°C, dilute to 9 mL with 50% aqueous acetone, and place in ice bath. After cooling, add aqueous K_2CO_3 (1.0 mL, 1.0M), with mixing, immediately before reading (blue color unstable) with a suitable spectrophotometer or colorimeter. Calculate concentration of episulfide from calibration graph similarly prepared, using known amounts of episulfide being tested.

Results and Discussion

In apparent contradiction to work published by Hammock et al. (3), the NBP reagent does respond to episulfides (syn. thiaranes) and may be used for their detection and analysis even in the presence of sulfur or other sulfur-containing substances such as glucosinolates, sulfonic acids, mercaptans, thiophenols, sulfides, disulfides,

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Table 1. Wavelength of maximal absorption (λ_{max}) and apparent molar absorptivity (ϵ) [a] for various episulfides and epoxides analyzed with 4-(*p*-nitrobenzyl)-pyridine

Compound	λ_{max} (nm)	ϵ
Episulfides		
2,3- Epithiopropyl methyl ether	565	206
1-Cyano-2,3-epithiopropane	560	3480
(2*S*)-1-Cyano-(2)-hydroxy-3,4-epithiobutane	570	1640
2,3-Epithiopropyl-*p*-methoxy phenyl ether	585	14 700
N-(2,3-Epithiopropyl)-phthalimide	585	9110
Styrene episulfide	562	5030
1,2-Epithiododecane	580	3020
1,2-Epithiocyclohexane	575	182
Epoxides		
2,3-Epoxypropyl-*p*-methoxy phenyl ether	585	22 300
n-(2,3-Epoxypropyl)-phthalimide	593	12 200
Styrene oxide	583	5620
1,2-Epoxydodecane	580	14 200
1,2-Epoxycyclohexane	585	720

[a] Apparent molar absorptivity values were obtained by reacting known amounts of each episulfide or epoxide with NBP reagent; however, NBP-episulfide and NBP-epoxide adducts have not actually been isolated, because of their reported instability (3).

sulfates, sulfoxides, sulfones, isothiocyanates, thioacids, and thioureas, which do not respond to the NBP reagent. Compounds tested were allylglucosinolate, 2-hydroxy-3-butenyl glucosinolate, *p*-toluene sulfonic acid, β-naphthalene sulfonic acid, ethyl mercaptan, 2-mercaptoethanol, dithiothreitol, thiophenol, thiodiphenylamine, methionine, cysteine, cystine, sodium sulfate, ferrous sulfate, benzidine sulfate, dimethyl sulfoxide, diethyl sulfone, ethyl phenyl sulfone, allyl isothiocyanate, thioglycolic acid, thiourea, phenyl thiourea, and elemental sulfur.

Absorbance is proportional to concentration, at least up to an absorbance (OD) of 1.0, for all episulfides or epoxides tested. Samples should be read immediately after K_2CO_3 addition because, after color development, a steady decrease in absorbance was observed. The rate varies with the episulfide being tested; however, loss of absorbance ranged from less than 1% up to 3%/min. Table 1 shows the wavelength of maximal absorption (λ_{max}) and apparent molar absorptivity (ϵ) for various episulfides and epoxides. The results show that the ϵ value is consistently lower for episulfides than for the corresponding epoxides (e.g., 3020 for 1,2-epithiododecane vs 14 200 for 1,2-epoxydodecane). The reason for such a difference in apparent ϵ value for 2 alkane compounds (e.g., 182 for 1,2-epithiocyclohexane vs 3020 for 1,2-epithiododecane) is not clear. These values are satisfactory for analytical purposes; they are reproducible and follow Beer's law. Also, some episulfides react with NBP reagent to form products that absorb at shorter wavelengths (λ_{max}) than those of the corresponding epoxides (e.g., 562 nm for styrene episulfide vs 583 nm for styrene oxide); this may provide a means of distinction between the two, at least in some cases.

Episulfides that were prepared enzymatically by the procedure of Petroski and Tookey (2), namely, 1-cyano-2,3-epithiopropane and (2*S*)-1-cyano-(2)-hydroxy-3,4-epithiobutane, could be detected at concentrations as low as 30 ppm in incubation mixtures. Detection limits for other episulfides and epoxides are, of course, inversely proportional to their respective ϵ values.

Recovery experiments were conducted to establish the precision of the quantitative episulfide method (Table 2). For these studies, known amounts of *N*-(2,3-epithiopropyl)-phthalimide (52.8 ppm or 105.6 ppm in 5 mL) or 2,3-epithiopropyl-*p*-methoxy phenyl ether (30.0 or 60.0 ppm in 5.0 mL) were added to enzyme incubation mixtures used to prepare 1-cyanoepithioalkanes, but lacking glucosinolate substrate, and extracted into methylene chloride before analysis (2). Recoveries of close to 100% were observed for all samples except the higher concentration of *N*-(2,3-epithiopropyl)-phthalimide where a recovery of about 91% was observed. Coefficients of variation were 3.4% for sample analysis and 3.1% for sample recovery.

Hammock et al. (3) found that NBP reagent does not respond to oxetanes, furans, tetrahy-

Table 2. Recovery of episulfides [a] added to enzyme incubation mixtures

Compound	Added, ppm	Found, ppm	Rec., %
N-(2,3-Epithiopropyl)-phthalimide	52.8	52.9	100.1
	105.6	95.6	90.5
2,3-Epithiopropyl-*p*-methoxy phenyl ether	30.0	31.1	103.8
	60.0	59.5	99.1
CV, [b] %		3.4	3.1

[a] See text for details of analysis.
[b] Coefficients of variation (CV) based on a total of 24 determinations: 6 determinations per concentration of each episulfide.

drofurans, acyclic aromatic or aliphatic ethers, olefins, conjugated dienes, aldehydes, ketals, ketones, carboxylic acids, esters, alcohols, or phenols. This finding was supported in this laboratory by a check of at least one substance in each chemical class. NBP reagent also does not respond to nitriles. Compounds tested in this laboratory were trimethylene oxide, furan, tetrahydrofuran, dioxane, *p*-methoxybenzene, ethyl ether, *p*-ethoxy benzene, 1-hexene, 1,3 cyclohexadiene, sorbic acid (2,4 hexadienoic acid), benzaldehyde, solketal (2,2-dimethyl-1,3-dioxolane-4-methanol), acetone, acetophenone, benzoic acid, acetic acid, *n*-propanol, ethanol, methanol, phenol, *p*-cresol, acetonitrile, and allyl cyanide.

It is well known that NBP reagent reacts with ethylenimines, organophosphorous compounds, and other alkylating agents (5–9), so care must be taken to avoid their presence in samples analyzed for episulfides or epoxides.

In summary, 4-(*p*-nitrobenzyl)-pyridine (NBP) is a useful reagent for the detection and colorimetric quantitation of episulfides as well as epoxides.

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PESTICIDE FORMULATIONS

High Performance Liquid Chromatographic Analysis of Diflubenzuron and Its Formulations: Collaborative Study

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Diflubenzuron 90% pre-concentrate and its 25% water-dispersible powder were analyzed by a high performance liquid chromatographic method. Six samples were extracted with 1,4-dioxane, linuron was added as internal standard, and diflubenzuron was separated on a 25 cm × 4.6 mm column packed with Zorbax BP-C_8 with acetonitrile-water-1,4-dioxane (450 + 450 + 100) at 1.3 mL/min and monitored at 254 nm. Results were obtained from 17 laboratories. Within-laboratory repeatability was 0.6% for both the pre-concentrate and the water-dispersible powder samples and the reproducibility was 1.2%. The method has been adopted as a full CIPAC method and was adopted official first action by AOAC.

Diflubenzuron, 1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea (PH 60-40) is an insecticide belonging to a group of compounds that acts by interfering with the deposition of chitin (1, 2). It is effective against a number of leaf-feeding larvae in forestry, top fruit, and field crops and against larvae of mosquitoes and flies. It is mainly formulated as 25% water-dispersible powder which is marketed under the trademark Dimilin® (Duphar B.V., The Netherlands).

The selection of high performance liquid chromatography (HPLC) as the technique to be used for the analysis of diflubenzuron was straightforward (3).

Infrared spectroscopy was unsuitable because of low solubility of diflubenzuron in common solvents and the expected interference from formulating aids. Possible interferences were also the reason to reject direct ultraviolet spectroscopy and titrimetry. Because of low volatility of diflubenzuron, gas chromatography of derivatives was the only likely possibility. However most derivatizations proved not to be sufficiently quantitative. Originally, straight phase liquid chromatography was tried but this system was sensitive to traces of water in the mobile phase. The low solubility of diflubenzuron in most organic solvents was another disadvantage. Consequently, reverse phase HPLC, most likely to comply with requirements of specificity, accuracy, shortness of analysis time, and availability of apparatus, was chosen as the best approach for developing a method.

Collaborative Study

Six diflubenzuron-containing samples, 3 pre-concentrates, 90% (Samples I, IIa, and IIb), and 3 water-dispersible powders, 25% (Samples III, IVa and IVb) were sent to 17 laboratories. Samples IIa and IIb were identical, as were Samples IVa and IVb. Collaborators were supplied with an analytical standard of the active ingredient, the internal standard (linuron), and an impurity (1,3-di(4-chlorophenyl)urea). Because the use of 3 alternative column fillings was allowed, e.g., μBondapak C_{18}, 10 μm, Spherisorb ODS, 5 μm, and Zorbax BP-ODS, 7 μm, the collaborators were instructed to check the column performance by determining resolution between diflubenzuron and 1,3-di(4-chlorophenyl)urea (Figure 1). Collaborators were also advised to make preliminary runs to become familiar with the procedure. The samples had to be analyzed once.

For each collaborator, the sequence of sample analyses was randomly assigned in advance. Each sequence of 3 sample analyses had to be preceded by 2 standard solutions. The average calibration factor, determined from the 2 standard solutions, had to be used for the calculation of the content of the 3 following sample solu-

The recommendation of the Associate Referee, B. Van Rossum, was approved by the General Referee and Committee A and was adopted by the Association. See the General Referee and Committee reports, *J. Assoc. Off. Anal. Chem.* (1983) **66**, March issue.

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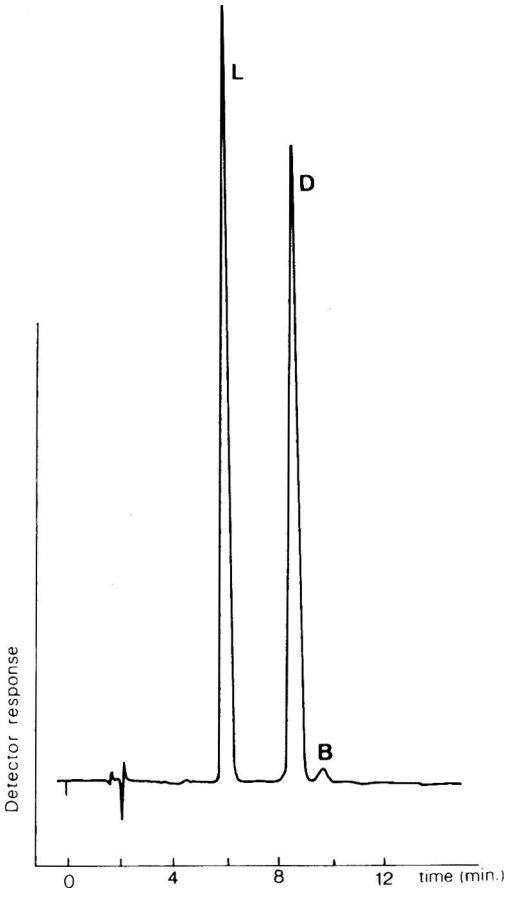

Figure 1. Typical chromatogram of diflubenzuron (D) with linuron (L) internal standard and impurity 1,3-di(4-chlorophenyl)urea (B).

tions. The calibration factor had to be recorded with 3 decimals, the contents with 2 decimals. The identity had to be checked simultaneously with the determination of diflubenzuron. The identity was taken to be confirmed if the difference between the relative retention time of diflubenzuron with respect to the internal standard for the sample solution deviated not more than 1.5% from that for the calibration solution.

Diflubenzuron (*N*-(((4-Chlorophenyl)amino)-carbonyl)-2,6-difluorobenzamide) in Formulations
Liquid Chromatographic Method
Official First Action
CIPAC-AOAC Method

Principle

Diflubenzuron is dissolved in 1,4-dioxane, sepd by HPLC, and detd from peak areas vs linuron internal std. Identity is confirmed by retention time.

Apparatus

(**a**) *Liquid chromatograph.*—Provided with const flow pump and 20 μL sample loop. Operating conditions: mobile phase flow rate, 1.3 mL/min; detector sensitivity, 128 × 10^{-3} AUFS; temp., ambient, should not fluctuate >2°; retention time of diflubenzuron relative to internal std, ca 1.36.

(**b**) *Detector.*—UV spectrophtr or fixed wavelength UV detector at 254 nm.

(**c**) *Recorder.*—Range to match output of HPLC detector.

(**d**) *Liquid chromatography column.*—Stainless steel, 250 × 4.6 mm, packed with Zorbax BP-C_8 (E.I. DuPont de Nemours & Co.), or equiv. (e.g., μBondapak C_{18}, 10 μm, Waters Associates, Inc.; Spherisorb ODS, 5 μm, Phase Separations Ltd, Deeside Industrial Estate, Queensferry Clwyd, UK; Zorbax BP-ODS, 7 μm, E.I. DuPont de Nemours).

(**e**) *Filter.*—Acrodisc disposable filter assembly, 1.2 μm (Gelman Sciences, Inc., 600 S Wagner Rd, Ann Arbor, MI 48106), or equiv.

Reagents

(**a**) *Mobile phase.*—Acetonitrile-H_2O-1,4-dioxane (45 + 45 + 10). Mix 450 mL acetonitrile, 450 mL H_2O, and 100 mL 1,4-dioxane and degas.

(**b**) *Solvent mixture.*—Acetonitrile-H_2O (45 + 55).

(**c**) *Internal std soln.*—Accurately weigh 25 mg linuron (National Physical Laboratory, Div. of Chemical Standards, Teddington, Middlesex, TW11 OLW, UK) into 100 mL vol. flask, dil. to vol. with acetonitrile, and mix.

(**d**) *Diflubenzuron std soln.*—Accurately weigh ca 50 mg pure diflubenzuron (Duphar B.V., PO Box 2, 1380 AA Weesp, The Netherlands) into 100 mL vol. flask. Add 50 mL dioxane and dissolve by heating 30 min in 80° H_2O bath. Swirl occasionally. Add 40 mL dioxane, cool, and dil. to vol. with dioxane. Pipet 5 mL into 50 mL vol. flask, add 5.00 mL internal std soln, (**c**), dil. to vol. with solv. mixt., (**b**), and mix.

(**e**) *1,3-Di(4-chlorophenyl)urea.*—Duphar B.V.

System Performance Check

Dissolve, in 100 mL vol. flask, 4.2 mg diflubenzuron and 1.8 mg 1,3-di(4-chlorophenyl)urea in 20 mL dioxane, dil. to vol. with solv. mixt., (**b**), and mix. Filter thru 1.2 μm filter before injection.

Table 1. Individual results, means, and ranges for pre-concentrate

	Sample				
Lab.	I	IIa	IIb	$\bar{x}$	R
1	90.85	91.04	90.93	90.98	0.11
2[a]	86.12	87.50	87.56	87.53	0.06
3	89.45	89.71	89.82	89.76	0.11
4	89.70	90.34	90.09	90.22	0.25
5	89.63	90.12	89.65	89.88	0.47
6	93.33	91.55	92.74	92.14	1.19
7	91.28	92.36	92.45	92.40	0.09
8	90.29	90.90	90.65	90.78	0.25
9	91.12	91.83	90.57	91.20	1.26
10	90.42	90.94	89.72	90.33	1.22
11	90.51	90.76	90.92	90.84	0.16
12	92.63	92.19	92.14	92.16	0.05
13	92.18	91.38	91.12	91.25	0.26
14	90.42	90.84	90.99	90.92	0.15
15	92.01	93.13	91.30	92.22	1.83
16	89.95	90.28	90.63	90.46	0.35
17	88.83	90.95	89.61	90.28	1.34
Mean	90.79			90.99	

[a] Excluded from statistical analysis, ranking test significant ($P < 0.05$).

Table 2. Individual results, means, and ranges for water-dispersible power

	Sample				
Lab.	III	IVa	IVb	$\bar{x}$	R
1	24.50	25.50	25.78	25.64	0.28
2[a]	23.64	24.60	24.47	24.54	0.13
3	24.53	25.28	25.39	25.34	0.11
4	24.48	25.24	25.15	25.20	0.09
5	24.37	25.47	25.45	25.46	0.02
6	25.04	25.95	25.82	25.88	0.13
7	25.02	25.99	25.92	25.96	0.07
8	24.48	25.46	25.42	25.44	0.04
9	24.00	24.09[b]	25.32[b]	24.70[b]	1.23[b]
10	23.32[c]	25.52	26.03	25.78	0.51
11	24.72	25.43	25.51	25.47	0.08
12	24.92	26.06	26.05	26.06	0.01
13	24.73	25.04	25.58	25.31	0.54
14	24.45	25.42	25.25	25.34	0.17
15	25.16	26.11	25.82	25.96	0.29
16	24.64	25.12	25.22	25.17	0.10
17	24.35	25.46	25.24	25.35	0.22
Mean	24.63			25.56	

[a] Rejected by ranking test for outlying laboratories ($P < 0.05$).
[b] Rejected by test for homogeneity of within-laboratory variation ($P < 0.05$).
[c] Rejected by Dixon test ($P < 0.05$).

Inject 20 μL onto column and det. resolution (R) by following formula:

$$R = 2d/(W_1 + W_2)$$

where R = resolution; d = distance between peak maxima; W_1 and W_2 = peak width at baseline of diflubenzuron and 1,3-di(4-chlorophenyl)urea, resp. Resolution should be >1. If necessary, resolution can be improved by slightly increasing H_2O content of mobile phase.

Preparation of Sample

(a) *Diflubenzuron pre-concentrate.*—Accurately weigh sample contg 1.0 g diflubenzuron into 200 mL vol. flask. Add 150 mL dioxane and heat 30 min in 80° H_2O bath. Swirl occasionally. Add 40 mL dioxane, cool, and dil. to vol. with dioxane. Pipet 10 mL into 100 mL vol. flask and dil. to vol. with dioxane. Pipet 5 mL dild soln into 50 mL vol. flask, add 5.00 mL internal std soln, (c), dil. to vol. with solv. mixt. (b), and mix. Filter thru 1.2 μm filter.

(b) *Water dispersible powder.*—Accurately weigh sample contg 0.5 g diflubenzuron into 200 mL vol. flask. Add 150 mL dioxane and heat 30 min in 80° H_2O bath. Swirl occasionally. Add 40 mL dioxane, cool, and dil. to vol. with dioxane. Immediately pipet 20 mL homogeneous suspension into 100 mL vol. flask and dil. to vol. with dioxane. Pipet 5 mL dild soln into 50 mL vol. flask, add 5.00 mL internal std soln, (c), dil. to vol. with solv. mixt. (b), and mix. Filter thru 1.2 μm filter.

Determination

Inject 20 μL diflubenzuron std soln, (d). Det. peak areas (or peak hts × retention times) of diflubenzuron and internal std. Repeat injections until response ratio (area diflubenzuron peak/area internal std peak) (F) varies <1% for successive injections. Inject 20 μL sample soln.

Calculation

$$\% \text{ Diflubenzuron} = RR \times W' \times V \times 100/(RR' \times W)$$

where RR and RR' = ratio of area of diflubenzuron peak to area of internal std peak for sample and std, resp.; W and W' = g sample and std, resp.; V = diln factor (= 20 and 10 for pre-concentrate and H_2O-dispersible powd., resp.).

Results and Discussion

Samples were sent to 18 laboratories; 17 collaborators returned their findings (single analyses on each sample). One collaborator had no capacity available to perform the study.

By scrutinizing the results, a number of calculation and integration errors were detected. In these cases the results were recalculated. These recalculated results are presented in Tables 1 and 2.

The means of the duplicate analyses on Sam-

Table 3. Analysis of variance for pre-concentrate diflubenzuron samples (I and II)

Source of variation	Degrees of freedom	Mean square	Expectation of mean square
Between samples	1	0.4334	
Between laboratories	15	2.7083	$\sigma_0^2 + 5/3\ \sigma LS + 3\ \sigma_L^2$
Lab. × sample interaction	15	0.2912	$\sigma_0^2 + 4/3\ \sigma_{LS}^2$
Between replicates	16	0.3205	σ_0^2
Estimate of repeatability standard deviation		s_0 = 0.55% w/w CV = 0.61% d.f. = 31	
Estimate of reproducibility standard deviation		s_x = 1.05% w/w CV = 1.16% d.f.[a] = 22	

[a] Approximated by Satterthwaite's formula (5).

ples II and IV and the ranges (absolute differences) are shown in Tables 1 and 2, together with the single values on Samples I and III.

To detect laboratories which show consistently high or low values, the ranking test described by Youden and Steiner (4) was applied. This was done by assigning ranks to the single determinations for Samples I and III, and to means of duplicates for Samples II and IV. The test shows, at the 5% level of significance, that Laboratory 2 produced consistently low values. The rank sum score for this laboratory is far beyond the 5% critical score, and therefore the results of this laboratory were excluded from further statistical analysis.

The results of the remaining 16 laboratories were examined, by means of Dixon's test, for incidental outlying individual results per sample. The test attained significance, at the 5% level, on the low value (23.32% w/w) for Laboratory 10 on Sample III. This value was omitted from further statistical analysis.

One of the requirements underlying the statistical analysis (analysis of variance) is homogeneity of within-laboratory variation (variation between replicates). The test described by Steiner (4) was applied to the ranges of the duplicates of Samples II and IV, separately. Significance (at the 5% level) was reached on the large difference (R = 1.23% w/w) between the duplicates on Sample IV reported by Laboratory 9. Both results for that sample were excluded from the subsequent statistical analysis.

Because precision of the analytical method (HPLC) may depend on the kind of sample, analyses of variance were performed for the pre-concentrate and the water-dispersible powder separately.

Table 3 displays the analysis of variance for the pre-concentrate samples. The laboratory × sample interaction mean square is slightly smaller than the between-replicates mean square (within-laboratory variation). Therefore, the interaction component σ_{LS} is assumed to be zero and the 2 mean squares are pooled to obtain the estimate of the repeatability standard deviation (see Steiner (4)).

$$s_0 = 0.55\%\ w/w,\ CV = 0.61\%$$

The mean square for laboratories in Table 3 has been compared with the pooled interaction and between replicates mean squares by means of the F-test. Significance is attained at the 1% level, indicating that the bias of the method is not the same for all laboratories.

The estimate of the reproducibility standard deviation (see Steiner (4)) is

$$s_x = 1.05\%\ w/w,\ CV = 1.16\%$$

The analysis of variance for the water-dispersible powder data is shown in Table 4. Because for Laboratories 9 and 10 the results on Samples IV and III, respectively, were to be excluded, we dropped the remaining data from these laboratories as well, to keep the analysis simple.

The same picture is seen as for the pre-concentrate samples. The laboratories × sample interaction mean square is slightly greater than the between-replicates mean square. Again, these 2 mean squares were pooled to obtain the estimate of the repeatability standard deviation

$$s_0 = 0.15\%\ w/w,\ CV = 0.59\%$$

Also for these samples, the between-laboratories

Table 4. Analysis of variance for water-dispersible powder diflubenzuron samples (III and IV)

Source of variation	Degrees of freedom	Mean square	Expectation of mean square
Between samples	1	7.0586	
Between laboratories	13	0.2318	$\sigma_0^2 + 5/3\ \sigma_{LS} + 3\ \sigma_L^2$
Lab. × sample interaction	13	0.0240	$\sigma_0^2 + 4/3\ \sigma_{LS}^2$
Between replicates	14	0.0211	σ_0^2
Estimate of repeatability standard deviation		s_0 = 0.15% w/w CV = 0.59% d.f. = 27	
Estimate of reproducibility standard deviation		s_x = 0.30% w/w CV = 1.20% d.f.[a] = 18	

[a] Approximated by Satterthwaite's formula (5).

mean square significance (at the 1% level) is greater than the pooled error mean square. The estimate of the reproducibility standard deviation is

$$s_x = 0.30\%\ w/w,\ CV = 1.20\%$$

The full analysis for the water-dispersible powder samples (including the incomplete data for Laboratories 9 and 10) yields as precision estimates s_0 = 0.64% (CV) and s_x = 1.36% (CV).

Apparently, the pre-concentrate samples and the water-dispersible powder samples are determined with equal relative precision. It is recommended that the method be adopted official first action.

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H. Tengler, Bayer A.G., Dormagen, FRG

K. Winsauer, Johannes Kepler Universität, Linz, Austria

M. Wisson, Sandoz Ltd, Basle, Switzerland

The authors also gratefully acknowledge the assistance of P. van Bemmel, Duphar B.V., Weesp, who did the statistical analysis.

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TECHNICAL COMMUNICATION

Modification of Klein's Wet Ashing Procedure for Determination of Mercury

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An investigation of the use of HCl in Klein's method (25.108-25.115) to prevent loss of mercury during wet digestion resulted in consistently good recoveries from jowar and fish samples. Recoveries for the modified method ranged from 90.9 to 97.0% compared with 14.4 to 35.5% for the original method.

Klein's method (1) for determination of mercury, as reported in *Official Methods of Analysis* of the AOAC (2), was investigated for its adaptability for mercury analyses in jowar (*Sorghum vulgare*) and fish. Initially, recovery of mercury added to jowar and fish was uniformly poor. However, complete recovery of mercury could be obtained if mercury was added to the acid jowar or fish digest subsequent to the wet ashing procedure, indicating that low recoveries may be due to either complex formation or volatilization during the wet oxidation procedure.

Mercuric chloride is not decomposed at elevated temperatures in water, compared with mercuric nitrate or mercuric sulfate which decompose even in cold water (3). Hydrochloric acid when added to mercuric chloride in dilute standard solutions stabilizes it. These 2 observations prompted an investigation of the use of HCl in Klein's method to prevent loss of mercury during wet digestion. Addition of HCl (approximately 0.1N concentration as HCl in the final volume of digest) during wet digestion resulted in consistently good recoveries of mercury from jowar and fish samples.

Experimental

Use weight of sample containing not less than 3 μg mercury. In the apparatus mentioned in the official AOAC method, up to 60 g fish sample and up to 10 g jowar sample, on a fresh weight basis, can be digested.

The modified wet ashing method is detailed below:

(*1*) In **25.112(a)**, add 20 mL HNO_3 to weighed sample in digestion flask, and then add 2.2 mL HCl (sp. gr. 1.13) for a final volume of 250 mL digest.

(*2*) Carry out wet digestion to complete oxidation. Do not filter off undigested fats and waxes but proceed to reflux with urea. Finally, filter off undigested fats and waxes and dilute to known volume.

Proceed with isolation and determination of mercury as reported in **25.108-25.115** (2).

Results and Discussion

Keeping in mind the presence of chloride ions in fish (especially sea fish), the quantity of HCl to be added is calculated such that the final digest contains approximately a 0.1N concentration as HCl. The concentration should not exceed 0.1N because, even though mercury can be completely extracted by dithizone in 1N acid solutions which are 0.2N in chloride ions, it is reported that high concentrations of chloride ions may hinder quantitative extraction of mercury (4).

Complete oxidation can be effected by single stage digestion and filtering undigested fats and waxes after final urea treatment. Compared with Klein's method, this modification reduces digestion time without affecting recovery.

Hydrochloric acid, H_2SO_4, and glacial acetic acid may contain mercury (5) and contribute to the analytical blank unless they are very pure. Therefore, mercury is determined in the reagent blank and subtracted during calculations.

Table 1 shows the recoveries for the Klein wet digestion method and also for the modified method. Recoveries of mercury by the original method were good for distilled water and fresh tomato juice, but the same method yielded poor recoveries for jowar and fish samples. More NO_2 was produced during wet digestion of jowar and fish samples compared with fresh tomato juice samples. NO_2 production was nil from distilled water.

The official AOAC method (2) cautions that during wet digestion the original reaction must not proceed violently or evolved NO_2 will carry vapors of digest mechanically through the condenser and cause loss of mercury. We followed

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Table 1. Recovery of mercury (added as $HgCl_2$) by wet ash and modified wet ash techniques [a]

Wet ash method			Modified wet ash method		
Added ppm	Rec., ppm	Rec., %	Added ppm	Rec., ppm	Rec., %
Distilled Water (50 mL)					
0.40	0.41	102.5	0.40	0.39	97.5
0.20	0.195	97.5	0.20	0.20	100.0
Fresh Tomato Juice					
0.00	0.00[b]	—	0.00	0.00[b]	—
0.96	0.85	88.5	0.96	0.90	93.8
0.48	0.45	93.8	0.48	0.46	95.8
Jowar					
0.00	0.00[b]	—	0.00	0.00[b]	—
2.02	0.29	14.4	2.01	1.95	97.0
0.46	0.15	32.6	0.41	0.39	95.1
1.83	0.51	27.9	1.76	1.60	90.9
1.69	0.60	35.5	1.72	1.65	95.9
Canned Sardines					
0.00	0.00[b]	—	0.00	0.00[b]	—
1.08	0.28	25.9	1.10	1.00	90.9
0.95	0.30	31.6	0.98	0.95	96.9
0.25	0.07	28.0	0.28	0.26	92.9
0.20	0.06	30.0	0.21	0.20	95.2

[a] Values reported are calculated after deducting reagent blank.

[b] It was assumed that the sample contained no mercury; results obtained at concentrations less than 0.05 ppm are not reliable.

all necessary precautions during the wet digestion, but good recovery could not be achieved for jowar and fish samples, possibly due to the large volumes of NO_2 evolved.

In the modified method, addition of HCl may have a stabilizing effect, preventing loss of mercury possibly by decomposition, complex formation, or volatilization. The dithizone method as modified offers a sensitive (0.05 ppm) and convenient method for the determination of mercury in foods.

Acknowledgments

The authors thank W. E. Eipeson for constructive reading of the manuscript and valuable suggestions, and C. P. Sri Natarajan, Director, Central Food Technological Research Institute, Mysore, for his keen interest in the work and constant encouragement.

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FOR YOUR INFORMATION

Warren R. Bontoyan and James P. Minyard, Jr

AOAC Annual International Meeting – Its 96th – A Success

After 18 years, with the 96th Annual International Meeting, AOAC returned to its former meeting place, the elegant and spacious Shoreham Hotel in Washington, DC. About 1100 analytical scientists attended the highly successful meeting held October 25–28, 1982.

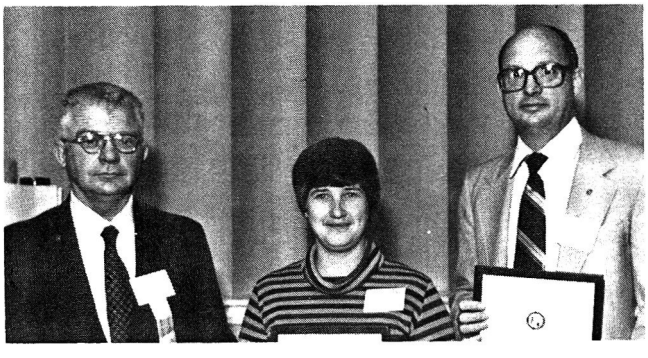
Many attendees enjoyed the festive wine and cheese party on Sunday evening, but the meeting officially opened with the General Session on Monday morning. James P. Minyard, the outgoing president, gave his presidential address, "From Introspection to New Horizons." He described AOAC expansion, past and future, as well as the Association's progress during his tenure.

Proposed bylaw amendments and provisional charters for regional sections were read and voted on. Incoming President Warren R. Bontoyan presented the Presidential plaque to Dr. Minyard, who, in turn, presented Peter F. Kane, Purdue University, West Lafayette, IN, with the 1982 award for the best Associate Referee Report of the Year. Dr. Minyard presented certificates to the 1982 Fellows of the AOAC: Wallace S. Brammell, Thomas Fazio, and Edward Smith, FDA, Washington, DC; D. Earle Coffin, Health and Welfare Canada, Ottawa, Ontario, Canada; Elmer George, Jr, NY Department of Agriculture and Markets, Albany, NY; Stanley E. Katz, Rutgers University-Cook College, New Brunswick, NJ; John W. Sherbon, Cornell University, Ithaca, NY; and Arthur H. Hofberg, Ciba-Geigy Corp., Greensboro, NC. The AOAC Wiley Scholarship winner, Carol Lynn Lasko of the College of the Siskiyous, was announced.

For organizing the regional sections, H. Michael Wehr, Oregon Department of Agriculture, Salem, OR; H. Michael Stahr, Iowa State Veterinary Diagnostic Laboratory, Ames, IA; and Audrey Gardner, NY State Agricultural Experiment Laboratory, Geneva, NY, received special awards, as did Nicole Hardin, FDA, New Orleans, LA, and Hershel F. Morris, Louisiana Department of Agriculture, Baton Rouge, LA, for planning and conducting the 1982 Spring Training Workshop and Exhibition.

In recognition and appreciation of their years of service, awards were presented to Marjorie D. Fuller, for 15 years as AOAC Assistant Business Manager and to Bernhard Larsen for 8 years as Treasurer of the AOAC.

The last item on the General Session agenda,

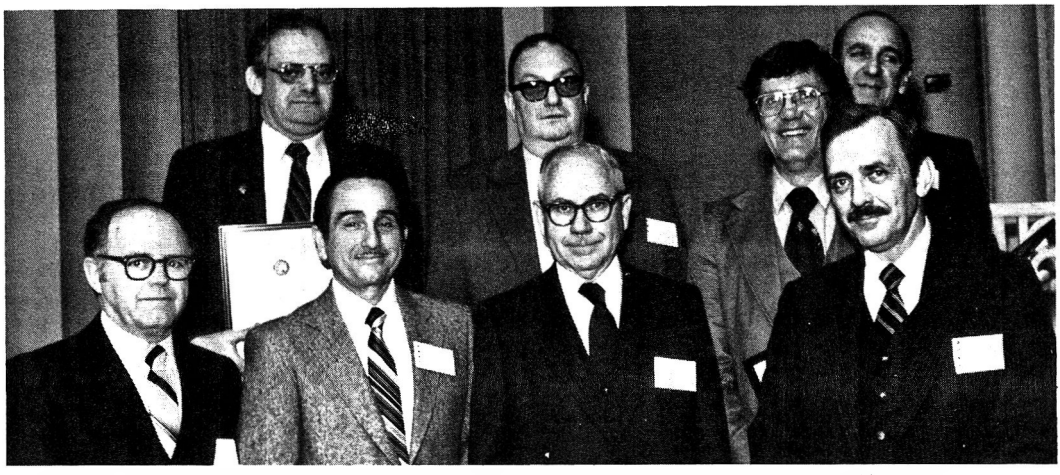


H. Michael Stahr,

Audrey Gardner,

and

H. Michael Wehr



Top row: Edward Smith, Stanley E. Katz, John W. Sherbon, and D. Earle Coffin; bottom row: Elmer George, Jr, Thomas Fazio, Wallace S. Brammell, and Arthur H. Hofberg

Odette L. Shotwell

Hershel F. Morris

and Nicole R. Hardin

Marjorie D. Fuller

Bernhard Larsen

Peter F. Kane

was an address, "Successful Interagency Cooperation: The Diehlstadt Story," by 1982 Harvey W. Wiley Award winner, Odette Shotwell, U.S. Department of Agriculture, Peoria, IL. She recounted a surprisingly successful emergency interagency effort to test 400,000 bushels of white corn suspected of aflatoxin contamination.

The Harvey W. Wiley Award banquet, featuring a 16-piece dance band, closed the first meeting day. For the balance of Monday and the next 3 days and evenings, attendees had their choice of over 200 papers, 48 poster presentations, 5 symposia, 58 equipment and supply exhibits, 2 seminars on automated analysis and 1 on microprocessor application for Kjeldahl and flow injection analysis, plus numerous reports, special discussion sessions, and special meetings.

The 5 timely symposia were titled: Detection of Deliberate Adulteration of Foods, Measurement of Water Activity in Foods, Advances in Nitrosamine Analysis, Analysis of Finished Drinking Water—Trace Contaminants, and Good Laboratory Practices.

A short course, Practice of Modern Liquid Chromatography, sponsored by the American Chemical Society, was held on the 2 days preceding the meeting.

1982 Associate Referee Report of the Year Award

Peter F. Kane, Purdue University, West Lafayette, IN, Associate Referee for potash, won the 1982 award for the best Associate Referee Report of the Year. He was nominated by Committee A for his report, "Collaborative Study of the Flame Photometric Determination of K_2O in Fertilizers."

Every year each Committee is asked to nominate one referee for this award. Other nominees this year were: Committee C—B. Denis Page, Health and Welfare Canada, Ottawa, Ontario, Canada; Committee D—Arthur Caputi, E. J. Gallo Winery, Modesto, CA; Committee E—Susan C. Hight, Food and Drug Administration, Washington, DC; Committee G—Michael H. Thomas, U. S. Department of Agriculture, Beltsville, MD.

Thanks to Reviewers

The work is difficult and time consuming, there is no pay, and little if any recognition—and yet few scientists refuse when asked to review a manuscript. As you can see from the following list, hundreds of men and women contribute their time and expertise to help ensure that published papers reflect work of a consistently high scientific value and technical quality and offer reliable information on the latest advances in their fields.

Providing a thoughtful review of a manuscript is no easy job. We wish it were possible to reward our reviewers, or at least to inform them of the disposition of the manuscripts they review and of the authors' responses to their comments, but our limited resources prohibit this.

What we can do and are happy to do is to tell you who they are. The following is a list of people who have reviewed manuscripts for the 1982 *Journal of the Association of Official Analytical Chemists.* Please forgive us if we have overlooked anyone.

Thank you: R. G. Achari, R. F. Addison, N. Ahmad, J. J. Akielaszek, H. Albert, R. H. Albert, L. V. Allen, Jr., D. Andrzejewski, C. Y. W. Ang, T. E. Archer, P. D. W. Areson, R. J. Argauer, J. A. Ault, J. E. Bailey, S. Barkan, S. A. Barnett, F. R. Batzer, J. R. Baxley, R. A. Beck, T. A. Bellar, G. A. Bennett, M. S. Bergdoll, S. W. Bingham, J. Bitman, H. R. Bolin, D. R. Boline, C. E. Boufford, J. A. Bowers, M. C. Bowman, W. G. Boyd, Jr., D. E. Bradway, J. A. Braswell, A. R. Brause, C. V. Breder, G. S. Brenner, C. D. Brokopp, S. M. Brown, J. V. Bruno, M. P. Bueno, L. B. Bullerman, B. C. Burros, R. J. Bushway, J. J. Byrne.

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Don't Miss the 8th Annual AOAC Spring Workshop and Exposition in Indianapolis

A wide range of subjects will be covered at the 8th Annual AOAC Spring Workshop and Exposition, to be held April 19–21, 1983, at the Sheraton West Hotel, Indianapolis, IN.

Topics and chairmen will be as follows: *Pesticide Formulations*, O. Dean Decker, Eli Lilly and Co., Greenfield, IN; *New Instrumentation*, Fred Regnier, Purdue Univ., West Lafayette, IN; *Pharmaceuticals*, Rafik Bishara, Eli Lilly and Co., Indianapolis, IN, and David Doedens, State Dept. of Toxicology, Indianapolis, IN; *Toxicology*, Rosemary Alstott, Central Testing Facility, Indianapolis, IN; *Microbiological Methods*, Dick Essex, Scott Laboratories, Zionsville, IN; *Chromatographic and Spectroscopic Techniques*, Ronald Hites, Indiana Univ., Bloomington, IN; *Antibiotic Analyses in Feeds and Foods*, H. S. Ragheb, Purdue Univ., West Lafayette, IN; *Toxicology*, Marina Stajic, M.D., Fairfax Hospital, Falls Church, VA;

Microbiology of Waste Waters, Robert H. Bordner, Environmental Protection Agency, Cincinnati, OH; *Mycotoxins*, John Tuite, Purdue Univ., West Lafayette, IN; *Monitoring for Organic Residues*, Jerry L. Hamelink, Eli Lilly and Co., Greenfield, IN; *Vitamin Analysis*, Coleman R. Seward, Food and Drug Admin. (FDA), Atlanta, GA; *High Pressure Liquid Chromatography*, Les J. Lorenz, Eli Lilly and Co., Indianapolis, IN; *Forensics*, Mohamed M. Gohar, State of Ohio Arson Crime Laboratory, Reynoldsberg, OH, and Howard L. Dobres, Drug Enforcement Admin., Chicago, IL; *Food Microbiology*, Robert M. Twedt, FDA, Cincinnati, OH; *Trace Metals Analysis*, Fred Fricke, FDA, Cincinnati, OH; *Problems Associated with Instrumentation and Automation*, Jack Zynger, Eli Lilly and Co., Indianapolis, IN; *Veterinary Toxicology*, Frank Ross, US Dept of Agriculture, Ames, IA; *Drug Residues in Foods and Feeds*, R. J. Noel, Purdue Univ., West Lafayette, IN; *Medical Devices*, Michael T. Kenny, Dow Chemical, Indianapolis, IN; and *Sampling Facets* (Chairman to be announced).

Registration fees are as follows: pre-registration (deadline April 4, 1983) $60, on-site registration $75, student registration $20, and registration by the day $35. Fees include 2 lunches and a wine and cheese party.

For further information, contact Lawrence Sullivan, Indiana State Board of Health, 1330 West Michigan St., Indianapolis, IN 46206 (317/633-0224).

Free Technical Assistance Available for Filling Out Premanufacture Notifications (PMN)

Chemical manufacturers in the northern midwest and east coast corridors receive, upon request, free technical assistance in filling out Premanufacture Notifications (PMNs), as required under Section 26(d) of the Toxic Substances Control Act (TSCA). Provided through the Field Technical Service and funded by the U.S. Environmental Protection Agency (EPA), this service is operated by a Washington-based management consulting firm, Triton Corp.

For further information in the east coast corridor, call Alan Schneider at (201) 277-0060 and in the northern midwest, call Joshua Phillips at (312) 454-0536. Both men have been trained and cleared by EPA to provide technical assistance and handle confidential business material (CBI).

SRMs for 2 Anticonvulsant Drugs and for Metal-on-Quartz Filters for Spectrophotometry Now Available from the National Bureau of Standards (NBS)

SRM 1599 Anticonvulsant Drug Level Assay Standard—This new standard reference material (SRM) certified for concentrations of 2 anticonvulsant drugs, valproic acid and carbamazepine, is available in a freeze-dried human serum base. Primary uses are for calibration and standardization of quantitative clinical analyses for these drugs in human serum, for critical evaluations of working or secondary standards prepared in-house, and for manufacturers of control materials, calibrators, and kits to assure the quality of such products.

This SRM consists of 4 vials of freeze-dried serum: 3 contain the 2 drugs at near, above, and lower concentrations than usually used to control convulsions caused by epilepsy, the 4th vial is a serum blank. The price is approximately $137 per 4-vial unit. Other clinical SRM's available from NBS include SRM 900 for the anticonvulsant drugs: phenytoin, ethosuximide, phenobarbital, and primidone, and SRM 909, a freeze-dried human serum certified for constituents found normally in blood.

SRM 2031 Metal-on-Quartz Filters for Spectrophotometry—Intended to be used as a reference standard for verifying the transmittance and absorbance scales of conventional spectrophotometers in the ultraviolet and visible regions of the electromagnetic spectrum, SRM 2031 consists of 3 individual filters in their metal holders and one empty filter holder. The transmittance and transmittance density (absorbance) of each filter have been measured and certified with an uncertainty of 1.0% for each of the following 10 wavelengths: 250, 280, 340, 360, 400, 465, 500, 546.1, 590, and 635 nm. The certified values are valid for 1 year from date of certification when the set of filters should be returned to NBS for free verification. The certificate issued with the SRM provides instructions for the use of the filters. Details of preparation and certification are issued with each set of filters. The price is $1260 per unit.

SRMs may be purchased from the Office of Standard Reference Materials, National Bureau of Standards, Room B311, Chemistry Building, Washington, DC 20234. Telephone 301/921-2045.

AOAC Gains a New Canadian Sustaining Member

The Ontario Ministry of Agriculture and Food, Toronto, Ontario, Canada, has become a Sustaining Member of AOAC. By doing so, they join several other Canadian agencies who, knowing the importance of independent methods validation, provide support for AOAC.

Meetings

April 7–8, 1983: Committee E-11 on Statistical Methods, ASTM Headquarters, Philadelphia, PA. Contact: Bill Hulse, 215/299-5507.

April 19–21, 1983: AOAC 8th Annual Spring Workshop and Exposition, Sheraton West Hotel, Indianapolis, IN. Contact: Lawrence Sullivan, Indiana State Board of Health, 1330 West Michigan St, Indianapolis, IN 46206; 317/633-0224. For more information, see article above.

May 17–19, 1983: 6th International Rapeseed Conference, Paris, France. Contact: Colza-Congres-Services, 1 rue Jules-Lefebvre, F. 75009 Paris, France.

June 1–4, 1983: Symposium-Flavour of Distilled Beverages; Stirling University, Scotland. Contact: Dr. J. R. Piggott, Dept. of Bioscience and Biotechnology, University of Strathclyde, 131 Albion St, Glasgow G1 1SD, Scotland

June 5–8, 1983: 66th Canadian Chemical Conference, Convention Centre, Calgary, Alberta, Canada. Contact: Dr. Arvi Rauk, MCIC, Department of Chemistry, Univ. of Calgary, Calgary, Alberta, T2N 1N4 Canada; 403/284-6247, or The Chemical Institute of Canada, 151 Slater St, Suite 906, Ottawa, Ontario, K1P 5N3 Canada; 613/233-5623.

June 7–10, 1983: 1st International Symposium on Drug Analysis, Free University of Brussels, Brussels, Belgium. Contact: C. Van Kerchove, Société Belge des Sciences Pharmaceutiques—Belgisch Genootschap voor Pharmaceutische Wetenschappen, rue Archimedesstraat 11, B-1040 Brussels, Belgium; telephone (02) 733 98 20 est. 33.

July 24–29, 1983: 35th Annual Meeting of the American Association for Clinical Chemistry, New York, NY. Contact: Marjorie Hack, AACC, 1725 K St, NW, Washington, DC 20006; 202/857-0717.

July 17–23, 1983: SAC 83—International Conference and Exhibition on Analytical Chemistry, University of Edinburgh, Edinburgh, Scotland. Contact: P. E. Hutchinson, Secretary, Analytical Div., Royal Society of Chemistry, Burlington, House, London, W1V OBN, UK.

July 18–21, 1983: 1983 National Conference of Standards Laboratories Workshop and Symposium: Metrology—Meeting the Challenge of Change, National Bureau of Standards, Boulder, CO. Contact: Gary Davidson, Co-chairman, 1983 NCSL Workshop and Symposium, TRW/DSSG, Bldg. S, Room 2767, One Space Park, Redondo Beach, CA 80278; 213/535-1684.

July 27–30, 1983: 3rd International Conference on Instrumental Analysis of Foods and Beverages—Recent Developments in Chemistry and Technology, Corfu Hilton Hotel, Corfu, Greece. Contact: D. J. Mussinan, IFF R & D, 1515 Highway 36, Union Beach, NJ 07735; 201/264-4500.

Chromatography Courses Offered

Varian Associates is offering 23 training courses in chromatography from October 1982 through September 1983. Courses will be conducted at Walnut Creek, CA; Park Ridge, IL; Houston, TX; and Florham Park, NJ. Topics range from basic courses in gas or liquid chromatography to advanced techniques, environmental applications, and special analyzer techniques.

Each course is a hands-on, laboratory oriented presentation, with personal instruction using the latest equipment and techniques. Course length ranges from 1 to 5 days and fees are from $150 to $450.

For more information, contact Varian Instruments, PO Box D-070, 220 Humboldt Court, Sunnyvale, CA 94086; telephone 408/734-5370, Ext. 415.

NEW PUBLICATIONS

Chlorinated Dioxins and Related Compounds – Impact on the Environment. Proceedings of the Workshop Instituto Superiore di Sanita, Rome, Italy. Edited by O. Hutzinger, R. W. Frei, E. Merian, and F. Pocchiari. Published by Pergamon Press, Headington Hill Hall, Oxford 0X3 OBW, England, 1982. Also available from Pergamon Press, Fairview Park, Elmsford, NY 10523, USA. 624 pp. 165 illus. Price: US$75.00. ISBN 0-08-0262562.

The workshop on which this book is based was organized to allow a multidisciplinary approach to problems posed by chlorinated dioxins. The most recent advances in analytical methodology, environmental fate and levels, incineration toxicology (metabolism), animal toxicology, and observations in man are covered.

Collaborative Interlaboratory Studies in Chemical Analysis. International Symposium on Harmonization of Collaborative Analytical Studies, Helsinki, Finland, September 1981. Edited by H. Egan and T. S. West. Published by Pergamon Press, Headington Hill Hall, Oxford 0X3 OBW, England, 1982. Also available from Pergamon Press, Fairview Park, Elmsford, NY 10523, USA. 178 pp, 27 illus. Price US$40.00. ISBN 0-08-026228-7.

There is great interest throughout the chemical sciences in the standardization and validation of those analytical methods which are the basis for specification and the legal enforcement of standards covering all aspects of human health and endeavor. Such standards and analytical methods should be compatible. Today, there are national and international research institutions whose primary goal is to develop such methods. This book summarizes the state of progress in this area. It will be of interest to analytical chemists and national and international organizations involved in the development and use of standard methods.

Mass Spectrometry in the Environmental Sciences. Theory and Applications. Edited by F. W. Karasek et al. Published by Pergamon Press, Headington Hill Hall, Oxford 0X3 OBW, England, 1982. Also available from Pergamon Press, Fairview Park, Elmsford, NY 10523, USA. 500 pp. Price: US$100.00. ISBN 0-08-026255-4.

A comprehensive, multi-author work, this book is intended to inform the chemist working in the pollution or pesticide area of the capabilities of mass spectrometry techniques and to inform the specialist mass spectroscopist of the background to various problems encountered in pollutant analysis.

Analytical Techniques in Environmental Chemistry II. Proceedings of the 2nd International Congress, 1981, Barcelona, Spain. Edited by J. Albaiges. Published by Pergamon Press, Headington Hill Hall, Oxford 0X3 OBW, England, 1982. Also available from Pergamon Press, Fairview Park, Elmsford, NY 10523, USA. 482 pp. 185 illus. Price: approx. US$75.00. ISBN 0-08-028740-9.

This book covers new methodologies and recent advances in analytical instrumentation for the identification of naturally occurring and man-made environmental chemicals. Emphasis is given to analytical strategies oriented to a better knowledge of ecosystem dynamics and to the formulation of environmental quality standards. This book will be of interest to research workers in the environmental sciences and analytical chemistry.

Confidentiality of Data and Chemicals Control. Published by the Organisation of Economic Co-operation and Development (OECD), 2, rue Andre-Pascal, 75775 Paris Cedex 16, France, 1982. Also available from OECD Publications Information Center, Suite 1207, 1750 Pennsylvania Ave, NW, Washington, DC 20006, USA. 94 pp. Price: US$10.00, £5.00, F50.00. ISBN 92-64-12365-2.

Prepared by an international group of experts, this report analyzes the problems associated with the confidentiality of data on chemicals submitted by industry to government. Public disclosure of data, exchange of information, and the protection of proprietary rights to data

are considered in detail as well as proposals that would permit governments to deal with these problems.

Good Laboratory Practice in the Testing of Chemicals. Published by the Organisation of Economic Co-operation and Development (OECD), 2, rue André-Pascal, 75775 Paris Cedex 16, France, 1982. Also available from OECD Publications Information Center, Suite 1207, 1750 Pennsylvania Ave, NW, Washington, DC 20006, USA. 62 pp. Price US$7.00, £7.80, F35.00. ISBN 92-64-12367-9.

Prepared by an international group of experts, this report addresses the issue of compliance at the national and international level with the OECD Principles of Good Laboratory Practice. Guidelines for national GLP inspections and study audits are proposed.

AOAC Regional Section Meetings

Midwest Regional Section Meeting

June 14-15, 1983
Ames, IA
Contact: H. Michael Stahr
Iowa State University
515/294-1950

Northwest Regional Section Meeting

June 15-16, 1983
Olympia, WA
Contact: H. Michael Wehr
Oregon Department of Agriculture
503/378-3793

Northeast Regional Section Meeting

June 28-29, 1983
Bennington, VT
Contact: Audrey Gardner
NY State Agriculture Experiment Station
315/787-2281

BOOK REVIEW

Developments in Meat Science – 2. Edited by Ralston Lawrie. Published by Applied Science Publishers, Ltd, 22 Rippleside Commercial Estate, Ripple Rd, Barking, Essex IG 11 OSA England, 1981. xxi + 299 pp. Price: $64.00. ISBN 0-85334-986-X.

A broad range of topics under the general heading of meat science is covered in this book. Although few individuals are likely to be interested in the entire book, select chapters should appeal to a large audience with diverse backgrounds such as analytical chemistry, veterinary medicine, human nutrition, and food production—the raising, transportation, slaughter, and processing of animals intended for human consumption, or even on the consumer level.

It was as a consumer of meat products that I found chapters on water activity and nutrition of meat of particular interest. Chapter 6 provides an in depth description of recent research in the production of intermediate moisture meat. The term water activity (a_w) is introduced, defined, and then used as a means of measuring the effectiveness of various additives in the production of a safe, stable, and appetizing meat product. Chapter 8 presents an unemotional discussion of the nutritional value and possible health related hazards associated with meat consumption and includes a table with an impressive summary of the nutritional value meat contributes to a daily diet.

As an analytical chemist involved with monitoring meat tissue for trace drug residues, I found several chapters of particular interest. Chapter 4, although primarily concerned with the production of an emulsion type meat product, provides valuable information on the biochemical changes occurring in muscle tissue following slaughter and the onset of rigor mortis. Factors such as pH change, ATP depletion, and water holding capacity are discussed. Chapter 5 discusses effects of freezing, frozen storage, and subsequent thawing of meat tissue—far too many analytical chemists need to be reminded that prolonged storage and repeated thawing of meat tissue can drastically alter both the nature of the tissue matrix and the component residue for which an assay is intended. Chapter 7 provides a good summary of analytical approaches currently being used for differentiating muscle tissue, connective tissue, and nonmeat protein.

This constitutes a valuable reference book for the analytical chemist, microbiologist, veterinarian, nutritionist, and others involved with the production and monitoring of meat products.

FRANCIS B. SUHRE
U.S. Department of Agriculture
Chemistry Division Laboratory Branch
Beltsville, Maryland 20705

GENERAL REFEREE REPORTS: COMMITTEE A

Report on Feeds

CLYDE E. JONES
State Department of Agriculture, 2331 W 31st Ave, Denver, CO 80211

Several new Associate Referees were appointed this year in areas where new methods or improved methods are needed by industry and regulatory agencies. Several collaborative studies were planned to start this year. An Associate Referee is still needed for Infrared Reflectance Techniques in Mixed Feeds.

Amino Acids in Mixed Feeds.—Wayne Stockland was appointed Associate Referee in August. He has a method ready for collaborative study that combines an amino acid analyzer with HPLC for complete amino acid profile. The Florida Department of Agriculture is interested in developing a chemical or HPLC method for specific amino acids.

Crude Fat in Pet Foods.—No Associate Referee. Some people are not aware of the editorial changes made last year in the fat method (**7.055-7.060**) instructions and are still having interpretation difficulties. The American Association of Feed Control Officials was requested by a few members of industry to investigate the matter, and the AAFCO report which followed endorsed the action taken by AOAC last year. No AOAC activity is recommended this year unless problems continue.

Crude Fiber.—Associate Referee David Holst worked as coordinator for the ISO Group on filtration problems of crude fiber and cell water determinations. No collaborative AOAC work is planned this year.

Crude Fiber in Milk Replacers.—Associate Referee Jim Pierce continues to experience problems with the method; a collaborative study is planned if problems can be solved.

Iodine in Feed.—Stuart Meridian, West Agro-Chemical, Inc., is the new Associate Referee. There is concern with the amount of iodine entering milk via animal feeds, which in turn may cause excessive iodine in the human diet. A method using ion selective electrodes is ready for collaborative study.

Non-Nutritive Residues.—Associate Referee Peter J. Van Soest participated in a collaborative study on an enzymatic method for dietary fiber, sponsored by Asp, University of Copenhagen; results are not yet available. He is considering an AOAC collaborative study on non-nutritive dietary fiber (NDF), and is developing a modified NDF procedure to measure soluble dietary fiber.

Crude Protein.—The new Associate Referee, Peter Kane, Office of the Indiana State Chemist, plans to conduct a collaborative study this year using copper catalyst for crude protein.

Water (Karl Fischer Method).—The new Associate Referee, David Wallace, Colorado Department of Agriculture, is conducting a collaborative study on automated KF for whole grains. If the study is successful, it may be expanded to mixed feeds.

Associate Referees on Minerals and Sampling reported no progress this year.

Recommendations

Continue study on all topics. Appoint an Associate Referee for IR Reflectance Technique in Mixed Animal Feeds.

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

The recommendations of the General Referee were approved by Committee A and were accepted by the Association. See the report of the Committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition.

Report on Fertilizers and Agricultural Liming Materials

ROBERT C. RUND
Office of the Indiana State Chemist, Purdue University, Department of Biochemistry, West Lafayette, IN 47907

Calcium and Magnesium; Copper; and Elemental Analysis of Liming Materials.—These subjects have been inactive for many years and no difficulty with existing methods has been brought to the attention of the Associate Referees or the General Referee. It is proposed that these topics be discontinued.

Iron.—A method for the determination of chelated iron in iron chelate concentrates has been collaboratively studied and evaluated by Associate Referee James Silkey. Following removal of oxidized non-chelated iron at pH 8.5, the chelated iron is determined by atomic absorption spectrophotometry. The Associate Referee proposes this method for adoption as official first action for concentrates of iron chelates. The General Referee concurs, providing an applicability statement limits the method to those chelating agents studied and notes nonapplicability to mixed fertilizers.

Nitrogen.—Associate Referee Paul R. Rexroad presents a compelling argument to advance the modified comprehensive nitrogen method (**2.061-2.062**) to official final action status. This method specifies copper instead of mercury as the catalyst during the Kjeldahl digestion and reduction step with chromium salts. Other modifications to the official final action comprehensive nitrogen method (**2.059-2.060**) include significant changes in the salt-to-acid ratio and the length of digestion. Magruder Check Sample results for the 4 years the modified comprehensive method has been published confirm its acceptance and comparative accuracy. The General Referee concurs in the recommendation to adopt **2.061-2.062** official final action.

Phosphorus.—Associate Referee Frank J. Johnson is leading a comparative evaluation by an investigative team of the AOAC official methods for available phosphoric acid and a method proposed by the European Economic Community. These methods are now under consideration by a working group of Technical Committee 134 of the International Standardisation Organization; no report is available at this time.

Potash.—Associate Referee Peter F. Kane has reported on a collaborative effort to express the manual flame method (**2.091-2.096**) and the automated method (**2.097-2.101**) as a single method, using performance parameters to judge instrument acceptability. This obviates the requirement to limit the application of common principles to specified instruments. Results of the collaborative study in which the proposed method was compared with the official sodium tetraphenyl boron method (**2.102**) were reported and used to substantiate the applicability of the proposal. The Associate Referee recommends adoption as official first action of the proposed flame photometric method collaboratively studied, and the General Referee concurs.

Sampling and Preparation of Sample.—Associate Referee Douglas Caine has been actively engaged in a joint effort with industry and regulatory officials to design a study of sampling procedures for bagged, blended fertilizers.

Slow-Release Mixed Fertilizers.—Associate Referee Stanley E. Katz proposes a new approach to evaluate the slow-release capabilities of water-insoluble nitrogen products. Samples of these products are currently being obtained for this work.

Sodium.—Associate Referee Luis F. Corominas presented a report at the previous AOAC meeting in which results of a collaborative study were given comparing the official first action flame emission spectrophotometric method with a method using atomic absorption. The 2 methods produced equivalent values and the Associate Referee recommended adoption of the proposed AAS method as official first action. Because timing of the report last year did not permit action, the General Referee now recommends adoption as official first action.

Soil and Plant Amendment Ingredients.—Associate Referee Clyde E. Jones has joined in a report describing a mini-collaborative study to deter-

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

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

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition.

mine the applicability of the official atomic absorption spectrophotometric method (**8.023-8.026**) for aluminum in baking powders and chemicals to aluminum contained in aluminum sulfate soil acidifiers. The results appear promising and the Associate Referee recommends adoption of the method as official first action. The General Referee concurs with this recommendation.

Water-Soluble Methyleneureas.—Associate Referee Allan D. Davidson reported the results of 9 collaborators using high pressure liquid chromatography for 5 sample pairs to establish the validity of this method. Coefficients of variation by HPLC for urea, methylenediurea, and dimethylenetriurea were acceptable. The Associate Referee recommends adoption of this HPLC method as official first action and the General Referee concurs.

Zinc.—A collaborative study specifying atomic absorption spectrophotometry is being planned. Preliminary work by Associate Referee Mary L. Hasselberger to determine criteria for the AAS method is under way.

Other Topics.—No progress is reported by the Associate Referees for the topics Biuret in Urea and Mixed Fertilizers; Boron; Free and Total Water; Sulfur. There is no Associate Referee for Molybdenum.

Recommendations

(1) Discontinue study on Calcium and Magnesium; Copper; and Elemental Analysis of Liming Materials.

(2) Adopt as official first action the proposed method for iron chelate concentrates with applicability statement as follows: "Applicable only to concentrates of FeEDTA, FeHEDTA, FeDTPA, FeEDDHA, Fe citrate, and FeDPS. Not applicable to mixed fertilizers."

(3) Adopt as official final action the modified comprehensive nitrogen method, **2.061-2.062**.

(4) Adopt as official first action the flame photometric method for potash proposed by the Associate Referee.

(5) Adopt as official first action the atomic absorption spectrophotometric method for sodium proposed by the Associate Referee.

(6) Adopt as official first action the atomic absorption spectrophotometric method for aluminum in aluminum sulfate soil acidifiers proposed by the Associate Referee.

(7) Adopt as official first action the HPLC method for water-soluble methyleneureas proposed by the Associate Referee.

(8) Appoint an Associate Referee for Molybdenum.

(9) Continue study on all other topics.

Report on Pesticide Formulations: Carbamate and Substituted Urea Insecticides

PAUL D. JUNG
Environmental Protection Agency, Office of Pesticide Programs, Beltsville, MD 20705

Once again, most of the Associate Referees in this area have been quite productive. All completed collaborative studies have used HPLC methods with an internal standard. Proposed methods all appear to be leaning heavily on this technique. The following is the present status of selected topics assigned to the Associate Referees:

Aldicarb; and Carbaryl.—William H. McDermott is working on a series of methods, both normal and reverse phase HPLC, directed to the analysis of these materials.

Bendiocarb.—Peter L. Carter has refined the method to a binary reverse phase system employing an internal standard, and plans a collaborative study.

Carbofuran; and Carbosulfan.—Edward J. Kikta has completed a collaborative study of an internal standard, reverse phase HPLC method for the determination of carbofuran. An interim first action adoption is anticipated. He also plans a collaborative study of a method for carbosulfan.

Methiocarb.—Newly appointed Steven C. Slahck plans to complete collaborative study of an HPLC method this year.

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

The recommendation of the General Referee was approved by Committee A and was accepted by the Association. See the report of the Committee, this issue.

Methomyl.—James E. Conaway, Jr, has completed a collaborative study of a reverse phase internal standard HPLC method. An interim first action approval is anticipated shortly.

Oxamyl.—Glenn A. Sherwood, Jr, has completed a collaborative study of a reverse phase internal standard HPLC method. An interim first action approval is anticipated shortly.

Pirimicarb.—Peter D. Bland indicates that this commodity is being phased out in the United States but will be marketed strongly throughout the world. A new Associate Referee will be sought.

Propoxur.—Newly appointed Steven C. Slahck plans to complete an HPLC collaborative study this year.

Recommendation

Continue study on all topics as outlined above.

Report on Pesticide Formulations: Fungicides and Disinfectants

THOMAS L. JENSEN
State Department of Agriculture, 3703 S 14th St, Lincoln, NE 68502

Although no collaborative studies were conducted in this area during the year, several section members were busy with preparation and followup work concerning collaborative studies.

Milton Parkins of Uniroyal has been appointed Associate Referee for Carboxin and Oxycarboxin. Many topics are still vacant, and new Associate Referees are being sought.

Alan Hanks reports followup work has been done in checking the stability of the pentachloronitrobenzene (**6.C08-6.C11**) internal standard. In-house work indicates the compound, *o*-terphenyl, is indeed stable over a period of time. He recommends continued use and adoption of the method as official final action.

A question has arisen on the status of triphenyltin methodology. Although both potentiometric titration and HPLC methods were collaboratively studied by CIPAC, only the potentiometric titration method holds AOAC status (**6.436-6.439**, final action). The General Referee has contacted Committee A regarding what work is required for the GLC method for triphenyltin to be acceptable for AOAC adoption.

Federal cutbacks have forced a change in priorities, and Lilia Rivera suggests a new referee be appointed for Benomyl.

Brian Korsch reports that a method for chlorothalonil is ready for collaborative study.

Recommendations

(1) Adopt as official final action the official first action GLC method for pentachloronitrobenzene, **6.C08-6.C11**.

(2) Continue study on the GLC method for triphenyltin.

(3) Continue study in all other areas.

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

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

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition; and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* 65, 450–521 (1982).

Report on Pesticide Formulations: Halogenated and Other Insecticides, Synergists, and Insect Repellants

JAMES E. LAUNER
State Department of Agriculture, Laboratory Services, Salem, OR 97310

These topics were very active this year; 3 collaborative studies were completed and recommended for official first action. Two Associate Referees were appointed during the past year: Dean Yeaman, Fumigants; Michael Sabbann, Perthane.

Because sabadilla alkaloids are no longer produced in this country, the General Referee recommends designating as surplus the final action method for dust formulations (**6.173-6.174**).

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

Chlordane and Heptachlor.—J. Forrette will initiate a collaborative study of the combination of total chloride and infrared spectroscopy to determine chlordane and heptachlor in mixtures.

Chlordimeform.—A. Hofberg will initiate a collaborative study of a GLC method.

Dicofol.—A. Rothman has completed a collaborative study of an HPLC method and recommends adoption as official first action. The General Referee concurs.

Diflubenzuron.—A. Van Rossum has completed a collaborative study of an HPLC method and recommends adoption as official first action. The General Referee concurs.

Endosulfan.—R. Watson investigated the CIPAC GLC method and recommends adoption as official first action. The General Referee concurs.

Fenvalerate.—R. Collins is cooperating with CIPAC on a GLC method.

Fumigants.—D. Yeaman is completing study of a GLC method for sulfuryl fluoride.

Permethrin.—H. Morris is cooperating with CIPAC on a capillary GLC method.

Piperonyl Butoxide and Pyrethrins.—D. Kassera recommends adoption of the official first action gas-liquid chromatographic method (**6.C22-6.C25**) as official final action. The General Referee concurs.

Rotenone.—R. Bushway has completed a collaborative study of an HPLC method and recommends adoption as official first action. The General Referee concurs.

Tetradifon.—A Martijn recommends discontinuing this topic with the satisfactory official final action method (**6.B09-6.B14**). The General Referee concurs.

Trichlorfon.—M. Sabbann is investigating an HPLC method.

Recommendations

(1) Continue official first action status of the following methods: (a) GLC method for technical allethrin (**6.149-6.154**); (b) radioactive tracer method for benzene hexachloride (**6.202**); (c) hydrolyzable chloride method for dicofol (**6.283-6.288**); (d) GLC method for fumigants (**6.143-6.148**); (e) infrared method for rotenone (**6.163-6.164**); (f) UV method for sulfoxide (**6.419**).

(2) Adopt as official first action the following methods described by the Associate Referees: (a) HPLC method for dicofol; (b) HPLC method for diflubenzuron; (c) CIPAC GLC method for endosulfan; (d) HPLC method for rotenone.

(3) Adopt as official final action the official first action GLC method for piperonyl butoxide and pyrethrins (**6.C22-6.C25**).

(4) Declare as surplus the final action method for sabadilla alkaloids (**6.173-6.174**).

(5) Initiate the topic Methyl Bromide and appoint an Associate Referee.

(6) Discontinue the topic Tetradifon.

(7) Continue the movement toward the goal of deletion of total halide methods as rapidly as possible.

(8) Continue study on all other topics.

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

The recommendations of the General Referee were approved by Committee A and were adopted by the Association, except 6.173-6.174 was not designated surplus, and the method for dicofol was not adopted. See the report of the Committee and "Changes in Methods," this issue.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition; and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **64**, 501-540 (1981); **65**, 450-521 (1982).

Report on Pesticide Formulations: Herbicides I, Other Organophosphate Insecticides, Rodenticides, and Miscellaneous Pesticides

G. MARSHALL GENTRY
State Department of Agriculture and Consumer Services, Tallahassee, FL 32301

Considerable activity by some Associate Referees during the past year has resulted in one method recommended for official first action and 2 collaborative studies planned for next year. Associate Referees are needed for Crufomate, Chlorophacinone, and Strychnine. Anyone interested in these areas should contact the General Referee.

The following is a summation of activities and recommendations from various Associate Referees:

Crotoxyphos.—Wendy King is investigating a GLC method.

Monocrotophos.—George Winstead is investigating normal phase HPLC to eliminate late, interfering peaks found with reverse phase HPLC and GLC.

Naled.—A. Aner Carlstrom is working on controls for the GLC method.

Brodifacoum.—Peter D. Bland is recommending a method for adoption as official first action.

Diphacinone—Violet M. Stephens is investigating HPLC columns for this compound.

Warfarin.—Elmer H. Hayes plans a collaborative study for the coming year.

Chlorophenoxy Herbicides and Dicamba.—Associate Referees Robert Grorud and John Forrette are working jointly on method development for combinations of 2,4-D, dicamba, and MCPP. A collaborative study of an HPLC method is planned.

Pentachlorophenol.—Elmer H. Hayes collaboratively studied a method, but results have not been presented.

Picloram.—The present AOAC method (**6.308-6.311**) is reliable and Associate Referee Timothy S. Stevens recommends continued use of this method.

No work is reported on Dichlorvos; Mevinphos; Tetrachlorvinphos; and Plant Growth Regulators.

Recommendations

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

(2) Discontinue the topic Picloram.

(3) Continue study on all other topics.

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

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

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition.

Report on Pesticide Formulations: Herbicides II

LASZLO TORMA
Montana Department of Agriculture, Laboratory Bureau, Montana State University, Bozeman, MT 59717

Progress to initiate and conclude collaborative studies for these compounds has been very slow during the past year. Many Associate Referees indicate that it is difficult to find time to carry out method development or collaborative study. Furthermore, some Associate Referees had to resign because they have accepted new positions.

Newly appointed Associate Referees and their topics are: P. Parkins, Alanap; J. E. Forrette, Barban; R. Stringham, Fluchloralin, Profluralin, Benefin, Trifluralin, and Penoxalin.

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

The recommendation of the General Referee was approved by Committee A and was accepted by the Association. See the report of the Committee, this issue.

The General Referee is still seeking Associate Referees for Benzoylpropethyl, Dinoseb, Monuron, and Oryzalin.

Recommendation

Continue study on all topics.

Report on Pesticide Formulations: Herbicides III

THOMAS L. JENSEN
State Department of Agriculture, 3703 S 14th St, Lincoln, NE 68502

Again, this year, much progress was made by Associate Referees in the pursuit of collaborated methods. Tim Stevens of Dow and Arnold Burns of Monsanto both completed successful HPLC studies. In addition, Associate Referees are planning at least 3 studies in the future, including work with propanil, metribuzin, and metolachlor. Other studies may also be initiated, depending on priorities. The following is a status report on selected topics in this section:

Alachlor, Propachlor, and Butachlor.—L. A. Furrer reports that a decision will be made soon about collaborative studies on these compounds.

Dalapon.—Timothy S. Stevens reports that a successful collaborative study was completed and recommends adoption as official first action.

Dichlobenil.—Edward E. Chapman reports that his company is no longer producing this compound and suggests that a new referee be appointed

Glyphosate.—Arnold J. Burns reports that a successful collaborative study was completed and recommends adoption as official first action.

Metolachlor.—Arthur H. Hofberg reports that an internal study of this compound proved successful and a full scale collaborative study is planned.

Metribuzin.—William Betker reports that a collaborative study is planned.

Propanil.—Delmas Pennington reports that a collaborative study is planned.

Terbuthylazine.—Arthur Hofberg reports that all communications received indicate that the first action method (**6.B20-6.B27**) is suitable for adoption as official final action.

Triazine Herbicides.—Arthur Hofberg reports that a study on replacement of the internal standards for these compounds will be undertaken in 1983.

Recommendations

(1) Adopt as official first action the HPLC methods for dalapon and glyphosate.

(2) Adopt as official final action the official first action CIPAC-AOAC method for terbuthylazine. Include terbuthylazine in the Triazine topic and delete as a separate topic.

(3) Continue study in all areas.

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

The recommendations of the General Referee were approved by Committee A and were adopted by the Association, except for dalapon. See the report of the Committee and "Changes in Methods", this issue.

Section numbers refer to "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **64**, 501-540 (1981).

Report on Pesticide Formulations: Organothiophosphate Pesticides

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Four new Associate Referees were appointed during the year to fill vacancies. In addition, a recent resignation has created a vacancy in one of the more important topics.

The following is a summation of activities and recommendations of the various Associate Referees:

Acephate.—A. Aner Carlstrom has been appointed Associate Referee for this pesticide. He hopes to conduct a collaborative study of a GLC method within the next 18 months.

Coumaphos.—Linda Ruiz as new Associate Referee has not had an opportunity to review the status of methodology in this area.

Diazinon.—Associate Referee A. H. Hofberg is studying a modification of the official first action GLC method for diazinon, **6.C12-6.C15.**

Dimethoate.—Associate Referee R. S. Wayne plans a collaborative study on an HPLC method.

Dioxathion.—Associate Referee W. H. Clark is studying an HPLC method in preparation for an AOAC-CIPAC study.

EPN.—Associate Referee J. E. Forrette plans both a GLC and an HPLC study as soon as it can be determined that impurities are not causing a bias in the GLC method.

Ethoprop.—Wallace Embry as new Associate Referee is currently studying a GLC method.

Fensulfothion.—Associate Referee Margie Owen has completed an HPLC study and recommends that the method be adopted official first action.

Fenthion.—Associate Referee W. G. Boyd, Jr, is studying a GLC method but has not been able to obtain a satisfactory standard.

Fonophos.—Herman Moya found it necessary to resign as Associate Referee for this pesticide.

Methidathion.—Tom Gale as new Associate Referee will investigate reverse phase HPLC for this pesticide.

Parathion and Methyl Parathion.—Both the HPLC and GLC methods for these pesticides are official first action (**6.379-6.387, 6.400-6.408**). Associate Referee E. R. Jackson plans a collaborative study of the parathion GLC method to include parathion dusts and methyl parathion-parathion mixtures.

Phorate.—Associate Referee Roman Grypa plans a collaborative study on a GLC method during the coming year.

No further work has been done on Azinphosmethyl; Chlorpyriphos; Demeton; Demeton-*S*-Methyl; Disulfoton; Encapsulated Organophosphorus Pesticides; Ethion; Malathion; Oxydemeton, and Sulprofos.

Recommendations

(1) Adopt as official first action the HPLC method for fensulfothion.

(2) Continue study on indicated topics.

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

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

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition; and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **65,** 450-521 (1982).

Report on Reference Materials and Standard Solutions

ROBERT ALVAREZ
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A Directory of Certified Reference Materials has been published recently by the International Organisation for Standardisation (ISO). It identifies hundreds of producers of certified reference materials (CRMs) throughout the world. CRM categories of special interest to AOAC include environmental; biological, botanical, foods; biomedical and pharmaceuticals; clinical chemistry; and chemicals-organic.

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

The recommendations of the General Referee were approved by Committee A and were accepted by the Association. See the report of the Committee, this issue.

Copies of the directory are available in the United States from the American National Standards Institute (ANSI), 1430 Broadway, New York, NY 10018, and in Europe from ISO, Case Postale 56, CH-1211, Geneve 20, Switzerland.

Two additional important publications of ISO dealing with reference materials are also available. ISO Guide 30-1981 (E) describes terms and definitions used in connection with reference materials, and ISO Guide 31-1981 (E) covers contents of reference material (RM) certificates. An RM is defined as a material or substance one or more properties of which are sufficiently well established to be used for the calibration of an apparatus, for the assessment of a measurement method, or for assigning values to materials. A CRM is an RM one or more of whose property values are certified by a technically valid procedure, accompanied by or traceable to a certificate or other documentation which is issued by a certifying body.

AOAC-related CRM activities of several organizations are noted below:

The Marine Analytical Chemistry Standards Program, National Research Council, Montreal Rd, Ottawa, Ontario, Canada K1A 0R9, has announced the availability of a seawater reference material for trace elements. The elemental concentrations reported are based on the analytical results by at least 2 independent methods. Marine sediment reference materials are also available.

The National Physical Laboratory, Teddington, Middlesex, UK, TW11 OLW, has issued a new catalog (July 1982) of CRMs. The catalog includes pesticides of certified purity, and a CRM for animal-feedstuff analysis.

The International Atomic Energy Agency, PO Box 590, A 1011 Vienna, Austria, issues both CRMs and RMs. Materials are issued as RMs, instead of as CRMs, either because the materials have not been analyzed by a sufficiently large number of different analytical techniques or because the individual intercomparison results are too divergent. Soil-5 (soil), SL-1 (lake sediment), and Air-3/1 (a simulated deposition of trace elements on filters) are available as CRMs for trace element determinations.

Community Bureau of Reference-BCR, Commission of the European Communities, 200 rue de la Loi, B-1049 Brussels, Belgium, issues various CRMs including high purity polycyclic aromatic hydrocarbons (carcinogens), thromboplastins, phosphate rock, and superphosphate fertilizers.

The National Institute of Environmental Sciences, Division of Chemistry and Physics, PO Yatabe, Tsukuba, Ibaraki 305, Japan, has characterized a pepperbush and pond sediment materials as CRMs for trace elements.

CRMs issued by the National Bureau of Standards (NBS) are known as Standard Reference Materials (SRMs). A new edition of the SRM catalog (1981–83) is available on request from the General Referee.

Recent developments in SRMs applicable to AOAC activities are listed below.

Biological, Botanical, Foods

(*1*) Citrus Leaves, SRM 1572, has been analyzed and issued. The Certificate of Analysis provides certified concentrations and uncertainties for 5 major and minor elements and for 16 trace elements. Included are the following trace elements of toxicological and nutritional significance: arsenic, cadmium, copper, lead, mercury, molybdenum, and zinc. The certificate is being revised to include a certified concentration for iodine, an essential trace element. The certified value is based on the concordant results by a recently developed isotope dilution, mass spectrometric method (IDMS) and by a second, independent analytical method. Because iodine is a mononuclidic element, radioactive iodine-129 was used to dilute the naturally occurring iodine in the SRM. This is the first time that a certified concentration value is being provided for iodine in a biological SRM.

(*2*) Bovine Liver, SRM 1577a, has been issued as a renewal for SRM 1577. The Certificate of Analysis for the new SRM lists certified concentrations for 22 elements. A number of these, namely, chlorine, phosphorus, sulfur, cobalt, molybdenum, silver, strontium, and uranium, were not certified in the original bovine liver.

A nonfat, dry milk powder is being analyzed for homogeneity and possible issue as an SRM.

Clinical

(*1*) Human Serum, SRM 909, was analyzed for urea by a recently developed IDMS method and for glucose by the IDMS method used previously for its certification. The stability of the lyophilized material has been monitored since it was issued. In particular, glucose has received special attention because its concentration in serum is known to change with time. The most recent determination of glucose at NBS showed a change in concentration. The change is within the range anticipated and, although small, is significant enough to warrant revision of the certificate dated January 13, 1981. The new re-

vised values for glucose are 7.73 ± 0.16 in Table 1 and 6.56 + 0.51/− 0.26 in Table 2. The revised certificate will also include a certified value for urea.

(2) Anticonvulsant Drug Level Assay Standard, SRM 1599, has been analyzed and certified for concentrations of the 2 anticonvulsant drugs, valproic acid and carbamazepine, in a freeze-dried human serum base. The certified concentrations of the drugs are at 3 different levels—near, above, and below the concentrations usually used to control convulsions caused by epilepsy. A serum blank is also supplied. SRM 1599 was prepared by weighing drugs of known purity into a human serum base. The certified concentrations were determined from independent analyses of the reconstituted products by gas chromatography and liquid chromatography. SRM 1599 was developed for use in calibrating and standardizing quantitative clinical analyses for these drugs in human serum. It is also useful in assuring the quality of laboratory working standards and manufactured control materials.

(3) Angiotensin I, SRM 998, has been analyzed and is being issued as a clinical material of known, high purity. It is intended primarily for use in the calibration and standardization of renin assays and as a reference peptide for amino acid determinations.

The certified angiotensin I purity was assessed by high performance liquid chromatography, and the peptide content was measured by the method of standard additions using phenylalanine as the added standard.

Environmental

(1) Polychlorinated Biphenyls in Oil, SRM 1581, has been issued. It was developed primarily for use in calibrating instrumentation and validating methodology for the determination of PCBs in oil. The Certificate of Analysis lists the certified concentration of Aroclor 1242 and Aroclor 1260 present individually at 100 μg/g in motor oil and transformer oil. SRM 1581 was prepared by accurately weighing and mixing each Aroclor and oil. The calculated concentrations of PCBs in the 4 ampuled solutions were confirmed by analysis. Preparative scale liquid chromatography was used to "fractionate" the oil solutions before gas chromatographic analysis. Interlaboratory testing of SRM 1581, as an unknown, showed widely divergent results. The use of SRM 1581 should assist analysts in identifying inaccurate PCB results and in developing more reliable methods.

(2) Urban Dust/Organics, SRM 1649, has been characterized and issued. The SRM was prepared from atmospheric particulate matter collected in the Washington, DC, area. The Certificate of Analysis provides certified concentrations (at μg/g levels) and estimated uncertainties for 5 polycyclic aromatic hydrocarbons. The compounds fluoranthene, benz(a)anthracene, benzo(a)pyrene, benzo(ghi)perylene, and ideno[1,2,3-cd]pyrene are classified as U.S. Environmental Protection Agency priority pollutants. Certification of the concentrations is based on agreement of results by independent methods based on gas chromatography and high performance liquid chromatography. The methods used are outlined and referenced in the certificate. In addition, the certificate lists, for information only, concentrations of 26 elements, 4 leachable anions, and 9 additional organic compounds of environmental interest.

(3) Urban Particulate Matter, SRM 1648, and River Sediment, SRM 1645, have been characterized further. Both SRMs are certified for chemical composition including trace elements of environmental importance. Revisions of the certificates in May 1982 reflect the additional characterization.

The Associate Referee on Stability of Organophosphorus Pesticide Standards, G. M. Doose, Food and Drug Administration, Los Angeles, CA, has studied the stability of acetone solutions of 10 pesticides: DMOA (Omethoate), Dimethoate, Naled, Acephate (Orthene), Azedrin (Monocrotophos), and Dursban (Chlorpyrifos). The solutions, prepared at mg/mL and μg/mL concentrations, were stored at room temperature and also refrigerated. Only the μg/mL working solutions of DDVP (Vapone) and Naled showed possible degradation after the 10-month storage at room temperature.

Recommendations

(1) Continue to investigate and report sources of available reference materials, especially certified reference materials, applicable for use in the development, testing, and validation of AOAC methods.

(2) Urge Associate Referees of all Committees to inform General Referees of their plans to conduct collaborative studies. In these studies, the use of test samples representative of larger lots of homogeneous material may enable the materials to be issued as CRMs, depending on the stability of analytes and matrix.

(3) Encourage the Associate Referee to complete his study by evaluating the purity of aged pesticide materials compared with recently produced materials from the manufacturers.

GENERAL REFEREE REPORTS: COMMITTEE B

Report on Drugs, Acidic and Neutral Nitrogenous Organics

JAMES W. FITZGERALD
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Acetaminophen in Drug Mixtures.—David J. Krieger presented his work on the development of an HPLC procedure for the identification and determination of acetaminophen in analgesic preparations at last year's Annual Meeting. During the past year, he submitted a paper for publication in *J. Assoc. Off. Anal. Chem.* describing his methodology, and plans to conduct a collaborative study on the method next year.

Amitriptyline HCl in Dosage Forms (HPLC).—Samuel Walker was appointed Associate Referee for the topic in August 1982. During the past year, he conducted a collaborative study on a slight modification of the procedure originally described by Butterfield and Sears (*J. Pharm. Sci.* (1977) **66,** 1117–1119). Results from 10 collaborators confirm the suitability of the procedure for the determination of amitriptyline in tablets and injections. Results of the collaborative study were presented at the Annual International Meeting.

Methyldopa.—Susan Ting has completed development of an HPLC procedure for determining methyldopa and methyldopa–thiazide combinations in dosage forms. A collaborative study on the methodology in under way. She gave a poster presentation on the methodology at this year's Annual International Meeting, and expects to complete her evaluation of the collaborative study by the end of 1982.

Primidone.—Stanley E. Roberts submitted his methodology to AOAC for publication. The paper has been published (*J. Assoc. Off. Anal. Chem.* (1982) **65,** 1063–1065), and the analytical work has been completed on the collaborative study. A statistical review of the data is under way and the Associate Referee expects to complete his evaluation of the collaborative study late in 1982.

Sulfamethoxazole in Tablets (HPLC).—John W. Robinson was appointed Associate Referee for the topic in August 1982. He presented his methodology and the results of a collaborative study at the Annual International Meeting. The method is reported to give good recoveries and has a coefficient of variation of less than 2%.

Sulfisoxazole in Dosage Forms (HPLC).—Robert W. Roos was appointed Associate Referee for the topic in August 1982. During the past year, he conducted a collaborative study on a minor variation of methodology previously described (*J. Assoc. Off. Anal. Chem.* (1981) **64,** 851–854). In the current methodology described at the Annual International Meeting, sulfadimethoxine was substituted for sulfabenzamide as the internal standard, and mobile phase rather than methanol was used for final dilution of the sample. Results from 7 collaborators on each of 4 commercial and 2 synthetic formulations were very good. Mean recoveries of sulfisoxazole from synthetic tablet powder and ophthalmic solution were 99.9 and 100.0%, respectively, and the mean coefficient of variation for all samples analyzed was less than 2%. Although nc synthetic ointments were included in the current study, previous work coupled with the current study indicates that the revised methodology is suitable for the analysis of sulfisoxazole in tablets, solutions, and ointments.

Other Associate Referees report little or no progress because of other commitments.

Recommendations

(1) Adopt as official first action the HPLC method described by the Associate Referee for the determination of amitriptyline hydrochloride in dosage forms.

(2) Adopt as official first action the HPLC method described by the Associate Referee for the determination of sulfisoxazole in dosage forms.

(3) Continue study on all other topics.

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

Recommendation 3 of the General Referee was approved by Committee B and was accepted by the Association. Committee B did not approve recommendations 1 and 2. See the report of the Committee, this issue.

Report on Drugs, Alkaloids

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Atropine in Morphine and Atropine Tablets and Injections.—Associate Referee Ira J. Holcomb reported that he is continuing his investigation of chromatographic procedures for the separation and determination of the atropine and morphine content of these pharmaceuticals. The results of a proposed gas chromatographic procedure show good resolution and adequate sensitivity for the simultaneous determination of the morphine and atropine content in its application to commercial samples.

Belladonna Alkaloids.—An Associate Referee is being sought. Methodology is needed for determining hyoscyamine and atropine individually when they are present together in the pharmaceutical. The work would also involve the determination of the other belladonna alkaloids that might be present, as well as ensuring the suitability of the method to detect decomposition products.

Colchicine in Tablets.—Associate Referee Richard D. Thompson reported the results of the application of his proposed liquid chromatographic method for the determination of colchicine. The HPLC system resolves colchicine from 13 associated compounds, including the related alkaloids and phototransformation products of colchicine. His study includes the comparison of an internal and external standard procedure. It was applied to samples of the bulk drug and dosage forms that contain colchicine alone and in combination with probenecid. A collaborative study of the proposed method is under way. One decomposition product, colchiceine, does not chromatograph under the proposed HPLC conditions. Either a TLC limits test or an alternative chromatographic procedure will have to be developed for that determination.

Curare Alkaloids.—Associate Referee John R. Hohmann reported that the intralaboratory validation of the revised HPLC procedure has been completed. The method was also applied to currently marketed samples of tubocurarine chloride injection. Results were in agreement with the current USP bioassay. He is seeking collaborators for studying the proposed HPLC procedure.

Ephedrine.—Associate Referee Charles C. Clark reported no further work on this topic. The Associate Referee recommends that the topic be discontinued.

Ergot Alkaloids.—Associate Referee Thomas C. Knott reported on the examination of the samples used in last year's collaborative study. Initial results indicate that low assays are obtained with samples placed in certain sample containers. The samples also show the presence of decomposition products. Aliquots of the same sample from the original sample container show good recovery and stability. Once the source of the low assays is confirmed and prevented a collaborative study is planned.

Neostigmine.—Associate Referee Rita E. Kling reported that no further work was done on this project.

Physostigmine and Its Salts.—Associate Referee Norlin W. Tymes reported the results of the collaborative study of his reverse phase HPLC procedure (1) for the analysis of physostigmine salicylate injection and solution and physostigmine sulfate ointment. Initially, the method was adopted official first action for the solution form only. Re-examination of the reports and data for the injection and ointment dosage forms disclosed the sources of errors that gave the divergence from the true values. After eliminating the erroneous results, the coefficient of variation was sufficiently reduced to warrant official first action status for these dosage forms also. The proposed HPLC method is a welcome improvement over the current official methods. Good resolution is obtained between physostigmine and any breakdown products, preservatives, and the internal standard fluazepam hydrochloride.

Pilocarpine.—Associate Referee I. W. Wainer reported no further progress on the proposed method for the simultaneous determination of pilocarpine, isopilocarpine, pilocarpic acid, and isopilocarpic acid, utilizing an alkylphenyl column and a UV detector. Following completion

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

The recommendations of the General Referee were approved by Committee B and were adopted by the Association, except for recommendation 2 which was adopted for the ointment form only. See the report of the Committee and "Changes in Methods," this issue.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition, and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **65**, 450–521 (1982).

of the intralaboratory study of the procedure reported last year (2), initiation of a collaborative study is recommended.

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

Rauwolfia serpentina.—Associate Referee Ugo Cieri reported the results of his development of a single tablet assay for determining the reserpine–rescinnamine content of *Rauwolfia serpentina* tablets. The large differences between the results obtained with the HPLC with fluorometric detector procedure and those obtained with the official fluorometric procedure warrant additional investigation on the source of the differences before any interlaboratory study is initiated.

Recommendations

(1) Declare as surplus the following official final action methods: **38.029–38.030,** infrared method for atropine in tablets, and **38.038,** Method II for ephedrine in inhalants.

(2) Adopt the official first action HPLC method for determining physostigmine and its salts in solutions, **38.C01–38.C06,** as official first action for the ointment and injection dosage forms.

(3) Discontinue the topic Ephedrine.

(4) Continue study on all topics as indicated.

REFERENCES

(1) Tymes, N. W. (1982) *J. Assoc. Off. Anal. Chem.* **65,** 132–137

(2) Smith, E. (1982) *J. Assoc. Off. Anal. Chem.* **65,** 300–301

Report on Drugs, Illicit

CHARLES C. CLARK

Drug Enforcement Administration, Southeast Regional Laboratory, Miami, FL 33166

The Associate Referee for benzodiazepines, Eileen Bargo, has completed a collaborative study on HPLC determination of oxazepam in dosage forms. The procedure and results appear satisfactory. The Associate Referee recommends adoption of the method as official first action.

The Associate Referee for cocaine, Charles C. Clark, recommends that the official first action method, **40.002–40.005,** for the determination of cocaine be adopted official final action. The method has been in official first action status for 5 years. It has been used successfully on many occasions and no adverse reports have been received concerning the method.

The Associate Referee for dimethyltryptamine, diethyltryptamine, and dipropyltryptamine, Jack Fasanello, plans no further method development in this area. The topic should be declared open.

The Associate Referee for heroin, Harold F. Hanel, states that a collaborative study will be initiated in the near future.

The Associate Referee for marihuana and synthetic tetrahydrocannabinol, Ivette Vallejo, and the Associate Referee for methadone, Eugene McConigle, express no interest in doing further work in their areas. The topics should be declared open.

The Associate Referee for methylphenidate, Stanley Schrieber, has retired and the topic should be declared open.

The Associate Referee for phencyclidine, Charles C. Clark, recommends that the gas-liquid chromatographic method for determining phencyclidine in powders, **40.016–40.018,** be adopted official final action. The method has been in official first action status for 4 years. It has been used successfully on numerous occasions and no adverse comments on the method have been received.

No reports were received on methaqualone

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Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition.

and optical crystallographic properties of drugs.

Because heroin tablets are no longer seen and are not likely to be seen in the near future, the General Referee recommends that the present official final action method for determining diacetylmorphine (heroin) in tablets, **40.006**, be repealed official first action.

Recommendations

(1) Adopt as official final action, the official first action method for the determination of cocaine, **40.002-40.005**.

(2) Adopt as official final action, the official first action method for determining phencyclidine in powders, **40.016-40.018**.

(3) Repeal official first action the official final action method for determining diacetylmorphine (heroin) in tablets, **40.006**.

(4) Adopt as official first action the HPLC procedure for the determination of oxazepam in dosage forms as described by the Associate Referee.

(5) Declare as open topics the refereeships on dimethyltryptamine, diethyltryptamine, and dipropyltryptamine; marihuana and synthetic tetrahydrocannabinol; methadone; and methylphenidate.

(6) Continue study on all other topics.

Report on Drugs, Miscellaneous

TED M. HOPES
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Benzoyl Peroxide.—The Associate Referee is planning no further work and recommends that the topic be discontinued.

Disulfiram.—A colorimetric method was adopted official first action at the 1980 meeting. The method (**36.B01-36.B04**) has been used a number of times without difficulty and the Associate Referee recommends that it be adopted official final action.

Ethylene Oxide.—The Associate Referee is conducting a stability study on samples prepared for collaborative study. If stability is found to be satisfactory, the method will be collaboratively studied this year.

Fluoride.—A collaborative study is planned for later this calendar year. Instructions to collaborators and collaborative samples are prepared.

Identification of Drugs (Mass Spectroscopy).—No reports have been received for over 4 years.

Medicinal Gases.—A progress report at this meeting describes a gas chromatographic method for assaying mixtures of carbon dioxide and oxygen. An in-house collaborative study was successful. A GC method for mixtures of nitrous oxide and oxygen has also been developed and will be tested in-house later this year. A collaborative study of both methods is planned.

Menadiol Sodium Diphosphate Injection.—A collaborative study is planned for late 1982.

Mercurial Diuretics.—The planned collaborative study was not conducted because the drug studied, mercaptomerin sodium, is no longer being manufactured. The Associate Referee will investigate other mercurial diuretics.

Mercury-Containing Drugs.—The Associate Referee has completed a collaborative study of his atomic absorption method for determining total mercury in mercury-containing drugs, and the results of that study are being presented at this meeting. The study was successful and it is recommended that this general method for the analysis of mercury-containing drugs be adopted official first action.

Metals In Bulk Drug Powders.—The Associate Referee has developed rapid tests for 8 metals in drug substances to replace the hydrogen sulfide test that is currently most often used. Separate limits tests have been developed for lead, arsenic, and selenium. The manuscript describing the procedures is in preparation, and a collaborative study will be conducted in 1983.

Microcrystalline Tests.—This refereeship has been vacant for several years. An Associate

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

Section numbers refer to "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **64**, 501-540 (1981); **65**, 450-521 (1982).

Referee is needed to review and improve these methods.

Protein Nitrogen Units in Allergenic Extracts.—An interim first action method was adopted as official first action at last year's meeting. The Associate Referee states that the method is being used routinely by manufacturers and she has received no comments. It is recommended that the method (**39.C01-39.C03**) be retained in official first action status for an additional year.

Thyroid by Differential Pulse Polarography—This project has been completed and the method was adopted official first action at last year's meeting. It is recommended that the method be retained in official first action status at least one additional year.

Recommendations

(1) Adopt as official final action the official first action colorimetric method, **36.B01-36.B04**, for determining disulfiram in tablets.

(2) Adopt as official first action the atomic absorption method for mercury-containing drugs as described by the Associate Referee.

(3) Discontinue the topics Benzoyl Peroxide and Identification of Drugs (Mass Spectroscopy).

(4) Continue study on all other topics.

Report on Drugs, Other Nitrogenous Bases

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Aminacrine.—The Associate Referee has had 2 papers published on the analysis of aminacrine preparations (*J. Assoc. Off. Anal. Chem.* (1983) **66**, 140-144 and 145-150). A promising method involving assay by visible spectrophotometry and identification by thin layer chromatography is ready for collaborative study.

Antihistamines, Adrenergic Combinations by HPLC.—This method, **38.B01-38.B06**, has been in official first action status for 2 years and appears to be quite satisfactory. Adoption as official final action is recommended.

Epinephrine-Lidocaine Combinations.—A paper has been submitted for publication on the analysis of these formulations by HPLC using an electrochemical detector. The Associate Referee hopes to conduct a collaborative study soon.

Phenothiazine Drugs, Identification by TLC.—A collaborative study has been conducted and is now being evaluated. A report will be prepared in the near future.

Phenothiazines in Drugs.—A recent intralaboratory study indicates that a revision of the procedure presented at last year's meeting is ready for an interlaboratory collaborative study. The Associate Referee expects to send the samples out soon, using 4 or 5 of the more widely used phenothiazines.

Recommendations

(1) *Antihistamines, Adrenergic Combinations by HPLC:* Adopt as official final action the official first action HPLC method, **38.B01-38.B06**, for the determination of antihistamine-adrenergic combinations in syrups or tablets.

(2) Continue study on all other topics.

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

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

Section numbers refer to "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **64**, 501-540 (1981).

GENERAL REFEREE REPORTS: COMMITTEE C

Report on Coffee and Tea

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The Associate Referee on Ash in Instant Tea, F. J. Farrell, has continued his work on a combined study of ash and moisture in instant tea. He has worked out a procedure which will overcome the difficulties previously encountered, and is now ready to start a collaborative study.

The Associate Referee on Moisture in Coffee and Tea, W. P. Clinton, reports that he has received no complaints or negative criticism on the method for mass loss determination on drying for instant coffee, which was adopted official first action (**15.012-15.013**). He recommends the method be adopted official final action.

The Associate Referee on Chlorogenic Acid in Coffee, J. A. Yeransian, has been unable to find enough collaborators for his automated method. In view of this difficulty and an apparent lack of interest in chlorogenic acid, he recommends that the subject be dropped.

The Associate Referee on Solvent Residues in Decaffeinated Coffee, B. D. Page, has developed a method which appears to be applicable to both decaffeinated coffees and teas. He hopes to subject it to collaborative study during the coming year.

The Associate Referee on Water Extract in Tea, E. de la Teja, reported the results of his collaborative study.

The Associate Referee on Caffeine in Coffee and Tea, J. M. Newton, did not work on this subject this year. However, he plans to study a method for theophylline in tea and should be appointed Associate Referee.

Recommendations

(1) Discontinue the topic Chlorogenic Acid in Coffee.

(2) Appoint an Associate Referee for Crude Fiber in Tea.

(3) Adopt as official final action the official first action method for moisture in instant coffee (**15.012-15.013**).

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

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

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition.

Report on Dairy Products

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The Referee has not received any reports from Associate Referees recommending official action. Some collaborative studies are in progress and there may be recommendations for interim approval, particularly from the studies on casein and whey protein in processed dairy products; lactose in milk, and total phosphorus content of cheese.

Progress continues to be made in Joint IDF/ISO/AOAC groups working on methods of analysis for milk and milk products. Work has been completed on the revision of the method for determination of chloride content of cheese

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(*J. Assoc. Off. Anal. Chem.* **65**, 1350–1356 (1982)). It is recommended that this potentiometric method be adopted official first action as a reference method to replace the present official final action method, **16.242–16.243**. It is also recommended that the present official final action method, **16.242–16.243**, be repealed official first action, and that method **16.224**, 12th edition (1975), be reinstated official first action as an alternative method.

Recommendations

(1) Adopt the interim first action method for determination of chloride content of cheese as official first action to replace official final action method **16.242–16.243**.

(2) Repeal official first action the present official final action method **16.242–16.243**; reinstate the official final action method **16.224**, 12th edition (1975), as an alternative method.

(3) Continue study on all other topics.

Report on Decomposition and Filth in Foods (Chemical Methods)

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Ammonia in Dogfish.—Associate Referee Beverly Smith reported that preparations for a collaborative study of an enzymatic procedure for determination of ammonia in dogfish are underway. As reported last year, the procedure compared favorably with the AOAC colorimetric method, **18.027–18.030**, and is more convenient for application in the field. Additional collaborators are needed. The Referee concurs that the enzymatic procedure be submitted to collaborative study.

Coprostanol.—Associate Referee James Stewart reported that additional work is required on the GLC procedure for coprostanol to apply it in practical situations. The search for a chemical indicator of fecal coliform contamination in oyster-producing waters and in foods remains a difficult analytical area. The Referee concurs that the study be continued.

Crabmeat.—Associate Referee Kurt Steinbrecher reported that work on the colorimetric determination of ammonia in crabmeat, **18.027–18.030**, is complete with regard to method development. The method, originally developed for the analysis of crabmeat, has also found applications to the determination of decomposition in shrimp, dogfish, and halibut. The Referee concurs that the method be adopted official final action.

Ethanol in Seafoods.—Associate Referee Harold R. Throm reported that 4 species of canned salmon—sockeye, pink, coho, and chum—were analyzed for ethanol content. A good correlation was found between ethanol content and organoleptic classification of decomposition for the 52 samples analyzed. The ranges found for each organoleptic class were—Class 1 (acceptable quality), 0–24 ppm ethanol; Class 2 (decomposed), 25–74 ppm; and Class 3 (advanced decomposed), 75 ppm and up. These values were obtained by chromatographing aliquots of the aqueous canning liquid on Porapak QS.

An improved analytical procedure for the determination of ethanol by headspace analysis is under development by T. Hollingworth of FDA and will be submitted to collaborative study. Collaborators are being sought. The Referee concurs that the study be continued.

Gas and Liquid Chromatography.—A collaborative study on the gas chromatographic method (*J. Assoc. Off. Anal. Chem.* **64**, 584–591 (1981)) for cadaverine and putrescine in fishery products has been completed. In the study, samples of canned albacore skipjack and yellowfin tuna and frozen shrimp and dolphin (mahimahi) were analyzed in duplicate. For acceptable quality canned tuna, the collaborators reported an average of 1.4 μg cadaverine/g sample (range 0.7–2.0) and an average of 0.5 μg putrescine/g (range 0.1–0.8). Increases in amines were reported for all decomposed samples with good precision. Recoveries of cadaverine added at a level of 10 μg/g to extracts of tuna averaged 105%

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with a range of 101–108%, and at a level of 20 μg/g averaged 102% with a range of 96–109%. Recoveries of putrescine added at a level of 5 μg/g averaged 102% with a range of 94–106%, and at a level of 10 μg/g averaged 101% with a range of 95–110%.

The GLC method has been applied to approximately 300 cans of tuna with the following results: For acceptable quality tuna, cadaverine averaged 0.6 μg/g (range 0.1–3.4) and putrescine averaged 0.5 μg/g (range 0.1–1.1). For borderline decomposed tuna, cadaverine averaged 20.4 μg/g (range 1.7–145.7), and putrescine averaged 2.0 μg/g (range 0.6–7.2). For advanced decomposed tuna, cadaverine averaged 55.5 μg/g (range 1.9–279.8) and putrescine averaged 3.6 μg/g (range 0.4–35.0). Cadaverine was the best indicator of decomposition with significant increases detected in more than 80% of the cans prepared from shipments of decomposed tuna. The Referee recommends that the GLC method for the determination of cadaverine and putrescine in fishery products be prepared for adoption as interim official first action and that study be continued.

Shellfish.—Associate Referee Theodore Chambers reported that the HPLC method for indole, **18.B01–18.B05,** has been used successfully by a number of laboratories during the past year. The applications included the following HPLC columns: Lichrosorb 10 RP8, 25 cm × 4 mm id; Micro Bondapak C18, 30 cm × 4 mm id; Spherisorb ODS 5 μm, 24 cm × 4 mm id; Zorbax ODS, 24 cm × 4.5 mm id; and Partisil 10 ODS, 25 cm × 4 mm id.

A study of the effects of cooking and chlorine treatment on indole, ammonia, acetic acid, and putrescine in shrimp was conducted. No increases in low levels of the compounds in shrimp were noted upon processing. In decomposed shrimp, cooking reduced the levels of ammonia and acetic acid while chlorination lowered the amounts of indole, ammonia, and acetic acid. Putrescine levels were not lowered by either cooking or chlorination. The Referee concurs that the study be continued.

GLC Determination of Volatile Amines—DMA and TMA.—Associate Referee Ronald C. Lundstrom reported on a method for the GLC determination of dimethylamine (DMA) and trimethylamine (TMA) in seafoods. These volatile amines are common degradation products of TMA-oxide in marine fish and have potential as indicators of fish quality. It is postulated that DMA is formed by the action of an endogenous enzyme in gadoid fish with the formation of an equimolar quantity of formaldehyde. TMA is produced in raw, unfrozen seafoods by a bacterial enzyme. In the analytical procedure, a perchloric acid extract of fish is neutralized with potassium hydroxide and extracted with benzene. The amines are then separated by gas chromatography on a porous polymer (Chromosorb 103) column and detected by a nitrogen-sensitive flame detector. The procedure has been applied to red hake, cod, haddock, cush, whiting, pollock, white hake, American plaice, blackback flounder, and squid. The Referee concurs that the procedure be submitted to collaborative study.

Although there were no progress reports on the topics Diacetyl in Citrus Products, TLC of Amines in Fishery Products, and Tomatoes, the Referee recommends that they be continued.

Recommendations

(1) Adopt as official final action the official first action method for the determination of ammonia in crabmeat, **18.027–18.030.**

(2) Submit the GLC method for the determination of cadaverine and putrescine in fishery products for adoption.

(3) Continue study on all other topics.

Report on Eggs and Egg Products

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Fat.—Associate Referee Lawrence E. Taber reports that he is continuing work on the monomolecular film method for yolk in egg white. An additional factor (an egg fat standard) was introduced in the method to eliminate problems caused by variations in reagents and oil which made it difficult to get good correlations between laboratories. He hopes that this additional feature will make it a standardized method instead of the empirical method used formerly and plans a collaborative study of the revised method. He also expects to report on a surfactant method for determining fat in whole egg and egg yolk products which will at least partially replace the acid hydrolysis method.

No reports were received from Associate Referees on any other topics.

An Associate Referee is needed for the topic Total Solids to replace former Associate Referee Herbert C. Sorensen. Interested persons should contact AOAC.

I am resigning as General Referee for this subject and thank all those Associate Referees who have worked with me over the past 15 years.

Recommendation

Appoint an Associate Referee on Total Solids, and continue study on all topics.

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

Committee C recommended that this General Refereeship be discontinued and that the Associate Referee topics be reassigned to other refereeships where practicable. See the report of the Committee, this issue.

Report on Fish and Other Marine Products

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Crabmeat Identification.—Associate Referee Judith Krzynowek continued her studies on this topic. Modifications were made on the official first action method, **18.B06-18.B11,** for generic identification of cooked and frozen crabmeat by substituting agarose for polyacrylamide as the gel medium. The advantages of agarose gel include use of nontoxic reagents, ease of gel preparation, and much more rapid focusing time. By excluding urea from agarose gel preparation, focusing could be completed in 0.5 h, using 30 watts constant power. This is a significant time saver compared with the overnight focusing at one watt constant power required in the polyacrylamide gel method. Although incorporating urea into the gel, as specified in the polyacrylamide method keeps the denatured proteins in solution in the gel, and some precipitation of proteins near the cathode end was observed in the agarose method, species of cooked crabmeat can be identified.

Determination of Fish Content in Coated Products (Breaded or in Batter).—H. Houwing and Frederick King were appointed Co-Associate Referees for this new topic, because of a need to develop uniform procedures to determine fish content of coated fish products that will be acceptable to the international community.

Drained Weight of Block Frozen Raw, Peeled Shrimp.—For the past 2 years, Associate Referee F. King has not been able to conduct drained weight measurements because needed samples were not received from the American Shrimp Canners and Producers Association. The Asso-

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ciation was unable to obtain funds to purchase and transport samples needed for the study.

Drip Fluid in Fish Fillets and Fish Fillet Blocks, Quantitation.—Associate Referee F. King is continuing to develop a procedure to measure "cook drip" (fluid obtained from cooked samples) by using a modified tuna press and by using moisture content (**24.003(a)**). However, his progress continues to be slow because of personnel and budget limitations.

Fish Species Identification, Thin Layer Isoelectric Focusing.—Associate Referee Ronald Lundstrom applied both low and high resolution conditions using isoelectric focusing (IEF) to determine whether various species of Pacific rockfish could be differentiated. He found that IEF was not able to differentiate several species, giving identical protein patterns. Although these observations are based on a limited study, the Associate Referee cautions users of IEF methods when identifying the particular species of rockfish.

The Associate Referee has also made some progress in evaluating micro-IEF gels. Some resolution is lost compared with that obtained on full-size gels, but reproducibility is improved. Cost of the smaller gels is much less, and separation time is reported to be 10–15 min. He has also made some preliminary comparisons of the reproducibility of different carrier ampholyte preparations available from several sources.

Nitrites in Smoked Fish.—Associate Referee Charles Cardile did not conduct the collaborative study planned for this year. Results from an intralaboratory study using the proposed specific ion electrode method compared favorably with those obtained using the official method.

Organometallics in Fish.—Associate Referee Walter Holak has developed a method for the determination of methyl mercury (or other organo-mercury compounds), using a simplified sample preparation procedure and atomic absorption or electrochemical detection of the HPLC eluates. A representative sample is blended with water to form a fine suspension, and methyl mercury is extracted by chloroform elution from a diatomaceous earth-hydrochloric acid column. The organo-mercury compound is then re-extracted into a small volume of 0.01N sodium thiosulfate solution. An aliquot is injected onto a reverse phase column (Zorbax ODS) and eluted with 60% methanol–ammonium acetate buffer, pH 5.5, containing mercaptoethanol. The organo-mercury compound in the eluate is converted to mercury vapor by passing it over a heated copper tube and then determined by AAS or by using an electrochemical detector equipped with a dropping mercury electrode. A collaborative study is planned for next year.

Other Topics.—The usefulness of AOAC procedure **18.003** for cooking seafood products is limited because only 4 techniques are described. It is proposed that this procedure be revised by omitting all detailed techniques of heating a product, and focusing on the requirement that a product be heated to an internal temperature of at least 160°F (70°C).

There is some confusion regarding the formula to calculate mg sodium or potassium/100 g sample. The formula given in **18.038** is correct. The formula in the 12th edition, **18.038,** as well as in the 11th edition, **18.021,** was in error and the correction was made in the 13th edition.

Recommendations

(1) Adopt as official first action the interim first action revision to **18.003,** Procedure for Cooking Seafocd Products, as recommended by the Associate Referee.

(2) Continue study on all topics.

Report on Food Additives

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Anticaking Agents.—The General Referee was unable to obtain an Associate Referee for this topic during the past year.

Antioxidants.—On December 28, 1981, B. Denis Page was appointed Associate Referee to evaluate analytical methods for antioxidants in foods. Dr. Page's method for determining 7 antioxidants in fats and oils has been collaboratively studied and adopted interim first action by Committee C. The General Referee recommends that the procedure now be adopted official first action. The method has been accepted by the International Union of Pure and Applied Chemistry and is being applied to a variety of food matrices.

Brominated Oils.—The General Referee accepted the recommendation of H. B. S. Conacher and on December 28, 1981, appointed J. F. Lawrence as the Associate Referee for this topic, replacing Dr. Conacher. The Associate Referee reports that the acid methanolysis transesterification procedure for determining brominated vegetable oils (BVOs) was reassessed and with certain precautions has been found to provide reliable results for dibromo-, tetrabromo-, and even hexabromostearate methyl esters. This is much more attractive than the official first action method, which uses sodium methoxide derivatization before analysis. The disadvantages of the latter are that corrosive reagents are required, multiple peaks are obtained for tetrabromostearate, and hexabromostearate cannot be determined. Because the acid-methanolysis technique is working well, we now use it for all BVO analyses. A paper is in preparation for publication which compares the method with the sodium methoxide method for the determination of BVOs in soft drinks.

In another project, high performance liquid chromatography (HPLC) was used to try to characterize BVOs for possible application to beverages and other foods. Although this work has potential, some problems were encountered when the technique was applied to beverage samples. Further work is planned this year.

Two feeding studies have been completed, and tissue and fluid analyses are almost complete. One study involves tissue distribution of BVO administered orally to rats. The second is related to mammary transfer of BVOs to weanling rats. Results (including some gas chromatographic/mass spectrometric (GC/MS) confirmations) should be completed by late 1982, at which time all the data will be assessed and further studies will be planned if necessary.

Dichlorodifluoromethane in Frozen Foods.—Little progress has been made by the Associate Referee during the past year because of higher priority commitments.

Dilaurylthiodipropionate.—The General Referee was unable to obtain an Associate Referee during the past year.

Dressings.—Associate Referee C. R. Warner reports that because of higher priority commitments, he has not been able to continue his studies and pursue a collaborative study of the method.

EDTA in Food Products.—Associate Referee G. A. Perfetti reports that work in the General Referee's laboratory has been directed towards extending the previously developed HPLC method to a variety of food matrices. A collaborative study involving mayonnaise and carbonated beverages was initiated but had to be terminated because of product spoilage and the extreme length of time taken by collaborators to complete the analysis after receipt of the sample. The study has been reinstated with a limited scope. The results have not all been received; they will be reported at the 1983 AOAC meeting.

Ethoxyquin in Foods.—A method has been developed for the determination of ethoxyquin in milk. Milk solids are precipitated by adding acetonitrile, and the water-acetonitrile supernate is washed with hexane to remove fats. Addition of sodium chloride causes the water-acetonitrile solution to separate into an aqueous phase and an acetonitrile phase, thus separating ethoxyquin from most water-soluble impurities. A large volume of water is then added to the acetonitrile layer and ethoxyquin is partitioned into hexane, which is removed at reduced pressure. The residue is dissolved in the mobile phase and analyzed by reverse phase HPLC with fluorescence detection (excitation wavelength 230 nm; 418 nm

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cutoff filter). The mobile phase is water–acetonitrile with a diethylamine–acetic acid buffer. Recoveries from samples fortified at 1, 5, and 10 ppb averaged 78% with a coefficient of variation (CV) of 5.0%. When the method was applied to commercial milk samples, extremely low levels (<1 ppb) of apparent ethoxyquin were found.

Gums.—The General Referee has been unsuccessful in finding an Associate Referee for this topic during the past year.

Indirect Additives from Food Packaging.—Associate Referee C. V. Breder reports the following progress:

Vinyl Chloride.—Work has continued in ASTM Committee D-20 on Plastics on the development of a rapid quality control method to determine residual vinyl chloride in polyvinyl chloride at the ca 5 ppb level. A second draft of such a method has been written and a round-robin evaluation has been held. The results showed the method to be satisfactory for its intended purposes. After minor editorial revisions of the method, it will be sent to the ASTM subcommittee for balloting. Following successful balloting, it will be published as an ASTM method.

Acrylonitrile.—A paper has been published by Timothy McNeal and Charles V. Breder (*J. Assoc. Off. Anal. Chem.* (1982) **65,** 184–187) entitled "Manual Headspace Gas-Solid Chromatographic Determination of Sub-Parts per Billion Levels of Acrylonitrile in 3% Acetic Acid."

Terephthalic Acid (TA).—Work continues on the development of a method to determine residual TA in polyethylene terephthalate (PET) beverage bottles. A method was developed in which PET was exhaustively extracted (ca 5–10 days) with refluxing tetrahydrofuran. The extract was concentrated, the acid was derivatized with *n*-propanol/BF_3, and the resulting ester was partitioned into isooctane and analyzed by gas-liquid chromatography (GLC). Excellent conversions and recoveries resulted. However, some question remains as to whether TA is being generated during the prolonged extraction. As a result, work is continuing.

Styrene.—A manuscript has been submitted to *J. Assoc. Off. Anal. Chem.* by Sandra L. Varner, Charles V. Breder, and Thomas Fazio on "Determination of Styrene Migration from Food-Contact Polymers into Margarine, Using Azeotropic Distillation and Headspace Gas Chromatography." A survey of 7 locally purchased margarines by the above method showed no styrene at a determination level of 25 ppb.

Toluenediamines.—A paper by Roger C. Snyder and Charles V. Breder on "High Performance Liquid Chromatographic Determination of 2,4- and 2,6-Toluenediamine in Aqueous Extracts" appeared in *J. Chromatogr.* (1982) **236,** 429–440. A report by Roger C. Snyder, William C. Brumley, Charles V. Breder, and Thomas Fazio on "Gas Chromatographic and Gas Chromatographic/Mass Spectrometric Confirmation of 2,4- and 2,6-Toluenediamine Determined by Liquid Chromatography in Aqueous Extracts" has been published in *J. Assoc. Off. Anal. Chem.* (1982) **65,** 1388–1394.

Volatiles.—ASTM Committee F-2 on Flexible Barrier Materials conducted a round-robin evaluation of ASTM Method F-151 for volatile solvents in laminated films. A final report of the results has not yet issued.

A paper by Henry C. Hollifield, Roger C. Snyder, and Charles V. Breder entitled "A Multiresidue Approach to the Identification of Food Packaging-Derived Volatiles in Foods and Containers" issued in *J. Chromatogr. Sci.* (1981) **19,** 514–517. A second paper by Richard C. Entz and Henry C. Hollifield on "Headspace Gas Chromatographic Analysis of Foods for Volatile Halocarbons" issued in *J. Agric. Food Chem.* (1982) **30,** 84–88.

Henry C. Hollifield gave two lectures to FDA Field Supervisors concerning "Headspace Analysis—Concepts and Quality Assurance Aspects."

Aliphatic Diamines.—A method, submitted by Toyo Seikan, to determine aliphatic diamines in aqueous retort pouch extracts has been validated in Food and Drug Administration (FDA) laboratories. The method was applied to the analysis of retort pouches from 2 different manufacturers. Approximately 1–4 ppb of the diamine used to prepare the pouch laminating adhesive was found in the extracts after the pouches were retorted for 2 h at 250°F.

Benzene.—A project is under way to validate a published method used to determine benzene in polystyrene polymers. After validation, the method will be used to analyze several styrene-containing polymer food-contact surfaces.

Organophosphorus-containing Antioxidants for Polyolefins.—A method to determine phosphorus-containing antioxidants, submitted by a petitioner, has been validated in FDA laboratories. The procedure involves an acid digestion of the polyolefin extracts followed by a color-forming reaction with the resulting inorganic phosphorus. Recoveries and results of actual extractions appear to be satisfactory.

New Migration Cell.—Extensive work is under

way to evaluate and apply a new migration cell used by both the National Bureau of Standards (NBS) and A. D. Little in their contracted work for FDA. The cell is comprised of a 24 mL vial topped with a Mininert cap. Polymer disks to be tested are threaded onto a wire support in the vial and solvent is added to the vial. The sealed vial is shaken in a thermostated water bath and aliquots of the solvent are periodically removed with a syringe through the Mininert septum and analyzed by appropriate methodology. An evaluation of the cell, using the migration of residual styrene from polystyrene into a number of food simulants and corn oil, is complete. The cell performed very well and will be applied to other polymer, migrant, and solvent systems.

ASTM Committee D-20 on Plastics sponsored a seminar in March 1982 on the "Migration of Additives from Food Contact Polymers." NBS and A. D. Little personnel presented results of their contracted work for FDA. FDA personnel presented papers on migration testing in general and on the regulatory aspects of work by NBS and A. D. Little. The seminar was very well attended and received.

Nitrates and Nitrites.—The General Referee has appointed J. B. Fox as the Associate Referee for this topic.

Nitrates (Selective Ion Electrodes).—Associate Referee S. L. Pfeiffer reports that the results received to date on the repeat collaborative study are good and acceptable for the intended purpose. The General Referee recommends that the method evaluated in the collaborative study be submitted for adoption as interim first action. He encourages use of the method, which is available from the Associate Referee, Sandra Pfeiffer, Gerber Products Co., Fremont, MI 49412 (telephone 616-928-2000).

Nitrosamines.—Associate Referee N. P. Sen reports excellent progress in this research area. A collaborative study has been conducted and new methods have been developed during the past year. The results of these efforts are summarized below.

1. "Determination of *N*-Nitrosodimethylamine in Nonfat Dry Milk: Collaborative Study" by N. P. Sen, S. Seaman, and R. Stapley. Ten laboratories participated in a collaborative study of a method for the determination of *N*-nitrosodimethylamine (NDMA) in nonfat dry milk. The method consisted of elution of NDMA with dichloromethane from a mixture of Celite, acidic sulfamic acid, and nonfat dry milk (all packed in a chromatographic column), concentration of the dichloromethane eluate in a Kuderna-Danish concentrator, and final analysis by a GLC/thermal energy analyzer (TEA) technique. Altogether 10 samples were used—6 were naturally contaminated (NDMA levels 0.38–3.56 ppb) and 4 were spiked with known levels (0.96 and 3.2 ppb) of NDMA. The complete data indicated overall CVs of 20.7 and 27.8% for repeatability and reproducibility, respectively. The pooled percent recovery of the overall method was 94.1 ± 2.0 (standard error). It is recommended that the method be adopted official first action.

2. "Estimation of Volatile *N*-Nitrosamines in Rubber Nipples for Babies' Bottles" by D. C. Havery and T. Fazio. A method has been developed for the estimation of volatile *N*-nitrosamines in baby bottle rubber nipples. In a study of rubber nipples from one manufacturer, NDMA, nitrosodiethylamine, and nitrosopiperidine were determined by GLC using a thermal energy analyzer and confirmed by MS at average levels ranging from 22 to 281 ppb. All 3 nitrosamines were also found to migrate from the nipple into milk or infant formula when sterilized together in a conventional sterilizer. Storing a bottle of milk containing a rubber nipple for 2 h at room temperature or overnight in a refrigerator after sterilization resulted in a 10–13% increase in nitrosamine levels migrating into milk. Upon repetitive sterilizations of a single nipple, the quantities of nitrosamines migrating into milk from rubber nipples were found to decline steadily but, after 7 sterilizations, nitrosamines were still readily detectable in the milk. Nitrosamine levels were found to be higher in rubber nipples after sterilization, indicating the presence of nitrosamine precursors. No nitrosamines were found in raw, uncured rubber. Chemical accelerators and stabilizers added during the vulcanization process are the source of the amine precursors in rubber nipples. This report has been published in *Food Chem. Toxicol.*

Polycyclic Aromatic Hydrocarbons in Foods.—An HPLC method has been developed to isolate basic azaarenes from smoked foods. The method involves alcoholic potassium hydroxide digestion of the sample, liquid-liquid extraction of the digestate to isolate the nonsaponifiable materials (e.g., polycyclic aromatic hydrocarbons, azaarenes, etc.), acid–base extraction to isolate the basic compounds from other materials, chromatography through basic alumina (10% deactivated with water), concentration, and HPLC analysis on a reverse phase C_{18} column. Samples analyzed included smoked salmon and smoked sausage. One unknown basic azaarene was indicated in

the ultraviolet chromatogram of the smoked salmon. Recovery of three azaarenes (5,7-dimethylbenzo(*a*)acridine, dibenzo(*a,j*)acridine (DBajAc), and dibenzo(*a,h*)acridine (DBahAc)) from samples spiked at 5 ppb ranged from 60% for DBajAc to 107% for DBahAc, with CVs ranging from 4.1 to 8.9 for these studies. Further studies are planned in which marine samples contaminated with petroleum products will be analyzed.

Sodium Lauryl Sulfate.—The Associate Referee reports no progress due to other higher priority commitments.

No other reports were received.

Recommendations

(1) Adopt as official first action the method for antioxidants in foods reported by B. D. Page; continue study.

(2) Adopt as official first action the method for *N*-nitrosodimethylamine in nonfat dry milk, collaboratively studied by the Associate Referee as Method I; continue study.

(3) Appoint Associate Referees on Anticaking Agents; Dichlorodifluoromethane in Frozen Foods; Dilaurylthiodipropionate; Gums; and Sodium Laury- Sulfate.

(4) Continue study on all other topics.

Report on Meat and Meat Products

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There has been a substantial increase in productivity this past year on topics related to meat, poultry, and products derived from meat and poultry. The conscientious performance and efforts of the Associate Referees have contributed substantially to collaborative study activities on nitrosopyrrolidine analysis in fried bacon, pentachlorophenol in poultry and swine, chlorinated hydrocarbons in poultry fat by gel permeation chromatography, calcium determination by EDTA titration, histologic procedure for detecting cardiac musculature, soy flour and partially defatted tissues in ground beef, automated method for total protein in meat products, and rapid microwave analysis for moisture. In addition, 2 procedures developed will be subjected to at least a 3-laboratory method validation, and the procedure for determination of crude protein in meat and meat products, **24.B01-24.B03,** is recommended for adoption as official final action.

The interest and range of responsibility noted above is representative of the diversity of the subject area covered by this Refereeship. Therefore, the title should be modified to more accurately reflect the subject area, for example, Meat, Poultry, and Meat and Poultry Products.

Interest continues in areas of nutritional analysis and food safety, suggesting more activities in areas related to sodium content in processed food and mechanically separated (species) products; recent comments by the Commissioner of the Food and Drug Administration on fats and sugars may increase activity in these areas. The Farm Bill of 1981 may result in more emphasis on animal drug residue methods as well as the U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) recently initiated Total Residue Avoidance Program (TRAP). This latter program is supporting animal drug residue projects in 31 states with 37 approved projects to date. The cost of performing residue analysis is resulting in more emphasis on developing multi-drug, multi-species, multi-tissue qualitative assays. Approaches include chemical, microbiological, histological, serological, and bioassay procedures. Others should not be discounted. The expanding development of new analytical instruments either alone or in tandem provides new approaches and capacity to detect analytes.

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The recommendations of the General Referee were approved by Committee C and were adopted by the Association, except under (2), only the method for calcium was adopted. See the report of the Committee and "Changes in Methods", this issue.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition; and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **64,** 501-504 (1981).

The diversity of needs will exert its influence on the Refereeship; and it is expected that several new topics will develop and some existing topics will be refined. In addition, some topics will be dropped through lack of need or interest.

Topics recommended to be dropped include Ashing Methods; Identification of Meat, Serological Tests; Moisture, Automated Karl Fischer Method; Fluoride in Deboned Meat and Poultry; and Proximate Composition Relationships, the first four because of lack of interest and the latter because the relationships normally would not be studied collaboratively. The incumbent Associate Referees concur with this recommendation. The topics Sodium and Potassium in Meat Products and Specific Ion Electrode Applications should be combined into Specific Ion Electrode Methods under the existing Associate Referee. Pending the successful evaluation of the collaboratively studied methods, they will be recommended for AOAC official first action status. The topic Detection and Identification of Cardiac Musculature, Soy Flour, and Partially Defatted Tissue in Ground Beef should be shortened to Histologic Identification Methods.

Ashing Methods.—A vacancy still exists for this topic. The topic should be discontinued because of low interest.

Automated Methods.—Associate Referee Jon Schermerhorn conducted an 8-laboratory collaborative study for total protein in meat products, using the Technicon AA-2 AutoAnalyzer. Additional data were collected on phosphorus from 4 laboratories. A collaborative study with the automated analyzer for phosphorus is planned.

Bone Content.—Paul Corrao, Associate Referee, successfully completed a collaborative study for calcium analysis using an EDTA titrimetric procedure. The method has an analytical range from 0.03 to 0.85% calcium with a minimum proficiency of 0.07%. Repeatability is ±0.016%, reproducibility is ±0.022%, with a recovery of 98–102%. The topic should continue because of interest with mechanically separated (species) products.

Chlorinated Hydrocarbon in Poultry.—Associate Referee James Ault has completed a collaborative study with 14 hydrocarbons in poultry fat, using gel permeation chromatography. Preliminary evaluation appears to be favorable. Coefficients of variation (CV) for reproducibility for all compounds is ≤20%. Levels evaluated ranged from approximately 0.1 to 1.0 ppm. The 14 compounds in the study were HCB, BHC, lindane, heptachlor epoxide, dieldrin, endrin, mirex, methoxychlor, chlordane, oxychlordane, *o,p'*-DDT, *p,p'*-DDT, *p,p'*-DDE, and *p,p'*-DDD. Work at USDA, FSIS, is directed at expanding the method for red meat fat samples.

Determination and Identification of Cardiac Musculature, Soy Flour, and Partially Defatted Tissue in Ground Beef.—This topic should be retitled *Histologic Identification Methods.* Associate Referee Al Carey conducted a collaborative study for the qualitative determination of these substances.

Fat in Meat Products.—Associate Referee Jon McNeal reports that no collaborative studies or methods are currently in progress. However, FSIS has decided to use the AOAC-approved Foss-Let methodology (**24.006-24.008**) in our laboratories. It is expected that most laboratories outside our program will continue to use the Soxhlet ether extraction procedure, **24.005.** A restudy of this method would be useful, especially since it has not been evaluated for products having a fat content <7% or on a protein fat-free basis. It has not been studied for products which have traditionally been regulated by their trimmable fat content, but which now have to be tested for total fat for total quality control requirements. These products often contain more than 50% fat. With these considerations in mind, we may wish to study the method on these types of products in the near future. McNeal also reports that Tecator Inc. is planning a collaborative study for their Soxtec System HT. They have 10 collaborators lined up. Their study will include products within the protein fat-free and total quality control categories as well as those for which we have traditionally performed fat analysis.

Fat and Moisture Analysis, Rapid Methods.—Julio Pettinati, Associate Referee, has completed an extensive 22-laboratory study on microwave rapid moisture analysis. The study included various types of microwave ovens. Evaluation by the Associate Referee shows equivalence to the AOAC procedure. Results from 4 laboratories for fat and 3 for protein in the above study may warrant consideration for a collaborative study.

Fluoride in Deboned Meat and Poultry.—Associate Referee Thomas Dolan reports no progress and suggests the topic be abolished or combined with other ion electrode technologies; the Referee concurs.

Identification of Meats, Serological Tests.—Associate Referee Richard Mageau reports that no studies have been conducted and that a new Associate Referee should be appointed who can assume the necessary responsibilities. The topic

should be discontinued unless a new Associate Referee can be found and activities can be scheduled in 1983.

Moisture, Automated Karl Fischer Titrator Method.—No activity or interest has been reported on this topic by Associate Referee Howard Bredimus; the topic should be discontinued.

Nitrates and Nitrites.—Associate Referee F. Bart Suhre has developed an ion chromatographic procedure for the simultaneous determination of nitrate and nitrite and plans to conduct at least a 3-laboratory validation within FSIS laboratories. The procedure can reliably detect both anions as low as 5 ppm and has been used to examine pump-, immersion-, and dry-cured bacon, breakfast sausage, ham, and emulsified products. He further reports a slackening interest in nitrate-nitrite methods because of changing priorities among scientists active in the field. Nitrite is still an issue and the topic should continue.

Nitrosamines in Bacon.—E. L. Greenfield, Associate Referee, reports a successful collaborative study of the dry column, thermal energy analyzer method for *N*-nitrosopyrrolidine in fried bacon, developed by W. Fiddler. A 3-analyst study has been completed in FSIS laboratories on a low temperature distillation procedure (a modification of the published Sen procedure) for quantitation and GC/MS confirmation of volatile nitrosamines in bacon. FSIS has also conducted studies for volatile nitrosamines in dry-cured products, comparing the mineral oil distillation, thermal energy analyzer AOAC method, the modified Sen procedure, and the dry column, thermal energy analyzer procedure. The Associate Referee also notes that the Food and Drug Administration (FDA) and FSIS tentatively plan a joint study on possible in vivo formation of nitrosamines in swine.

Non-Meat Proteins in Meat.—Julio Pettinati, Chairperson for the 3-member Associate Referee committee, reports that method development to date has been limited to qualitative techniques only. His research priorities have shifted, but the topic should continue because of economic considerations and the recently published USDA regulation approving use of whey protein in certain meat products. Several types of nonmeat proteins are of keen interest to FSIS scientists. Emphasis will be placed on increasing method development in this area.

Pentachlorophenol in Animal and Poultry Tissues.—Associate Referee Douglas Gillard has successfully completed a collaborative study on detection of PCP in poultry, beef, and swine, using a method with an internal standard. The detectability range is 30–1000 ppb; the study range was 50–500 ppb. The method is quite rapid; all laboratories analyzed 48 samples over a 2-day period. Statistical evaluation indicates that for the range of 50–500 ppb, the method has a coefficient of variation (CV) for reproducibility of less than 20%, and for repeatability, a CV of less than 15%. The data do not indicate a species or day effect on the analytical results.

Protein in Meat.—F. Bart Suhre, Associate Referee, reports that the collaborative study for the determination of crude protein in meat products appears in *J. Assoc. Off. Anal. Chem.* **65,** 1339–1345 (1982). No collaborative studies are planned for 1983. He recommends that method **24.B01–24.B03,** the block digestion-steam distillation method, be adopted official final action. The Referee concurs.

Proximate Composition Relationships.—There has been no activity on this topic and the relationships would not normally be studied collaboratively. Associate Referee A. Malanoski recommends the topic be discontinued and the Referee concurs.

Sodium and Potassium in Meat Products.—Associate Referee R. Simpson has developed an automated specific ion electrode method for sodium and potassium in 11 processed meat products. Samples are prepared by a simple aqueous extraction followed by filtration and assay, using an automated NOVA Biomedical ion analyzer. The range of analysis is 25–1000 mg%. Products included bologna, ham, pork sausage, pump-cured bacon, pepperoni pizza, lunch meat, beef noodle soup, beef stew, turkey pot pie, spaghetti with meat balls, and frankfurters. There are no current plans for a collaborative study; however, a 3-laboratory validation study is anticipated. The Associate Referee recommends this topic be combined with the topic Specific Ion Electrode Methods and the current method be extended to include chloride ion determination. The Referee concurs.

Specific Ion Electrode Methods.—Associate Referee R. Simpson reports no new studies are currently under way with this topic. It should be combined with Sodium and Potassium in Meats into a single topic.

Sugar and Sugar Alcohols.—A new Associate Referee has been recommended, Douglas Burr of the New York State Food Laboratory. He plans a collaborative study using HPLC in an automated procedure. Sugars and antioxidants are to be included; compounds, e.g., propyl gallate and some sugar alcohols, may be added.

Temperature, Minimum Processing.—There has been no activity by Associate Referee Julio Pettinati and he does not wish to continue. The Referee thanks him for his efforts on this topic. A new Associate Referee has been recommended James Eye, FSIS, Science, Microbiology Division. Currently studies are being conducted by the Agriculture Research Center in Athens, GA, on improving the coagulation test, a size exclusion HPLC method, isoelectric focusing techniques, and a method for correlation of temperature with the loss of solubility of biuret-positive water-extractable (species) muscle proteins. Dr. Eye has developed a test for cooked and roast beef, using the bovine catalase test.

Recommendations

(1) Adopt as official final action the official first action method, **24.B01-24.B03,** block digestion steam distillation for protein in meat.

(2) Adopt as official first action the automated method for total protein; calcium analysis by EDTA titration; chlorinated hydrocarbons in poultry tissue by gel permeation chromatography; determination of cardiac musculature, soy flour, and partially defatted tissue in ground beef; microwave rapid moisture analysis, dry column-thermal energy analyzer method for *N*-nitrosyopyrrolidine in fried bacon; and pentachlorophenol in animal and poultry tissues.

(3) Conduct collaborative studies for phosphorus in meat products using the Technicon AA-2 AutoAnalyzer; HPLC automated method for sugars; antioxidants and sugar alcohols in meat products. Conduct validation or collaborative studies on nitrate-nitrite analysis of meat products by ion chromatography; sodium and potassium in meat and poultry products by specific ion electrode methods; fats in meat products using the Soxtec System.

(4) Extend the gel permeation chromatography analytical method for chlorinated hydrocarbons in poultry to red meat animals.

(5) Re-evaluate the AOAC Soxhlet procedure for fats, **24.005,** to include products with low fat, protein fat-free determinations, and total quality control products.

(6) Initiate the following topics: HPLC Methods for Meat and Poultry Products, Bioassay Methods for Meat and Poultry Products, Chemical Antibiotic Methods, Steroid Analysis in Meat and Poultry Products.

(7) Discontinue the topics Ashing Methods; Fluoride in Deboned Meat and Poultry; Identification of Meats, Serological Tests; Moisture, Automated Karl Fischer Titrator Method; and Proximate Compositional Relationships.

(8) Continue study on all other topics.

(9) Change title of the refereeship to Meat, Poultry, and Meat and Poultry Products.

Report on Microchemical Methods

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No work was done this year on the HPLC study on vegetable material (Laverne H. Scroggins) because of difficulty with the apparatus and difficulty finding collaborators.

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

Committee C recommended that this General Refereeship be discontinued and that the Associate Referee topic be reassigned to another Referee if practicable.

Recommendation

Continue study on the HPLC of vegetable materials.

Report on Mycotoxins

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A score of years have passed since the AOAC Referee on Nuts and Nut Products recommended that "chemical methods for identifying mycotoxins in moldy nuts be studied" (1) and 16 years since the topic "Mycotoxins" was established under the refereeship of Alfred D. Campbell, who saw the topic through its first 4 formative years and continued to participate as an Associate Referee ever since. We meet again to take account of the progress that has been made, and to set the course for the future, but this time without the participation of Al Campbell, who on February 22, 1982, while on assignment in Saudi Arabia, suffered a fatal heart attack. In recognition of Al's contributions to the refereeship and to the AOAC, it is fitting that these sessions on mycotoxins be devoted to his memory.

A memorial event normally evokes retrospection, and this occasion provides even more reason for that direction of thought: This will be my last General Referee report. I have retired from the Food and Drug Administration (FDA) and subsequently have resigned as General Referee, effective at the end of this meeting. Twelve years ago Al transferred to me an enthusiastic team and an evolving topic. The evolution of the team and the topic over the years has created many changes, but I transmit to the next General Referee on Mycotoxins that which I received from Al, an enthusiastic team and an evolving topic, plus the following advice distilled from the experiences they engendered.

First, keep in mind that the mycotoxin topic is peculiar in that the development of analytical methods precedes the determination of the need to regulate. Each Associate Referee has accepted, in addition to the explicit task of method development, the implied task of determining whether regulation is needed; note the coverage of occurrence and toxicology data in their reports. Thus each associate refereeship has within it the basis for its own elimination, as witness the dropping of Cocoa (2), Coffee (2), and Tea (3) as active topics, the recommendation to that effect in this report of the Associate Referee for Patulin, and the cautious probing in that direction of the Associate Referee for Sterigmatocystin (4).

Second, keep in mind that the more expensive or esoteric the equipment required for an analysis, the fewer the laboratories that will have the analytical capability, the fewer the analyses that will be performed, and the smaller the data base from which to make decisions. The simplicity and low cost of thin layer chromatography (TLC) should not be given up lightly for other more glamorous, and expensive, tools of separation science. The promised improvements in precision from the use of more instrumentation have not materialized for between-laboratory comparisons.

Finally, keep alive the system for making mycotoxin reference standards readily available. This is the heart of analytical method transfer. Without reliable reference standards, the best of methods is worthless.

And now, what do the Associate Referees have to say?

Aflatoxin M—Robert D. Stubblefield (U.S. Department of Agriculture (USDA), Northern Regional Research Center (NRRC), Peoria, IL) reports that a packed column procedure (5) for the extraction of aflatoxin M_1 from milk, as an alternative to the blender procedure in the current methods (**26.A10-26.A14, 26.090-26.094**), is undergoing testing at several laboratories. After evaluation of reports from the laboratories early in 1983, a decision will be made whether to recommend adoption.

The Associate Referee has concluded that the method proposed by Foos and Warren (6) should be the high performance liquid chromatographic (HPLC) method evaluated by collaborative study. He plans to conduct the study this fall and winter.

The Associate Referee has extended his studies on aflatoxin residues in bovine tissue. He has been working in cooperation with J. L. Richard and A. C. Pier of the National Animal Disease Center in Ames, IA. (Dr. Pier is now at the University of Wyoming, Laramie, WY.) In the first

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

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

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition; and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* 63, 374-423 (1980); 65, 450-521 (1982).

[1] Retired.

study, 2 cows were dosed orally for 3 consecutive days at a level of 0.35 mg B_1/kg body weight/day. One cow was slaughtered 24 h after the last dose; the second cow was fed normal aflatoxin-free dairy ration for 7 more days and then slaughtered. Except for the thymus, all samples of tissue, meat, milk, blood, and stomach contents contained aflatoxins B_1 and M_1. Kidney, liver, and mammary glands had the highest levels of total aflatoxins (58, 13, and 25 ng/g) with the M_1 level 40 times the B_1 level in kidney. Aflatoxin B_1 and M_1 residues were present (0.02–0.11 ng/g) in only kidney, liver, and intestine samples from the second cow, which had been withdrawn to an uncontaminated feed for 7 days, and only M_1 (0.10 and 1.5 ng/mL) was found in the final milk and urine samples from the same cow.

In another study, 3 groups of 5 steers each (average weight 403 lb (183 kg)/steer) were used. The first group was given only aflatoxin-free rations for 17.5 weeks; the second group was fed a diet containing naturally contaminated corn for 17.5 weeks; and the third group was fed the same diet as the second group for 15 weeks and then changed to the aflatoxin-free diet for 2.5 weeks. The amount of feed for all groups increased during the study, resulting in a dietary aflatoxin concentration that ranged from 352 to 455 ng/g. No differences in weight gains between groups were found during the study, and no signs of illness attributable to aflatoxin were seen in any of the steers. Aflatoxins B_1 and M_1 were readily detected in urine collected throughout the feeding trial, with M_1 concentrations ranging from 4 to 41 times the B_1 concentrations during the time the steers were on aflatoxin-containing diets. No aflatoxin was found in tissues or urine from the third group of steers 2.5 weeks after withdrawal of aflatoxin-containing feed.

No aflatoxin was present at necropsy in tissues or fluids of steers from the first group and the only sample from steers of the third group that had aflatoxin present was the rumen contents (low levels of B_1). All tissues of steers from the second group had B_1 and M_1 present (B_1 at 0.002–0.37 ng/g and M_1 at 0.10–4.82 ng/g), except that no B_1 was found in the round skeletal muscle. In most samples, M_1 levels were higher than B_1 levels.

The Associate Referee recommends that the official first action methods for aflatoxin M_1 in milk and cheese (**26.A10-26.A14**) and in dairy products (**26.090-26.094**) and for aflatoxins B_1 and M_1 in liver (**26.C01-26.C08**) be retained in that status, that evaluation continue of the Fukayama et al. column extraction method (5) for aflatoxin M_1 in milk, and that the Foos and Warren HPLC method (6) for aflatoxin M_1 in milk be validated by collaborative study. The Referee concurs.

Aflatoxin Methods.—Before his untimely death, Alfred D. Campbell (FDA, Washington, DC) had initiated a collaborative study of 2 HPLC methods for aflatoxins in peanut butter, one using a normal phase column, the other a reverse phase column. In this undertaking he had the assistance of 2 people who had been involved in the development of the methods, Octave J. Francis, Jr (FDA, New Orleans, LA) and Roberta M. Beebe (FDA, San Francisco, CA, retired). The data collected from the collaborators by Mr. Francis and Mrs. Beebe were transmitted to the General Referee for collation and statistical interpretation. The final report, as prepared for publication, shows recoveries of aflatoxins B_1, B_2, G_1, and G_2 from spiked samples by the normal phase HPLC method of 79, 92, 74, and 88%, respectively; by the reverse phase method the recoveries were 103, 105, 89, and 163%. Pooled repeatabilities for B_1, B_2, G_1, and G_2 by the normal phase HPLC method were 20, 25, 28, and 17%, respectively; by the reverse phase method repeatabilities were 19, 22, 38, and 31%. Pooled reproducibilities for B_1, B_2, G_1, and G_2 by the normal phase HPLC method were 34, 33, 39, and 34%, respectively; by the reverse phase method reproducibilities were 32, 46, 51, and 52%. Although both methods show an improved limit of detection and better within-laboratory precision over current AOAC official methods, the between-laboratory precision is no better and the recovery of G_2 by the reverse phase method shows evidence of interferences being measured. For these latter adverse reasons and because of no compensating benefits of present value, neither method was submitted for adoption.

Because of reports, both published and unpublished, that, in spite of evidence to the contrary from collaborative studies, the CB and BF methods did not produce the same quantitative answers, Dr. Campbell, in cooperation with J. W. Dickens and T. W. Whitaker (USDA, Raleigh, NC), had initiated a study (7) to resolve the difference in observations and at the same time provide a test of an improved version of the BF method that Dickens and Whitaker had developed. Dickens and Whitaker have agreed to follow through on the plans for the study.

In the context of this associate refereeship, the General Referee calls attention to that part of the report of the Associate Referee on Tree Nuts relating to method improvement and his recom-

mendation for continued activity in the approaches taken.

The General Referee also calls attention to an interim recommendation that zinc acetate–aluminum chloride solution be allowed as an alternative to lead acetate solution in the method for aflatoxins in cottonseed products (**26.A01-26.A09**). Theoretically, and on the basis of performance data submitted, the addition of the alternative creates no substantive change in the method and does allow those concerned with the disposal of lead-containing wastes a choice of a more benign residue. The Referee, therefore, repeats the recommendation that sections **26.A02** and **26.A04** be revised as follows:

26.A02(e): *Lead acetate soln* . . . and dil. to 1 L, or optional *zinc acetate–aluminum chloride soln.*—Dissolve 200 g $Zn(OAc)_2$ and 5 g $AlCl_3$ in H_2O and dil. to 1 L.

26.A04: Measure 100 mL filtrate . . . and refilter as above, or, optionally, replace $Pb(OAc)_2$ soln with $Zn(OAc)_2$–$AlCl_3$ soln.

The General Referee has also considered the use of the water slurry procedure for obtaining the analytical sample from the lot sample as constituting no substantive change in method II (**26.032-26.036**) for aflatoxins in peanut products, and therefore recommends that secs. **26.034** and **26.035** be revised as follows:

26.034: See **26.028,** or prep. peanut samples by H_2O slurry procedure [*J. Am. Oil Chem. Soc.* **57,** 269 (1980)].

26.035: . . . and ca **4** g NaCl to peanuts or meal. If peanut sample is prepd by H_2O slurry method, weigh 130 g slurry into blender jar. Add 50 mL 2.2% salt soln, 150 mL MeOH, and 100 mL hexane.

Alternaria Toxins.—A. Douglas King, Jr (USDA, Western Regional Research Center, Albany, CA) reports that an important paper (8) was published this past year dealing with the isolation of the minor *Alternaria* toxins, altenuisol and altertoxins I and II. These **3** often amount to <**0**.1% of the crude extract, whereas alternariol, alternariol methyl ether, and tenuazonic acid are often produced in the 5–10% range. The importance of this paper describing the separation of the 3 minor toxins is related to their suspected toxicity and mutagenicity, both of which should be further studied. This paper is a companion to one on the isolation of alternariol, alternariol methyl ether, and altenuene (9).

The structure of altertoxin I has been elucidated (10). A combination of TLC and silicic acid column chromatography was used to purify the compound isolated from culture on rice. It was identified as 1,2,7,8,12*b*-pentahydro-1,4,6*b*,10-tetrahydroxyperylene-3,9-dione. There was no evidence for the dehydro form, called altertoxin II by Harvan and Pero (11), in decomposition products isolated from the highly purified altertoxin I.

Another recently published study (12) describes the toxicity of molds isolated from grain in India. Cultures of *Alternaria* species were toxic (3 of 10 tested). Although no deaths occurred when mice were injected intraperitoneally with the culture extracts, lesions were produced in livers or livers and spleens of the mice. Cultures of several species of *Aspergillus* and *Penicillium* had similar toxicity. *Fusarium, Chaetomium,* and *Paecilomyces* were slightly more toxic. These observations are similar to those involving *Alternaria* in foodstuffs.

The presence of *Alternaria* mycotoxins in foodstuffs still has not been adequately demonstrated, and further work on the stability of these compounds is needed. The demonstrated toxicity of several of the *Alternaria* toxins requires that work be continued to determine the potential for their occurrence in foods. The Referee concurs in these goals.

Citrinin.—David M. Wilson (University of Georgia, Coastal Plain Station, Tifton, GA) notes that extraction of citrinin has been a difficult problem that is matrix-dependent. In the last few years, detection of citrinin by TLC has been improved by the addition of chelating agents to the plates or developing solvent, and HPLC on a reverse phase column is relatively straightforward. However, the extraction recovery is not consistent. Extracts from grains, such as wheat or barley, give more consistent results than from corn or peanuts. There seem to be at least 3 obstacles to good extraction and recovery. One is the chelating ability of citrinin, one is the effect of pH on citrinin properties, and another is the effect of temperature on the stability of citrinin. Currently under test is a fluorometric method for estimating citrinin after acid/base partition cleanup and no evaporation step. This approach, it is hoped, will improve the recovery of citrinin. The Associate Referee recommends continued effort in this direction. The Referee concurs.

Ergot Alkaloids.—Colette P. Levi (General Foods Corp., White Plains, NY) has found it impossible to devote the necessary time and effort to work on the ergot alkaloids, and in the current climate of retrenchment has no idea when that situation might change. She has, therefore, offered her resignation as Associate Referee, which is re-

gretfully accepted. Other investigators, however, have been active. The studies on content and composition of Canadian ergot noted in last year's report (4) have been extended to triticale and barley (13), and ergot has been included among the mycotoxins for which Canadian feedstuffs were surveyed (14, 15). A study has also been carried out on losses of ergot alkaloids during the making of bread and pancakes (16). These studies had been made possible by the application of HPLC, with a plethora of methods from which to choose (17–20). Mass spectrometric (MS) techniques have also been used for further characterization (19, 21). With the extent of interest in ergot alkaloids, as evidenced by recent publications, the Referee recommends that the topic be retained, Dr. Levi be thanked for her past efforts, and a replacement be sought.

Grains.—Odette L. Shotwell (USDA, NRRC, Peoria, IL) reports that studies on the preservation of corn samples collected for aflatoxin analysis continued. Attempts in 1980 to prevent mold growth in corn samples by the addition of 5% Monoprop (propionic acid on vermiculite (1 + 1)) had not been successful; aflatoxin levels were lower in the stored, treated samples than they had been before addition of Monoprop. In 1981, corn samples were collected in Georgia, shipped to Peoria, and stored 6 weeks with no treatment and with 1, 2, and 5% Monoprop (equivalent to 0.5, 1.0, and 2.5% propionic acid) added. All treatments with Monoprop resulted, as in the 1980 experiment, in a decrease of aflatoxin levels. Although aflatoxin B_{2a} was the expected reaction product, it could not be extracted from treated samples after storage, using 4 different extraction methods. In the 1981 experiment, corn samples had not been shucked before shipment to Peoria, and aflatoxin levels increased considerably during shipment. It would appear that corn to be analyzed for aflatoxin should be shucked immediately after collection from the field to facilitate drying.

Attempts are still being made to estimate possible aflatoxin levels in corn lots by weighing the bright greenish-yellow fluorescing (BGYF) particles associated with the presence of *Aspergillus flavus* or *A. parasiticus*. A study of 1971 white corn grown in southeastern Missouri indicated that the correlation between weight percent BGYF and aflatoxin levels in corn lots was not sufficiently good to use BGYF to estimate toxin levels (22). Since then, Dickens and Whitaker (23) have presented evidence indicating that aflatoxin levels in yellow corn marketed in North Carolina can be estimated by weight percent BGYF. They are still investigating the possibility, using corn collected in a cooperative state effort, to determine the best method of field inoculation to form aflatoxin in corn.

At the University of Wisconsin the determination of aflatoxin in corn by an enzyme-linked immunoassay (ELISA) was compared to determination by a solid phase radioimmunoassay (RIA) (24). At a level of 5.8 ng/g or higher, recoveries of aflatoxin B_1 from corn samples were 80.0% by ELISA and 61% by RIA. Overall, the ELISA technique gave more reproducible results than did RIA. ELISA is considerably faster, safer, and less expensive than RIA, and is adaptable for use in test kits.

An MS technique, based on negative ion (NI) chemical ionization (CI) was developed for confirming aflatoxin identity (25, 26). Tandem MS (27), or MS-MS, also promises to be a sensitive method for confirming the identity of aflatoxins and other mycotoxins in relatively crude extracts. Although the mass spectrometer is a relatively expensive analytical tool, it can be of value in special situations. MS—MS has been investigated to confirm the identity of *Fusarium* toxins in corn, and is now being used to confirm the identity of deoxynivalenol (DON) extracted from scabby wheat. The use of MS—MS is also being explored for screening crude extracts of scabby wheat for DON.

Studies are continuing on the potential exposure of agricultural workers to aflatoxin by inhalation of airborne dusts from aflatoxin-contaminated corn (28, 29). In these studies, corn delivered to and unloaded from an elevator contained an average total aflatoxin level of 153 ng/g. The average aflatoxin level in dusts collected during delivery and unloading was 1600 ng/g. Dusts collected in personnel samplers worn by workers in the elevators had an average level of 1200 ng/g. Airborne dusts were also collected on one farm. Corn harvested from the farm had an average aflatoxin level of 1600 ng/g, and dusts collected during harvesting, grinding, and feeding had average aflatoxin levels of 3210, 1250, and 1500 ng/g, respectively. Dust from personnel samplers on the farm had even higher levels. John Lacey (Rothamsted Experimental Station, Harpenden, Hartfordshire, UK, personal communication), who has studied farmer's lung in Great Britain, noted that many of the dust particles collected at harvest are conidia (mold spores). Accordingly, a determination was made of the aflatoxin content of the conidia of 9 isolates of *A. flavus* and *A. parasiticus* (30). Conidia of the 5 *A. flavus* isolates had 94 400, 36, 84 000, and

2930 ng/g and nondetectable levels of aflatoxin B_1, and the conidia of one isolate had a 565 000 ng/g level of G_1. Conidia of the 4 *A. parasiticus* isolates had 1106, 2510, 5140, and 54 300 ng/g levels of B_1 and 3050, 23 500, 11 200, and 7270 ng/g levels of G_1. The high aflatoxin levels in airborne dusts collected in personnel samplers, therefore, could be due to conidia of molds of the *A. flavus* group.

The Associate Referee recommends continued study along the lines reported above. The Referee concurs.

Mixed Feeds.—Thomas R. Romer (Romer Labs, Inc., Washington, MO) calls attention to his new affiliation, which follows 14 years of mycotoxin research experience at Ralston Purina Co. As a result of this move, he was unable to carry out the planned collaborative study of a method for aflatoxins in mixed feeds, nor will he be able to continue to perform the functions of an Associate Referee. He has, therefore, tendered his resignation, with the recommendation that the topic Mixed Feeds be discontinued, since Associate Referees for the specific mycotoxins should be able to follow through on each substrate-specific area. The Referee accepts the resignation with an understanding of the circumstances that brought it about, and with appreciation for Mr. Romer's 12 years of valuable service and contributions to the Refereeship. He also concurs in the recommendation that the topic be dropped.

Ochratoxin.—Stanley Nesheim (FDA, Washington, DC) reports that ochratoxin contamination of grains, especially barley, continues to be a problem in parts of Europe, and that some of the ochratoxin from the contaminated grain used for feed is found in animal tissue. For this reason, the European countries have expressed an interest in having the International Union of Pure and Applied Chemistry (IUPAC) validate a TLC method for ochratoxin in tissue. No consensus exists among the interested laboratories as to which method should be collaboratively studied. The Associate Referee therefore undertook an evaluation of 2 official AOAC methods (**26.014-26.019, 26.096-26.102**), 3 personally communicated methods (A. Cantafora, Institute for Health, Rome, Italy; K. Hult, Royal Institute of Technology, Stockholm, Sweden; H. P. van Egmond, National Institute of Public Health, Bilthoven, The Netherlands), and 4 published methods (31-34) to either select the best one or to devise a new method consisting of the best features of the different methods. The extraction and cleanup steps of the 9 methods were evaluated, using TLC to determine recovery and effectiveness of extract cleanup. The conclusion was that none of the evaluated methods was a significant improvement over the present AOAC ochratoxin methods for barley (**26.096-26.102**) and for green coffee (**26.104-26.109**). Of the 2 AOAC methods, the green coffee method is the better. The barley method has been applied extensively to animal tissue in a number of laboratories, but has not been collaboratively studied using animal tissue as a substrate. The Associate Referee has recommended to IUPAC that they cooperate with the AOAC in a collaborative study using a modification of the green coffee method as published in a volume of selected methods for mycotoxins (31) and using exposure to ammonia vapor to enhance the fluorescence of the ochratoxin spots on the TLC plate.

Under the National Toxicology Program, acute and subchronic toxicity tests of ochratoxin A in rats have been completed in preparation for a full chronic study for which FDA has prepared 50 g ochratoxin A. The acute and subchronic studies showed the anticipated renal toxicity, in addition to which the acute study showed bone marrow hypoplasia.

The Associate Referee recommends a collaborative study of the modified green coffee method (31) applied to swine tissue. The Referee concurs.

Patulin.—Peter M. Scott (Canadian Health Protection Branch, Ottawa, Ontario, Canada) reports that an IUPAC collaborative study on the determination of patulin in apple juice by HPLC was unsuccessful because patulin decomposed in the samples. In view of the availability of the official final action AOAC method (**26.111-26.116**) for determination of patulin in apple juice by TLC, the recently confirmed lack of tumorigenic activity of patulin administered orally to rats (35), and resultant lack of regulatory interest in North America, the Associate Referee recommends that the topic be dropped. The Referee concurs.

Penicillic Acid.—Charles W. Thorpe (FDA, Washington, DC) reports that penicillic acid was included among the mycotoxins recovered by 3 different multimycotoxin screening methods (36-38) described in the literature. All 3 methods use acetonitrile for extraction, TLC for the ultimate separation, and exposure of the developed TLC plate to ammonia vapors for visualization. In one method (36) the toxins are fractionated on a silica gel column, in the other methods (37, 38) on a polyamide column. A method for the determination of penicillic acid

in chicken tissues (39) has also been described. This method used reverse phase HPLC with an ultraviolet absorption detector at 254 nm; extraction is with ethyl acetate after digestion of the tissues with 3N HCl. The Associate Referee has also developed a method for penicillic acid (40) that uses HPLC with post-column derivatization and a fluorescence detector. The extraction and cleanup is the same as in a previously described method (41) that uses gas-liquid chromatography (GLC) of the trifluoroacetate derivative of penicillic acid. This latter method has been successfully tested in a minicollaborative study as a prelude to a proposed full collaborative study.

An investigation of means for prevention of penicillic acid formation (42) revealed that orsellinic acid derivatives could inhibit production of penicillic acid by *Penicillium cyclopium*.

The Associate Referee recommends that the proposed collaborative study of the GLC method (41) be carried out. The Referee concurs.

Sterigmatocystin.—Octave J. Francis, Jr (FDA, New Orleans, LA) reports no activity on this topic in the past year, either on his part, or, as far as can be determined from the published literature, in any other laboratory. Since the only demonstrated natural occurrence of sterigmatocystin in an edible foodstuff has been in ripening cheese (43), he recommends validation of the method (44) that was used for that demonstration, and a determination of the extent to which that contamination can occur. The Referee concurs.

Tree Nuts.—Vincent P. DiProssimo (FDA, Brooklyn, NY) reports successful application of an NI CI MS method for the confirmation of identity of aflatoxin B_1 isolated from contaminated Brazil nuts, filberts, pistachio nuts, and melon seeds. The method, first described by Brumley et al. (25), will be validated by collaborative study as soon as method details have been agreed to between the operating and originating laboratories. Work has also progressed on adaptation to the CB method of the silica gel column chromatographic procedure (**26.A05**) used in the cottonseed method, and its further use as part of a general purpose method for aflatoxins that incorporates presently available information on economies of scale and solvent usage. In this pursuit, Mr. DiProssimo has confirmed the observation (45) of the Associate Referee for Aflatoxin M that silica gel column wash solvents containing acid ingredients must be completely removed from the column before elution of the aflatoxins is attempted, if untimely aflatoxin elution or undesirable aflatoxin reactions are to be avoided. The Associate Referee recommends collaborative study of the Brumley et al. method (25) for confirmation of aflatoxin identity as soon as operating details have been settled, and continued study toward a general aflatoxin method. The Referee concurs.

Trichothecenes.—Robert M. Eppley (FDA, Washington, DC) reports that the last year has been an exciting time for those interested in the trichothecenes. The news media have given considerable coverage to the purported use of trichothecenes as chemical warfare agents, calling on everyone whose name has been associated with the compounds for technical background. More recently, locally severe infections by *Fusarium roseum* of winter wheat in some parts of the United States have resulted in the occurrence of DON (vomitoxin) in the affected grain. This is a finding similar to that for Canadian wheat included in last year's report (4).

The Canadian findings had stimulated method development, with added impetus from the more recent findings in the United States. Two of the proposed methods for DON show considerable promise. The GLC method of Scott et al. (46), modified by substitution of a 30-min shake for a 3-min blend to extract the toxins (G. A. Bennett, USDA, NRRC, Peoria, IL, personal communication) has provided a reported 85–95% recovery, with a limit of determination around 0.1 μg/g, and is adaptable to direct coupling to a mass spectrometer for confirmation of compound identity. The major drawback of this method is that it is labor intensive, and takes a relatively long time for completion. Another and simpler TLC method (47) has been used by the Associate Referee, and with minor modifications shows considerable promise for general use. Methods using HPLC have been under development in several groups, but none is yet at the stage for interlaboratory evaluation. An excellent review of methods for trichothecenes, presented at the 1981 AOAC meeting, has been updated and published (48), which, together with the recommendations of the panel on trichothecenes (49) from the same meeting, should serve as a basis for further trichothecene method development.

Some of the new techniques in MS have been applied to trichothecenes. One application reported (50) is the use of NI CI for confirmation of identity. MS–MS shows considerable promise for screening crude extracts (G. A. Bennett, USDA, NRRC, Peoria, IL, personal communication).

The Associate Referee recommends that both the Scott et al. (46) and Romer et al. (47) methods for DON be pushed to final development for collaborative study, that the efforts to implement the trichothecene panel recommendations of last year (49) be continued, and that those recommendations be modified to give top priority to DON. The Referee concurs.

Zearalenone.—Glenn A. Bennett (USDA, NRRC, Peoria, IL) reports that method development for the determination of zearalenone and its metabolites has slowed considerably, providing a respite for method evaluation and refinement. Modifications of the HPLC method of Ware and Thorpe (51) were necessitated by low recoveries of zearalenone and zearalenol from obviously moldy grain samples, especially oats. Suggested improvements (R. Bagnaris, FDA, New Orleans, LA, personal communication) involved reducing the sample size, changing the concentrations of acid and base for cleanup, adding saturated salt solution before extraction with base, and adding a chloroform backwash. An HPLC method for zearalenone in chicken tissue (52) was reported to be rapid, with recoveries of 83–95% and a limit of determination around 50 ng/g. Two rapid TLC methods for zearalenone have been developed. One author (53) claims a limit of determination of 130–140 ng/g in extracts from corn, sorghum, and wheat. Another (R. A. Corley, University of Illinois, Urbana, IL, personal communication) claims the capability for analysis of 10 samples in less than 2 h with limits of determination around 80 and 150 ng/g for zearalenone and zearalenol, respectively, on TLC plates sprayed with Fast Violet Blue salt.

Since cereal grains are susceptible to attack by a variety of molds, some of which can elaborate more than one toxic metabolite, multitoxin methods have been developed (54–56). One of these methods (56) uses TLC and GLC to systematically screen for 10 toxins, including zearalenone, produced by *Fusaria*. Less ambitious, in terms of the number of toxins sought, is ongoing research in the Associate Referee's laboratory where GC/MS is being studied for the simultaneous detection and quantitation of zearalenone, zearalenol, and DON, and tandem quadrupole MS for zearalenone and DON. The latter technique can be used without sample cleanup or derivatization.

Studies have continued on the effects of zearalenone on animals and its mode of action. Free zearalenone was found in the blood, feces, and urine of female pigs dosed with 3.5–11.5 mg zearalenone/kg body weight (55). Zearalenone (up to 59 ng/mL), α-zearalenol (up to 155 ng/mL), and some β-zearalenol were detected in their urine by HPLC. Other animal studies (56) showed that zearalenone had little effect (except reduced libido scores) on the sexual development of boars. There have been studies on the enzymes responsible for the transformation of zearalenone to zearalenol and their subcellular location (57). Despite their nonsteroidal structure, zearalenol and its derivatives exhibit estrogenic activity toward target uterine tissue by inducing specific protein synthesis (58).

Studies on the occurrence and stability of zearalenone have demonstrated its presence in grain dust at 50–100 ng/g (M. P. Whidden, USDA, New Orleans, LA, personal communication). In Taiwan, 6.9% of swine feedstuffs contained zearalenone at levels of 162–1203 ppb (59). Ammoniation only partially destroys zearalenone (60). Some reduction of zearalenone level can be expected in the processing and preparation of foods (61).

The Associate Referee recommends that work continue toward selection of an HPLC method for collaborative study, and on rapid screening methods, particularly methods that will detect other mold toxins that may occur with zearalenone in grains. The Referee concurs.

Recommendations

(1) Adopt the following section revisions as constituting no substantial change in the methods:

26.A02(e): *Lead acetate soln* . . . and dil. to 1 L, or optional *zinc acetate–aluminum chloride soln.*—Dissolve 200 g $Zn(OAc)_2$ and 5 g $AlCl_3$ in H_2O and dil. to 1 L.

26.A04: Measure 100 mL filtrate . . . and refilter as above, or, optionally, replace $Pb(OAc)_2$ soln with $Zn(OAc)_2$–$AlCl_3$ soln.

26.034: See **26.028,** or prep. peanut samples by H_2O slurry method [*J. Am. Oil Chem. Soc.* **57,** 269 (1980)].

26.035: . . . and ca 4 g NaCl to peanuts or meal. If peanut sample is prepd by H_2O slurry method, weigh 130 g slurry into blender jar. Add 50 mL 2.2% salt soln, 150 mL MeOH, and 100 mL hexane.

(2) Initiate collaborative studies on the Foos-Warren HPLC method for aflatoxin M_1 in milk, a comparison of the CB, BF, and modified BF methods, the modified method for ochratoxin in green coffee applied to swine tissue, the Thorpe method for penicillic acid, the van Eg-

mond et al. method for sterigmatocystin in cheese, the Brumley et al. method for confirmation of aflatoxin identity by CI NI MS, the Scott et al. and Romer et al. methods (as modified) for DON in grains, and a selected HPLC method for zearalenone in grains.

(3) Discontinue the topics Mixed Feeds and Patulin.

(4) Continue study on all other topics.

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Report on Oils and Fats

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Antioxidants.—B. D. Page has successfully completed a collaborative study of the high pressure liquid chromatographic (HPLC) method for determining 7 antioxidants in oils and fats (1). The method has been granted interim first action status and it is recommended that it be adopted as official first action.

The Associate Referee has developed methodology for determining nonphenolic antioxidants involving (a) use of HPLC to isolate the antioxidants followed by gas chromatographic (GC) determination, or (b) solvent extraction followed by GC determination. Additional investigation of the methodology will be followed by a collaborative study.

Cyclopropene Fatty Acids.—G. S. Fisher is planning a collaborative study of methodology (2) for cyclopropene fatty acids.

Chromatographic Methods.—W. G. Doeden, Jr, is proceeding with plans for collaborative study of methods for determining fatty acid composition by capillary column GC and determining triglyceride composition (carbon number determination) by programmed temperature GC. The International Union of Pure and Applied Chemistry (IUPAC) Commission on Oils, Fats and Derivatives has completed an initial collaborative study of methodology for determination of triglycerides by GC with 50-70 cm conventional packed columns (3). Palm oil, coconut oil, and cocoa butter triglycerides were examined by 8 collaborators (one collaborator used a 10 m capillary column). Additional study will involve analysis of animal fats.

Emulsifiers.—H. Brüschweiller is completing a joint AOAC-IUPAC collaborative study of a GC procedure for a number of emulsifiers. The procedure involves separation of emulsifiers from an oil sample by column chromatography as described in secs. **28.133-28.138** (4). The emulsifier fraction is hydrolyzed in alcoholic KOH solution and the components are silylated and determined by either packed or capillary column GC. The emulsifiers determined in the current collaborative study are monoglycerides, diacetyl tartaric acid monoglycerides, and sorbitan monostearate.

Lower Fatty Acids.—G. Bigalli is planning a collaborative study of methodology for quantitation of lower fatty acids by GC using either pentane as the solvent with 20% DEGS columns or decane with 3% methyl silicone columns.

Marine Oils.—R. G. Ackman is continuing the study of methodology for analysis and identification of fish oils, including the use of fused silica capillary columns for GC determination of fatty acid composition. Six fused silica columns with polar liquid phases will be evaluated with various fish oils in a program to develop standard columns for use in capillary column GC. There

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

The recommendations of the General Referee were approved by Committee C and were adopted by the Association, except recommendation (2). See the report of the Committee and "Changes in Methods", this issue.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition.

is current interest in analyzing fish oil for eicosapentenoic acid (20:5) content because of reports that this polyunsaturated fatty acid has desirable antithrombotic properties (5).

The Associate Referee has completed an additional collaborative study of a capillary column GC method for determination of erucic acid in edible fats and oils (6). Four samples (mixtures of partially hydrogenated marine oil and rapeseed oil with or without added methyl erucate or corn oil and containing approximately 5 or 11% erucic acid) were sent to 9 collaborators. The results appeared to be satisfactory with adequate interlaboratory reproducibility. The ranges in coefficients of variation for the 3 samples containing approximately 5% erucic acid were similar to those obtained in the first collaborative study (7), which estimated erucic acid at the 10% level. The Associate Referee also observed that the capillary column GC method is at least as reproducible and convenient to perform as the low-temperature thin layer chromatographic (TLC) method (8, 9). However, there are questions about the stability of the Silar-5CP capillary columns and the ability of these columns to determine erucic acid in the presence of mixtures of cis and trans acids. Accordingly, no recommendation for adoption will be made pending additional review and publication of the collaborative results.

Olive Oil Adulteration.—E. Fedeli has supplied the Referee with a number of methods used in Italy that were validated by collaborative studies; the collaborative results are under review.

Oxidized Fats.—A. E. Waltking is continuing study of procedures for oxidation products (polymers) in vegetable oils. The interim first action column chromatographic method for oxidation products in frying fats (10) has been adopted as official first action (11). A method was developed for determination of polymers in abused vegetable oils by gel permeation chromatography. The method employs μStyragel columns to separate methyl esters of the fatty acids of vegetable oils from polymer (dimer) acids. A joint AOCS-AOAC-IUPAC collaborative study of the method is planned.

Pork Fat in Other Fats.—L. El-Sayed has submitted methodology for detection of lard in hydrogenated fats and shortenings. Lard is detected by determination of the palmitic acid enrichment factor (mole % palmitic acid in the 2-monoglycerides isolated by pancreatic lipase hydrolysis and TLC divided by the mole % palmitic acid in the triglycerides). Lard samples exhibit a factor of about 3 or more, whereas the factor is always <1 with hydrogenated vegetable fats.

Spectrophotometric Methods.—A. J. Sheppard is planning a collaborative study of the enzymatic method for *cis,cis*-methylene interrupted polyunsaturated fatty acids in various foods.

Sterols and Tocopherols.—H. J. Slover has initiated a collaborative study of the GC method for determination of tocopherols and sterols in vegetable oils.

Trans Isomers by Infrared Spectrophotometry.—B. L. Madison, Procter and Gamble Co., Cincinnati, OH, has described a procedure (12) for determining the trans unsaturated fatty acid content of fats and oils that is more accurate than the current infrared spectrophotometric method (secs. **28.075-28.080,** 4). Increased accuracy is obtained by using a 2-component calibration standard (methyl linoleate-methyl elaidate) and measuring the samples as methyl esters. Dr. Madison is planning a joint AOCS-AOAC-IUPAC collaborative study.

Water Content.—R. Bernetti has completed evaluation of the results of an international collaborative study on the determination of water content in vegetable oil products by the Karl Fischer method recommended by the International Standards Organization (ISO/TC 34/SC 11, N 99). The Associate Referee recommends adoption of the method.

Commission on Oils, Fats and Derivatives, Applied Chemistry Division, IUPAC.—The General Referee chaired the annual meeting of the IUPAC Commission on Oils, Fats and Derivatives on September 6–10, 1982, in Zeist, The Netherlands. The Commission reviewed 13 projects, including methodology for glycerine products and alkaline soaps, polyunsaturated fatty acids, emulsifiers, plastic monomers in oils, polycyclic aromatic hydrocarbons, industrial lecithin products, erythrodiol in grapeseed and olive oils, total sterols, mineral oil residues, solvent residues in oils and oilseed cakes, thiobarbituric acid value, and triglyceride composition by GC. A method for determination of solvent residues in oilseed cakes based on the headspace method of Dupuy and Fore (13) will be subjected to collaborative study. Methodology for determination of erythrodiol in olive oil and grapeseed oil (useful primarily for detection of olive residue oil in olive oil), total sterols, lecithins, and triglycerides (carbon number) will be subjected to additional study. A project was initiated on determination of N-3 polyunsaturated fatty acids (linolenic, etc.). Methods adopted earlier for identification and determination of tocopherols and determi-

nation of polar compounds in frying fats have been published (14). Methods for analysis of commercial glycerine products and alkaline soaps have also been published (15).

Recommendations

(1) Adopt as official first action the HPLC method for antioxidants in fats and oils.

(2) Adopt as official first action the Karl Fischer method for determination of water content in vegetable oil products.

(3) Continue study on all other topics.

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Report on Plant Toxins

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The broad scope of this new General Refereeship has necessitated considerable discussion of the needs for methods of analysis for plant toxins. These discussions have indicated some joint interests with other Referees, particularly those for Veterinary Analytical Toxicology and for Oils and Fats. Close coordination will be maintained with these Referees.

Two problem areas which deserve immediate attention with regard to methods of analysis are the pyrrolizidine alkaloids and the solanaceous glycoalkaloids. The pyrrolizidine alkaloids occur in many plant genera, including *Senecio* and *Crotalaria*. They have been implicated in human liver diseases, including primary liver cancer, and venooclusive disease and have been involved in substantial losses of livestock. The glycoalkaloids of potatoes (*Solanum tuberosum*) can result in human and livestock poisonings. The varietal variation of alkaloid content has necessitated the monitoring for these toxins in newly developed cultivars. In addition, other species of *Solanum* present as pasture and range weeds have caused considerable livestock losses. Several of these species are common weeds in cultivated crops, and their berries have been known to contaminate pea and bean crops. Steps to establish Associate Refereeships in these areas have been initiated.

Recommendations

(1) Establish Associate Refereeship for Pyrrolizidine Alkaloids.

(2) Establish Associate Refereeship for Solanaceous Alkaloids.

(3) Continue evaluation of other plant toxin problems and assess needs for analytical methodology.

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

The recommendations of the General Referee were approved by Committee C and were accepted by the Association. See the report of the Committee, this issue.

Report on Processed Vegetable Products

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Fibrous Material in Frozen Green Beans.—The method on fibrous material in frozen green beans was adopted official final action (**32.059-32.061**). Associate Referee George W. Varseveld is soliciting further comments on this topic.

pH Determination.—The method for pH determination in acidified foods was adopted official final action (**32.B01-32.B08**). Associate Referee Frederick E. Boland recommends that work continue on this topic.

Sodium Chloride.—The potentiometric method (Method III) for sodium chloride in foods was adopted official final action (**32.025-32.030**). Associate Referee Wallace S. Brammell reports he is completing preparation of a review paper on methods for determining sodium chloride in foods and recommends that the study be continued.

As recommended in the previous AOAC meeting, the following official final action methods were declared surplus: sodium chloride (Method I), **32.023**; sodium chloride (Method II, rapid method), **32.024**.

Water Activity Determination.—The method on water activity determination was adopted official final action (**32.004-32.009**).

Associate Referee William H. Stroup reported that a quality assurance sample containing 2 salt slushes (both $(NH_4)_2SO_4$) and 2 portions of soy sauce (both naturally brewed soy sauce) were analyzed by 13 FDA district laboratories using the hair hygrometer and 5 FDA laboratories using various electrical hydrometers. All test results were deemed acceptable for use in the statistical analyses of the quality assurance sample. Interested persons are invited to participate in continued study on this topic.

There was no response to the request to initiate a new topic for Volume of Entrapped Air in Flexible Retort Pouches.

Recommendation

Continue study on all topics.

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

The recommendation of the General Referee was approved by Committee C and was accepted by the Association. See the report of the Committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition; and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **64**, 501-540 (1981).

Report on Seafood Toxins

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Ciguatoxin—Biochemical Methods.—(Associate Referee Yoshitsugi Hokama, School of Medicine, University of Hawaii at Manoa, Honolulu, Hawaii.) A previous report to AOAC on ciguatoxin by the General Referee (1) presented the background of ciguatera poisoning and the recent developments of assays for the detection of ciguatoxin in fish tissue. This report presents the completion of a 2-year study of the radioimmunoassay method (2-5) for detection of ciguatoxin in fish tissue and of the development of an enzyme-linked immunosorbent assay (ELISA) previously mentioned in the General Referee's report (1).

RIA Evaluation and Study: A 2-year study of the RIA procedure for the detection of ciguatoxin (CTX) directly from fish tissue has been completed with *Seriola dumerili* (amberjack, kahala) as the targeted fish. *S. dumerili* is one of the major fish species implicated in ciguatera poisoning in

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Hawaii (2–5). The following is a summary of the results of this study of the RIA method: 1. RIA detected CTX in 93% of fish tissues obtained from clinically documented ciguatera poisoning which involved several species (3). 2. The immunoglobulin nature of the reactive proteins in sheep serum previously immunized with CTX-HSA conjugate (2, 5) was confirmed by DEAE-cellulose fractionation and immunoelectrophoresis characterization with specific rabbit anti-sheep IgG. 3. A longitudinal study with commercial *S. dumerili* proved the RIA effective in protecting the consumer from ciguatera poisoning by removal of the potentially hazardous fish from the markets. Fifteen percent of the total 5529 fish weighing a total of 90 360 lb was rejected. During this 2-year period, no incident of ciguatera poisoning due to marketed *S. dumerili* occurred, although poisonings due to other fish species were reported to the Hawaii State Department of Health (30 cases involving 88 individuals).

This study (3) suggested that the RIA is useful, but is costly and time consuming. The cost factor can be minimized if only large *S. dumerili* are sampled. It is suggested that the RIA is also applicable and useful for developing and surveying fishing grounds in areas where ciguatera outbreaks can and do occur. The RIA procedure is very sensitive, and shows specificity to polyether lipid residues. Thus cross reactivity occurs with the following compounds: okadaic acid, brevetoxin, monensin, and other polyether-containing lipid residues. Other disadvantages of the RIA include expensive equipment and handling of radioisotopes (short-life of reagents). For these reasons the enzyme-linked immunosorbent assay has been developed in our laboratory and is presently being evaluated.

ELISA Method: This method is similar to that of the RIA in which CTX is measured directly from fish tissues. The sheep anti-CTX is conjugated to horseradish peroxidase (HRP) and incubated directly with a tissue sample of standard size (a disc 3 mm in diameter × 2 mm thick having a surface area of 37.7 sq mm and weighing 11.0 ± 2.0 mg). The subsequent steps involve washing of the tissue samples followed by addition of the freshly prepared 4-chloro-1-naphthol substrate for HRP. The violet-blue color which develops is then examined at 405 nm, after removal of the tissue in a Titertek Multiskan spectrophotometer (Flow Laboratories). The critical factors are: 1, Tissue sample size should be consistent and uniform; 2, fixation of tissues with freshly prepared H_2O_2–MeOH for 3 min is necessary; 3, sample to be tested should be reasonably fresh and maintained frozen until tested; and 4, if tissues are difficult to cut, they should be frozen with Cryokwik Freon.

Presently, the ELISA method is being assessed by following a procedure similar to that used successfully for the RIA: 1, Examination of fish tissues from clinically documented ciguatera poisoning from the Hawaii State Department of Health and the Food and Drug Administration through the courtesy of the General Referee; 2, testing of commercial *S. dumerili* (amberjack, kahala); 3, survey of fish from Hawaii and the Northwest Hawaiian Islands for fishing development; and 4, assessment of fish caught by the sport fishermen. Corresponding examinations of some of the same fish will be carried out with the mouse bioassay (4) and the guinea pig atrium test (6).

It is planned to have the ELISA procedure evaluated within a year as to its practicality, specificity, and sensitivity.

The General Referee notes that under an FDA contract, the Associate Referee has delivered ELISA kits to FDA 5 for evaluation. Results of this evaluation should be available in early 1983.

In addition to the above methodology for the detection of ciguatoxic fish, a counterimmunoelectrophoresis (CIEP) technique has been developed which appears to have the ability to distinguish toxic from nontoxic fish (D. L. Emerson et al., Medical University of South Carolina, Charleston, SC, private communication). Differentiation between toxic and nontoxic fish was supported by an in vivo mouse bioassay. The CIEP procedure is being submitted for publication.

Shellfish Poison.—Associate Referee William L. Childress (FDA, Boston, MA) resports that, although during the past year experimental work on chemical methods for PSP has been minimal, valuable information has been obtained. In his laboratory, an HPLC method was investigated by pre-column derivatization of the PSP toxins through $NaOH/H_2O_2$ oxidation, column separation of the oxidized toxin, and fluorometric detection. The oxidation procedure is similar to the fluorometric assay for the detection of saxitoxin (STX) reported by Bates et al. (7). The HPLC system consisted of a C_{18} analytical column (Ultrasphere IP, Beckman, 5 μm, 15 cm × 4.6 mm) with a pellicular C_{18} guard column (Co-Pell ODS, Whatman, 7 cm × 2.1 mm). The mobile phase was 2% CH_3CN in 0.02M NH_4Ac, pH 5.0. The detector was a Perkin-Elmer MPF-3L fluo-

rescence spectrophotometer with a 20 μL flow cell (Alltech); excitation was at 335 nm with emission at 392 nm. For sample preparation, 1.0 mL of the toxin solution (ca 80 μg/mL) was mixed with 1.0 mL of 1.0N NaOH and 50 μL of 10% H_2O_2. After 40 min, the pH was adjusted to ca 5.0 with acetic acid and 50 μL was injected into the HPLC system.

No response was obtained for neosaxitoxin (neoSTX), gonyautoxin-I (GTX-I), and gonyautoxin-IV (GTX-IV). However, multiple peaks were observed in the chromatograms for saxitoxin, gonyautoxin-II (GTX-II), and gonyautoxin-III (GTX-III). The Associate Referee concludes that, because multiple products are formed and not all the toxins respond, the Bates et al. approach is not suitable for the quantitation of the individual PSP toxins. The Referee concurs.

The Associate Referee reports reviewing a manuscript entitled "High Pressure Liquid Chromatographic Determination of Toxins Associated with Paralytic Shellfish Poisoning" by J. Sullivan and W. Iwaoka, University of Washington, Seattle, WA. The paper describes an HPLC separation of STX, neoSTX, GTX-I to IV and separation of B_1, B_2, C_1, and C_2, N-sulfocarbamoyl derivatives of STX, neoSTX, GTX-II, and GTX-III, respectively, found in *Protogonyaulax* sp. (8). Partial separation was achieved on a bonded phase cyano column with detection by fluorescence following post-column alkaline oxidation (NH_4^+, periodic acid). Separation of GTX-I from IV and C_1 from C_2 was not attained; both pairs co-elute. Application of the procedure to a toxic (mouse assay) mussel (*M. edulis*) extract revealed the presence of GTX-II, GTX-III, and STX with fair resolution. It is evident that the procedure has promise, but further refinement is necessary. The Referee concurs after reviewing a copy of the manuscript (9).

In view of the above results, the Associate Referee will be investigating HPLC/electrochemical detection of PSP, since it is known that STX can be reduced at −0.8 to −0.9V on a dropping mercury electrode (R. Gajan, FDA, private communication, 1982) and the analogs of saxitoxin can be chemically reduced to STX (10). The chromatography reported by Sullivan and Iwaoka will be used in evaluating the electrochemical behavior of these toxins.

The General Referee reports that at a recent PSP management workshop sponsored by the California Department of Health Services and FDA in Berkeley, CA, September 21-23, 1982, 2 new significant approaches were presented for the detection of PSP. The first was a fly bioassay, a modification of a procedure reported to detect insect toxins (11), being developed at the University of Southern California, Los Angeles, CA, as an alternative method to the AOAC mouse bioassay. The method, presented by A. Siger and demonstrated at the workshop by A. Andrasi, utilizes the domestic house fly (*Musca domestica*) and appears to be comparable in sensitivity, if not greater, to the mouse assay. It has the advantage of being much less costly and is able to detect very small (fmole) amounts of toxin. The fly assay is currently being evaluated against a number of East/West Coast extracts of toxic shellfish previously analyzed by the mouse bioassay. If results are shown to be compatible, the Refereee recommends that the assay be collaboratively studied.

The other approach was an RIA double antibody method for the detection and quantitation of STX presented by P. Guire, Bio-Metric Systems, Inc., Eden Prairie, MN. The RIA procedure, a forerunner of an eventual Ciba EMIT® field test kit for PSP, was shown to quantitate STX (detection limit 4 ng ± 10%).

The method was able to detect only 13-33% of the total amount of toxin in West Coast mussel extracts determined by the mouse assay, but when STX is added to these shellfish extracts it is recovered quantitatively (~99%). Since STX is usually a minor constituent in toxic West Coast mussels compared to the gonyautoxins (J. Meyer & Y. Shimazu, unpublished results), it is reasoned that the method appears specific only for STX. Cross reactivity with the gonyautoxins is being investigated.

In a prior report (1), the Referee noted that a sodium channel binding exchange assay with tritium-labeled STX was being investigated by Richard Rogart, Harvard Medical School, Children's Hospital Medical Center, Boston, MA. Dr. Rogart has standardized his procedure and completed assaying a number of East Coast (Maine) shellfish extracts previously examined by the mouse assay; his results are forthcoming.

Tetrodotoxin.—Associate Referee Yuzuru Shimizu (College of Pharmacy, University of Rhode Island, Kingston, RI) reports on headway towards the development of a chemical assay for tetrodotoxin (TTX). However, he is still investigating the biosynthetic origin of TTX, which remains a mystery. The toxin, as mentioned in the last report (1), occurs in pufferfish, gobies, newts, blue-ring octopus, and marine snails. Even within identical species, the toxicity levels

vary greatly. This rather whimsical pattern of toxin occurrence raises the question about the true origin of the toxin (12): It is produced by the organism itself or not, and how is it biosynthesized? Our attempt to study the biosynthesis of TTX in Atlantic pufferfish, *S. maculatus*, and toxic newts, *Taricha* sp., by studying the uptake of common precursors (^{14}C-labeled acetate, citrulline, arginine, and D-glucose) were unsuccessful. In experiments with toxic TTX containing *Taricha* sp., significant incorporation of radioactivity, into the amino acid and sterol make-up of the organism was observed but no radioactivity associated with the TTX control.

The apparent lack of de novo synthesis of the toxin by the newts can be interpreted in many ways: 1, The toxin is synthesized during a very limited developmental stage and its turnover is very slow; 2, the toxin is synthesized only under certain stressed conditions as a self-defense mechanism; 3, the toxin or its key precursor comes from a special dietary source; 4, the toxin stored by the newt is synthesized by a symbiotic microorganism, but in captivity, the conditions are not suitable for the organism or toxin production; 5, the precursors cannot reach the synthesis site; etc. Our results have a remarkable resemblance to those observed in the biosynthetic study of another potent neurotoxin, batrachotoxin, in the frog, *Phyllobates aurotaenia* (13). The frog failed to synthesize the toxin from [1-^{14}C]-acetate, and the F1 frogs reared in captivity completely lacked the toxin. Interestingly, the puffer fish, *Fugu rubripes rubripes*, commercially grown in Japan are completely devoid of toxicity when they are hatched from eggs and hand-raised. Furthermore, the feeding of pure tetrodotoxin to nontoxic fish did not result in the toxin being accumulated in the body (14). The need for analytical methods is evident in our search for the origin of TTX and for its detection in pufferfish, which is prized as a delicacy. The Referee concurs.

Recommendation

Continue study on all topics.

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GENERAL REFEREE REPORTS: COMMITTEE D

Report on Alcoholic Beverages

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Felipe Alfonso of the Bureau of Alcohol, Tobacco and Firearms has been appointed Associate Referee for Vanillin and Ethyl Vanillin in Alcoholic Beverages. Duane H. Strunk of J. E. Seagram & Sons Co., Inc., has been appointed Associate Referee for Alcohol Content of High Solids Distilled Spirits and has completed a collaborative study. The method utilizes a smaller sample size (50 mL), an all-glass distillation apparatus, and the Mettler/Parr density meter for determination of proof. Ten collaborators participated; results indicate very good precision and accuracy. The Associate Referee recommends the method for adoption as official first action, and the General Referee concurs.

Arthur Caputi, Jr, of the E. & J. Gallo Winery, Associate Referee for Ethanol in Wine by GLC, reports a collaborative study involving 14 collaborators. In addition, analysts were requested to provide information on the type of dilution techniques used. Overall, results were good and the Associate Referee recommends adoption of the method as official first action; the General Referee concurs. In the final version of the method, information on the new National Bureau of Standards Standard Reference Material 1590 (stabilized wine with ethanol content of 18.57% ± 0.16% at 20°C) will be incorporated for reference and its availability for use by analysts will be noted.

A. J. Cutaia with the Stroh Brewery and AOAC-ASBC (American Society of Brewing Chemists) Liaison Officer and the Associate Referee for Brewing Materials has proposed an ASBC collaboratively studied GLC method for ethanol in beer for adoption as official first action. The ASBC collaborative study compared the standard ASBC reference method (distillation-pycnometer) with the proposed GLC method; the results indicate comparability, with the GLC method having the advantage of being much faster. The General Referee concurs with the recommendation for adoption as official first action.

A collaborative study of the alcohol determination in wine by dichromate oxidation has been carried out and the results are being evaluated.

The Associate Referee for Carbon Dioxide in Wine, Arthur Caputi, recommends that the volumetric method (**11.063-11.065**) be surplused; the General Referee concurs.

The Associate Referee for Color in White Wine, Robert Dowrie, reports that no work is being carried out and there are no plans for future effort; he recommends that the topic be dropped, and the General Referee concurs. Likewise, the topic Reducing Sugars should be dropped. A collaborative study is being planned for malic acid in wine.

Recommendations

(1) Delete the topics Color in White Wine, and Reducing Sugars.

(2) Surplus the volumetric method (**11.063-11.065**) for carbon dioxide in wine.

(3) Carry out collaborative study of malic acid in wine.

(4) Adopt as official first action the method for alcoholic content of high solids distilled spirits.

(5) Adopt as official first action the method for ethanol in wine by GLC.

(6) Adopt as official first action the method for ethanol in beer by gas chromatography.

(7) Continue study on all topics.

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

The recommendations of the General Referee were approved by Committee D and were adopted by the Association, except for ethanol in beer. See the report of the Committee and "Changes in Methods," this issue.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition

Report on Cereal Foods

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Phytates.—Associate Referee Barbara Harland is continuing study on a new phytate method reported at the last annual meeting.

Iron.—James Martin, FDA, Atlanta Center for Nutrient Analysis, was appointed Associate Referee for this topic. He is planning a mini-collaborative study for modification of method **14.012**.

Starch.—Bert D'Appolonia, North Dakota State University, was appointed Associate Referee to replace Robin Saunders. No new methods are presently under study.

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

The recommendation of the General Referee was approved by Committee D and was accepted by the Association. See the report of the Committee, this issue.

Recommendation

Continue study of methods for phytate and iron.

Report on Fruits and Fruit Products

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Adulteration of Orange Juice by Pulpwash and Dilution.—The Referee recommends that work continue on this subject.

Fruit Acids.—During the past year, Associate Referee E. D. Coppola has been working with high pressure liquid chromatography (HPLC) using 2 reverse phase C_{18} columns to separate the different acids in fruits. He has been able to separate the most important acids in cranberry, apple, and grapefruit juices, but has had difficulty obtaining reproducibility because of column deterioration. Accordingly, the Referee recommends continued study.

Fruit Juices, Identification and Characterization.—This refereeship is vacant. The Referee recommends that an Associate Referee be appointed.

Iso-Ascorbic Acid (Erythorbic Acid-Antioxidant) in Fruit Purees.—This refereeship is vacant. The Referee recommends that an Associate Referee be appointed.

Orange Juice Content.—Associate Referee Carl Vandercook reports that there is a continuing interest in the problem of detecting adulteration in citrus juices on the national and international levels. The Associate Referee has been in contact with several leading scientists working on the problem. Data have been shared and plans for future cooperation are underway. Two different pattern recognition techniques are being considered. It is recommended that the study on orange juice content be continued.

Soluble Solids in Citrus Fruit Juices as Degrees Brix.—A final rule on the standard of identity and fill of container for grapefruit juice was published in the Federal Register of January 27, 1981 (46 FR 8462). This standard provided for a correction factor based on acidity to be added to the sucrose value obtained by the refractometer to yield true % soluble solids. An objection to this standard pointed out that no similar provision was made to convert the refractometer reading to true degrees Brix. Most citrus juices are purchased by the amount of fruit solids present as determined by degrees Brix. Hence there is a valid need for a correction factor, based on acidity, to convert the refractometer reading to true degrees Brix.

Yeatman, Senzel, and Springer (*J. Assoc. Off. Anal. Chem.* **59**, 368 (1976)) developed two cor-

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Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition.

rections based on acidity to be added to the sucrose values obtained by the refractometer to yield true soluble solids and true degrees Brix. The correction factor for obtaining true soluble solids is published in the current edition of *Official Methods of Analysis* (**22.025**).

During the past year Committee D, on the Referee's recommendation, granted interim first action status to the method described below. The method should be adopted official first action.

Soluble Solids in Citrus Fruit Juices as Degrees Brix

Proceed as in **22.024.** Correct values for sucrose by refractometer for acidity by adding $(0.012 + 0.193x - 0.0004x^2)$ to sucrose value, where x = % anhyd. citric acid in sample as detd in **22.060** or **22.061.**

Recommendations

(1) Appoint Associate Referees on the following topics: Fruit Juices, Identification and Characterization; and Iso-Ascorbic Acid (Erythorbic Acid-Antioxidant) in Fruit Purees.

(2) Adopt as official first action the method for soluble solids in citrus fruit juices as degrees Brix.

(3) Continue study on all other topics.

Report on Nonalcoholic Beverages

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The Associate Referee for caffeine and methyl xanthenes in nonalcoholic beverages conducted a collaborative study on caffeine in cola beverages last year. The method was not approved because data did not give a measure of accuracy. The Associate Referee plans to determine caffeine content in cola extracts and compare the proposed method with existing official methods.

Recommendations

(1) Determine the accuracy of the proposed method for caffeine in cola beverages and further study other methyl xanthenes in nonalcoholic beverages.

(2) Appoint an Associate Referee on Lasiocarpine and Pyrrolizidines in Herbal Beverages.

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

The recommendations of the General Referee were approved by Committee D and were accepted by the Association. See the report of the Committee, this issue.

Report on Preservatives and Artificial Sweeteners

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Benzoates, Saccharin, and Caffeine (High Pressure Liquid Chromatography).—Associate Referee B. Woodward and others (1, 2) previously reported a successful collaborative study of a method which was subsequently adopted official first action for soda beverages (**12.050–12.053**) in 1979. During the past year, she conducted some additional studies on fruit drinks. The mobile phase was modified to increase efficiency of the analysis, and results appear to be promising. It is recommended that study be continued.

Formaldehyde in Olives.—Associate Referee R.

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

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

Section numbers refer to *Official Methods of Analysis* (1980) 13th edition; and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **63**, 374–423 (1980).

J. Reina has improved the sensitivity of his previous method by conducting a recovery study for formaldehyde in olives at a level of 1.02 ppm. The following procedure was used: The drained olives were blended in a Hobart blender to a paste-like consistency. One hundred g of this composite was spiked with formaldehyde at 1.02 ppm and acidified with HCl. A derivative of formaldehyde was formed by adding a solution of $CHCl_3$ containing 2,4-DNPH. The mixture was refluxed under a water condenser, cooled, and filtered. A portion of the $CHCl_3$ extract was placed on an acidic alumina column which also contained $NaHCO_3$ and anhydrous Na_2SO_4. The column was eluted with a mixture of $CHCl_3$, hexane, and ethyl ether to separate the hydrazone derivative from the interfering oils. The eluate containing the HCHO-2,4-DNPH derivative was evaporated to dryness, and the residue was dissolved in hexane and transferred to a separatory funnel. The derivative was extracted from the $CHCl_3$ with acetonitrile. A portion of this acetonitrile extract was further cleaned up through a C_{18} Sep-Pak cartridge prior to injecting it into a liquid chromatograph. A 10 μm C_{18} column was used with a mobile phase of chloroform–water (55 + 45) and a UV detector at 254 nm. The recovery was 95.1% at the 1.02 ppm level. It is recommended that a limited within-laboratory study be conducted to determine its reproducibility prior to a collaborative study.

Meats, Ground, Screening Method for Chemicals and Added Blood.—Associate Referee J. J. Maxstadt (3) previously reported on a method which was adopted official first action for chemical preservatives in ground meats (**20.A01-20.A05**). A limited study was subsequently conducted to improve the procedure for added blood. Although no work was performed during the past year because of other commitments, additional studies are planned for the coming year. It is recommended that study continue.

Organic Preservatives (Thin Layer Chromatography).—Associate Referee C. P. Levi previously conducted a limited within-laboratory study with 6 types of food spiked with 9 common preservatives: *p*-hydroxybenzoic acid, methyl paraben, ethyl paraben, propyl paraben, butyl paraben, salicylic acid, potassium sorbate, benzoic acid, and dehydroacetic acid. The results were promising and additional work was planned; however, no report was received during the past year. It is recommended that study continue.

Saccharin in Foods (Differential Pulse Polarography).—Associate Referee W. Holak previously reported on a successful collaborative study which was subsequently adopted official final action (**20.A06**) in 1982. No report was received during the past year. It is recommended that the study be continued.

Benzoic Acid and Sorbic Acid in Foods (Gas-Liquid Chromatography).—B. K. Larsson conducted a collaborative study for the simultaneous determination of benzoic acid and sorbic acid in foods by gas-liquid chromatography. The study was performed by 8 laboratories on 3 food products: almond paste at 2 levels (1–2 g/kg), fish homogenate at 2 levels (0.5–2 g/kg), and apple juice at 3 levels (0.04–1 g/kg). The acids are converted to trimethylsilyl (TMS) esters and then determined by gas-liquid chromatography. Phenylacetic acid and caproic acid are used as internal standards for benzoic acid and sorbic acid, respectively. A statistical evaluation of the data excluded 6 of the results as outliers, failure to follow instructions, or not reported. Three of these excluded results were for the lowest level (0.04 g/kg) of benzoic acid and sorbic acid in apple juice. Excluding these values, the overall accuracy for the method ranged from 94.2 to 105.9% for benzoic acid, and from 96.4 to 109.1% for sorbic acid. The results appear to be satisfactory in almond paste, fish homogenate, and apple juice at levels ranging from 0.5 to 2 g/kg; however, at the lowest level for apple juice (0.04 g/kg), the relatively poor reproducibility (14.7–23.3% CV) and the low number of determinations (5 laboratories) indicate that further study is needed at this level. It is recommended that the method be adopted official first action for almond paste, fish homogenate, and apple juice at levels ranging from 0.5 to 2 g/kg and that additional studies be conducted to improve the reproducibility at the 0.04 g/kg level, using the modifications suggested by the author.

Recommendations

(1) Appoint Associate Referees for the topics Benzoates and Hydroxybenzoates in Foods; Preservatives (Quantitative Methods).

(2) Continue official first action status of the method for determination of sodium saccharin, sodium benzoate, and caffeine by HPLC (**12.050-12.053**); continue study for the applicability to other compounds and for quantitating potassium sorbate.

(3) Continue official first action status of the method for chemical preservatives in ground meat (**20.A01-20.A05**); continue study of the method for added blood.

(4) Adopt as official first action the method for GLC determination of benzoic acid and sorbic acid in almond paste, fish homogenate, and apple juice at levels ranging from 0.5 to 2 g/kg; continue study of the method to improve the reproducibility at a level of 0.04 g/kg.

(5) Continue study on all other topics.

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Report on Spices and Other Condiments

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Having been appointed General Referee near the end of May 1982, I cannot report much progress in this area. It is recommended that Associate Referees be appointed to the 2 vacant topics: Ash and Pungent Principals in Mustard; Vinegar.

Recommendations

(1) *Ash and Pungent Principals in Mustard.*—Continue study to determine if there is continued interest.

(2) *Characterization of Natural Spices.*—Discontinue topic, based on discussions with members of the Executive Committee of the Technical Group of the American Spice Trade Association. This committee represents a cross section of the technical expertise of the spice industry and it is their opinion that there is little likelihood of the industry agreeing on any characterization over that published in Title 21 of the *Code of Federal Regulations*, 101.22(a)(2).

(3) *Extractable Color in Capsicum Spices and Oleoresins.*—Continue study, based on the recommendation of the Associate Referee, J. E. Woodbury.

(4) *Moisture in Dried Spices.*—Continue study, based on recent interest generated within the spice industry.

(5) *Vinegar.*—Continue study to determine if need and interest exist.

(6) *Pungency of Capsicums and Oleoresins.*—Establish a new Associate Refereeship.

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

The recommendations of the General Referee were approved by Committee D and were accepted by the Association. See the report of the Committee, this issue.

Report on Sugars and Sugar Products

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Chromatographic Methods.—Associate Referee Michael Gray reports no new developments.

Color, Turbidity, and Reflectance—Visual Appearance.—Frank Carpenter reports no new developments on the attempts to revise the AOAC method for color in sugars to reflect the ICUMSA methods.

Corn Syrups and Corn Sugars.—Last year, Raffaele Bernetti presented the results of a collaborative study (1) to expand the scope of the official first action method for minor saccharides in corn syrup by HPLC, **31.228-31.236**, to include products containing dextrose levels in excess of 98%. This study, containing recommended amendments, was adopted interim official first action in April 1982. Dr. Bernetti now recommends adoption as official first action.

This year, Dr. Bernetti presented the results of a study, "Titratable Acidity in Corn Syrups: A Collaborative Study," co-authored by Owens and Bernetti. The statistical results show no agreement between the visual end point and pH 8.3. They recommend electrometric titration to pH 6.0 because highly refined corn syrups have a very low titre and titration curves show the inflection point at pH 6.0. Therefore, he recommends adoption as official first action of the electrometric titration at pH 6.0 to replace the present official visual phenolphthalein end point method, **31.217**.

New tables relating refractive indices, densities, and dry substance for corn syrups, including high fructose corn syrups, will be proposed for interim action to replace the present tables **31:09** and **31:10**. Refractive index information is available and documented (2) and a density study is being reviewed for submission for publication in the *Journal of Chemical and Engineering Data*.

Dry Substance.—Associate Referee Joseph Dowling had no new developments to report.

Enzymatic Methods.—Associate Referee Marc Mason has presented a paper dealing with the determination of glucose, sucrose, and lactose by using immobilized enzyme electrodes. He recommends further work with the enzymatic procedures.

Honey.—Jonathan White reports that in cooperation with Frank Robinson at the University of Florida a study has been completed on the carbon isotope ratio values for citrus honey and citrus nectar to determine if the heavier $\delta^{13}C$ values for citrus honeys are natural to the species or induced by beekeeping practices. The study shows that the average $\delta^{13}C$ value of Florida citrus honey is not significantly different from the nectar, but does differ from the mean for all U.S. honeys (3).

The Associate Referee recommends adoption as official final action the following official first action methods for honey: **31.115**, nitrogen; **31.116**, proline; **31.119(a)(b)(c)**, direct polarization; **31.133**, sugars by alternative method, II (4); **31.138**, sugars by HPLC; **31.148**, high fructose corn syrup by TLC; **31.A01**, spectrophotometric method for hydroxymethylfurfural. He also recommends deletion first action of the official first action method **31.119(d)**, direct polarization at 87°; deletion of the procedures **31.122**, dextrin (approximate) and **31.146**, commercial invert sugar—resorcinol test; adoption as official final action the official first action amendment to **31.150-31.153**, the carbon isotope ratio method for adulteration in honey.

Dr. White reports that he plans to test a convenient rapid procedure for determining diastase number using a commercially available kit.

Maple Sap and Syrup.—Kelly Baggett presented the results for Associate Referee Maria Franca Morselli of a collaborative study on the mass spectrophotometric determination of cane sugar and corn syrup in maple syrup by the $^{13}C/^{12}C$ carbon isotope ratio. Based on the study results, official first action is recommended. They report that a manuscript on sodium and chloride in sugar maple sap as a source of off-flavor has been submitted for publication in the AOAC *Journal*. A study on the GLC analysis of organic acids in maple sap has been completed to replace the existing AOAC method for malic acid. A study in cooperation with Paul Krapcho, University of

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

The recommendations of the General Referee were approved by Committee D and were adopted by the Association, except recommendations (3) and (6) and second part of (1). See the report of the Committee and "Changes in Methods," this issue.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition; and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **63**, 374-423 (1980); **64**, 501-540 (1981); **65**, 450-521 (1982).

Vermont, is planned on the detection of beet sugar in maple products by using nuclear magnetic resonance spectroscopy. Dr. Morselli, with her staff, will continue to study proposed revisions of methods for maple products appearing in the 13th edition. Some preliminary suggestions were presented at the symposium on the Detection of Deliberate Adulteration of Foods.

Stable Carbon Isotope Ratio Analyses.—Associate Referee Landis Doner reports that the carbon isotope ratio method is being used extensively for apple juice and orange juice with no adverse comment (5). He recommends adoption as official final action of the official first action stable carbon isotope ratio methods, **22.B01-22.B04** and **22.C01-22.C04** for the detection of corn syrups in apple juice and orange juice, respectively.

Standardization of Sugar Methods of Analysis.—Whitney J. Newton has resigned as Associate Referee, and Margaret A. Clark of Sugar Processing Research, Inc., has been appointed to fill the position. She plans to continue with the evaluation of methods commonly used for sugar analyses.

Sugars in Cereals.—Associate Referee Lucian Zygmunt reports that he has received no objections regarding the official first action HPLC method for the determination of sugars in sweetened cereals, **14.C01-14.C04** (6), and recommends adoption as official final action. He reports that he has been evaluating a series of cation exchange columns for use in the determination of sugars, alcohols, and organic acids in food products; glycerol and sorbitol determination appears feasible by the procedure.

Sugars, Reducing.—Since this topic has no Associate Referee, it is recommended that the topic be discontinued.

Sugar in Sugar Cane.—Luis Vidaurreta reports that he has been studying the retention of sugars by the cellulose in cane bagasse method for increased liberation of the sugars and analyses by HPLC.

Weighing, Taring, and Sampling.—Associate Referee Melvin Lerner reports changes in the U.S. Customs handling of raw sugar importations. The changes effectively reduce the number of raw sugar samples per ton submitted for analysis by about 90%, and also reduce routine Customs supervision of weighing, taring, and sampling of the raw sugar importations at the importers' premises. The changes also require that entries not scheduled for sampling may be assessed duty based on a settlement value expressed in sugar degrees obtained by the current official method for raw sugar recognized by the International Commission for Uniform Methods of Sugar Analysis (Raw Sugar, page 25) (7).

Other Topic.—Saucerman and Winstead presented the study of the rapid HPLC method for the quantitative determination of lactose at high levels of purity. The results look promising. Moreover, the authors have presented the additional precision and reproducibility data, applicability data, and a study by one collaborator using 3 different instrument systems. The authors also state that ruggedness and interlaboratory tests were conducted. The Referee recommends adoption of the HPLC method as official first action, and appointment of one of the authors as Associate Referee.

Recommendations

(1) Adopt as official first action the interim official first action amendment to HPLC method **31.228-31.236** for the determination of minor saccharides in corn syrups to include products with dextrose levels in excess of 98% as described the Associate Referee; adopt as official first action the electrometric titration method at pH 6 for the determination of titratable acidity in corn syrups to replace the official visual phenolphthalein end point method, **31.217**.

(2) Adopt as official final action the following official first action methods for honey: **31.115**, nitrogen; **31.116**, proline; **31.119**(a)(b)(c), direct polarization; **31.133**, sugars by alternative method II; **31.138**, sugars by HPLC; **31.148**, high fructose corn syrup by TLC; **31.A01**, spectrophotometric method for hydroxymethylfurfural. Adopt as official final action the official first action amendment to **31.150-31.153**, the carbon isotope ratio method for adulteration in honey: "Samples with a $\delta^{13}C$ value between −23.4 and −21.5‰ are considered adulterated only if a positive result is obtained from TLC method **31.148-31.149**." Delete first action the official first action method **31.119(d)**, direct polarization at 87°C. Delete procedures **31.122**, dextrin (approximate), and **31.146**, commercial invert sugar—resorcinol test.

(3) Adopt as official first action the mass spectrometric stable carbon isotope ratio analysis for determination of cane sugar and corn syrups in maple syrups as presented by the Associate Referee.

(4) Adopt as official final action the official first action mass spectrometric stable carbon isotope ratio analysis **22.B01-22.B04** for deter-

mination of corn syrup in apple juice. Adopt as official final action the official first action mass spectrometric stable carbon isotope ratio analysis **22.C01-22.C04** for determination of corn syrups in orange juice.

(5) Adopt as official final action the official first action method, **14.C01-14.C04**, for HPLC determination of sugars in sweetened cereals.

(6) Adopt as official first action the HPLC determination of lactose at high purity levels; appoint an Associate Referee.

(7) Discontinue the topic Sugars, Reducing.

(8) Continue study on all topics unless specifically cited.

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Report on Vitamins and Other Nutrients

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At the 96th Annual Meeting a "Brain Storming Session on HPLC Oil-Soluble Vitamin Assays of Foods" was conducted. Many HPLC assays have been published but we only have an official status method for one of these vitamins. This was a 4 h working session of international scientists considering applicable methodology for initiation of a collaborative study for the AOAC in this analytical area.

Associate Referee reports and contributed papers were presented at the Vitamin and Other Nutrients section meeting.

Harry Lento of Campbell Soups resigned as Associate Referee for Fat in Food by Chloroform-Methanol Extraction, and Chester E. Daugherty of that organization replaced him.

Edgar Elkins of National Food Processors Association was appointed Associate Referee for Sodium in Foods.

Recommendations

(1) Adopt as official first action the method for fat in food by chloroform-methanol extraction as recommended by the Associate Referee.

(2) Adopt as official first action the collaboratively studied semi-automated method for vitamin C in food products.

(3) Adopt as interim official action the net protein ratio evaluation of protein recommended by the Associate Referee.

(4) Adopt as official final action the HPLC method, **43.C01-43.C09**, for vitamin D in mixed feeds, premixes, and pet foods.

(5) Continue study on all other topics.

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

The recommendations of the General Referee were approved by Committee D and were adopted by the Association, except recommendations (2) and (3). See the report of the Committee and "Changes in Methods," this issue.

Section numbers refer to "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **65**, 450-521 (1982).

GENERAL REFEREE REPORTS: COMMITTEE E

Report on Carbamate Pesticides, Fumigants, and Miscellaneous

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Associate Referee for Sodium Monofluoroacetate (1080), H. M. Stahr, Iowa State University, is attempting to select the best method for study. The NMR detection method has been eliminated because of insensitivity. The best determinative approaches appear to be HPLC (with or without derivatization), TLC using selective derivatization, and GLC. Work is continuing along these lines.

R. Krause, FDA, Associate Referee for Carbamate Insecticides, Liquid Chromatographic Method, recently began a 2-phase collaborative study of his HPLC method for *N*-methyl carbamate insecticides. Phase I will provide recovery data and example chromatograms to assure that the HPLC equipment is operating properly and that the 8 collaborators have become proficient in using the method. Phase II will provide accuracy and precision data for samples fortified with unknown carbamate solutions furnished by the Associate Referee.

R. W. Young of Virginia Polytechnic Institute, Associate Referee for Carbamate Insecticides, Gas-Liquid Chromatographic Methods, and Sujit Witkonton, FMC Corp., Associate Referee for Carbofuran, were involved in laboratory moves this year and therefore did not report any work or progress.

T. Dumas of Agriculture Canada, Associate Referee for Phosphine, has developed a trapping method in conjunction with E. J. Bond (*J. Chromatogr.* (1981) **206,** 384–386) for GLC determination of low levels of phosphine in cargo ships after fumigation of their grain cargoes. The next study should be an adaptation of the method for residue work on grain crops.

All other Associate Referees had little or no progress to report. The topics Fenvalerate, Fumigants, and Permethrin remain open. This is my last report to AOAC because I have resigned as General Referee.

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The recommendations of the General Referee were approved by Committee E and were accepted by the Association. See the report of the Committee, this issue.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition.

Recommendations

(1) *Carbamate Insecticides, Gas-Liquid Chromatographic Methods.*—Prepare write-up of the on-column silylation gas-liquid chromatographic method for determining carbamate insecticide residues as their silylated derivatives for review and comment by the General Referee, and if the write-up is approved, conduct interlaboratory trial of the method.

(2) *Carbamate Insecticides, Liquid Chromatographic Methods.*—Complete the collaborative study of the HPLC method for determining *N*-methyl carbamate residues in crops (*J. Assoc. Off. Anal. Chem.* (1980) **63,** 1114–1124).

(3) *Carbofuran.*—Continue study of analytical methods for determining carbofuran, its carbamate metabolites, and its phenolic metabolites in foods.

(4) *Ethylene Oxide and Its Chlorohydrin.*—Continue study of the gas-liquid chromatographic method of Scudamore and Heuser (*Pestic. Sci.* (1971) **2,** 80–91) for determining ethylene oxide, ethylene chlorohydrin, and ethylene bromohydrin in foods.

(5) *Fenvalerate.*—Appoint an Associate Referee; continue study to evaluate analytical methods for determining residues of fenvalerate in foods.

(6) *Fumigants.*—Appoint an Associate Referee; continue study to extend the official final action gas-liquid chromatographic method for determining volatile fumigants in grain, **29.056–29.057,** to cover additional fumigants (1,2-dichloroethane, methyl bromide, and tetrachloroethylene) and additional food (citrus fruits, milled products, and baked goods).

(7) *Inorganic Bromides in Grains.*—Continue study of the gas-liquid chromatographic method of Heuser and Scudamore (*Pestic. Sci.* (1970) **1,** 244–249) for determining inorganic bromides after conversion to 2-bromoethanol, as tested in interlaboratory studies on grain (*Analyst* (1976) **101,** 386–390) and lettuce (*J. Assoc. Off. Anal. Chem.* (1979) **62,** 1155–1159).

(8) *Permethrin.*—Appoint an Associate Referee; continue study to evaluate analytical methods for determining residues of permethrin in foods.

(9) *Phosphine.*—Continue study of methods for determining residual phosphine in fumigated products, including the Associate Referee's modified gas chromatographic determination of phosphine (*J. Assoc. Off. Anal. Chem.* (1978) **61,** 5–7) and the gas-liquid chromatographic method reported by T. W. Nowicki (*J. Assoc. Off. Anal. Chem.* (1978) **61,** 829–836) and the most recent method by Dumas and Bond (*J. Chromatogr.* **206,** 384–386 (1981) for determining the total residue of intact phosphine and phosphine derived from residual aluminum phosphide in wheat.

(10) *Resmethrin.*—Continue study to evaluate analytical methods for determining residues of resmethrin in foods.

(11) *Sodium Monofluoroacetate.*—Continue study of the methods described by the Associate Referee in 1982, with gas-liquid chromatography, thin-layer-chromatography, and high performance liquid chromatography for determining residues of sodium monofluoroacetate in foods.

Report on Fungicides, Herbicides, and Plant Growth Regulators

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Six Associate Referee reports were received. There were two resignations: W. P. Cochrane, Associate Referee for 1-Naphthaleneacetic Acid and 1-Naphthaleneacetamide; and H. A. McLeod, Associate Referee for Diquat and Paraquat. Three new Associate Referees were appointed: Brian Worobey replaces H. A. McLeod, while Dalia Gilvydis is the Associate Referee for Captan and Related Fungicides, and Mikio Chiba is Associate Referee for Benzimidazole-Type Fungicides.

Benzimidazole-Type Fungicides.—Associate Referee Mikio Chiba reports that the high pressure liquid chromatographic procedure previously described for benomyl and methyl-2-benzimidazole carbamate (*J. Assoc. Off. Anal. Chem.* (1980) **63,** 1291–1295) is being studied for possible modifications to simplify the extraction and determinative steps. These modifications include replacement of the 0°C extraction with one at room temperature and substitution of a reverse phase system for the normal phase silica column.

Captan and Related Fungicides.—Associate Referee Dalia Gilvydis reports that gas-liquid chromatographic systems for the quantitation of captan, folpet, and captafol have been evaluated and a 5% SP-2401 column on 100–120 mesh Supelcoport was selected. PAM I and II procedures are being studied and recovery data are being accumulated for different substrates.

Chlorophenoxy Alkyl Acids.—Associate Referee Alan Smith has initiated collaborative study of a gas-liquid chromatographic method for 2,4-(dichlorophencxy)acetic acid in wheat straw and dried green wheat with 12 laboratories participating. The method, based on that by Jensen and Glas (Dow Chemical Co., Midland, MI), is being applied to duplicate samples of each material fortified at 3 levels.

Diquat and Paraquat.—Associate Referee Brian Worobey reports that diquat and paraquat were analyzed simultaneously in potatoes by a modification of the borohydride reduction method of King (*J. Agric. Food Chem.* (1978) **26,** 460). Samples which were fortified and aged yielded recoveries of 36–63% at levels from 0.05 to 5.0 ppm. Precision was good (SD ± 3%). An intralaboratory study is being conducted.

1-Naphthaleneacetic Acid and 1-Naphthaleneacetamide.—Associate Referee William Cochrane reports that because of insufficient collaborators for the high pressure liquid chromatographic method, he is resigning his position as Associate Referee and recommends that the topic be discontinued.

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

The recommendations of the General Referee were approved by Committee E and were accepted by the Association. See the report of the Committee, this issue.

Organotin Fungicides.—Associate Referee Richard Cannizzaro will attempt to initiate a collaborative study of his method for organotin fungicides within the next year.

Pentachlorophenol.—Associate Referee Arnold Borsetti reports that an intralaboratory study of his method for pentachlorophenol in seafood, eggs, and gelatin has been completed. Overall recoveries below 0.10 ppm were 72% and averaged 101% at 0.5 and 1.0 ppm. The presence of negative peaks in the chromatograms affected quantitation at lower levels and requires rectification before the method is ready for interlaboratory trial.

Recommendations

(1) *Anilazine.*—Appoint an Associate Referee to conduct a collaborative study of the high pressure liquid chromatographic method for anilazine described by Lawrence and Panopio (*J. Assoc. Off. Anal. Chem.* (1980) **63,** 1300–1303).

(2) *Benzimidazole-Type Fungicides.*—Continue study of room temperature extraction and reverse phase HPLC modifications of the method reported by the Associate Referee for benomyl and methyl-1-benzimidazole carbamate (*J. Assoc. Off. Anal. Chem.* (1980) **63,** 1291–1295).

(3) *Captan and Related Fungicides.*—Continue study of methods for extraction and cleanup of captan, captafol, and folpet and of the variability of GLC response of these compounds.

(4) *Carbamate Herbicides.*—Appoint an Associate Referee to study residue methods for carbamate herbicides in crops.

(5) *Chlorophenoxy Alkyl Acids.*—Evaluate results of collaborative study of the gas-liquid chromatographic method for 2,4-(dichlorophenoxy)acetic acid in wheat and wheat straw.

(6) *Chlorothalonil.*—Appoint an Associate Referee to study a gas-liquid chromatographic method for chlorothalonil in crops.

(7) *Dinitro Compounds.*—Appoint an Associate Referee to study methods for dinitro aryl herbicides.

(8) *Diquat and Paraquat.*—Continue intralaboratory study of the Associate Referee's modification of the borohydride reduction method (*J. Agric. Food Chem.* (1978) **26,** 1460) for diquat and paraquat in potatoes.

(9) *Dithiocarbamates, General Residue Method.*—Appoint an Associate Referee to test a method for dimethyl dithiocarbamates and ethylenebis(dithiocarbamates) in foods.

(10) *Maleic Hydrazide.*—Appoint an Associate Referee to test high pressure liquid or gas-liquid chromatographic methods for determining maleic hydrazide.

(11) *1-Naphthaleneacetic Acid and 1-Naphthaleneacetamide.*—Discontinue topic.

(12) *Organotin Fungicides.*—Initiate collaborative study of the gas-liquid chromatographic method for fenbutatin oxide and fentin in foods.

(13) *Pentachlorophenol.*—Continue study of means to eliminate negative peaks and improve quantitation at levels below 0.10 ppm.

(14) *Sodium o-Phenylphenate.*—Appoint an Associate Referee to study high pressure liquid or gas-liquid chromatographic methods for *o*-phenylphenol in foods.

(15) *Substituted Ureas.*—Appoint an Associate Referee to investigate methods for urea herbicides suitable for collaborative study.

(16) *Succinic Acid 2,2-Dimethylhydrazide.*—Appoint an Associate Referee; continue study.

(17) *Thiocarbamate Herbicides.*—Appoint an Associate Referee; continue study.

(18) *s-Triazines.*—Appoint an Associate Referee to conduct collaborative study of the gas-liquid chromatographic method for atrazine and cyanazine (*J. Assoc. Off. Anal. Chem.* (1980) **63,** 273).

(19) *Trifluralin.*—Appoint an Associate Referee to conduct collaborative study of a method for trifluralin in crops.

Report on Metals and Other Elements

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Atomic Absorption Spectrophotometry (AAS).—Milan Ihnat, Associate Referee, is continuing investigation of factors affecting the reliability of flame AAS measurements before consolidating AOAC and other AAS methods for individual elements into a general analytical scheme for elemental analysis of biological materials. The contributions of digestion acid-insoluble residue to total element levels in NBS Standard Reference Materials and use of matrix-matched standards have been studied, and recommendations have been made on steps necessary to improve the accuracy of AAS determinations. This work is expected to lead to a collaborative study of a general AAS analytical scheme for several elements.

Cadmium and Lead in Earthenware.—Benjamin Krinitz, Associate Referee, reports that the International Standards Organisation committees on glass and ceramics have adopted the hot leach method for leachable Pb and Cd from cookware. As reported in last year's General Referee report (*J. Assoc. Off. Anal. Chem.* (1981) **65,** 342), the method successfully underwent an international collaborative study coordinated by John Gould, Division of Chemical Technology, FDA.

Because of the relatively high variability encountered in the results of the hot leach collaborative study, questions have been raised regarding the relative variability of the determinative step of the method compared to the variability in the levels of cadmium and lead extracted from the cookware. Standard solutions, which would have allowed this comparison to be made, were not sent to collaborators in the hot leach study. However, in the collaborative study for AOAC method **25.031-25.034** conducted by Krinitz and Franco (*J. Assoc. Off. Anal. Chem.* (1973) **56,** 869), the average relative standard deviation for 7 leach solutions sent to 13 collaborators and analyzed by atomic absorption spectroscopy was 5.2% for lead and 3.9% for cadmium. One could reasonably expect comparable results in the hot leach study. Krinitz and Franco did not send actual ceramic ware samples to the collaborators, only leach solutions. However, in preparing the leach solutions they leached 54 high level and 54 low level cups for lead. Because none of the cups contained leachable cadmium, one of the lead leach solutions was spiked at 1 μg/mL Cd to *simulate* cadmium leaching. The within-laboratory coefficients of variation for the cups ranged from 17.2 to 80.0% for 24 h leaching. No between-laboratory coefficients of variation are possible because all the leaching was carried out in one laboratory. Typically the between-laboratory variability is about 1.5 times larger than the within-laboratory variability. Thus the coefficients of variation for the hot leach collaborative study, which ranged from 11.3 to 53.7% for within-laboratory (repeatability) and 26.6 to 57.6% for between-laboratory (reproducibility) fall within the range of those of the study conducted by Krinitz and Franco, which resulted in official final action adoption of method **25.031-25.034.**

Carbon Rod Atomization.—Robert Dabeka, Associate Referee, reports that a collaborative study of a rapid screening method for Pb in canned milks and infant formulas (Dabeka, R. W. (1980) "Rapid Screening Determination of Lead in Canned Milks and Infant Formulas Using Graphite-Furnace Atomic Absorption Spectrometry," Health Protection Branch Laboratory Procedure LPFC-114, Ottawa, Ontario, Canada) will be repeated within the next few months. The previous study was not successful because some collaborators used instrumentation with inadequate background correction capability. The Associate Referee has developed a technique for each collaborator to independently determine that the background correction capability of the AAS instrument used is adequate before participating in the study. Collaborators with graphite furnace/carbon rod AAS capabilities and experience in low level Pb determination are being sought to participate in the collaborative study of the method.

Emission Spectrochemical Methods.—Fred Fricke, Associate Referee, reports no progress during the past year toward conducting a collaborative

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The recommendations of the General Referee were approved by Committee E and were adopted by the Association, except recommendation (2). See the report of the Committee and "Changes in Methods," this issue. Subsequent to the Annual Meeting, the Committee adopted the hot leach method interim official first action.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition, and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **63,** 374-423 (1980); **65,** 450-521 (1982).

study of an inductively coupled plasma (ICP) method for multielement analysis of foods. However, as more ICP instruments come into general use, there is strong interest in having a collaboratively studied ICP method. During the coming year the Associate Referee will be reviewing candidate methods and seeking collaborators for such a study.

Fluorine.—Robert Dabeka, Associate Referee, reports that the fluoride method and collaborative study reported in 1981 (Dabeka, R., & McKenzie, A. (1981) *J. Assoc. Off. Anal. Chem.* **64,** 1021–1026) has not been recollaborated because of other priorities. After the recommended improvements are made to extend the analytical range and to adapt the method to fresh rather than freeze-dried foods, the method will be restudied collaboratively.

Hydride Generating Techniques.—Stephen Capar, Associate Referee, reports that various hydride generating systems presented in the literature with AAS detection are being reviewed for suitability for possible future collaborative study. Elements being considered for initial study using this technique are As, Se, and Sn.

Mercury.—Ronald Suddendorf, Associate Referee, reports no progress for the year and has resigned. A replacement Associate Referee is being sought for this topic.

Methyl Mercury in Fish and Shellfish.—Susan Hight, Associate Referee, reports that the collaborative study of the GC method (Watts, J., et al. (1976) *J. Assoc. Off. Anal. Chem.* **59,** 1226–1233; O'Reilly, J. (1982) *J. Chromatogr.* **238,** 433–444) for determination of methyl mercury in fish and shellfish has been completed successfully. Nine laboratories analyzed blind duplicates of 4 sample types (swordfish, tuna, oysters, and shrimp) at 2 levels each between 0.15 and 2.48 μg Hg/g. The overall reproducibility coefficient of variation was 10% and the overall average recovery (compared to reference laboratory values) was 108%. Data from one laboratory were not included in the statistical analysis because that laboratory did not follow the method as written.

Multielement Analysis of Infant Formula by ICP.—Ronald Suddendorf, Associate Referee, reports that 6 laboratories have completed the collaborative study of a method (unpublished) for determination of Ca, Na, P, K, Mg, Mn, Cu, Fe, and Zn in infant formula by ICP. Two other laboratories that were to have participated have dropped out and will not conduct the study because of other commitments. A preliminary review of the data indicates that the results of the study are acceptable and that the method will be recommended for interim official first action status after a complete statistical analysis of the data.

Multielement Determination after Closed System Digestion.—Walter Holak, Associate Referee, has extended the official first action closed system digestion method (**25.A01–25.A05**) for Pb, Cd, As, Se, and Zn to include Cu, Ni, and Cr. Ni and Cr are determined by differential pulse polarography and Cu by anodic stripping voltammetry. A modification of the closed system digestion allows all operations, including digestion and determination, to be carried out in a single Teflon decomposition vessel. This greatly minimizes contamination and is especially useful for determining low levels of Pb. Participants are being sought for collaborative study to extend the method to include Cu, Ni, and Cr and the modification discussed above.

Multielement Residues by Resin Column Separation.—Richard Baetz, Associate Referee, reports no progress for the year, but is conducting a literature search for a method suitable for collaborative study.

Organometallics.—Ronald Suddendorf, Associate Referee, reports no progress for the year and has resigned this Associate Refereeship. John Jones, FDA, Division of Chemical Technology, is actively conducting research in this area and has been recommended as the replacement.

Polarography.—Raymond Gajan, Associate Referee, reports no progress for the year. The dry ash–anodic stripping voltammetric method adopted as official first action last year (**25.C01–25.C07**) is being actively used for determination of lead and cadmium in foods.

Tin.—Edgar Elkins, Associate Referee, will work with Robert Dabeka in conducting a collaborative study of a rapid and simple method (Dabeka, R., & McKenzie, A. (1981) *J. Assoc. Off. Anal. Chem.* **64,** 1297–1300) for Sn in canned foods using nitric and hydrochloric acid sample digestion and nitrous oxide-acetylene flame AAS determination of Sn. Collaborators are actively being sought for conduct of this study within the next few months.

Voltammetric Methods.—Eric Zink, Associate Referee, reports no progress for the year. Recommendation of the official first action method, **25.080–25.082** (determination of Pb in milk and fruit juices by ASV), for official final action is postponed pending publication of the collaborative study results.

Recommendations

(1) Continue assessment of AAS methods and develop a comprehensive AAS analytical scheme for collaborative study.

(2) Adopt as official first action (after conversion to AOAC format) the hot leach method for leachable Pb and Cd from cookware adopted by the International Standards Organisation committees on glass and on ceramics.

(3) Collaboratively study the rapid screening method for Pb in canned milks and infant formula by carbon rod AAS.

(4) Collaboratively study an ICP method for multielement analysis of foods.

(5) Collaboratively study the fluoride method of Dabeka and McKenzie again after incorporating recommended improvements.

(6) Adopt as official first action the GC method for determination of methyl mercury in fish and shellfish studied collaboratively by Hight and Capar.

(7) Complete the statistical analysis of the ICP collaborative study of the method for multielemental analysis of infant formula. Make appropriate recommendations regarding interim official first action status.

(8) Collaboratively study the modified closed system digestion method by collaborative study to extend the official first action method (**25.A01-25.A05**) to include Cu, Ni, and Cr. Continue official first action status for this method.

(9) By way of clarification, change the title of the Associate Refereeship "Organometallics" to "Organometallics in Foods."

(10) Continue official first action status of the dry ash ASV method for Pb and Cd in foods, **25.C01-25.C07.**

(11) Collaboratively study the AAS method for Sn in foods. Continue official first action status for method **25.136-25.138.**

(12) Continue official first action status of voltammetric method **25.080-25.082,** pending publication of the collaborative study results.

(13) Continue work on all other topics.

Report on Multiresidue Methods (Interlaboratory Studies)

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Five Associate Referees have reported on the status of work on their topics. The topic Pesticides in Meat and Meat Products remains vacant.

Comprehensive Multiresidue Methodology.—Associate Referee J. Froberg will attempt in 1983 to obtain additional assistance as necessary to proceed toward a collaborative study on the improved pesticide multiresidue method of Luke et al. (*J. Assoc. Off. Anal. Chem.* (1981) **64,** 1187-1195). It will first be necessary to conduct a ruggedness test and intra- and interlaboratory trials of the method. In these trials, the standard additions approach proposed by the Associate Referee after the publication of the method will be used to offset the quantitation problems resulting from greater gas-liquid chromatographic responses for some analytes in the presence of crop extractives than in pure standard solutions. The Associate Referee should then prepare protocols for collaborative study of the method using standard additions for quantitation, if warranted by the results of the intra- and interlaboratory trials, for review and comment by the General Referee.

Gas-Liquid Chromatography (Alkaline Pre-column).—G. Miller expects during 1983 to be able to complete a publication covering the overall information on the effectiveness of the alkaline precolumn (*J. Assoc. Off. Anal. Chem.* (1969) **52,** 548-564) in providing quantitative results in actual food analyses. A collaborative study involving solutions of pesticides (dichloro-bis-(phenyl) ethane derivatives) has been completed along with subsequent intralaboratory studies to

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show effectiveness with actual food analyses.

Organophosphorus Pesticide Residues.—Associate Referee R. Laski is working toward extension of method **29.039-29.043** to include additional polar organophosphorus pesticides and metabolites of current interest. During 1983, he hopes to investigate recoveries of approximately 14 chemicals.

Pesticides in Meats and Meat Products.—This is an important topic which has been vacant for many years. An Associate Referee should be appointed to collaborate the extension of method **29.001-29.028** to the determination of organochlorine pesticide residues in meat and meat products. It is recognized that other methods may also be widely used, but an official method is needed to serve as a benchmark for the variety of methods likely to be used on meats in the future.

Pollutant Phenols in Fish.—Associate Referee Larry Smith has developed a method involving a dual column chromatographic operation to recover purified phenolic residues, derivatization, and an electron capture GLC determinative step. A wide range of phenolic and other acidic residues are recoverable by this approach. The Associate Referee is carrying out an interlaboratory evaluation of the method, with a future collaborative study if the interlaboratory evaluation is successful. The Referee concurs with this course of study.

Whole Blood.—Associate Referee H. M. Stahr has initiated a collaborative study of his method for chlorinated pesticides in whole blood (*J. Assoc. Off. Anal. Chem.* (1980), **63**, 965-969). Six samples of freeze-dried blood with lindane and mirex added by feeding a bovine animal were distributed to 8 laboratories. The Associate Referee earlier demonstrated the stability of the pesticides in the freeze-dried blood. The Associate Referee should obtain the results from the remainder of the collaborative study participants and prepare a report, including his recommendation. The method will be considered for interim adoption, depending upon the findings of the study.

Recommendations

(1) Conduct ruggedness test and intra- and interlaboratory trials of the improved pesticide multiresidue method of Luke et al. (*J. Assoc. Off. Anal. Chem.* (1981) **64**, 1187-1195), possibly using the standard additions approach subsequently proposed by the Associate Referee; conduct collaborative study as indicated by results of these studies.

(2) Submit manuscript on the performance of the alkaline precolumn gas-liquid chromatographic method for identifying and determining *o,p'*-DDT, *p,p'*-DDT, *p,p'*-TDE, methoxychlor, and Perthane as their dehydrohalogenated derivatives (*J. Assoc. Off. Anal. Chem.* (1969) **52**, 548-553) in the collaborative study of the quantitative dehydrohalogenation and determination of these pesticides in the absence of a food sample matrix, and in the intralaboratory studies of the method in actual food analyses.

(3) Continue study to extend the coverage of **29.039-29.043** to additional organophosphorus pesticides and additional crops; continue study to compile and summarize available data on the recovery of organophosphorus pesticides by this method.

(4) Appoint an Associate Referee for Pesticides in Meat and Meat Products; initiate collaborative study to test the applicability of the AOAC multiresidue methodology, **29.001-29.028**, for determining organochlorine pesticide residues in meat and meat products.

(5) Continue study on topic of Pollutant Phenols in Fish.

(6) Finalize and report the collaborative study on pesticides in whole blood.

Report on Organochlorine Pesticides

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Associate Referees are appointed to 10 topics. The topics Dicofol and Root-Absorbed Residues are vacant. A brief summary of the status and recommendations for each topic follow.

Chlordane (Wilbur Saxton, FDA, Seattle).—The Associate Referee has invested his research time this year in experiments with capillary column gas chromatography, which he sees as the most practical tool for determination of chlordane residues. Quantitation by capillary GLC would still require the current choice between use of technical chlordane or individual components as reference standards. Further work is needed on the choice of columns and investigation of temperature programming. The Associate Referee intends to continue these studies of the determinative step so that he will be able to recommend a particular technique. At that point, the technique will be tested in another laboratory.

It is recommended that work continue on the application of capillary column gas chromatography to the analysis of residual chlordane. It is also recommended that the Associate Referee initiate a collaborative study of a method combining multiresidue extraction, **29.011-29.012,** acetonitrile partitioning cleanup, **29.014,** Florisil column chromatographic cleanup and residue separation, **29.031-29.033,** and electron capture gas-liquid chromatography, **29.018,** for determining residues of *cis*-chlordane, *trans*-chlordane, octachlor epoxide (oxychlordane), and heptachlor epoxide in butter, eggs, fish, and poultry fat.

Chlorinated Dioxins (David Firestone, FDA, Washington).—Work is continuing on development and evaluation of cleanup procedures suitable for low level (parts per trillion) examination of foods for the presence of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Results from the 1981 international (U.S./Canada) interlaboratory study comparing methods for TCDD in fish have been evaluated and a report was presented at the 1982 AOAC meeting. The various methods used were capable of detecting 1-10 ppt TCDD. The great variation (29-109%) in recovery of an internal standard demonstrated the need for including a correction for recoveries as part of the quantitation technique for TCDD.

Work on a multi-column HPLC method by R. Niemann has resulted in the description of a method in which TCDD is determined by capillary column gas chromatography with electron capture detection and confirmed by multiple ion GC/mass spectrometry with 12 ions monitored. The FDA Detroit district has begun an evaluation of the individual steps of the method described by Niemann and of the method used by the Dow Chemical Co.

It is recommended that work on methods for TCDD continue with a goal of choosing a method which can be recommended for TCDD analysis at ppt levels.

Chlorobenzilate, Chloropropylate, and Bromopropylate (Roy Brosdal, FDA, Chicago).—No Associate Referee report was received.

Dicofol (Vacant).—It is recommended that an Associate Referee be appointed to develop a suitable method for dicofol residues.

Kepone (F. D. Griffith, Jr, Virginia Division of Consolidated Laboratory Services, Richmond, VA).—The Associate Referee has been unable to spend time on the project during the past year. He expects to renew his involvement in analysis of Kepone in the upcoming year because of a resurgence of interest in Virginia, now that the James River has been reopened for fishing. It is recommended that the Associate Referee continue to study methods for Kepone with a goal of identifying a method suitable for interlaboratory use.

Low Moisture-High Fat Samples (Leon Sawyer, FDA, Minneapolis).—The method for extraction of oils and pesticide residues from oilseeds and other low moisture-high fat samples, which was presented at the October 1981 AOAC meeting, has been published (Sawyer, L. D. (1982) *J. Assoc. Off. Anal. Chem.* **65** 1122-1128). It is recommended that this method be collaboratively studied during the upcoming year.

Multiresidue Methodology, Miniaturization (Ronald Erney, FDA, Detroit).—The Associate Referee completed his evaluation of the results of a collaborative study and presented it at the 1982 meeting. The method which was tested

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involves solvent extraction of fish, followed by cleanup of an aliquot of the extract on a small (4 g) column of Florisil. Residues are eluted from the Florisil column with 6% and 15% ethyl ether-petroleum ether eluants. Seven collaborators participated in the study. Recoveries of 5 added pesticides and 2 added PCB mixtures ranged from 79.3 to 100.6%, and between-laboratory coefficients of variation ranged from 6.3 to 20.6%. Two types of fish were included in the study: perch with approximately 3.8% fat and cod with approximately 0.16% fat. A supplemental cleanup step was included in the study for the 15% eluate of the perch sample to which dieldrin had been added but not to the equivalent eluate from cod. Comparison of recoveries of dieldrin through the 2 trials showed little loss of dieldrin through the supplemental cleanup (76% average recovery from perch, 73–80% range for 7 values, vs 82% average recovery from cod, 73–86% range for 7 values). The Associate Referee feels that the supplemental cleanup should be included as part of the method, and that it should be recommended for cleanup of 15% eluates of fish samples with more than 5% fat content to prevent deterioration of the GC column.

He believes that the similar micro-scale alkaline hydrolysis method which already has official status (**29.017**, "Changes in Methods" (1980) *J. Assoc. Off. Anal. Chem.* **63**, 395) is not a suitable substitute for the technique used in this collaborative study, because only a single partitioning with hexane of the alkaline-treated extract is used in that procedure, compared with a double partitioning in the procedure tested during this collaborative study. Because method **29.017** was originally developed for dehydrohalogenation of Perthane to its olefin (1), it was not tested on 15% eluates of fatty foods. Another reference to micro-scale alkaline hydrolysis methods (2) points out that ethanol-water (1 + 1), as used by the Associate Referee during the final double partitioning step, is preferable to the water used in method **29.017** in its ability to prevent emulsions during partitioning of fatty extracts.

It is recommended that the method be adopted as official first action for *p,p'*-DDE, *p,p'*-TDE, *p,p'*-DDT, dieldrin, heptachlor epoxide, and PCBs in fish, and that the supplemental cleanup step as described by Erney be included for optional use on 15% eluates and recommended for samples of fish of more than 5% fat content.

Photochemical Derivatization for Confirmation of Residue Identity (Paul Ward, FDA, Atlanta).—The Associate Referee presented at this meeting a report on his method for confirming pesticide identity based on the GLC relative retention times of pesticide photoderivatives compared with those of standard solutions. Of 35 organochlorine compounds which were found to produce usable photoderivatives when irradiated with UV light, 10 were chosen for further study. Two of the 10 were added to each of 5 food or feed products (celery, fish, eggs, cheese, and pelletized animal feed) and analysis performed by the appropriate AOAC method. Photolysis was then performed on the extracts from those methods to determine how the photoderivatization method would be affected by the presence of sample co-extractives. Derivatives formed in the sample extract were the same as those formed in pure solution (based on GLC retention times), but usually the degree of conversion was reduced by the presence of co-extractives. In no case did the presence of co-extractives prevent the formation of the derivatives. The Associate Referee recommends that the photoderivatives of selected pesticides be identified by mass spectrometry and that the results of these identifications be published.

The General Referee recommends that mass spectrometric identification of photoderivatives be performed for selected compounds, such as endosulfan I, *cis-* and *trans*-nonachlor, and octachlor epoxide, whose derivative formation was shown to be excellent, even in the presence of sample extract; results should be published.

Polychlorinated Biphenyls (Leon Sawyer, FDA, Minneapolis).—No further work has been done by the Associate Referee on this topic. It is recommended that the Associate Refereeship be maintained so that a focal point will exist for future questions about methodology for PCB.

Root-Absorbed Residues, Extraction Procedure (Vacant).—It is recommended that an Associate Referee be appointed to (*1*) evaluate recently developed information on the extraction of root-absorbed residues; (*2*) develop and test an appropriate method for root-absorbed residues based on these findings, with the goal of incorporating such a procedure into the multiresidue method, **29.001-29.018**; and (*3*) undertake interlaboratory and collaborative testing of the method.

Tetradifon, Endosulfan, Tetrasul (Lawrence Mitchell, FDA, Atlanta).—The Associate Referee has now collected samples of all 11 nonfatty foods for which method **29.029-29.034** has not yet been validated. It is recommended that the intralaboratory trials be completed with method **29.029-29.034** to validate the use of this method

for tetradifon, endosulfan I, II, and sulfate, and tetrasul in all foods now covered by **29.001-29.018.**

Toxaphene (Larry Lane, Mississippi State University).—The Associate Referee has continued to study the application of a technique in which toxaphene residues are initially separated by TLC into 15 fractions, after which each fraction is examined by capillary column GLC with electron capture detection. The purpose of this approach is to attempt to identify which components of technical toxaphene are likely to persist and appear as residues in various foods. A survey being done in the Associate Referee's laboratory will allow 31 different fish samples (composites representing 127 individual fish) to be examined by this technique and provide further information about this approach. Components which persist as residues will be analyzed by mass spectrometry to determine identity. It is recommended that the work on this topic continue in order to develop a practical means of quantitating residues of toxaphene.

Recommendations

(1) Appoint Associate Referees on Dicofol and Root-Absorbed Residues to fill vacant topics.

(2) Create a Refereeship on Gel Permeation Chromatography Cleanup for Organochlorine Residues and appoint an Associate Referee.

(3) Create an Associate Refereeship on PCBs in serum and appoint an Associate Referee.

(4) Adopt as official first action the miniaturized method for *p,p'*-DDE, *p,p'*-DDT, *p,p'*-TDE, dieldrin, heptachlor epoxide, and PCBs in fish.

(5) Collaboratively study the method for oilseeds and some related low moisture-high fat samples (Sawyer, L. D. (1982) *J. Assoc. Off. Anal. Chem.* **65,** 1122-1128).

(6) Collaboratively study a method combining multiresidue extraction, **29.011-29.012,** acetonitrile partitioning cleanup, **29.014,** Florisil column chromatographic cleanup and residue separation, **29.031-29.033,** and electron capture gas-liquid chromatography, **29.018,** for determining residues of *cis*-chlordane, *trans*-chlordane, octachlor epoxide (oxychlordane), and heptachlor epoxide in butter, eggs, fish, and poultry fat.

(7) Continue the ongoing work on chlorinated dioxins; chlorobenzilate, chloropropylate, and bromopropylate; Kepone; photochemical derivatization; polychlorinated biphenyls; tetradifon, endosulfan, and tetrasul; and toxaphene.

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Report on Organophosphorus Pesticides

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The published literature on the determination of organophosphorus pesticide residues has been monitored by the General Referee during the past year. Szeto et al. (1) determined acephate and methamidophos residues in fish, asparagus, sediment, and water. Residues were extracted with acetonitrile except for fish samples which were extracted with ethyl acetate. Samples were cleaned up on a mixed column of charcoal-cellulose (2 + 5) followed by analysis by gas-liquid chromatography (GLC) with a nitrogen-phosphorus detector (NPD).

Yamazaki et al. (2) described a method for the determination of acephate and its metabolite methamidophos in tobacco plants. The residues were extracted with ethyl acetate, cleaned up on a silica gel column, and determined by GLC with flame photometric detection (FPD). Wilson and Bushway (3) reported a high performance liquid chromatographic (HPLC) method for the determination of azinphos-methyl and azinphos-methyl oxon in tomatoes, green beans, potatoes, apples, and blueberries. Residues were extracted with methanol, partitioned into methylene chloride, and cleaned up on an activated C_{18}

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Sep-Pak cartridge. Watters (4) determined bromophos and malathion residues in rapeseed by GLC-FPD. Samples were Soxhlet-extracted with ethyl acetate–hexane (2 + 8) and cleaned up on a Sephadex LH-20 gel column.

Guinivan et al. (5) simultaneously determined chlorpyrifos and its metabolite, 3,5,6-trichloro-2-pyridinol by electron capture GLC after preparative gel permeation chromatography of ethyl acetate extracts of Southern Pea vines. The pyridinol was analyzed as its *N,O*-bis(trimethylsilyl)acetamide derivative. Chlorpyrifos was stable to the derivatization procedure. A later publication by the same authors (6) reported refinements to the method to avoid chromatographic interference in the determination of chlorpyrifos residues in peaches and Bahia grass samples. Chlorpyrifos-methyl residues were determined in balsam fir foliage, forest litter, soil, sediment, and fish by GLC-FPD and NPD (7). Samples were extracted by 20% acetone in hexane, and cleaned up on a Florisil column by elution with 40% benzene in hexane. Average recoveries ranged from 91 to 102%. Simonaitis et al. (8) developed a method for the determination of chlorpyrifos-methyl in dry cat food by GLC-FPD, including extraction with acetone and cleanup on an acetonitrile-on-Florisil column. This procedure was compared with and found to be superior to 7 other cleanup procedures.

Iwata et al. (9) reported analysis of residues of chlorthiophos and its sulfoxide, sulfone, oxon, oxon sulfoxide, and oxon sulfone in citrus. Residues were extracted with acetone, partitioned into benzene, and cleaned up on a silica gel column. Chlorthiophos was eluted with 50% hexane in benzene, the sulfone with benzene, the sulfoxide and the oxon with 1% acetone in benzene, the sulfone oxon with 5% acetone in benzene, and, finally, the sulfoxide oxon with 15% acetone in benzene. Residues were determined by GLC-NPD except for the sulfone oxon and sulfoxide oxon fractions which contained interfering background peaks. These compounds were each separately hydrolyzed to diethylphosphoric acid and then analyzed as the pentyl ester after treatment with ethereal diazopentane. These authors (9) carried out some residue stability studies and reported that chlorthiophos and its sulfoxide were stable in laboratory-treated whole fruit stored at 8°C for 2 and 7 days. The oxon sulfoxide levels decreased significantly. Residues of chlorthiophos, its sulfoxide, and its oxon sulfoxide were stable in chopped rind samples prepared from laboratory-treated fruits and kept under frozen storage for up to 6 weeks. Residues of all compounds (chlorthiophos, the sulfoxide, the sulfone, the oxon, the oxon sulfoxide, and the oxon sulfone) were stable in the acetone extract stored at 8°C for up to 6 weeks.

Residues of crufomate were determined in milk and milk products by GLC-NPD following acetone extraction, re-extraction into methylene chloride, and cleanup by hexane–acetonitrile partitioning (10). Seguchi and Asaka (11) reported a method for the determination of diazinon and its metabolites in fish. Following extraction with chloroform–2-propanol (1 + 1), one portion of the extract was cleaned up on silica gel, derivatized with 2,5-dichlorobenzenesulfonyl chloride and TMS for GLC-electron capture determination of pyrimidine metabolites. The other portion of the extract was cleaned up on alumina for GLC-FPD determination of diazinon and related compounds. Dimethoate and dimethoxon residues were determined in wheat plants by GLC-FPD (12). Residues were extracted with chloroform, and cleaned up on a silica gel column with elution by acetone. Leidy and Sheets (13) determined residues of ethoprop and disulfoton and its metabolites in soils and tobacco. Extracts (chloroform for ethoprop and ethyl acetate for disulfoton and its metabolites) were cleaned up on Florisil and residues were determined by GLC-FPD. Hunt et al. (14) reported the determination of ethoprop in vegetables by GLC-FPD following hexane extraction and cleanup on a silicic acid column. Brown (15) developed a GLC-FPD method for fenamiphos, its sulfoxide, and its sulfone in plants and soil. Samples were extracted with acetone and residues were partitioned into chloroform. Cleanup was accomplished on a silica gel and Nuchar C-cellulose columns in series. Fenamiphos and its sulfone were eluted with acetone–hexane (2 + 1). Fenamiphos sulfoxide was eluted from the silica gel column with acetone. Haché et al. (16) described a method for residues of fenitrothion and its metabolites (fenitroxon and *S*-methyl-fenitrothion) in soil, chicken liver, urine, clams, and pine needles. Following extraction with acetonitrile and filtration, the diluted filtrate was passed through an Amberlite XAD-7 resin column. Residues were eluted with ethyl acetate and determined by GLC-FPD. Abdel-Kader and Webster (17) determined residues of fenitrothion and its metabolites (fenitrooxon, *S*-methyl fenitrothion, *O*-demethyl fenitrothion, *O*-demethyl *S*-methyl fenitrothion, 3-methyl-4-nitrophenol, dimethyl phosphorothioc acid) in stored wheat. Residues were extracted with acidified acetone

or methanol, derivatized with diazoethene, cleaned up on a silica gel column, and determined by GLC-FPD. The 3-methyl-4-nitrophenol metabolite was determined by GLC-electron capture detection of the acetone extract. In cases where high levels of residues were present, it was necessary to separate the residues into neutral and acidic compounds to quantitate *O*-demethyl fenitrothion.

Hansen et al. (18) reported the GLC-mass spectrometric characterization of malathion residues in stored rice. They pointed out the necessity of careful interpretation of results because the methyl ethyl succinate ester of malathion co-elutes with malaoxon from a nonpolar GLC column. Malathion and methidathion residues were determined in oranges following acetone extraction and Florisil column cleanup (19). Malathion was eluted with 5% acetone in hexane and methidathion with 25% acetone in hexane. Residues were stable under frozen storage or in acetone extracts for up to 21 days. Residues in fruit stored at 8°C declined after 2 days, demonstrating that samples should be processed within 2 days.

Sonobe et al. (20) studied the extraction (blending, washing and leaching) efficiency of methanol or acetonitrile of biologically incorporated ^{14}C-phorate residues in potatoes, carrots, and radishes. Methanol was a better extractant than acetonitrile for all the crop-time combinations. The major residues in the methylene chloride extracts (organic extractable) of potatoes and radishes were phorate sulfone and phorate sulfoxide. In carrots, phorate sulfoxide, phorate sulfone, and phorate were the major residues.

The HPLC separation and UV detection of phosfolan, mephosfolan, and 5 of their metabolites was reported (21). Generally poor recoveries were obtained in experiments with tomato leaves and human plasma. Barry et al. (22) used the AOAC general multiresidue method for non-fatty foods to determine pirimiphos-methyl in imported dried foods. Residues were eluted from a Florisil column in the 15% ethyl ether-petroleum ether fraction and identified by GLC-mass spectrometry. Ivey et al. (23) determined stirofos residues in eggs by GLC-FPD, including extraction with acetonitrile followed by cleanup on a silicic acid column with elution of residues by dichloromethane-hexane (1 + 1). Waliszewski (24) described a method for the determination of triazophos in rapeseeds. Following acetonitrile extraction and partitioning with hexane, residues were cleaned up on an aluminum oxide column, eluted with hexane-acetone (90 + 10), and determined by GLC with an alkali flame ionization detector.

McIntyre et al. (25) reported a GLC-FPD method for residues of diazinon, malathion, and parathion in sewage sludge. Residues were extracted with dichloromethane-hexane (1 + 1) and cleaned up on an alumina column.

Luke et al. (26) modified the multiresidue procedure of the same authors (27) by eliminating the Florisil cleanup. Samples were extracted with acetone and partitioned with petroleum ether and methylene chloride. Double concentration with petroleum ether was used to remove the last traces of methylene chloride. Recoveries were reported for 44 organophosphorus compounds determined by GLC-FPD.

Funch (28) reported the analysis of 6 organophosphorus compounds in fruits and vegetables by HPLC with UV detection following acetonitrile extraction and partitioning into dichloromethane. Cabras et al. (29) determined dimethoate, phosalone, and tetrachlorvinphos residues in grapes by HPLC with UV detection following extraction with benzene and without further cleanup.

Schulten and Sun (30) reported the field desorption mass spectrometry of 21 organophosphorus pesticides and their identification in crude extracts of waste water. Parker et al. (31) studied the HPLC-negative chemical ionization mass spectrometry of 18 organophosphorus pesticides and reported some preliminary work on the analysis of residues in water samples. Bushway (32) determined residues of azinphos-methyl and its oxon in water by HPLC with UV detection.

Lewis and MacLeod (33) developed a portable sampler using polyurethane foam as the trapping medium for pesticides in air. Seven organophosphorus pesticides were included and their collection efficiency was above 72%. Andrews and Good (34) investigated the extraction of pesticides (phosalone was the organophosphorus compound tested) from water using bonded-phase sorbents. The optimum bonded-phase silica for the extraction was C_8.

During the past years there has been disappointing progress in the area of multiresidue methods for organophosphorus pesticide residues. However, there has been considerable work on methods for residues of individual compounds as evidenced by the published literature. The Food and Drug Administration is developing a Surveillance Index for Pesticides. Each pesticide is classified as to the potential hazard posed by its residues. To date, 24 orga-

nophosphorus pesticides have been included in the index; none have been classified as Class I. Six (azinphos-methyl, disulfoton, EPN, methamidophos, monocrotophos, phorate) have been classified as II. Of these, only EPN is included in AOAC official methods **29.039-29.043.** In an attempt to stimulate activity and provide validated methods, the Referee will try to locate Associate Referees to develop/evaluate/validate residue methods for azinphos-methyl, disulfoton, methamidophos, monocrotophos, and phorate. Where possible, it would be preferable if these could be integrated into existing multiresidue methods.

A brief summary of each Associate Referee topic follows:

Confirmation Procedures.—The previous Associate Referee resigned. New Associate Referee Bill Lee has re-evaluated the pentafluorobenzyl bromide derivatization procedure, made some minor improvements, and plans to initiate the collaborative study recommended last year.

High Fat Samples.—Associate Referee Ronald Scharfe was unable to work on the assignment this year. He plans to evaluate available gel systems and their applicability to organophosphorus pesticide residues in high fat samples.

Sweep Codistillation.—Associate Referee Randall R. Watts reports that the status of the subject is unchanged from last year. It is reported that a commercial unit has been developed in Australia and information is being sought on this unit.

Thin Layer Chromatography.—Associate Referee Melvin E. Getz reports that the status of the subject is essentially unchanged from last year. He has been unable to organize an interlaboratory study because the laboratories with quantitative thin layer chromatographic equipment do not have time to participate in this study. The Associate Referee plans to carry out a study comparing the quantitative thin layer chromatographic method with GLC and HPLC residue quantitations.

Other Topics.—The topics Extraction Procedures; General Method for Organochlorine and Organophosphorus Pesticides; and Soils are vacant.

Recommendations

(1) Collaboratively study the Coburn and Chau (35, 36) confirmation procedure for organophosphorus pesticides recovered from water by a suitable method such as that of Ripley et al. (37).

(2) Appoint an Associate Referee to study the efficiency of extraction procedures for residues of organophosphorus pesticides in crops.

(3) Appoint an Associate Referee to evaluate the applicability of the AOAC multiresidue method, **29.001-29.018,** for determining additional organophosphorus pesticide residues in fatty and nonfatty foods and include the use of the nitrogen-phosphorus detector as an alternative GLC detector.

(4) Continue studies to evaluate gel chromatography as a cleanup procedure for the determination of organophosphorus pesticides and their metabolites in fatty foods and utilize the nitrogen-phosphorus detector as one of the GLC detectors for residue determination.

(5) Appoint an Associate Referee to develop multiresidue extraction and cleanup methods for organophosphorus pesticides and their metabolites in soils.

(6) Evaluate any commercial sweep-codistillation apparatus that becomes available and extend the applicability of the method to the determination of organophosphorus pesticide residues in fatty foods.

(7) Carry out an interlaboratory study to evaluate the quantitative thin layer chromatographic approach and carry out a study to compare quantitative thin layer chromatographic method with GLC and HPLC residue quantitations.

(8) Appoint Associate Referees for Azinphos-methyl, Disulfoton, Methamidophos, Monocrotophos, and Phorate to develop methods for residues of these compounds.

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Report on Radioactivity

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The main activity in the next few months will be to recommend new Associate Referees for the various radionuclides. Over the past years, the number has dwindled without any significant replacement. Frederick G. D. Shuman, a newly appointed Associate Referee on Barium-140, resigned. Jacqueline Michel, Director, Geological Sciences, Division of the Research Planning Institutes, Inc., has agreed to take over as Associate Referee for Radium-228.

Neutron Activation Analysis.—William B. Stroube, Jr, has reported on the progress of his collaborative study on determination of sodium by NAA on biological media. Two collaborators have sent in data, two others are in the process of reporting their results, and the remaining two have the samples in process. After this study is evaluated, the Associate Referee will see if it is feasible to conduct a study on more than one metal, possibly three. He has made contacts with others and looks forward to recruiting more collaborators.

Iodine 131.—The study on quantitative determination of I-131, Cs-137, and Ba-140 in milk was reported in the September 1982 issue of the *Journal*. The method was adopted official first action. This method has a limit of detection of 10 pCi/L. The NRC has required that iodine-131 be determined at a level of detection of 0.5 pCi/L. There is a method that reportedly has this sensitivity. In addition, there are one or two variations. Associate Referee David G. Easterly will conduct a ruggedness test on this method during

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the coming year and plans to conduct a collaborative study on the tested method.

Barium-140.—The method was adopted official first action (see Iodine-131). The Associate Referee has resigned, but will not be replaced at present, because there appears to be little interest in this radionuclide.

Strontium-89 and 90.—No activity is reported on this radionuclide for this year. A new Associate Referee will be recommended during the coming year.

Cesium-137.—The results of the collaborative study for the determination of cesium-137 in foods have been received and are being evaluated. A new Associate Referee will be recommended during the coming year.

Radium-228.—A method on determination of radium-228 in foods and water has been published (*J. Assoc. Off. Anal. Chem.* (1982) **65**, 1424–1428). A new Associate Referee has been recommended and it is expected that activity in this area will continue.

Recommendations

(1) Adopt as official final action the official first action extension, for quantitative determination of I-131, Cs-137, and Ba-140 in milk, **48.025–48.029**

(2) Continue study on all topics.

Report on Water

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Chemical Pollutants in Aquatic Biota.—This topic should be deleted because it is covered elsewhere in Committee E.

Chemical Pollutants in Water and Wastewater.—Continue review of analytical methods collaboratively studied by ASTM, EPA, and others for possible adoption by AOAC as official methods; continue study.

Chlorinated Solvents in Water.—Appoint an Associate Referee; continue study to evaluate and collaboratively study methods for determining chlorinated solvents in water and wastewater.

Organophosphorus Pesticides in Water.—Appoint an Associate Referee to evaluate method proposed by the U.S. Environmental Protection Agency or other methods for determining organophosphorus pesticides in water and wastewater and to initiate a collaborative study.

Triazine Herbicides in Water.—Appoint an Associate Referee; continue study to evaluate and collaboratively test methods for determining triazine herbicides in water and wastewater.

Herbicides in Water and Sediment.—Initiate topic to evaluate existing methods for phenoxy alkanoic and other acidic herbicides in sediment. Submit protocol for collaborative study of the selected method to the General Referee for review and comment and if approved initiate the collaborative study.

Nutrients and Major Ions in Water.—Initiate topic, particularly to study sulfate and aluminum in water.

Other Topic.—Continue official first action status of the atomic absorption spectrophotometric method for determining cadmium, chromium, copper, iron, lead, manganese, silver, and zinc in water, **33.089–33.094.**

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GENERAL REFEREE REPORTS: COMMITTEE F

Report on Analytical Mycology of Foods and Drugs

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The mold determination procedure for canned tomatoes, **44.098**, involves sieving and performing separate mold counts on the drained liquid and the pulped tomatoes. Canned, crushed tomatoes may consist of finely chopped tomatoes packed in puree, which makes separation by sieving impossible. Wayne T. Smith of the FDA Philadelphia District has standardized the procedure for canned, crushed tomatoes by pulping the entire contents of the can and handling the pulp as a puree, **44.096**.

The official method for tomato rot fragments, **44.099**, specifies that each slide be examined at 30–45X with transmitted light. The wide-field microscope has traditionally been the only choice because of its large stage and field of view. However, the lack of fine focusing and a mechanical stage results in a considerable amount of eye strain and fatigue for the analyst. Don J. Vail of the FDA Atlanta District has developed a slide-holding adapter which replaces the stock slide holder on the latest generation of compound microscopes. The large mechanical stages on these microscopes permits smooth movement of the slide, and the wide-field optics readily accommodate the rot fragment slide, **44.002(t)**. In addition, the confirmation of suspected rot fragments is made easier by switching to 100X magnification instead of having to move the slide to another microscope. A study is being initiated to compare rot fragment counts, using both the compound and wide-field microscopes.

A limited interlaboratory collaborative study on a modified Howard mold count method for whole berries was conducted. A coarsely pulped berry sample (0.040 in. screen) was used in the study to simulate whole berries and to assure an even distribution of the mold among the sample aliquots. Each aliquot was then pulped (0.027 in. screen) by the collaborator as if it were a mixture of whole berries.

The results indicate a wider variation in mold counts among collaborators than expected. The average mold counts ranged from 11.8 to 36.7%. This variation appeared to be a systematic counting error which resulted in higher than expected counts from 2 of the collaborators. Work on this method will continue.

The laboratory cyclone, **44.004(h)**, commonly called a pulper, is specified in the product preparation step of Howard mold count methods for whole fruits or vegetables. Whole products must be pulped before mold counting; however, the laboratory cyclone is no longer commercially made. Machine shop estimates for custom-made cyclones range from $2000 to $3000. A commercially available home fruit strainer has been modified by the addition of a 0.027 in. screen. A laboratory study has been initiated to compare mold counts of fruits prepared by a laboratory cyclone and the modified Squeezo strainer.

The description of the centrifuge (see *Procedural Changes*) used in the method for standardizing fruit tissue concentrations, **44.C08**, **44.C09**, and **44.082**, is being updated and expanded. This will make it easier to locate equivalent centrifuges.

Before the fruit tissue standardization method, **44.C08-44.C09**, was adopted, fruit nectars were being counted "as is" with no preparation other than mixing. To facilitate regulatory comparability, the mold count results of fruits diluted 1 + 1, **44.C09**, are being adjusted to correspond to fruits diluted 1 + 3 (see *Procedural Changes*).

Procedural Changes

In method **44.082**, Mold Count, Citrus and Pineapple Juices, Canned, Single Strength, it is recommended that the third sentence be changed to read: "Centrf. 10 min at 2200 rpm, using International type EXD centrf. (Damon/IEC Div., 300 Second Ave, Needham Hts, MA 02194) with 8-place No. 240 head, No. 320 shield, No. 325 trunion ring, and No. 571 cushion, or other centrf. giving equip. max. relative centrifugal force of 1062.4768 × g as computed by following formula: $RCF_{max.} = 1.118 \times 10^{-5} N^2 r$

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= × g, where N = rpm and r = radius of centrf. arm in cm (distance from center of centrf.-head to bottom of horizontal centrf. tube). The following formula may be used to det. equiv. centrf.: $N_1^2 r_1 = N_2^2 r_2$, where N_1 = 2200 rpm and r_1 = 19.6 cm."

In method **44.C09**, the following statement should be added: "Before calcg % mold counts for fruits dild 1 + 1, divide number of positive fields by 2."

Recommendations

(1) Revise **44.082** and **44.C09** as described.
(2) Continue study on all other topics.

Report on Extraneous Materials in Foods and Drugs

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A collaborative study was conducted on a brine saturation technique for the extraction of light filth from whole, rubbed, and ground sage. The General Referee recommends that any adoption actions be deferred until the Associate Referee resolves all identified critical issues.

The General Referee concurs with the recommendation that method **44.A04-44.A07** (TLC method for urine strains) be editorially revised by adding a precleaning technique for Analtech MN 300 cellulose TLC plates. The technique removes a colored impurity that occasionally interferes with visualization of the urinary indican spot.

The General Referee recommends that method **44.183-44.185** (TLC method for uric acid from bird and insect excreta) be editorially revised by modifying the marker dye mixture to eliminate the masking effect of the upper dye spot on the uric acid spot.

The General Referee recommends that method **44.B08-44.B11** (chemical test for mammalian feces) be editorially revised to permit the alternative use of gelled working test media (WTM) plugs to cover sample material on the gelled WTM base instead of requiring the use of freshly prepared liquid WTM. The revision also includes the addition of an alkaline phosphatase positive control to check the activity of the gelled or freshly prepared WTM agar. These changes will provide a test medium alternative that will save analytical time and reduce false negatives.

Collaborative studies will be initiated on methods for filth in selected botanicals, unground marjoram, chocolate liquor, whole or equivalent insects in oats, a modification of the TLC method for uric acid, and the automated method for uric acid in flour.

New Associate Referees were appointed to the following topics: Marvin Nakashima (FDA, Washington, DC) as a co-Associate Referee on Botanicals; Alan R. Olsen (FDA, Los Angeles) on the newly established topic of Filth in Shrimp; and Russell G. Dent (FDA, Washington, DC) on the existing topic of Canned Mushrooms.

The following topics are recommended for discontinuation of study: Asbestos Measurements in Foods, Drugs, and Cosmetics; Ready-to-Eat Breakfast Cereals; Canned Fish; Canned and Dehydrated Soups; Water-Insoluble Inorganic Residues in Peanut Butter; Mammalian Excreta Fragments in Milled Food Products; and Automated Filth Analysis.

Methods development research will continue on the following existing topics: Adulteration of Botanical Drugs by Foreign Plant Materials; Botanicals; Brine Extraction Techniques; Chocolate Products; Cocoa Powder and Press Cake; Food Supplement Tablets; Cracking Flotation Methods for Whole Grains; Insect Excreta in Flour; Processed Meats; Methods for Urine Detection; Mite Contamination Profiles and Characterization of Damage of Foods; Mites in Stored Foods; Dried Mushroom Products; Particulates in Large-Volume Parenterals; Rye Bread; Soluble Insect and Other Animal Filth; Spices; Isolation of Extraneous Filth from Dehydrated Vegetable Products; Chemical Identification Test for Vertebrate Excreta; and Fecal Sterols.

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An Associate Referee is needed to study methods for light filth in mole paste.

Recommendations

(1) Discontinue the following topics: Asbestos Measurements in Foods, Drugs, and Cosmetics; Automated Filth Analysis; Cereals, Breakfast, Ready-to-Eat; Fish, Canned; Mammalian Excreta Fragments in Milled Food Products; Soup, Canned and Dehydrated; Peanut Butter, Water-Insoluble Inorganic Residues.

(2) (a) Editorially revise the TLC method for urine stains (**44.A04-44.A07**) by adding the following instructions for pre-cleaning Analtech MN 300 cellulose TLC plates before use: "**44.A06(d)** *Pre-cleaning Analtech MN 300 cellulose plates.*—Develop unused plate in either std developing tank or sandwich chamber using developing solv. **44.A05(f)**. Develop to 15 cm above lower edge of plate. Thoroly dry plate using either hair dryer, forced draft oven at ≤80° for ca 15 min, or overnight in fume hood. Plate must be room temp. and completely free of solv. odor before use." Pre-cleaning is necessary with these commercial plates because a colored impurity has been observed to travel with the solvent front and occasionally interfere with visualization of the urinary indican spot.

(b) Editorially revise the TLC method for uric acid from bird and insect excreta (**44.183-44.185**) by modifying the marker dye mixture as follows: "**44.183(e)** Dissolve 16 mg FD&C Red No. 2 and 32 mg FD&C Yellow No. 6 in 50 mL H_2O, and mix well. These dyes serve as visual markers during development, with R_f for Red No. 2 at 0.38–0.40; uric acid, 0.41–0.43; and Yellow No. 6, 0.65, using Analtech plate and sandwich chamber. Merck plates have lower R_f values and do not sep. Red No. 2 and uric acid when overspotted." The dye mixture, which is overspotted on the sample unknown, brackets the uric acid spot after TLC development. The present dye mixture results in the upper marker dye sometimes masking in the uric acid spot. The proposed dye mixture results in compact and adequately intense marker spots that do not interfere with visualization of the uric acid spot.

(3) Establish topic Shrimp, Filth in; appoint an Associate Referee.

(4) Editorially revise the chemical test for mammalian feces as follows: In **44.B09**, add "(d) *Spatula.*—Curved on one end, knob on the other end (Arthur H. Thomas Co. No. 8340-H10, or equiv.)." In **44.B10(c)**, change the last paragraph to read "Long term storage: Add ca 1 mL portions to cups. Gelled plugs, in cups, may be stored for up to 4 months if sealed in a plastic bag, held at room temp., and protected from direct sunlight. Discard any gels showing pink color and/or vol. loss." In **44.B11**, revise the first paragraph to read "Transfer suspect feces . . . to cup contg 1 mL gelled WTM. Cover with addnl 1 mL cool (40–41°) WTM or, alternatively, with plug of gelled WTM. (Use clean spatula, **44.B09(d)**, to manipulate covering plug of WTM and to press plug into close contact with sample.) Place cup in 40–41° H_2O bath. Check for development of red color near particles." In **44.B11**, add as a new third paragraph "*Positive control preparation.*—Using calf intestine alkaline phosphatase (AKP) (Calbiochem No. 52457, or equiv.), prep. 1 mg/mL soln in borate buffer (stock test reagent **44.B10(b)**, without phthln diphosphate). Add 20 μL AKP soln to 1 mm diam. filter paper disks (Whatman No. 1, or equiv.). Use positive control disks with either liq. WTM or alternative gelled plug WTM. Note: Positive control disks may be stored up to 4 months if held at room temp. and protected from light." The paragraph beginning "*Test response.*—" will be the fourth paragraph in **44.B11**. The changes described will provide a test medium alternative that will save analytical time and reduce false negatives.

Report on Microbiological Methods

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This report is the final one to be written for the General Refereeship on Microbiological Methods as an entity. Beginning with the 1983 Annual Meeting, all collaborative studies, Associate Referee reports, and Referee recommendations concerning the microbiology of foods will be presented in the report of the General Referee for Food Microbiology. Studies concerned with the microbiology of drugs, cosmetics, and medical devices will be reported to a separate General Referee. The need for this subdivision is a consequence of the rapid and continued expansion of the General Refereeship for Microbiological Methods which now includes 29 Associate Refereeships. Most of these refereeships focus on a highly specialized area, yet collectively they represent too broad a spectrum to be managed efficiently by a single General Referee.

Collaborative Studies

Hydrophobic Grid Membrane Filter Methods.—Fourteen laboratories participated in a comparative collaborative study of the official first action method (**46.013-46.016**) and the hydrophobic grid membrane filter (HGMF) method for the enumeration of total coliforms in nonfat dry milk (NFDM) and canned custard. The HGMF is imprinted with a grid pattern which subdivides the membrane surface into a large number of separate growth compartments of known and equal size. An appropriate dilution of the blended samples is filtered and the HGMF is placed on the surface of m-FC agar for incubation at 35°C for 24 ± 2 h. An enumeration is made of all squares containing one or more colonies, and this occupied square count is converted to a most probable number (MPN) determination by the formula:

$$\text{MPN} = [N][\ln (N/N - x)],$$

where N = total number of squares and x = number of occupied squares. This MPN value is multiplied by the appropriate dilution factor and expressed as the number of total coliforms per g food.

Six artificially inoculated samples each of NFDM and of canned custard were analyzed by each of 14 laboratories. Results of 7 of the laboratories were not included in the statistical analyses, however, for one of the following reasons: significant numbers of coliforms were detected in negative control samples, collaborators failed to follow instructions carefully, or collaborators submitted statistically outlying data. Statistical analysis of the data from the other collaborators showed that the random errors associated with the HGMF method were significantly lower than the errors for the official first action method, and the total coliform counts obtained with the HGMF method were within the 95% confidence interval of the official method. Total coliform counts obtained with the HGMF method did not differ significantly from those counts obtained by the official method in a 3-way analysis of variance. The Associate Referee recommends that the HGMF procedure be adopted official first action for the enumeration of total coliforms in NFDM and canned custard, and the General Referee concurs.

Associate Referee Reports

Bacillus cereus Isolation and Enumeration.—In 1981, the Associate Referee collaboratively studied a scheme for differentiating typical strains of *Bacillus cereus* from other members of the *B. cereus* group: the insect pathogen, *B. thuringiensis;* the mammalian pathogen, *B. anthracis;* and rhizoid strains of *B. cereus* variant *mycoides* (*J. Assoc. Off. Anal. Chem.* (1982) **65,** 1134-1139). The scheme includes tests in addition to those of the official first action method (**46.A10-46.A15**) for making this species differentiation. These additional tests were for motility, hemolytic activity on trypticase-soy-sheep blood agar, rhizoid growth on nutrient agar, and detection of protein toxin crystals by carbol fuchsin stain. The differential tests were adopted as interim official first action in March 1982. The Associate Referee recommends that the original official first action method (**46.A10-46.A15**) be revised to incorporate the

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Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition, and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* 63, 374-423 (1980).

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

additional differential tests, and the General Referee concurs.

Enteropathogenic Escherichia coli.—Associate Referee Ira J. Mehlman reports the development of an improved method for *Shigella sonnei* in foods. The method consists of enrichment of *Shigella* broth containing, per liter: 20 g tryptone, 2 g KH_2PO_4, 2 g K_2HPO_4, 1 g glucose, 5 g NaCl, 0.5 mg novobiocin, and 1.5 mL Tween 80. After anaerobic incubation for 20 h at 44°C, the enrichment broths are streaked to MacConkey agar and suspicious colonies are screened biochemically and tested for motility. When the method was used for the examination of lettuce, celery, brussel sprouts, mushrooms, and ground beef, the sensitivity of the method varied from 0.3 to 100 *Shigella sonnei* cells per g food. The current procedure in the *Bacteriological Analytical Manual* (BAM) can detect *Shigella* species if 10^6 cells per g are present. The improved procedure is applicable to other *Shigella* species if enrichments are incubated at 42°C. The Associate Referee reports that additional refinements to increase specificity and sensitivity are needed before conducting a collaborative study.

Helium Leaks in Canned Foods.—The Associate Referee reports that bacteria in the cooling water can enter canned foods through holes larger than 5 μm. The microleak test, as described in the BAM, has difficulty in locating holes smaller than 15 μm, but a proposed helium leak test will detect holes as small as 1 μm. Plans are currently under way for conducting a comparative collaborative study of the 2 methods for detecting micro leaks in a variety of canned foods.

Salmonella.—The enhanced recovery of *Salmonella* from NFDM pre-enriched under conditions of reduced rehydration rates has been demonstrated in the Associate Referee's laboratory. The official final action method of NFDM pre-enrichment (**46.056**) consists of mixing well, by swirling, a 25 g sample at a 1:9 sample/distilled water ratio and equilibrating the mixture at room temperature for 60 min. The pH is adjusted to 6.8 ± 0.2, and brilliant green dye is added to a final concentration of 0.002% before incubation at 35°C. The improved procedure consists of gently adding a 25 g sample to 225 mL sterile distilled water and letting the sample soak undisturbed for 60 min before incubation. The pH is not adjusted for samples analyzed by the soak method. Analysis of sample sizes larger than 25 g, e.g., 100 g and 375 g, did not result in any decrease in sensitivity of the soak method. Associate Referee Paul L. Poelma reports that a comparative collaborative study of the official and soak methods for NFDM sample rehydration is currently in progress and that the results will be reported at the 1983 Annual Meeting.

Somatic Cell Automated Optical Counting Method.—Associate Referee Wesley N. Kelley is planning a collaborative study of the Coulter milk cell counter (CMCC), a rapid automated system for detecting somatic cells in fresh and preserved milk. The CMCC system counts all particles above a certain size by interrupting an electrical impulse. Ten collaborators with CMCC systems have agreed to participate in a collaborative study planned for FY 83. Three practice samples in duplicate sent to potential collaborators revealed initial problems of lack of uniformity of sample preparation and difficulty in calibration of the system to a level low enough so that primarily somatic cells are counted. A progress report was presented at the 1982 Annual Meeting with recommendations for resolutions of these problems.

Spore Formers and Non-spore Formers in Low Acid Canned Foods.—It has been estimated (Segner (1979) *Food Technol.* **33,** p. 55) by one canning company that 90% or more of customers' spoilage problems probably are due to leaker spoilage, a type of spoilage which involves post process recontamination of foods sterilized in hermetically sealed containers. This type of spoilage is caused most commonly by non-sporeforming bacilli of nonhealth significance. Another type of spoilage, less common but potentially more hazardous than leaker spoilage, is caused by underprocessing. In this case, organisms such as *Clostridium botulinum* and other sporeformers survive and multiply. In many cases, the organisms that multiply autosterilize, which makes conventional microbiological procedures useless in determining whether spoilage was caused by leakage or underprocessing. There is a need for a screening procedure for rapidly differentiating these 2 types of spoilage. Associate Referee Mary L. Schafer has developed a technique based on gas-liquid chromatographic identification in the can contents of 2 chemical compounds characteristic of sporeformers. Practice samples have been distributed to potential collaborators in anticipation of a collaborative study.

Staphylococcus Toxin.—Associate Referee Reginald W. Bennett reports that plans are under way to conduct a collaborative study of a screening process (Meyer & Palmieri (1980) *Appl. Environ. Microbiol.* **40,** 1080–1085) for detecting enterotoxin production by large numbers of foodborne staphylococcal isolates. The screening procedure uses polyvalent (serotypes A, B, C,

D, and E) immunodiffusion slides to test for enterotoxin production of pooled cultures that have been inoculated separately on the surface of a plate of semisolid agar.

Testing Biological Sterility Indicators.—A procedure for sterility testing of 10% fat emulsions was studied. The procedure consists of solubilizing the emulsion in dimethyl sulfoxide and filtering the mixture through a polyester membrane. In addition to being rapid, this procedure avoids the problems of turbidity and membrane filter plugging associated with other methods.

Yeasts, Molds, and Actinomycetes.—Associate Referee Phillip B. Mislivec has initiated a survey of yeast and mold flora of a variety of frozen fruits (blackberries, blueberries, cherries, raspberries, and strawberries). One hundred of these samples were plated in potato dextrose agar with and without NaCl added to final concentrations of 4, 6, and 7.5% to determine the effect of the added NaCl on mold spreader inhibition and on viable mold counts. Preliminary results indicate that 7.5% NaCl effectively inhibits mold spreaders and that viable counts are not affected adversely. The effect of 7.5% NaCl on viable mold counts of other foods, particularly foods containing heat-injured mold organisms, will be determined in FY 83 before a collaborative study is conducted.

Recommendations

(1) Adopt as official first action the hydrophobic grid membrane filter method for the enumeration of total coliforms in nonfat dry milk and canned custard.

(2) Revise the official first action method for the enumeration and confirmation of *Bacillus cereus* in foods (**46.A10-46.A15**) to incorporate additional tests for differentiating *B. cereus* from other *Bacillus* species.

(3) Discontinue the following topics: Cereal Products (Microbiological); Microbe Identification by Capillary Gas Chromatography; Somatic Cell, Rolling Ball Viscometer Procedure; and Somatic Cell, Millipore-DNA Assay.

(4) Consolidate the following topics:

(a) Automated Method for Fungi; Yeast and Mold Counts; and Pathogenic Yeasts, Molds, and Actinomycetes into the single topic Yeasts, Molds, and Actinomycetes.

(b) Coliform Bacteriology; Enteropathogenic *Escherichia coli;* and *Escherichia coli* and Coliform Bacteria into the single topic *Escherichia coli* and Other Coliforms.

(c) *Bacillus cereus* Enterotoxin and *Bacillus cereus* Toxin into the single topic *Bacillus cereus* Enterotoxin.

(d) *Staphylococcus* and *Staphylococcus aureus* into the single topic *Staphylococcus*.

(5) Transfer the following topics from the General Refereeship for Food Microbiology to a separate General Refereeship: Testing Biological Sterility Indicators; Sterility Testing of Medical Devices; and Cosmetic Microbiology.

(6) Appoint Associate Referees for the existing topics of *Staphylococcus* and of *Yersinia enterocolitica*.

(7) Initiate the topic Genetic Methods for the Detection of Bacterial Pathogens and the topic Identification of Microorganisms by Biochemical Kits.

(8) Continue study on all other topics.

GENERAL REFEREE REPORTS: COMMITTEE G

Report on Antibiotics

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This year has been one of relatively modest activity, as measured by collaborative studies. However, there appear to be significant advances in methodology in the formative stage, as indicated in some of the contributed papers. The 2 areas that portend significant forward movement are chromatographic assays for bacitracin and chlortetracycline in premixes and feeds.

β-Lactam Antibiotics in Milk: Qualitative Procedures.—A collaborative study of the Delvotest system was performed with the ampule method to establish the common lower limits of sensitivity in a variety of fluid milk products. The results indicated that levels as low as 0.0045 IU/mL could be determined essentially 100% of the time in skim milk, chocolate skim milk, homogenized milk, and half-and-half. Associate Referee W. N. Kelley recommended acceptance of this procedure for β-lactam residues (penicillin) in milk and milk products at 0.0045 IU/mL. The General Referee concurs and recommends the adoption of the Delvotest ampule system as official final action with a lower limit of detectability of 0.005 IU/mL.

The Associate Referee also recommended the adoption of the multitest system as official final action, excluding use in chocolate products. Since this qualitative assay system is essentially the same as the ampule system and is primarily used for raw fluid milk, and no adverse comments have been received, the General Referee concurs with the recommendation. The recommended lower limit of detection for both variations is 0.005 IU/mL.

Recommendations

(1) Adopt as official final action the official first action qualitative color reaction tests for β-lactam in fluid milk products, **16.C17-16.C22,** for residues ≥0.005 IU/mL, excluding use in chocolate products for the multitest system. (2) Continue study on all other topics.

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

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

Section numbers refer to "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **65,** 450-521 (1982).

Report on Biochemical Methods

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Work on the development of a general method and protocol for the assessment of performance of immunochemical pregnancy test kits has been completed (*J. Assoc. Off. Anal. Chem.* (1982) **65,** 592-597). This procedure is a practical evaluation method which should be of use to laboratories using direct agglutination and agglutination inhibition tests.

One of the main priorities of this refereeship is the establishment of more rigorous validation data and methods for immunoassays. One of the problems in the assessment of specificity of immunoassays has been the lack of alternative, independent analytical procedures whereby the performance of immunoassays can be checked, but with the advent of both HPLC and sensitive mass spectrometric techniques, this problem is being obviated.

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In 1981, the Associate Referee for immunochemical methods for estrogen analysis completed a study on the validation of a radioimmunoassay for 17β-estradiol by gas chromatography–mass spectrometry. Given the importance of accurate validation of immunoassays, this work has been extended to a review on this subject and has been submitted to the *Journal of Clinical Chemistry*.

Preliminary work on the development of a colorimetric assay for heparin reveals stability problems with the color development. Work on this phase of the investigation has been postponed while the feasibility of an HPLC approach is evaluated. The HPLC approach has the potential of separating heparin into compounds and thereby allowing evaluation of the effect of type and concentration of component on anticoagulant activity. This would be useful in product quality control.

The Associate Refereeship for immunochemical methods for meat products has recently been reassigned due to retirement of Alonza Hayden, USDA. The method currently involves the use of agar-gel diffusion techniques to speciate meat protein. The new Associate Referee, David Berkowitz, USDA, will review existing methodology on use of enzyme-linked immunosorbent assay (ELISA) technology as a detection tool. Collaborative study of this technique will await further development in this area.

A new Associate Refereeship added in 1981 is Immunochemical Methods for Staphylococcal Enterotoxin: Development of Monoclonal Antibodies. At this time, hybridomas have been achieved which produce antibodies of the IgG type against SEA only and SEA and SED in combination. Work is proceeding in the stabilization and cloning of these cells. This work has excellent potential for leading to a source of antibodies capable of distinguishing enterotoxin types. This will open the way for the development in sensitive, specific, and, most of all, rapid immunochemical toxin measurement tests.

Work continues on the development of rapid immunochemical screening methods for sulfa drugs. Initial assessment of antibody achieved was 1/5000 titer in New Zealand rabbits. There is, however, a problem with stability of antibody; hence, no specificity studies were performed. Work on this project continues.

The General Referee is collaborating with the planning group for the 1984 Spring Workshop. We are designing a major session on analytical immunology. This presentation will include both didactic and applications session. We are also considering a workshop session on selected immunochemical methods of analysis.

Recommendation

Continue study on all topics.

Report on Color Additives

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The Associate Referee on Atomic Absorption in Color Analysis, L. Moten, is continuing his study of methods for the determination of trace elements in color additives exempt from certification.

The Associate Referee on Color in Other Foods, N. Adamo, has completed writing a method for the extraction of FD&C colors from cookies and has been analyzing specially prepared cookies to test the suitability of the method for collaborative study.

The Associate Referee on High Pressure Liquid Chromatography, E. Cox, recommends on the basis of work to be prepared for publication by N. Richfield-Fratz that the method for the determination of intermediates and side reaction products in FD&C Red No 40 (**34.B01–34.B06**) be modified by changing the first sentence of **34.B06**

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to read: "Weigh 0.250 g sample, add 10 mL 0.1M $Na_2B_4O_7$ first, then add ca 50 mL H_2O, and, when dissolved, dil. to 100 mL with H_2O." Failure to add the sodium borate first may result in decomposition of DMMA.

The Associate Referee on Inorganic Salts, W. Brammell, has presented a paper on an ion selective electrode method for the determination of sodium iodide in FD&C Red No. 3. He is developing ion selective electrode methods for the determination of iodide and chloride in color additives and is planning collaborative studies on these methods.

The Associate Referee on Intermediates, Uncombined, in Certifiable Triphenylmethane Colors, A. Scher, has presented a discussion of the determination of intermediates, leuco base, and subsidiary colors in FD&C Blue No. 1 by reverse phase HPLC, and is planning a collaborative study of this method.

The Associate Referee on Intermediates, Uncombined, in Certifiable Water-Soluble Azo Colors, D. Marmion, plans to study improved methods for the detection of impurities in sulfonic acids used to prepare color additives.

The Associate Referee on Subsidiary Colors in Certifiable Color Additives, J. Bailey, reported on a reverse phase HPLC method for the determination of intermediates and subsidiary colors in D&C Green No. 5. Also under this topic, A. Goldberg and R. Calvey contributed a paper on the determination of 2,4-dinitro-1-naphthol and 1-naphthol in Ext. D&C Yellow No. 7.

Recommendations

(1) On the basis of routine use by the Division of Color Technology for the past year, adopt as official final action the official first action methods for the HPLC determination of intermediates and reaction by-products in FD&C Yellow No. 5 (**34.C01-34.C06**).

(2) As recommended by the Associate Referee, editorially revise the official first action method for intermediates and reaction by-products in FD&C Red No. 40 (**34.B01-34.B06**) as described above.

(3) Continue study on all topics.

Report on Cosmetics

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Essential Oils and Fragrance Materials, Composition.—Associate Referee Harris H. Wisneski has nearly completed work on the development of an analytical method for the determination of cinnamaldehyde in fragrances. The proposed procedure, which uses HPLC-fluorometry as the respective separation and detection techniques, is selective and sensitive. The aldehyde fraction of the fragrance is first isolated by extraction with amino caproic acid sodium salt. An aliquot of the extract is reacted with 1,2-diaminonaphthalene under acid conditions to form the fluorophore. The reaction mixture is then analyzed by reverse phase HPLC-fluorometry to determine cinnamaldehyde. The method is selective for cinnamaldehyde and other unsaturated aldehydes. Although saturated aldehydes form fluorescent derivatives, the excitation and emission bands are displaced enough to fall outside the range of the filters used for the cinnamaldehyde fluorophore. Preliminary studies indicate recoveries in the 85–95% range.

No Associate Referee reports were received on the remaining topics.

Recommendations

(1) Continue official first action status of method **35.019-35.023,** and method **35.001-35.006.**

(2) Continue study on all other topics.

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Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition.

Report on Drug Residues in Animal Tissues

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Carbadox.—Associate Referee Jose E. Roybal, FDA, Denver, CO, has been investigating a fluorescence HPLC derivative of the carboxylic acid hydrolytic product of carbadox. The derivatizing agent is 4-bromo-methyl-7-methoxycourmarin.

Dimetridazole.—Associate Referee Maritza Colon Pullano, FDA, Denver, CO, was unable to pursue work on this drug in tissue because of work on the same drug in feed.

Screening Methods.—Associate Referee Michael H. Thomas, USDA, Beltsville, MD, reported a successful collaborative study of a TLC fluorescence densitometric screening method for sulfaquinoxaline in turkey tissues; sulfadimethoxane in turkey, swine, and duck tissues; sulfamethazine in turkey and swine tissues.

Committee G, during the summer 1982, adopted the screening method for the 3 applications listed as interim first action. It was also noted that the collaborative study showed the TLC procedure to be quantitative for the drug-tissue combinations listed.

Considering the number of animal drugs for which screening procedures would be useful, several Associate Referees could be appointed in this subject area.

Sulfonamide Drugs.—The Manuel-Steller GLC and FSIS GLC/MS methods were adopted first action last year (**41.C09-41.C12** and **41.C01-41.C08**). S. J. Stout, W. A. Steller, and A. J. Manuel have developed a gas chromatography-chemical ionization-mass spectrometry procedure for the confirmation of sulfamethazine in cattle and swine tissues. This procedure uses the GLC extract from the Manuel-Steller GLC method. With these 2 methods and the TLC screening procedure (also qualitative), excellent procedures for determining several of the sulfonamide (parent) drugs are now available.

Recommendations

(1) Adopt as official first action the interim screening method for sulfonamide drugs.

(2) Appoint Associate Referees on the topics Diethylstilbestrol; Dinitrobenzamide; Nitrofurans; Sulfonamide drugs.

(3) Continue study on all topics.

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Report on Drugs in Feeds

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Amprolium.—(Kathleen Eaves, Associate Referee). A high performance liquid chromatographic procedure using the paired-ion technique is being developed. A possible mini-collaborative is scheduled.

Carbadox.—(Mark A. Litchman, Associate Referee). Long-range plans call for converting present methodology to high performance liquid chromatography.

Ethopabate.—(Kathleen Eaves, Associate Referee). A high performance liquid chromatographic method is being developed.

Furazolidone and Nitrofurazone.—(Robert L. Smallidge, Associate Referee). A method is ready for collaborative study.

Pyrantel Tartrate.—(James A. Braswell, Asso-

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ciate Referee). A high performance liquid chromatographic method is being developed.

Roxarsone.—(Glenn M. George, Associate Referee). A collaborative study is under way.

Sulfa Residues.—(Robert K. Munns, Associate Referee). A collaborative study is under way.

Sulfamethazine and Sulfathiazole.—(Dwight M. Lowie, Associate Referee). A high performance liquid chromatographic method has been submitted for publication and a collaborative study is planned.

No plans were reported for work on other topics. Associate Referees are needed on the following topics: Arsanilic Acid; Rabon; Phenothiazine.

Recommendation

Continue study on all topics.

Report on Veterinary Analytical Toxicology

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The General Refereeship in Veterinary Analytical Toxicology was initiated at the 1981 AOAC meeting. Since that time, all Associate Referee topics mentioned in this report have been assigned. The Referee is grateful to the Associate Referees for their willingness to serve and for their efforts during the last year.

The activities of the General Referee area are coordinated with those of professional scientists who are associated with the American Association of Veterinary Laboratory Diagnosticians (AAVLD) and the American College of Veterinary Toxicologists (ACVT). Members from both groups serve on a committee established in 1981 by the AAVLD. The specific makeup and function of the committee and its working relationship with the General Referee has recently been reported (1).

The Associate Referees currently assigned cover only a portion of the topics of interest in veterinary analytical toxicology. The topics not currently assigned are either presently covered by other General Referee areas or will be assigned in the future.

There have been some recent books and manuals published on the subject of veterinary analytical toxicology (2–4). Only a few of the methods in these publications are substantiated by collaborative studies. These sources, and others, can serve as a guide for toxicological analysis and also has a basis for further study and collaboration.

The typical toxicology analyst is presented with specimens of a wide variety and type. The samples are of a biological nature and in varying states of decomposition. The procedures followed in analysis are usually adapted from methods developed for entirely different types of samples. The toxicologist and analyst must keep in mind the limitations of the methods and that the methods should provide data that can be used in support of the diagnosis of toxicosis.

Specifically, several publications have recently appeared with this goal in mind. Reynolds (5) has developed an HPLC screening method for toxic levels of 12 or more anticoagulant rodenticides in baits and feeds. Ray et al. have reported (6) on an HPLC procedure for diagnosing "blister beetle" poisoning. Casper (7) gave a recent report on dicoumarol levels associated with sweet clover poisoning. Stahr et al. (8) have reported on the use of TLC for analysis of several metals of diagnostic interest. These few examples point out the diverse nature and broad range of interest that exists in veterinary analytical toxicology.

There has been a great deal of effort by all Associate Referees to determine what methods are now being used in the field. Several methods surveys, interlaboratory studies, and questionnaires have been completed or are now in process. The response by members of the veterinary diagnostic community has been excellent. A great deal of information on sampling and methods has been compiled. In addition,

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

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition.

participants for the collaborative process have been secured.

A brief summary of the status and recommendations for each topic follows.

Cholinesterase.—Associate Referee Richard Pfeiffer has resigned due to a job change. It is recommended a new appointment be made and study be continued.

Copper in Animal Tissues.—David Osheim (National Veterinary Services Laboratories, Ames, IA) reports that a survey of methods for copper analysis in serum has been completed. The survey was conducted to determine what methods are currently being used and to aid in selection of a method for collaborative study. Thirty-six laboratories agreed to participate and 29 of those reported results on 2 bovine serum samples provided to them. The mean values were 76 and 74 μg copper/100 mL serum with CVs of 47% and 58%, respectively, for the 2 samples.

A variety of methods were used, including direct dilution, wet and dry ashing for sample preparation, and ICP, AAS, and flame emission for detection.

Even though there was a wide range of results, a method from the Perkin-Elmer "Cookbook" (9) was used by 5 laboratories and gave good agreement. The method is simple, short, and economical. It involves a direct dilution of the serum sample with water, preparation of standards in 10% glycerol to compensate for viscosity difference, and determination by AAS.

Based on the survey results and further study in his laboratory, the Associate Referee conducted a collaborative study of the above method and recommends it be adopted official first action.

Lead in Animal Tissues.—Associate Referee Robert J. Everson (Purdue University, West Lafayette, IN) reports that he has received the results from a questionnaire concerning lead analysis which was sent to industrial and state laboratories to find out what methods are currently being used, the frequency of analysis, and the types of samples currently being analyzed.

For blood lead analysis, organic extraction/AAS, furnace/AAS, and Delves cup/AAS are used by the majority. Analysis of other diagnostic samples for lead is done by a variety of techniques ranging from dry ash/AAS to wet ash/ICP.

The Associate Referee recommends that an interlaboratory study be conducted on lead analysis in blood. All participants will be allowed to use their method of choice. Based on the results from this study, a collaborative study will be planned.

Multiple Anticoagulant Screening.—Associate Referee John David Reynolds (Animal Disease Laboratory, Centralia, IL) has sent a questionnaire to several state and federal diagnostic laboratories requesting information on screening methods currently used for anticoagulant rodenticides. Although the number of laboratories that are actually screening for anticoagulants is small, several expressed interest in participating in a collaboration. Currently HPLC, TLC, UV, GLC, and GLC/MS are used.

The Associate Referee recommends that an interlaboratory study be conducted followed by a collaborative study of his HPLC method for screening baits and feeds (5). Although the method is designed for the detection of 12 or more anticoagulants, the initial phase of the study will include only warfarin, fumarin, racumin, and coumachlor. Other studies that will incorporate 12 or more anticoagulants are planned for the future. The lack of enough laboratories with gradient HPLC capabilities requires that several isocratic systems be used. The main emphasis of the initial study will be identification and quantitation.

Multielement Analysis by ICP.—Associate Referee W. Emmett Brazelton (Michigan State University, East Lansing, MI) reports difficulty in finding enough laboratories with ICP capabilities interested in doing analysis of animal tissues. If enough laboratories and instruments are found, the Associate Referee recommends that his wet ash/ICP method for trace elements in animal tissue undergo ruggedness testing and further study toward a collaborative study.

Nitrate/Nitrite.—Associate Referees Michael Carlson and Norman Schneider (University of Nebraska, Lincoln, NE) report significant progress during the year toward a collaborative study. An interlaboratory study conducted by Casper (10) on nitrate analysis in forage has given support for the nitrate electrode in the determinative step. In this study, 16 participating laboratories were allowed to use their method of choice on 5 forage samples. The methods employed included nitrate electrode, hydrazine reduction/colorimetric, cadmium column reduction/colorimetric, and others. The laboratories using the nitrate electrode obtained results which were in good agreement, while other methods gave a broad range of results. The samples used for the study contained levels of nitrate as KNO_3 ranging from 0.30% to greater than 5.0%. The high level represents that which would be expected

in the case of nitrate poisoning (11). The Associate Referees recommend that a collaborative study be conducted on nitrate determinations in forage samples, using the nitrate electrode.

Selenium in Animal Tissues.—Associate Referee James Roof (Bureau of Animal Industry, Harrisburg, PA) reports on the completion of a survey of methods/interlaboratory study of selenium in blood. One porcine blood sample and one bovine blood sample were analyzed by 14 different laboratories. The laboratories provided their method when results were reported.

The results from the bovine sample gave a mean of 0.09 μg/mL blood with a standard deviation of 0.02, while the porcine sample mean was 0.12 μg/mL blood with a standard deviation of 0.04. The methods used included the AOAC method for plant tissue (**3.097-3.101**), dry ash/fluorometric, wet and dry ash/hydride generation AAS, dry ash/GLC and wet ash/ICP-AES with hydride generation. The Associate Referee recommends further study and evaluation of methods toward a collaborative study.

Poisonous Plants.—Associate Referee George Rottinghaus (University of Missouri, Columbia, MO) is currently conducting a survey of veterinary diagnostic laboratories to determine the needs for poisonous plant analysis. Because a wide variety of compounds are encountered in various types of poisoning from plants, there is an almost limitless number of procedures needed. The Associate Referee recommends when the survey is completed that the needs be assessed and methodology be developed and/or studied.

Arsenic in Animal Tissues.—Associate Referee Tracy Hunter (Bureau of Consolidated Laboratories, Richmond, VA) reports on the completion of a survey of methods for arsenic in animal tissues. The survey was aimed at finding what methods are currently being used for cases of suspected arsenic poisoning in domestic animals. Seventeen laboratories were provided a kidney sample and a liver sample. Each contained a high level of arsenic. The means and standard deviations were 10.2 ppm and 2.5 for the kidney and 21.2 ppm and 3.7 for the liver, respectively.

The majority of the laboratories used arsine generation (**41.011**) for detection and quantitation. Other detection techniques included hydride/AAS, ICP, and graphite furnace/AAS. Roughly an equal number of laboratories use wet and dry ashing. The Associate Referee recommends that a collaborative study be conducted to expand the existing method for arsenic residues in animal tissues (**41.009-41.012**) to include levels that would be encountered in animal poisonings.

Molybdenum.—Associate Referee Howard Casper (North Dakota State University, Fargo, ND) is currently conducting a literature review of methods. He recommends that the existing method for molybdenum in plants (**3.056-3.058**) be investigated for application to animal tissues.

Rumensin.—Associate Referee Ronda Moore (Iowa State University, Ames, IA) has reviewed the literature and contacted several laboratories to find which methods are currently used to screen feed samples related to animal poisonings for rumensin. Methods being used include a TLC method (12) and a colorimetric method (13), both of which employ a reaction with vanillin for detection. The Associate Referee recommends that studies on sensitivity of the TLC method and on the stability of rumensin at low levels in feeds be conducted. Interlaboratory and collaborative studies will then be conducted based on results of these studies.

Recommendations

(1) Adopt as official first action the method for copper in serum as described by the Associate Referee.

(2) Conduct an interlaboratory study followed by collaborative study on the multiple anticoagulant screening method.

(3) Conduct a collaborative study on nitrate in forage, using the nitrate electrode.

(4) Conduct a collaborative study to expand the existing arsenic method (**41.009-41.012**) to include levels encountered in animal poisonings.

(5) Continue study on all topics.

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TRANSACTIONS OF THE ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS

The ninety-sixth annual meeting of the Association of Official Analytical Chemists was held at the Shoreham Hotel, Washington, DC, on October 25, 26, 27, and 28, 1982. The following reports, along with the actions of the Association, were given at the business meeting held Thursday, October 28, 1982, J. P. Minyard presiding.

Report of the Official Methods Board

ELMER GEORGE, JR, *Chairman*
State Department of Agriculture and Markets, Food Laboratory, Albany, NY 12235

Other members: R. Brunelle, J. A. Burke, D. E. Coffin, A. R. Hanks, J. C. Kissinger, E. Sarnoff, H. M. Wehr

The 96th Annual Meeting marks the second year of the Official Methods Board existence. The majority of business during the year was conducted by correspondence. The Board met once at the Annual Meeting.

Nominations received for the Best Associate Referee Report award were: Peter F. Kane, Committee A; B. Denis Page, Committee C; Arthur Caputi, Committee D; Susan C. Hight, Committee E; and Michael H. Thomas, Committee G. Nominations were not submitted by Committees B and F. The award was presented to Peter F. Kane by president James P. Minyard at the General Session of the Annual Meeting, October 25, 1982.

The 7 Official Methods Committees received recommendations from General Referees for adoption of 30 methods or revisions during the year; 7 were recommended for interim official first action and 23 for official first action approval. Last year 40 collaborative study reports were submitted. The average per year for the decade ending 1980 was 48.

The customary semi-annual letters of instruction from the chairman of the Board were sent to Associate and General Referees in January and June. General Referees were requested to complete a special Associate Referee survey form and return it by September 20. Approximately 500 forms were received. Information requested included: address and telephone changes, status of collaborative studies started and/or plans for the coming year, Associate Referee special needs, and suggestions for improving the collaborative study process. Pertinent information obtained from the survey will appear in *The Referee*.

The Board of Directors requested comments and suggestions on the Long-Range Planning Committee's (LRPC) recommendations for increasing methods output. Specifically, the request included review of Part 2, identification of method needs and available sources of methods for collaborative study; Part 3, study of "candidate method" approach; and Part 4, identification of ways for expediting implementation of the collaborative study process. The assignment was divided among 3 subcommittees. In summary, the majority of the Board Members recommend that the Board of Directors exercise extreme caution in approving and implementing the LRPC's report. Furthermore, the majority of the Board believes that the present approach to methods development and testing should not be basically changed; in addition, care must be taken to not usurp the current responsibilities of General and Associate Referees.

On October 24, 1982, the Official Methods Board established a new collaborative study policy. Namely, whenever an Associate Referee or any author submits a report of a collaborative study with a method recommended for adoption, a separate, formal copy of the method, written in substance although not necessarily in style for publication in *Official Methods of Analysis*, must be attached. The required statistical parameters must also be included with the report as a separate, distinct item.

Accepted

Report of Committee A on Recommendations for Official Methods

ALAN R. HANKS (Office of the Indiana State Chemist, Purdue University, Department of Biochemistry, West Lafayette, IN 47907), *Chairman;* WARREN R. BONTOYAN (Environmental Protection Agency, Office of Pesticide Programs, Beltsville, MD 20705); A. ANER CARLSTROM (Chevron Chemical Co., 940 Hensley St, Richmond, CA 94804); LOUIS W. FERRARA (IMC Corp., 1401 S 3rd St, Terre Haute, IN 47808); FRANK J. JOHNSON (TVA/National Fertilizer Development Center, Muscle Shoals, AL 35660); HOWARD P. MOORE (Ohio Department of Agriculture, Consumer Analytical Laboratory, Feed and Fertilizer Section, 8995 E Main St, Reynoldsberg, OH 43068); RICHARD H. COLLIER (Department of Biochemistry, Purdue University, West Lafayette, IN 47907), *Secretary;* and EDWIN M. GLOCKER (14697 Roxbury Rd, Glenelg, MD 21737), *Statistical Consultant*

The Committee is pleased to report positive recommendations for 11 of 13 collaborative studies this year. Because of a slight misunderstanding, one of the studies, dalapon, did not reach the Committee for consideration; however, review for interim action approval should be completed shortly. The second study not acted on will be considered for interim action as soon as complete data can be obtained. In addition to the full collaborative studies, positive action was taken on 2 changes in status as a result of strong supportive data and information provided by Associate Referees and communicated by General Referees to the Committee.

For the first time in many years, the Committee met individually with the General Referees. These meetings, although extending the Committee time in session to 7 h, were productive and filled 2 important needs: informal information exchange and interactive review. The practice of individual General Referee meetings will be continued in the future.

The Committee agrees with the Methods Board recommendation that reports of collaborative studies received for review be accompanied by a separate copy of the method, as determined by the Associate Referee, that should be published in *Official Methods of Analysis;* furthermore, the Committee agrees with the Methods Board recommendation that method performance data, required by the Association from 1982 forward, should be included as a distinct item in the report of a collaborative study.

The Committee wishes to express a concern and make a suggestion for consideration by the Association. *Background:* It is found that methods may be adopted interim first action and be recommended for first action at the next Annual Meeting without having been either published in full in a vehicle readily available to the membership, or presented at an Annual Meeting prior to the Association's vote for adoption. The same course of events, especially during periods of restricted travel, appears to be occurring in some instances for collaborative studies received for initial review at the same Annual Meeting at which they are adopted. *Concern:* Under the circumstances as stated, are interested members afforded adequate opportunity to review collaborative studies before voting for adoption? *Suggestion:* Consideration should be given to the development of a policy covering this concern with possible inclusion of a requirement for either publication of the complete study in a vehicle readily available to the membership, or presentation at an Annual Meeting prior to the Association's vote for adoption. General Referees meeting with the Committee this year agree with the concern and concur with the suggestion.

Two General Referee vacancies currently exist. Of particular concern is the vacancy for Hazardous Substances. Since the Consumer Product Safety Commission has closed its field laboratories, the availability of well qualified scientists to fill this General Refereeship has greatly diminished. The Committee is initiating an active search for a qualified General Referee for Hazardous Substances. Persons interested in serving in this capacity and/or knowing of persons who might be interested in serving should contact Methods Committee A.

Finally, the Committee takes great pleasure in publicly expressing its sincere thanks and appreciation to Howard P. Moore of the Ohio Department of Agriculture for his outstanding service as a member and his strong leadership as chairman of Methods Committee A. Howard, good luck in retirement and watch out for Indians in Tennessee.

FEEDS

(1) *Amino Acids in Mixed Feeds:* Continue study.
(2) *Fat, Crude, in Pet Foods:* Continue study.
(3) *Fiber, Crude:* Continue study.
(4) *Fiber, Crude, in Milk Replacers:* Continue study.
(5) *Infrared Reflectance Techniques in Mixed Animal Feeds:* Appoint an Associate Referee; continue study.
(6) *Iodine in Feeds:* Continue study.
(7) *Minerals:* Continue study.
(8) *Non-Nutritive Residues:* Continue study.
(9) *Protein, Crude:* Continue study.
(10) *Sampling:* Continue study.
(11) *Water (Karl Fischer Method):* Continue study.

FERTILIZERS

(1) *Biuret in Urea and Mixed Fertilizers:* Continue study.
(2) *Boron:* Continue study.
(3) *Calcium and Magnesium:* Discontinue topic.
(4) *Copper:* Discontinue topic.
(5) *Elemental Analysis of Liming Materials:* Discontinue topic.
(6) *Free and Total Water:* Continue study.
*(7) *Iron:* (a) Adopt as official first action the atomic absorption method for chelated iron in iron chelate concentrates, as reported by the Associate Referee, with the provision that an applicability statement be added to limit the method to the chelating agents studied and to note that the method is not applicable to mixed fertilizers. (b) Continue study.
(8) *Molybdenum:* Appoint an Associate Referee; continue study.
*(9) *Nitrogen:* (a) Adopt as official final action the official first action modified comprehensive nitrogen method (**2.061-2.062**). (b) Continue official first action status of water-insoluble nitrogen, Method II (**2.073-2.074**). (c) Continue study.
(10) *Phosphorus:* Continue study.
*(11) *Potash:* (a) Adopt as official first action the flame photometric method described by the Associate Referee. (b) Continue study.
(12) *Sampling and Preparation of Sample:* Continue study.
(13) *Slow-Release Mixed Fertilizers:* Continue study.
*(14) *Sodium:* (a) Adopt as official first action the atomic absorption method described by the Associate Referee at the 95th Annual Meeting, October 1981. (b) Continue study.
*(15) *Soil and Plant Amendment Ingredients:* (a) Adopt as official first action the atomic absorption method for aluminum in aluminum sulfate soil acidifiers described by the Associate Referee. (b) Continue study.
(16) *Sulfur:* (a) Continue official first action status of the sulfur method (**2.A01**). (b) Continue study.
*(17) *Water-Soluble Methyleneureas:* (a) Adopt as official first action the high performance liquid chromatographic method described by the Associate Referee. (b) Continue study.
(18) *Zinc:* Continue study.

HAZARDOUS SUBSTANCES

(1) *Ammonia as a Product Ingredient:* Continue study.
(2) *Benzene in Consumer Products:* Continue study.
(3) *Carbolic Acid (Phenolic) Compounds:* Continue study.
(4) *Chlorinated Hydrocarbons:* Continue study.
(5) *Diethylene Glycol and Ethylene Glycol:* Appoint an Associate Referee; continue study.
(6) *Flammable Substances in Pressurized Containers:* Continue study.
(7) *Flash Point of Solids and Semisolids:* Appoint an Associate Referee; continue study.
(8) *Formaldehyde:* Continue study.
(9) *Hazardous Components in Resin Systems:* Appoint an Associate Referee; continue study.
(10) *Pentachlorophenol in Toy Paints:* Continue study.
(11) *Petroleum Distillates in Mixtures:* Continue study.
(12) *Selenium:* Appoint an Associate Referee; continue study.

* An asterisk before a recommendation indicates that official action on that recommendation resulted in its appearance in "Changes in Methods."

The recommendations submitted by Committee A were adopted by the Association.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition; and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **63**, 374-423 (1980); **64**, 501-540 (1981); 65, 450-521 (1982).

(13) *Toxic Metals in Paints:* Continue study.
(14) *Turpentine:* Continue study.
(15) *Viscosity of Liquids:* Appoint an Associate Referee; continue study.
(16) *Other Topic:* Continue official first action status of the method for volatile denaturants in alcoholic products (**5.012-5.013**); continue study.

PESTICIDE FORMULATIONS: CARBAMATE INSECTICIDES AND SUBSTITUTED UREA INSECTICIDES

(1) *Aldicarb:* Continue study.
(2) *Carbaryl:* Continue study.
(3) *Carbofuran and Carbosulfan:* Continue study.
(4) *2,2-Dimethyl-1,3-benzodioxol-4-yl Methylcarbamate (Bendiocarb):* Continue study.
(5) *4-Methylthio-3,5-xylyl Methylcarbamate (Methiocarb):* Continue study.
(6) *o-Isopropoxyphenyl Methylcarbamate (Propoxur):* Continue study.
(7) *Methomyl:* Continue study.
(8) *Oxamyl:* Continue study.
(9) *Pirimicarb:* Appoint an Associate Referee; continue study.

PESTICIDE FORMULATIONS: FUNGICIDES AND DISINFECTANTS

(1) *Benomyl:* Appoint an Associate Referee; continue study.
(2) *Captan:* Continue study.
(3) *Carboxin and Oxycarboxin:* Continue study.
(4) *Chlorothalonil:* Continue study.
(5) *Copper Naphthenate:* Continue study.
(6) *Dinocap (2,4-Dinitro-6-octyl Phenyl Crotonate):* Continue study.
(7) *Dithiocarbamate Fungicides:* Continue study.
(8) *Folpet:* Continue study.
*(9) *Pentachloronitrobenzene:* (a) Delete the statement, "Discard after 3 days," of paragraph **6.C09(c)** of the official first action GLC method (**6.C08-6.C11**) and adopt the modified method official final action. (b) Continue study.
(10) *o-Phenylphenol:* Continue study.
(11) *Triphenyltin:* Continue study.

PESTICIDE FORMULATIONS: GENERAL METHODS

(1) *Atomic Absorption Spectroscopy:* Continue study.
(2) *Contaminants in Pesticide Formulations:* Continue study.
(3) *Dioxins (2,3,7,8-Tetrachloro-p-dibenzo-p-dioxin in 2,4,5-T):* Continue study.
(4) *Nitrosamines:* Continue study.
(5) *Pesticides in Spray Tank Dispersions:* Continue study.
(6) *Physical Properties of Pesticides:* Continue study.
(7) *Sampling:* Continue study.
(8) *Sampling of Pressurized Cans (Aerosols):* Appoint an Associate Referee; continue study.
(9) *Volatility of Hormone-Type Herbicides:* Continue study.
(10) *Water-Soluble Copper in Water-Insoluble Copper Fungicides:* Continue official first action status of the CIPAC-AOAC atomic absorption and bathocuproine methods (**6.B01-6.B08**).

PESTICIDE FORMULATIONS: HALOGENATED

(1) *Benzene Hexachloride and Lindane:* Continue official first action status of the benzene hexachloride radioactive tracer method (**6.202**); continue study.
(2) *Chlordane:* Continue study.
(3) *Chlordimeform:* Continue study.
(4) *Dicofol (1,1-Bis(chlorophenyl)-2,2,2-trichloroethanol):* Continue official first action status of the hydrolyzable chloride method (**6.283-6.288**); continue study.
*(5) *Diflubenzuron:* (a) Adopt as official first action the CIPAC high performance liquid chromatographic method described by the Associate Referee. (b) Continue study.
(6) *Dipropyl Isocinchomeronate:* Continue study.
*(7) *Endosulfan:* (a) Adopt as official first action the CIPAC gas-liquid chromatographic method described by the Associate Referee. (b) Continue study.
(8) *Fenvalerate:* Continue study.
(9) *Heptachlor:* Continue study.
(10) *Methoxychlor:* Continue study.
(11) *Perthane:* Continue study.
(12) *Tetradifon:* Discontinue topic.
(13) *Toxaphene:* Continue study.
(14) *Trichlorfon (Dylox):* Continue study.

PESTICIDE FORMULATIONS: HERBICIDES I

(1) *Chlorophenoxy Herbicides:* (a) Continue official first action status of the following high performance liquid chromatographic methods: 2-methyl-4-chlo-

rophenoxyacetic acid (**6.A18-6.A22**); 2,4,5-trichlorophenoxyacetic acid (**6.A23-6.A26**); 2,4-dichlorophenoxyacetic acid esters and amine salts (**6.275-6.279**). (b) Continue study.

(2) *Dicamba:* Continue study.
(3) *Pentachlorophenol:* Continue study.
(4) *Picloram:* Discontinue topic.
(5) *Plant Growth Regulators:* Continue study.
(6) *2,3,6-Trichlorobenzoic Acid:* Appoint an Associate Referee; continue study.

PESTICIDE FORMULATIONS: HERBICIDES II

(1) *Alanap:* Continue study.
(2) *Barban:* Continue study.
(3) *Bensulide (S-(O,O-Diisopropyl) Phosphorodithioate Ester of N-(2-Mercaptoethyl Benzenesulfonamide):* Continue study.
(4) *Benzoylprop-ethyl:* Appoint an Associate Referee; continue study.
(5) *Bromacil and Lenacil:* Continue study.
(6) *Chloroxuron:* Continue study.
(7) *Dimethyl Tetrachloroterephthalate:* Continue study.
(8) *Dinoseb:* Appoint an Associate Referee; continue study.
(9) *Diuron:* Continue study.
(10) *S-Ethyl Dipropylthiocarbamate:* Appoint an Associate Referee; continue study.
(11) *Fluchloralin, Profluralin, Benefin, Trifluralin, and Penoxalin:* Continue study.
(12) *Fluometuron:* Continue study.
(13) *Linuron:* Continue study.
(14) *Methazole:* Continue study.
(15) *Monuron:* Appoint an Associate Referee; continue study.
(16) *Oryzalin (3,5-Dinitro-N,N-(dipropyl)sulfanilamide):* Appoint an Associate Referee; continue study.
(17) *Paraquat:* Continue study.
(18) *Siduron:* Continue study.
(19) *Thiocarbamate Herbicides:* Continue official first action status of the gas-liquid chromatographic method for thiocarbamate herbicides (**6.426-6.430**); continue study.

PESTICIDE FORMULATIONS: HERBICIDES III

(1) *Alachlor, Butachlor, and Propachlor:* Continue study.
(2) *Amitrol:* Appoint an Associate Referee; continue study.
(3) *Bentazone:* Appoint an Associate Referee; continue study.
(4) *Bromoxynil:* Continue study.
(5) *Cacodylic Acid:* Appoint an Associate Referee; continue study.
(6) *Cyanazine (Bladex®):* Appoint an Associate Referee; continue study.
(7) *Dalapon:* Continue study.
(8) *Dichlobenil:* Appoint an Associate Referee; continue study.
(9) *Disodium Methane Arsenate:* Appoint an Associate Referee; continue study.
*(10) *Glyphosate:* (a) Adopt as official first action the high performance liquid chromatographic method described by the Associate Referee. (b) Continue study.
(11) *Metolachlor:* Continue study.
(12) *Metribuzin (4-Amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5-(4H)-one):* Continue study.
(13) *Monosodium Methane Arsenate:* Appoint an Associate Referee; continue study.
(14) *Propanil (3′,4′-Dichloropropionanilide):* Continue study.
*(15) *Terbuthylazine:* (a) Adopt as official final action the official first action gas-liquid chromatographic method (**6.B20-6.B27**). (b) Include this topic under Triazine Herbicides.
(16) *s-Triazine Herbicides:* Continue study.

PESTICIDE FORMULATIONS: INORGANIC PESTICIDES

(1) *Aluminum Phosphide:* Continue study.
(2) *Sodium Chlorate:* Appoint an Associate Referee; continue study.

PESTICIDE FORMULATIONS: ORGANOTHIOPHOSPHATE PESTICIDES

(1) *Acephate (Orthene®):* Continue study.
(2) *Azinphosmethyl (O,O-Dimethyl S-[(4-Oxo-1,2,3-benzotriazin-3(4H)-yl)methyl]phosphorodithioate):* Continue study.
(3) *Chlorpyriphos:* Continue study.
(4) *Coumaphos:* Continue study.
(5) *Demeton:* Appoint an Associate Referee; continue study.
(6) *Demeton-S-Methyl:* Appoint an Associate Referee; continue study.
(7) *Diazinon (O,O-Diethyl O-(2-Isopropyl-6-methyl-4-pyrimidinyl) Phosphorodithioate):* Continue official first action status of the gas-liquid chromatographic method for diazinon (**6.331**); continue study.
(8) *Dimethoate:* Continue study.
(9) *Dioxathion:* Continue study.
(10) *Disulfoton:* Continue study.
(11) *Encapsulated Organophosphorus Pesticides:*

Continue official first action status of the gas-liquid chromatographic method for encapsulated diazinon (**6.C12-6.C15**); continue study.

(12) *EPN:* Continue study.

(13) *Ethion:* Continue study.

(14) *Ethoprop:* Continue study.

(15) *O-Ethyl O-(4-Methylthio) Phenyl S-Propyl Phosphorothioate (Bolstar®):* Continue study.

*(16) *Fensulfothion:* (a) Adopt as official first action the high performance liquid chromatographic method described by the Associate Referee. (b) Continue study.

(17) *Fenthion:* Continue study.

(18) *Fonophos:* Appoint an Associate Referee; continue study.

(19) *Malathion:* Continue study.

(20) *Methidathion (Supracide®):* Continue study.

(21) *Parathion and Methyl Parathion:* Continue official first action status of the volumetric (**6.388-6.394**), colorimetric (**6.395-6.399**), gas-liquid chromatographic (**6.379-6.383**), and high performance liquid chromatographic (**6.384-6.387**) methods for parathion, and the gas-liquid chromatographic (**6.400-6.404**) and high performance liquid chromatographic (**6.405-6.408**) methods for methyl parathion; continue study.

(22) *Phorate:* Continue study.

(23) *Temephos:* (a) Continue official first action status of the CIPAC high performance liquid chromatographic method (**6.C16-6.C21**). (b) Appoint an Associate Referee. (c) Continue study.

PESTICIDE FORMULATIONS: OTHER INSECTICIDES, SYNERGISTS, AND INSECT REPELLANTS

(1) *Allethrin:* Continue official first action status of the gas-liquid chromatographic method for technical allethrin (**6.149-6.154**); continue study.

(2) *2,3:4,5-Bis(2-butylene)tetrahydro-2-furaldehyde (MGK Repellant 11®):* Continue study.

(3) *Dipropyl Isocinchomeronate (MGK Repellant 326®):* Continue study.

(4) *Fumigants:* Continue official first action status of the gas-liquid chromatographic method for fumigant mixtures (**6.143-6.148**); continue study.

(5) *Nicotine:* Continue study.

(6) *Permethrin:* Continue study.

*(7) *Piperonyl Butoxide and Pyrethrins:* (a) Adopt as official final action the official first action gas-liquid chromatographic method (**6.C22-6.C25**). (b) Continue study, including low levels and mixed formulations.

(8) *Resmethrin:* Continue study.

*(9) *Rotenone and Other Rotenoids:* (a) Adopt as official first action the high performance liquid chromatographic method described by the Associate Referee. (b) Continue official first action status of the infrared method for rotenone (**6.163-6.164**). (c) Continue study.

(10) *Other Topics:* (a) Continue official first action status of the UV method for sulfoxide (**6.419**). (b) Establish the new topic Methyl Bromide and appoint an Associate Referee.

PESTICIDE FORMULATIONS: OTHER ORGANOPHOSPHATE INSECTICIDES

(1) *Crotoxyphos:* Continue study.

(2) *Crufomate:* Appoint an Associate Referee; continue study.

(3) *Dichlorvos (2,2-Dichlorovinyl Dimethyl Phosphate):* Continue official first action status of the infrared methods (**6.324-6.327** and **6.328-6.330**); continue study.

(4) *Mevinphos:* Continue study.

(5) *Monocrotophos (Dimethyl Phosphate of 3-Hydroxy-N-Methyl-cis-Crotonamide):* Continue study.

(6) *Naled:* Continue study.

(7) *Tetrachlorvinphos (2-Chloro-1-(2,4,5-trichlorophenyl)vinyl Dimethyl Phosphate):* Continue study.

PESTICIDE FORMULATIONS: RODENTICIDES AND MISCELLANEOUS PESTICIDES

*(1) *Brodifacoum (Talon®):* (a) Adopt as official first action the high performance liquid chromatographic method presented by the Associate Referee. (b) Continue study.

(2) *Chlorophacinone:* Appoint an Associate Referee; continue study.

(3) *Diphacinone:* Continue study.

(4) *α-Naphthylthiourea:* (a) Continue official first action status of the surplus method (**6.139**). (b) Appoint an Associate Referee. (c) Continue study.

(5) *N-3-Pyridyl-N'-p-nitrophenyl Urea*

(*Vacor®*): Appoint an Associate Referee; continue study.

(6) *Strychnine:* Appoint an Associate Referee; continue study.

(7) *Warfarin:* Continue study.

PLANTS

(1) *Ashing Methods:* Continue study.

(2) *Atomic Absorption Methods:* Continue official first action status of the atomic absorption method for calcium, copper, iron, magnesium, manganese, potassium, and zinc (**3.006-3.009**); continue study.

(3) *Boron:* Continue study.

(4) *Chromium:* Continue study.

(5) *Copper and Cobalt:* Continue study.

(6) *Fluoride:* Continue official first action status of the potentiometric method (**3.077-3.082**) and the semi-automated method (**3.083-3.095**) for fluoride; continue study.

(7) *Nitrogen, Non-Protein:* Appoint an Associate Referee; continue study.

(8) *Plasma and Spark Emission Spectroscopy:* Continue study.

(9) *Selenium:* Continue study.

(10) *Starch:* Continue study.

(11) *Sulfa:* Continue study

(12) *Sulfur:* Appoint an Associate Referee; continue study.

(13) *Zinc:* Appoint an Associate Referee; continue study.

REFERENCE MATERIALS AND STANDARD SOLUTIONS

Stability of Organophosphorus Pesticide Standards: Continue study.

TOBACCO

(1) *Differentiation of Cigar and Cigarette Tobacco (Sequential Differential Solvent Extraction):* Continue study.

(2) *Humectants in Cased Cigarettes:* Continue official first action status of the method for glycerol, propylene glycol, and triethylene glycol in cased cigarette cut filler and ground tobacco (**3.147-3.150**); continue study.

(3) *Nicotine, Gas Chromatography:* Continue official first action status of the method for nicotine on Cambridge filter pads (**3.158-3.161**); continue study.

(4) *Tar and Nicotine in Cigarette Smoke:* Continue study.

Report of Committee B on Recommendations for Official Methods

EVELYN SARNOFF (Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232), *Chairman;* ANTHONY ROMANO, JR (Drug Enforcement Administration, Southeastern Regulatory Laboratory, Miami, FL 33166); JOSEPH V. THOM (State Department of Health, Laboratory Services Program, Berkeley, CA 94704); WILLIAM W. WRIGHT (U.S. Pharmacopeial Convention, 12601 Twinbrook Pkwy, Rockville, MD 20852); THOMAS LAYLOFF (Food and Drug Administration, 1114 Market St, St. Louis, MO 63101); JAMES B. KOTTEMANN (Food and Drug Administration, Office of Drugs, Washington, DC 20204), *Secretary;* CHANG S. LAO (Food and Drug Administration, 5600 Fishers Ln, Rockville, MD 20857), *Statistical Consultant*

At the meeting of Committee B on October 26, 1982, the subject of general refereeships was discussed. The refereeship on Drugs, Steroids and Terpinoids has been vacant for many months. There are now two General Referees whose subjects are contained in one chapter, Chapter 38, of *Official Methods of Analysis.*

Committee B proposes that the refereeship of Thomas Alexander, Drugs, Other Nitrogenous Bases be merged with that of Edward Smith, Drugs, Alkaloids, as Drugs, Nitrogenous Bases, and that Mr. Alexander take over the vacant position of General Referee on Drugs, Neutral (Steroids and Terpinoids). Both Dr. Smith and Mr. Alexander concurred in this proposal.

Committee B proposes that all antibiotics topics be assigned to Ted Hopes, General Referee on Drugs, Miscellaneous. If antibiotics topics increase greatly in number, a new refereeship can be established as Drugs, Antibiotics.

DRUGS, ACIDIC AND NEUTRAL NITROGENOUS ORGANICS

(1) *Acetaminophen in Drug Mixtures:* Continue study.
(2) *Amitriptyline Hydrochloride in Dosage Forms (HPLC):* Continue study.
(3) *Aspirin, Phenacetin, and Caffeine with Other Drugs:* Continue study.
(4) *Aspirin and Salicylic Acid in Aspirin Products (Semiautomated Analysis):* Continue study.
(5) *Barbiturates:* Continue study.
(6) *Benzothiazine Derivatives:* Appoint an Associate Referee; continue study.
(7) *Benzthiazide by HPLC:* Continue study.
(8) *Methyldopa:* Continue study.
(9) *Primidone:* Continue study.
(10) *Probenecid:* Continue study.
(11) *Sulfonamides (Thin Layer Chromatography):* Continue study.
(12) *Sulfamethoxazole (HPLC):* Continue study.
(13) *Sulfisoxazole (HPLC):* Continue study.
(14) *Thiazide Diuretics, Semiautomated Individual Dosage Units Analysis:* Continue study.

DRUGS, ALKALOIDS

(1) *Atropine in Morphine and Atropine Tablets and Injections:* Continue study.
(2) *Belladonna Alkaloids:* Appoint an Associate Referee; continue study.
(3) *Colchicine in Tablets:* Continue study.
(4) *Curare Alkaloids:* Continue study.
(5) *Ephedrine:* Discontinue topic.
(6) *Ergot Alkaloids:* Continue study.
(7) *Neostigmine:* Continue study.
*(8) *Physostigmine and Its Salts:* Adopt as official first action, for the ointment dosage form, the HPLC method for solutions, **38.C01-38.C06.** Make appropriate changes in method **38.C01** to include the ointment form. Continue study of method for injection dosage form.
(9) *Pilocarpine:* Continue study.
(10) *Rauwolfia Alkaloids:* Continue study.
(11) *Rauwolfia serpentina:* Continue study.
*(12) *Other Topics:* Declare as surplus the following official final action methods: **38.029-38.030,** infrared method for atropine in tablets; **38.038,** Method II for ephedrine in inhalents.

DRUGS, ILLICIT

(1) *Amphetamines in Mixtures:* Appoint an Associate Referee; continue study.
*(2) *Benzodiazepines:* Adopt as official first action the HPLC method for the determination of oxazepam in dosage forms as described by the Associate Referee; continue study.
(3) *Chemical Microscopy:* Continue study.
*(4) *Cocaine:* Adopt as official final action the official first action method, **40.002-40.005,** for the determination of cocaine.
(5) *Dimethyltryptamine (DMT), Diethyltryptamine (DET), and Dipropyltryptamine (DPT):* Continue study.
*(6) *Heroin:* Repeal official first action the official final action method, **40.006,** for determining diacetylmorphine (heroin) in tablets; continue study.
(7) *Lysergic Acid Diethylamide (LSD):* Appoint an Associate Referee; continue study.
(8) *Marihuana and Synthetic Tetrahydrocannabinol (THC):* Continue study.
(9) *Methadone:* Continue study.
(10) *Methamphetamine:* Appoint an Associate Referee; continue study.
(11) *Methaqualone Hydrochloride:* Continue study.
(12) *Methylphenidate Phenidine Hydrochloride:* Appoint an Associate Referee; continue study.
(13) *Optical Crystallographic Properties:* Continue study.
*(14) *Phencyclidine Hydrochloride (PCP):* Adopt as official final action the official first action method, **40.016-40.018,** for determining phencyclidine in powders.

DRUGS, MISCELLANEOUS

(1) *Benzoyl Peroxide:* Discontinue topic.
*(2) *Disulfiram:* Adopt as official final action the official first action colorimetric method, **36.B01-36.B04,** for determining disulfiram in tablets.
(3) *Ethylene Oxide:* Continue study.
(4) *Fluoride:* Continue study.
(5) *Identification of Drugs (Mass Spectroscopy):* Discontinue topic.
(6) *Medicinal Gases:* Continue study.

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* An asterisk before a recommendation indicates that official action on that recommendation resulted in its appearance in "Changes in Methods."

The recommendations submitted by Committee B were adopted by the Association

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition, and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **64,** 501-540 (1981); **65,** 450-521 (1982).

(7) *Menadiol Sodium Diphosphate Injection:* Continue study.
(8) *Mercurial Diuretics:* Continue study.
*(9) *Mercury-Containing Drugs:* Adopt as official first action the atomic absorption method for determining total mercury in mercury-containing drugs as described by the Associate Referee.
(10) *Metals in Bulk Drug Powders:* Continue study.
(11) *Microcrystalline Tests:* Appoint an Associate Referee; continue study.
(12) *Protein Nitrogen Units in Allergenic Extracts:* Continue study.
(13) *Thyroid and Thyroxine-Related Compounds:* Continue study.
(14) *Thyroid by Differential Pulse Polarography:* Continue study.

DRUGS, OTHER NITROGENOUS BASES

(1) *Aminacrine:* Continue study.
*(2) *Antihistamines, Adrenergic Combinations by HPLC:* Adopt as official final action the official first action HPLC method, **38.B01-38.B06,** for determination of antihistamine-adrenergic combinations in syrups or tablets; discontinue topic.
(3) *Chlorpromazine:* Continue study.
(4) *Dicyclomine Capsules:* Continue study.
(5) *Epinephrine-Lidocaine Combinations:* Continue study.
(6) *Epinephrine and Related Compounds by HPLC-Electrochemical Detectors:* Continue study.
(7) *Homatropine Methyl Bromide in Tablets:* Continue study.
(8) *Phenethylamine Drugs—Semiautomated Individual Unit Analysis:* Continue study.
(9) *Pheniramine with Pyrilamine, Phenylpropanolamine, and Phenylephrine:* Continue study.
(10) *Phenothiazine Drugs, Identification by TLC:* Continue study.
(11) *Phenothiazines in Drugs:* Continue study.

DRUGS, STEROIDS AND TERPINOIDS

(1) *Automated Corticosteroid Methods:* Continue study.
(2) *Automated Methods for Progestins in Tablets:* Continue study.
(3) *Digitoxin, Automated Individual Tablet Analysis:* Continue study.
(4) *Estrogens:* Continue study.
(5) *Estrogens (Fluorometric Method):* Continue study.
(6) *Ethinyl Estradiol, Automated Individual Tablet Analysis:* Continue study.
(7) *Steroid Acetates:* Continue study.
(8) *Steroid Phosphates:* Continue official first action status of method **39.047-39.051;** continue study.

Report of Committee C on Recommendations for Official Methods

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This Committee has reviewed the activities of the assigned refereeships. Because of inactivity and lack of interest in certain areas, the Committee has recommended that certain General Referee topics and accompanying Associate Refereeships be discontinued. We recommend reassignment of the Referees to other topic areas wherever practicable.

COFFEE AND TEA

(1) *Ash in Instant Tea:* Continue study.
(2) *Caffeine:* Continue study.
(3) *Chlorogenic Acid:* Discontinue topic.
(4) *Crude Fiber in Tea:* Appoint an Associate Referee; continue study.
*(5) *Moisture in Coffee and Tea:* Adopt as official final action the official first action method, **15.012-15.013,** for the determination of loss on drying of instant coffee; continue study.
(6) *Solvent Residues in Decaffeinated Coffee:* Redesignate topic as *Solvent Residues in Decaffeinated Coffee and Tea;* continue study.
(7) *Solvent Residues in Decaffeinated Tea:* Discontinue topic; combine under new topic, Solvent Residues in Decaffeinated Coffee and Tea.
(8) *Theophylline in Tea:* Appoint an Associate Referee; continue study.
(9) *Water Extract in Tea:* Continue study.

DAIRY PRODUCTS

(1) *Casein and Caseinates:* Continue study.
(2) *Chocolate Milk, Fat Test:* Continue study.
(3) *Cryoscopy of Milk:* Continue study.
(4) *Fat, Automated Methods:* Continue study.
(5) *Fat in Milk (AutoAnalyzer):* Continue study.
(6) *Infrared Milk Analyzer (IRMA):* Continue study.
(7) *Lactose in Dairy Products (Chromatographic Determination):* Continue study.
(8) *Lactose in Dairy Products (Enzymatic Determination):* Continue study.
(9) *Moisture in Cheese (Karl Fischer Method):* Continue study.
(10) *Nitrates in Cheese:* Continue study.
(11) *Phosphatase, Rapid Method:* Continue study.
(12) *Phosphatase, Reactivated:* Continue study.
(13) *Phosphorus:* Continue study.
(14) *Protein Constituents in Processed Dairy Products:* Continue study.
(15) *Protein in Milk, Rapid Tests:* Continue study.
(16) *Protein Reducing Substance Tests:* Continue study.
(17) *Solids-Not-Fat:* Continue study.
(18) *Vapor Pressure Osmometry:* Continue study.
*(19) *Other Topics:* Adopt as official first action the interim first action potentiometric method for the determination of chloride in cheese (*J. Assoc. Off. Anal. Chem.* **65,** 1350–1356 (1982)) to replace the present official final action method, **16.242-16.243.** Repeal official first action the present official final action method, **16.242-16.243.** Reinstate official final action method, **16.224** of the 12th edition (1975), as an alternative method.

DECOMPOSITION AND FILTH IN FOODS (CHEMICAL METHODS)

(1) *Ammonia in Dogfish:* Initiate collaborative study of enzymatic procedure for ammonia determination; continue study.
(2) *Coprostanol:* Continue study.
*(3) *Crabmeat:* Adopt as official final action the official first action method, **18.027-18.030,** for the determination of ammonia in crabmeat; continue study.
(4) *Diacetyl in Citrus Products:* Continue study.
(5) *Ethanol in Seafoods:* Continue study.
(6) *Gas and Liquid Chromatography:* Initiate proceedings for adoption as interim official first action of the GLC method for the determination of cadaverine and putrescine in fishery products; continue study.
(7) *GLC of Volatile Amines—TMA and DMA:* Continue study.
(8) *Shellfish:* Continue study.
(9) *TLC Identification of Amines in Fishery Products:* Continue study.
(10) *Tomatoes:* Continue study.

EGGS AND EGG PRODUCTS

(1) *Color:* Discontinue topic.
(2) *Fat:* Discontinue topic.
(3) *Phosphorus:* Discontinue topic.
(4) *Sterols (Gas Chromatography):* Discontinue topic.
(5) *Total Solids:* Discontinue topic.
(6) *Other Topic:* Discontinue Refereeship; reassign Associate Referees to other

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The recommendations submitted by Committee C were adopted by the Association.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition; and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **63,** 374-423 (1980); **64,** 501-540 (1981); **65,** 450-521 (1982).

General Refereeships, wherever practicable.

ENZYMES

(1) *Amylase Activity in Cereal and Cereal Products:* Discontinue topic.
(2) *Catalase in Frozen Vegetables:* Discontinue topic.
(3) *Papain:* Discontinue topic.
(4) *Peroxidase in Frozen Vegetables:* Discontinue topic.
(5) *Proteolytic Enzymes in Treated Meats:* Discontinue topic.
(6) *Rennet:* Discontinue topic.
(7) *Other Topic:* Discontinue Refereeship; reassign Associate Referees to other General Refereeships, wherever practicable.

FISH AND OTHER MARINE PRODUCTS

(1) *Crabmeat Identification:* Continue study.
(2) *Determination of Fish Content in Coated Products (Breaded or in Batter):* Continue study.
(3) *Drained Weight of Block Frozen, Raw, Peeled Shrimp:* Continue study.
(4) *Drip Fluid in Fish Fillets and Fish Fillet Blocks (Quantitation):* Continue study.
(5) *Fish Species Identification (Thin Layer Isoelectric Focusing):* Continue study.
(6) *Nitrites in Smoked Fish:* Continue study.
(7) *Organometallics in Fish:* Continue study.
*(8) *Other Topic:* Adopt as a procedure the interim revision to **18.003,** *Procedure For Cooking Seafood Products,* as recommended by Associate Referee Frederick J. King.

FOOD ADDITIVES

(1) *Anticaking Agents:* Appoint an Associate Referee; continue study.
*(2) *Antioxidants:* Adopt as official first action the interim official first action method for the HPLC determination of antioxidants in fats and oils; continue study.
(3) *Brominated Oils:* Continue study.
(4) *Chloride Titrator:* Continue study.
(5) *Chlorobutanol in Milk:* Continue study.
(6) *Dichlorodifluoromethane in Frozen Foods:* Continue study.
(7) *Dilauryl Thiodipropionate:* Appoint an Associate Referee; continue study.
(8) *Dimethylpolysiloxane:* Continue study.
(9) *Dressings:* Continue study.
(10) *EDTA in Food Products:* Continue study.
(11) *Ethoxyquin in Meat and Eggs:* Continue study.
(12) *Gums in Foods:* Appoint an Associate Referee; continue study.
(13) *Indirect Additives from Food Packages:* Continue study.
(14) *Mineral Oil in Raisins:* Continue study.
(15) *Nitrates (Selective Ion Electrodes):* Continue study.
(16) *Nitrates and Nitrites:* Continue study.
(17) *Nitrosamines:* Continue study.
(18) *Polycyclic Aromatic Hydrocarbons in Foods:* Continue study.
(19) *Polysorbates:* Continue study.
(20) *Propylene Chlorohydrin:* Continue study.
(21) *Sodium Lauryl Sulfate:* Continue study.

GELATIN, DESSERT PREPARATIONS, AND MIXES

(1) *Gel Strength:* Discontinue topic.
(2) *Other Topic:* Discontinue General Referee subject.

MEAT AND MEAT PRODUCTS

(1) *Ashing Methods:* Discontinue topic.
(2) *Automated Methods:* Continue study.
*(3) *Bone Content:* Adopt as official first action the collaboratively studied method for the determination of calcium in mechanically separated poultry and beef, using an EDTA titrimetric procedure; continue study.
(4) *Chlorinated Hydrocarbons in Poultry:* Continue study.
(5) *Determination and Identification of Cardiac Musculature, Soy Flour and Partially Defatted Tissue in Ground Beef:* Retitle topic *Histologic Identification Methods;* continue study.
(6) *Fat in Meat Products:* Continue study.
(7) *Fat and Moisture Analysis, Rapid Methods:* Continue study.
(8) *Fluoride in Deboned Meat and Poultry:* Discontinue topic.
(9) *Identification of Meats, Serological Tests:* Appoint an Associate Referee; continue study.
(10) *Moisture, Automated Karl Fischer Titrator Method:* Discontinue topic.
(11) *Nitrates and Nitrites:* Continue study.
(12) *Nitrosamines in Bacon:* Continue study.
(13) *Non-Meat Proteins in Meat:* Continue study.
(14) *Pentachlorophenol in Animal and Poultry Tissue:* Continue study.
*(15) *Protein in Meat:* Adopt as official final

action the official first action block digestion-steam distillation method for the determination of crude protein in meat, **24.B01-24.B03;** continue study.

(16) *Proximate Composition Relationships:* Discontinue topic.

(17) *Sodium and Potassium in Meat Products:* Combine under single topic *Specific Ion Electrode Applications;* discontinue topic.

(18) *Specific Ion Electrode Applications:* Combine with topic, *Sodium and Potassium in Meat Products* under single topic; continue study.

(19) *Sugars and Sugar Alcohols:* Appoint an Associate Referee; continue study.

(20) *Temperature, Minimum Processing:* Appoint an Associate Referee; continue study.

(21) *Other Topics:* Change the title of the General Referee topic to Meat, Poultry, and Meat and Poultry Products; initiate new topics: HPLC Methods for Meat and Poultry Products, Bioassay Methods for Meat and Poultry Products, Chemical Antibiotic Methods, and Steroid Analysis in Meat and Poultry Products.

MICROCHEMICAL METHODS

(1) *HPLC of Vegetable Material:* Discontinue topic.

(2) *Other Topic:* Discontinue Refereeship; assign Associate Referee to other General Refereeship if practicable.

MYCOTOXINS

(1) *Aflatoxin M:* Retain first action status of the methods for aflatoxin M_1 in milk and cheese (**26.A10-26.A14**), and in dairy products (**26.090-26.094**), and for aflatoxins B_1 and M_1 in liver (**26.C01-26.C08**). Continue evaluation of the Fukayama column extraction method for aflatoxin M_1 in milk. Collaboratively study the Foos-Warren HPLC method for aflatoxin M_1 in milk.

*(2) *Aflatoxin Methods:* (a) Revise sections **26.A02** and **26.A04** to read as follows:
26.A02(e). *Lead acetate soln.* . . . and dil. to 1 L, or optional *zinc acetate-aluminum chloride soln.*—Dissolve 200 g $Zn(OAc)_2$ and 5 g $AlCl_3$ in H_2O and dil. to 1 L.
26.A04: Measure 100 mL filtrate . . . and refilter as above, or, optionally, replace $Pb(OAc)_2$ soln with $Zn(OAc)_2$-$AlCl_3$ soln.

(b) Revise the BF method, **26.032-26.036,** as follows:
26.034: See **26.028,** or prep. peanut samples by H_2O slurry method [*J. Am. Oil Chem. Soc.* **57,** 269(1980)].
26.035: . . . and ca 4 g NaCl to peanuts or meal. If peanut sample is prepd by H_2O slurry method, weigh 130 g slurry into blender jar. Add 50 mL 2.2% salt soln, 150 mL MeOH, and 100 mL hexane.

(c) Compare by collaborative study the CB (**26.026-26.031**) and BF (**26.032-26.036**) methods with a modified version of the BF method [*J. Am. Oil Chem. Soc.* **57,** 269(1980)].

(3) *Alternaria Toxins:* Continue study.

(4) *Citrinin:* Continue study.

(5) *Ergot Alkaloids:* Continue study.

(6) *Grains:* Continue study.

(7) *Mixed Feeds:* Discontinue topic.

(8) *Ochratoxins:* Collaboratively study a modification (in IARC Publication No. 44) of the method for ochratoxin in green coffee, **26.104-26.109,** applied to swine tissue.

(9) *Patulin:* Discontinue topic.

(10) *Penicillic Acid:* Collaboratively study the Thorpe GLC method for penicillic acid.

(11) *Sterigmatocystin:* Collaboratively study the van Egmond et al. method [*J. Assoc. Off. Anal. Chem.* **63,** 110(1980)] for sterigmatocystin in cheese, and apply the method to a survey of moldy cheese rind from U.S. cheese plants.

(12) *Tree Nuts:* Collaboratively study the Brumley et al. method [*Anal. Chem.* **53,** 2003(1981)] for confirmation of aflatoxin identity, and continue study toward a general aflatoxin method.

(13) *Trichothecenes:* Continue study of the Scott [*J. Assoc. Off. Anal. Chem.* **64,** 1364(1981)] and Romer methods for DON in grains in preparation for collaborative study; continue implementation of the trichothecene panel recommendations [*J. Assoc. Off. Anal. Chem.* **65,** 892(1982)] with the modification that DON be given top priority.

(14) *Zearalenone:* Continue study of HPLC methods towards selection of the best for collaborative study; continue study toward a rapid screening method.

NUTS AND NUT PRODUCTS

(1) *Antioxidants:* Discontinue topic.

(2) *Composition (Ash, Fat, Fiber, Protein, Water):* Discontinue topic.
(3) *Moisture and Water Activity:* Discontinue topic.
(4) *Oil, Hydrogenated, in Peanut Butter:* Discontinue topic.
(5) *Other Topic:* Discontinue General Refereeship; reassign Associate Referees to other General Refereeships wherever practicable.

OILS AND FATS

*(1) *Antioxidants:* Adopt as official first action the interim official first action method for the HPLC determination of antioxidants in oils and fats; continue study.
(2) *Chromatographic Methods:* Continue study.
(3) *Cyclopropene Fatty Acids:* Continue study.
(4) *Emulsifiers:* Continue study.
(5) *Karl Fischer Method for Water in Oils and Fats:* Continue study.
(6) *Lower Fatty Acids:* Continue study.
(7) *Marine Oils:* Continue study.
(8) *Olive Oil Adulteration:* Continue study.
(9) *Oxidized Fats:* Continue study.
(10) *Pork Fats in Other Fats:* Continue study.
(11) *Spectrophotometric Methods:* Continue study.
(12) *Sterols and Tocopherols:* Continue study.

PLANT TOXINS

(1) *Pyrrolizidine Alkaloids:* Initiate topic; appoint an Associate Referee.
(2) *Solanaceous Alkaloids:* Initiate topic; appoint an Associate Referee.
(3) *Other Topic:* Continue evaluation of additional plant problems and assess the needs for analytical methodology.

PROCESSED VEGETABLE PRODUCTS

(1) *Fibrous Material in Frozen Green Beans:* Continue study.
(2) *pH Determination:* Continue study.
(3) *Sodium Chloride:* Continue study.
(4) *Volume of Entrapped Air in Flexible Retort Pouches:* Continue study.
(5) *Water Activity Determination:* Continue study.

SEAFOOD TOXINS

(1) *Ciguatoxins:* Continue study.
(2) *Ciguatoxins; Biochemical Methods:* Continue study.
(3) *Paralytic Shellfish Poisoning; Immunoassay Method:* Continue study.
(4) *Shellfish Poisons:* Continue study.
(5) *Tetradotoxins:* Continue study.

Report of Committee D on Recommendations for Official Methods

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ALCOHOLIC BEVERAGES

(1) *Acetate in Wines and Fruit Juices (Enzymatic Assay):* Continue study.
(2) *Alcoholic Content by Oscillating U-Tube Density Meter:* Continue study.
*(3) *Alcohol Content of High Solids Distilled Spirits:* Adopt as official first action the Mettler/Parr density meter method for determining the proof of liqueur products containing dissolved solids as de-

scribed by the Associate Referee; continue study.

(4) *β-Asarone:* Continue study.

(5) *Bromide Ion in Wine:* Continue study.

*(6) *Carbon Dioxide in Wine:* Surplus the official first action volumetric method for determining carbon dioxide, **11.063-11.065;** continue study.

(7) *Citric Acid in Wine:* Continue study.

(8) *Color in White Wine:* Discontinue topic.

(9) *Color Intensity for Distilled Alcoholic Beverage Products:* Continue study.

(10) *Coumarin in Wine:* Continue study.

(11) *Diethylpyrocarbonate in Beverages:* Continue study.

*(12) *Ethanol in Wine by GLC:* Adopt as official first action the GLC method for the determination of ethanol in wine as described by the Associate Referee; continue study.

(13) *Flavor Compounds in Malt Beverages:* Continue study.

(14) *Glycerol in Wine:* Continue study.

(15) *Hydrogen Cyanide:* Continue study.

(16) *Malic Acid in Wine:* Continue study.

(17) *Malt Beverages and Brewing Material:* Continue study.

(18) *Sorbic Acid in Wine:* Continue study.

(19) *Sugar, Reducing:* Discontinue topic.

(20) *Sulfur Dioxide in Wine:* Continue study.

(21) *Tartrates in Wine:* Continue study.

(22) *Vanillin and Ethyl Vanillin in Alcoholic Beverages:* Continue study.

(23) *Volatile Acidity in Wine:* Continue study.

(24) *Volatile Congeners in Alcoholic Beverages:* Continue study.

(25) *Other Topics:* Continue official first action status of the following methods: **9.094,** artificial colors; **9.101-9.103,** cyanide; **9.119,** total acidity; **9.123,** total malic acid; **9.129,** thujone; **10.148,** aphids; **10.182-10.185,** yeast; **11.047,** citric and malic acids; **11.057,** cyanide; **11.058-11.062** and **11.063-11.065,** carbon dioxide, manometric and volumetric methods, respectively.

CACAO PRODUCTS

(1) *Caffeine and Theobromine:* Continue study.

(2) *Carbohydrates in Chocolate Products:* Continue study.

(3) *Moisture in Cacao Products:* Continue study.

(4) *Shell in Cacao Products, Micro Methods:* Continue study.

(5) *Other Topics:* Continue official first action status of the following methods: **13.002,** moisture; **13.045,** lecithin; **13.050,** glucose; **13.040,** unsaponifiable matter in cocoa butter.

CEREAL FOODS

(1) *Iron:* Continue study.

(2) *Phytates:* Continue study.

(3) *Starch in Raw and Cooked Cereals:* Continue study.

(4) *Other Topics:* Continue official first action status of the following methods: **14.049-14.053,** α-amylase; **14.103-14.115,** lactose; **14.117-14.120,** mineral oil; **14.150-14.153,** sterols.

FLAVORS

(1) *Additives in Vanilla Flavorings:* Continue study.

(2) *Citral:* Appoint an Associate Referee; continue study.

(3) *Essential Oils:* Appoint an Associate Referee; continue study.

(4) *Glycyrrhizic Acid and Glycyrrhizic Acid Salts:* Continue study.

(5) *Imitation Maple Flavors, Identification and Characterization:* Appoint an Associate Referee; continue study.

(6) *Organic Solvent Residues in Flavorings:* Appoint an Associate Referee; continue study.

(7) *Vanillin and Ethyl Vanillin in Foods:* Continue study.

(8) *Other Topics:* Continue official first action status of the following methods: **12.031,** essential oils; **12.032-12.036,** caffeine; **19.001-19.002,** alcohol; **19.033-19.035,** vanilla resins; **19.067,** oils of lemon and orange in extracts; **19.070,** oils of lemon, orange, or lime in oil-base flavors; **19.096, 19.097,** and **19.098,** almond extract; **19.099,** benzaldehyde; **19.104,** benzoic acid; **19.113, 19.114,** and **19.115,** ginger extract; **19.117, 19.118, 19.119,** peppermint, spearmint, and wintergreen extracts; **19.124,** anise and nutmeg extracts; **19.125, 19.126,** and **19.127-19.128,** other extracts and toilet preparations.

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* An asterisk before a recommendation indicates that official action on that recommendation resulted in its appearance in "Changes in Methods."

The recommendations submitted by Committee D were adopted by the Association.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition; and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **63,** 374-423 (1980); **64,** 501-540 (1981); **65,** 450-521 (1982).

FRUITS AND FRUIT PRODUCTS

(1) *Adulteration of Orange Juice by Pulp Wash and Dilution:* Continue study.
(2) *Fruit Acids:* Continue study.
(3) *Fruit Juices, Identification and Characterization:* Appoint an Associate Referee; continue study.
(4) *Isoascorbic Acid (Erythorbic Acid-Antioxidant) in Fruit Purees:* Appoint an Associate Referee; continue study.
(5) *Orange Juice Content:* Continue study.
*(6) *Other Topics:* Adopt as official first action the interim official first action method for soluble solids in citrus fruit juices as degrees Brix as described by the General Referee.

NONALCOHOLIC BEVERAGES

(1) *Caffeine and Methyl Xanthenes in Nonalcoholic Beverages:* Continue study.
(2) *Lasiocarpine and Pyrrolizidines in Herbal Beverages:* Appoint an Associate Referee; continue study.

PRESERVATIVES AND ARTIFICIAL SWEETENERS

(1) *Benzoates and Hydroxybenzoates in Food:* Appoint an Associate Referee; continue study.
(2) *Benzoates, Saccharin, and Caffeine, High Pressure Liquid Chromatography:* Continue official first action status of method **12.050-12.053.**
(3) *Formaldehyde:* Continue study.
(4) *Meats, Ground, Screening Methods for Chemical Preservatives:* Continue official first action status of method **20.A01-20.A05.**
(5) *Organic Preservatives (Thin Layer Chromatography):* Continue study.
(6) *Preservatives (Qualitative Methods):* Appoint an Associate Referee; continue study.
(7) *Saccharin and Its Salts:* Continue study.
*(8) *Other Topics:* (a) Adopt as official first action the official NMKL GLC method for determination of benzoic and sorbic acids in apple juice, almond paste, and fish homogenate at levels of 0.5–2 g/kg. (b) Continue official first action status of the following methods: **20.024-20.028,** benzoic acid by TLC; **20.042-20.045,** boron by atomic absorption spectrophotometry; **20.056-20.057,** soluble fluorides by fluorescence quenching of aluminum 8-hydroxyquinolate; **20.062-20.064,** formaldehyde; **20.073-20.075,** nitrites; **20.077-20.078,** qualitative tests for quaternary ammonium compounds (QAC); **20.090-20.092,** eosin yellowish method for QAC; **20.098-20.101,** sorbic acid oxidation method; **20.121-20.122,** thiourea in frozen peaches; **20.157-20.161,** identification of non-nutritive sweeteners; **20.162,** cyclohexylsulfamate qualitative test; **20.168-20.172,** cyclohexylamine in cyclamates; **20.173-20.176,** dulcin; **20.177,** P-4000; **20.187,** saccharin by sublimation; **20.A01-20.A05,** preservatives in ground beef.

SPICES AND OTHER CONDIMENTS

(1) *Ash and Pungent Principles in Mustard:* Appoint an Associate Referee; continue study.
(2) *Characterization of Natural Spices and Flavors:* Discontinue topic.
(3) *Extractable Color in Capsicum Spices and Oleoresins:* Continue study.
(4) *Moisture in Dried Spices:* Continue study.
(5) *Monosodium Glutamate in Foods:* Appoint an Associate Referee; continue study.
(6) *Pungency of Capsicums and Oleoresins:* Initiate topic; appoint an Associate Referee.
(7) *Vinegar:* Appoint an Associate Referee; continue study.

SUGARS AND SUGAR PRODUCTS

(1) *Chromatographic Methods:* Continue study.
(2) *Color, Turbidity, and Reflectance—Visual Appearance:* Continue study.
*(3) *Corn Syrup and Corn Sugar:* Adopt as official first action the interim official first action HPLC method described by the Associate Referee to extend method **31.228-31.236** for the determination of minor saccharides in corn syrups to include products with dextrose levels in excess of 98%; continue study.
(4) *Dry Substance:* Continue study.
(5) *Enzymatic Methods:* Continue study.
*(6) *Honey:* (a) Adopt as official final action the following official first action methods for honey: **31.115,** nitrogen; **31.116-31.118,** proline; **31.119(a), (b), (c),** direct polarization; **31.133-31.137,** sugars by alternative method II; **31.138-31.142,** sugars by HPLC; **31.148-31.149,** high fructose corn syrup by TLC; **31.A01-31.A02,** spectrophotometric method for hydroxymethylfurfural. (b) Adopt as

official final action the official first action amendment to **31.150-31.153,** carbon isotope ratio method for honey.
(c) Delete the official first action method **31.119(d),** direct polarization at 87°.
(d) Delete procedures **31.122,** dextrin (approximate); and **31.146-31.147,** commercial invert sugar—resorcinol test.
(e) Continue study.

(7) *Maple Sap and Syrups:* Continue study.

*(8) *Stable Carbon Isotope Ratio Analysis:* Adopt as official final action the following official first action mass spectrometric stable carbon isotope ratio analysis methods: **22.B01-22.B04,** for the determination of corn syrups in apple juice; and **22.C01-22.C04,** for the determination of corn syrups in orange juice; continue study.

(9) *Standardization of Sugar Methods of Analysis:* Continue study.

*(10) *Sugars in Cereals:* Adopt as official final action the official first action method, **14.C01-14.C04,** for the HPLC determination of sugars in sweetened cereals; continue study.

(11) *Sugar in Sugar Cane:* Continue study.

(12) *Sugars, Reducing:* Discontinue topic.

(13) *Weighing, Taring, and Sampling:* Continue study.

(14) *Other Topic:* Initiate the topic HPLC Determination of Lactose at High Purity Levels, and appoint an Associate Referee.

VITAMINS AND OTHER NUTRIENTS

(1) *Amino Acids:* Continue study.

(2) *Automated Nutrient Analysis:* Continue study on thiamine.

(3) *Biotin:* Continue study.

(4) *Carotenoids:* Continue study.

(5) *Choline in Feeds:* Continue study.

(6) *Dietary Fiber:* Continue study.

(7) *Energy Value of Foods (Biological):* Continue study.

*(8) *Fat in Food by Chloroform-Methanol Extraction:* Adopt as official first action the method described by the Associate Referee; continue study.

(9) *Folic Acid:* Continue study.

(10) *HPLC Assay for Total Vitamins A, D, and E Content in Foods, Feeds, and Pharmaceuticals:* Continue study.

(11) *Iodine in Foods:* Continue study.

(12) *Niacinamide (Polarography):* Continue study.

(13) *Pantothenic Acid, Total Activity in Foods:* Continue study.

(14) *Protein Quality Evaluation in Foods:* Continue study.

(15) *Sodium in Foods:* Continue study.

(16) *Thiamine Column Packing Material and Enzyme:* Continue study.

(17) *Vitamin A in Foods and Feeds:* Continue study.

(18) *Vitamin C in Milk-Based Foods:* Continue study.

*(19) *Vitamin D:* Adopt as official final action the official first action HPLC method, **43.C01-43.C09,** for Vitamin D in mixed feeds, premixes, and pet food; continue study.

(20) *Vitamin E in Foods and Feeds:* Continue study.

(21) *Vitamin E in Pharmaceuticals (Gas Chromatography):* Continue study.

(22) *Vitamin K_1 in Foods and Feeds:* Continue study.

Report of Committee E on Recommendations for Official Methods

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The U.S. Environmental Protection Agency and many state agencies require well validated analytical methods and standardized test procedures for evaluating chemical/physical properties of industrial process residuals in their hazardous waste management programs. Committee E recommends the establishment of a General Referee topic for Industrial Process Wastes and has agreed to oversee the development of this topic area.

The present arrangement of studies on analytical methods for pesticide residues in foods provides two General Referees for pesticides classified by chemical type (viz., Organochlorine Pesticides; Organophosphorus Pesticides) and two General Referees for pesticides classified, at least in part, by function (viz., Carbamate Pesticides, Fumigants, and Miscellaneous; Fungicides, Herbicides, and Plant Growth Regulators). Committee E recommends the restructuring of these pesticide residue General Refereeships according to chemical type by effecting the following changes in the General Referee topics upon the publication of this report in the *Journal:*

(1) *Organochlorine Pesticides:* Change title of topic to Organohalogen Pesticides.

(2) *Fungicides, Herbicides, and Plant Growth Regulators:* Change title of topic to Organonitrogen Pesticides.

(3) *Carbamate Pesticides, Fumigants, and Miscellaneous:* Discontinue General Referee topic and transfer related Associate Referee topics to the General Referees for Organohalogen Pesticides, Organonitrogen Pesticides, and Organophosphorus Pesticides, as specified in the Committee's recommendations for the specific Associate Referee topics.

CARBAMATE PESTICIDES, FUMIGANTS, AND MISCELLANEOUS

(1) *Carbamate Insecticides, Gas-Liquid Chromatographic Methods:* Transfer topic to the General Referee for Organonitrogen Pesticides; prepare write-up of the on-column silylation gas-liquid chromatographic method for determining carbamate insecticide residues as their silylated derivatives for review and comment by the General Referee and, if the write-up is approved, conduct interlaboratory trial of the method.

(2) *Carbamate Insecticides, Liquid Chromatographic Methods:* Transfer topic to the General Referee for Organonitrogen Pesticides; complete the collaborative study of the Associate Referee's method for determining residues of *N*-methylcarbamate insecticides in crops by high performance liquid chromatography with post-column fluorescent derivative formation (*J. Assoc. Off. Anal. Chem.* (1980) **63**, 1114–1124).

(3) *Carbofuran:* Transfer topic to the General Referee for Organonitrogen Pesticides; continue study of analytical methods for determining carbofuran, its carbamate metabolites, and its phenolic metabolites in foods.

(4) *Ethylene Oxide and Its Chlorohydrin:* Transfer topic to the General Referee for Organohalogen Pesticides; continue study of the gas-liquid chromatographic method of Scudamore and Heuser (*Pestic. Sci.* (1971) **2**, 80–91) for determining ethylene oxide, ethylene chlorohydrin, and ethylene bromohydrin in foods.

(5) *Fenvalerate:* Transfer topic to the General Referee for Organohalogen Pesticides; appoint an Associate Referee to evaluate and collaboratively study analytical methods for determining residues of fenvalerate in foods.

(6) *Fumigants:* Transfer topic to the General Referee for Organohalogen Pesticides and appoint an Associate Referee; continue study to extend the official final action gas-liquid chromatographic method for determining volatile fumigants in grain, **29.056-29.057,** to cover additional fumigants (1,2-dichloroethane, methyl bromide, and tetrachloroethylene) and additional foods (citrus fruits, milled products, and baked goods).

(7) *Inorganic Bromides in Grains:* Transfer topic to the General Referee for Organohalogen Pesticides; continue study of the gas-liquid chromatographic method of Heuser and Scudamore (*Pestic. Sci.* (1970) **1,** 244–249) for determining inorganic bromides after conversion to 2-bromoethanol, as tested in interlaboratory studies on grain (*Analyst* (1976) **101,** 386–390) and lettuce (*J. Assoc. Off. Anal. Chem.* (1979) **62,** 1155–1159).

(8) *Permethrin:* Transfer topic to the General Referee for Organohalogen Pesticides; appoint an Associate Referee to evaluate and collaboratively study analytical methods for determining residues of permethrin in foods.

(9) *Phosphine:* Transfer topic to the General Referee for Organophosphorus Pesticides; continue study of methods for determining residual phosphine in fumigated products, including the gas chromatographic method reported by the Associate Referee (*J. Assoc. Off. Anal. Chem.* (1978) **61,** 5–7) and the gas-liquid chromatographic method reported by T. W. Nowicki (*J. Assoc. Off. Anal. Chem.* (1978) **61,** 829–836) for determining the total residue of intact phosphine and phosphine derived from residual aluminum phosphide in wheat.

(10) *Resmethrin:* Transfer topic to the General Referee for Organohalogen Pesticides; continue study to evaluate analytical methods for determining residues of resmethrin in foods.

(11) *Sodium Monofluoroacetate:* Transfer topic to the General Referee for Veterinary Analytical Toxicology (Committee G).

FUNGICIDES, HERBICIDES, AND PLANT GROWTH REGULATORS

(1) *Anilazine:* Appoint an Associate Referee; initiate collaborative study of the method reported by Lawrence and Panopio (*J. Assoc. Off. Anal. Chem.* (1980) **63,** 1300–1303) for determining anilazine residues by high pressure liquid chromatography.

(2) *Benzimidazole-Type Fungicides:* Continue study of the method reported by the Associate Referee (*J. Assoc. Off. Anal. Chem.* (1980) **63,** 1291–1295) for the simultaneous determination of benomyl *per se* and its degradation product methyl 2-benzimidazole carbamate in crops by high performance liquid chromatography.

(3) *Captan and Related Fungicides:* Continue study to improve the reproducibility of gas-liquid chromatographic response for captan, folpet, and captafol and to evaluate extraction/cleanup procedures for residues of these fungicides in crops.

(4) *Carbamate Herbicides:* Appoint an Associate Referee; continue study to evaluate methods for determining residues of carbamate herbicides in crops.

(5) *Chlorophenoxy Alkyl Acids:* Transfer topic to the General Referee for Organohalogen Pesticides; complete the collaborative study of the Associate Referee's improved version of the gas-liquid chromatographic method reported by Jensen and Glas (*Chem. Anal. (N.Y.)* (1981) **58,** 223–261) and evaluate the effectiveness of the modified method for determining residues of 2,4-dichlorophenoxyacetic acid in dried green wheat and wheat straw.

(6) *Chlorothalonil:* Appoint an Associate Referee; continue study to evaluate gas-liquid chromatographic methods for determining residues of chlorothalonil in crops.

(7) *Dinitro Compounds:* Appoint an Associate Referee; continue study of methods for determining dinitro aryl herbicide residues in foods.

(8) *Diquat and Paraquat:* Complete the intralaboratory study of the Associate Referee's modification of R. R. King's gas-liquid chromatographic method for determining diquat residues after reduction with sodium borohydride (*J. Agric.*

Food Chem. (1978) **26,** 1460–1463) to evaluate the performance of the modified method for the simultaneous determination of diquat and paraquat residues in potatoes.

(9) *Dithiocarbamates, General Residue Methods:* Appoint an Associate Referee; continue study to develop methods for distinguishing dialkyldithiocarbamates from ethylenebisdithiocarbamates and for determining the parent fungicides and their metabolites in foods.

(10) *Maleic Hydrazide:* Appoint an Associate Referee; continue study of gas-liquid chromatographic or high pressure liquid chromatographic methods for determining maleic hydrazide in foods.

(11) *1-Naphthaleneacetic Acid and 1-Naphthaleneacetamide:* Discontinue topic.

(12) *Organotin Fungicides:* Prepare protocol for collaborative study of the gas-liquid chromatographic method proposed by the Associate Referee for determining residues of triphenyltin hydroxide and fenbutatin-oxide in foods for review and comment by the General Referee; if approved by the General Referee, initiate the collaborative study of this method.

(13) *Pentachlorophenol:* Transfer topic to the General Referee for Organohalogen Pesticides; initiate interlaboratory study of the improved gas-liquid chromatographic method for determining pentachlorophenol, underivatized, in gelatin, fish, and eggs, based on the method reported by the Associate Referee for the analysis of milk and blood (*J. Agric. Food Chem.* (1980) **28,** 710–714); continue study of this method to improve quantitation of pentachlorophenol residues at levels below 0.1 ppm if such residues are determined to be of regulatory significance.

(14) *Sodium o-Phenylphenate:* Appoint an Associate Referee; continue study to evaluate gas-liquid chromatographic and high pressure liquid chromatographic methods for determining *o*-phenylphenol in foods.

(15) *Substituted Ureas:* Appoint a new Associate Referee; continue study to evaluate methods for determining substituted urea herbicides and their metabolites in foods.

(16) *Succinic Acid, 2,2-Dimethylhydrazide:* Change title of topic to Daminozide and appoint an Associate Referee; continue study to develop a gas-liquid chromatographic or high pressure liquid chromatographic method for determining residues of daminozide in foods.

(17) *Thiolcarbamate Herbicides:* Appoint an Associate Referee; continue study to evaluate methods for determining residues of thiolcarbamate herbicides in crops.

(18) *s-Triazines:* Appoint an Associate Referee; initiate collaborative study of the gas-liquid chromatographic method outlined by the General Referee in *J. Assoc. Off. Anal. Chem.* (1980) **63,** 273 for determining atrazine and cyanazine in corn and potatoes.

(19) *Trifluralin:* Appoint an Associate Referee; continue study to evaluate methods for determining residues of trifluralin in crops.

METALS AND OTHER ELEMENTS

(1) *Atomic Absorption Spectrophotometry (AAS):* Continue study to consolidate present AOAC official methods for determining individual elements by AAS into a unified atomic absorption spectrophotometric analytical scheme for multielement analysis of foods and other biological substrates.

(2) *Cadmium and Lead in Earthenware:* (a) Defer decision on approval of the recommendation by the General Referee for adoption as official first action of the hot leach method for determining extractable cadmium and lead in ceramic cookware and enamel cookware by atomic absorption spectrophotometry, pending submission of a revised manuscript on the collaborative study reported by J. H. Gould et al. or a supplementary report by the Associate Referee for Cadmium and Lead in Earthenware and/or the General Referee for Metals and Other Elements that includes the following: (*i*) a description of the method as intended for publication in *Official Methods of Analysis;* (*ii*) a summary of the performance of the method in the collaborative study in terms of the statistical parameters previously requested by Committee E for use in all collaborative study reports (see *J. Assoc. Off. Anal. Chem.* (1979) **62,** 413); (*iii*) available information on the relative variability of the determina-

tive step of the method compared with the variability in the levels of cadmium and lead extracted from the cookware; (*iv*) clarification on the presence or absence of light during the hot leaching of the product and on the number of units of product to be included for analysis of a product in the proposed official method; (*v*) clarification of the product application of the hot leach method and its relationship to that of the official final action method, **25.031-25.034.** (b) Continue study on the effect of light on the extractability of cadmium from earthenware and submit an evaluation of data supporting the editorial revisions made in 1980 to make the official final action AOAC-ASTM method for determining cadmium and lead in earthenware, **25.031-25.034,** conform to the revised ASTM procedure.

(3) *Carbon Rod Atomization Techniques:* Initiate second collaborative study (after approval of the study design by the General Referee) of the method outlined by the Associate Referee (*J. Assoc. Off. Anal. Chem.* (1982) **65,** 1005-1009) for the graphite-furnace atomic absorption spectrophotometric determination of lead in canned milk and infant formulas, with revised method instructions to ensure that the instruments used by the collaborators have adequate background correction capability for use in the study.

(4) *Emission Spectrochemical Methods:* Continue study to select a method and develop a protocol for collaborative study of multielement analysis of raw agricultural commodities by inductively coupled plasma (ICP) emission spectroscopy; upon approval of the protocol by the General Referee, initiate collaborative study of the method.

(5) *Fluorine:* Prepare protocol for second collaborative study of the microdiffusion and fluoride-specific electrode method previously studied by the Associate Referee (*J. Assoc. Off. Anal. Chem.* (1981) **64,** 1021-1026) for determining fluoride in infant foods, incorporating the improvements suggested in the report on the first study; after approval of the study protocol by the General Referee, initiate collaborative study.

(6) *Hydride Generating Techniques:* Continue study to evaluate hydride generators and to select a specific method and hydride generator for collaborative study of the hydride evolution atomic absorption spectrophotometric determination of arsenic, selenium, and tin in foods.

(7) *Mercury:* Appoint an Associate Referee; continue study to evaluate and collaboratively test methods for determining mercury in foods.

*(8) *Methyl Mercury in Fish and Shellfish:* Adopt as official first action the electron capture gas-liquid chromatographic method collaboratively studied and reported by the Associate Referee in 1982, for the determination of methyl mercury in fish and shellfish.

(9) *Multielement Analysis of Infant Formula:* Initiate topic to collaboratively study the inductively coupled argon plasma (ICP) emission spectroscopic method (unpublished) for determining calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc in infant formulas; complete the statistical analysis of data from the collaborative study and, if appropriate, submit report with recommendation for adoption under the interim first action approval procedure.

(10) *Multielement Determination After Closed System Digestion:* Continue official first action status of the multielement method, **25.A01-25.A05** (*J. Assoc. Off. Anal. Chem.* (1980) **63,** 388-391), for the determination of arsenic, cadmium, lead, selenium, and zinc in foods; initiate collaborative study to extend the applicability of **25.A01-25.A05** to the determination of copper by anodic stripping voltammetry and to the determination of chromium and nickel by differential pulse polarography, using the modifications developed by the Associate Referee to conduct the digestion and determination in the same vessel to minimize contamination.

(11) *Multimetal Residues by Resin Column Separations:* Continue study of the resin column chromatographic technique re-

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ported by the Associate Referee for isolating transition elements and heavy metals from alkali and alkaline earth metals (*J. Agric. Food Chem.* (1975) **23,** 44–45), for use in conjunction with multielement detection systems.

(12) *Organometallics:* Change title of topic to Organometallics in Foods; continue study to develop methods for determining organometallic compounds in foods.

(13) *Polarography:* Continue official first action status of the dry ash, anodic stripping voltammetric method for determining cadmium and lead in foods other than fats and oils, **25.C01–25.C07** (*J. Assoc. Off. Anal. Chem.* (1982) **65,** 476–478); continue study.

(14) *Tin:* Continue official first action status of the atomic absorption spectrophotometric method for determining tin in canned fruits, vegetables, and juices, **25.136–25.138;** in conjunction with the Associate Referee for Fluorine, conduct and evaluate a collaborative study of the nitrous oxide–acetylene flame atomic absorption spectrophotometric method reported by Dabeka and McKenzie (*J. Assoc. Off. Anal. Chem.* (1981) **64,** 1297–1300) for determining tin in canned foods after nitric acid–hydrochloric acid digestion of the sample.

(15) *Voltammetric Methods:* Continue official first action status of the anodic stripping voltammetric method for determining lead in evaporated milk and fruit juice, **25.080–25.082;** submit revised report for publication of results from collaborative study supporting the official status of this method.

MULTIRESIDUE METHODS (INTERLABORATORY STUDIES)

(1) *Comprehensive Multiresidue Methodology:* Conduct ruggedness test and intra- and interlaboratory trials of the improved pesticide multiresidue method of Luke et al. (*J. Assoc. Off. Anal. Chem.* (1981) **64,** 1187–1195), using the standard additions approach proposed by the Associate Referee to offset the quantitation problems resulting from the enhancement of gas-liquid chromatographic responses for some analytes in the presence of crop extractives; if warranted by the results of the intra- and interlaboratory trials, incorporate the technique for standard additions into the procedure and initiate collaborative study of the modified method, after approval of the study design by the General Referee.

(2) *Gas-liquid Chromatography (Alkaline Precolumn):* Prepare report describing the performance of the alkaline precolumn gas-liquid chromatographic method for identifying and determining *o,p'*-DDT, *p,p'*-DDT, *p,p'*-TDE, methoxychlor, and Perthane as their dehydrohalogenated derivatives (*J. Assoc. Off. Anal. Chem.* (1969) **52,** 548–553) in the collaborative study of the quantitative dehydrohalogenation and determination of these pesticides in the absence of food sample matrix, and in the intralaboratory studies of the method in actual food analyses.

(3) *Organophosphorus Pesticides Residues:* Continue study of the official final action carbon cleanup method for residues of parathion, paraoxon, EPN, and carbophenothion and its oxygen analog in apples and green beans, **29.039–29.043,** to extend the coverage of this method to additional organophosphorus pesticides and additional crops; continue study to compile and summarize available data on the recovery of organophosphorus pesticides by this method.

(4) *Pesticides in Meat and Meat Products:* Appoint an Associate Referee; initiate collaborative study to test the applicability of the AOAC multiresidue methodology, **29.001–29.028,** for determining organochlorine pesticide residues in meat and meat products.

(5) *Pollutant Phenols in Fish:* Complete the interlaboratory study of the electron capture gas-liquid chromatographic method proposed by the Associate Referee for the analysis of fish for residues of phenols classified as priority pollutants by the U.S. Environmental Protection Agency; if this method, in which phenols are converted to pentafluorobenzyl bromide derivatives after cleanup by gel permeation chromatography and cesium silicate adsorption chromatography, is successful in the interlaboratory study, develop a protocol for collaborative study of the method for review and comment by the General Referee.

(6) *Whole Blood:* Complete the collaborative

study of the Associate Referee's method for determining organochlorine pesticides in whole blood (*J. Assoc. Off. Anal. Chem.* (1980) **63,** 965–969); if warranted by an evaluation of the results, submit a report on the collaborative study with a recommendation for adoption of the method under the interim first action adoption procedure.

ORGANOCHLORINE PESTICIDES

(1) *Chlordane:* Initiate collaborative study of the method combining multiresidue extraction, **29.011–29.012,** acetonitrile partitioning cleanup, **29.014,** Florisil column chromatographic cleanup and residue separation, **29.031–29.033,** and electron capture gas-liquid chromatography, **29.018,** for determining residues of *cis*-chlordane, *trans*-chlordane, octachlor epoxide (oxychlordane), and heptachlor epoxide in butter, eggs, fish, and poultry fat; continue study of capillary column gas-liquid chromatography for determining the terminal residues of chlordane in foods.

(2) *Chlorinated Dioxins:* Continue study to develop and evaluate analytical methods for determining and confirming the identity of residues of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) at parts per trillion levels in fish, milk, and other foods; continue study to evaluate methods for determining hexachloro-, heptachloro-, and octachloro-substituted dibenzo-*p*-dioxins and dibenzofurans in foods.

(3) *Chlorobenzilate, Chloropropylate, and Bromopropylate:* Prepare a report on the work done to incorporate the determination of chlorobenzilate, chloropropylate, and bromopropylate residues at tolerance levels in fruits and nuts into the multiresidue method, **29.001–29.018,** and to convert these compounds to their trifluoroacetic acid derivatives for enhanced sensitivity in the electron capture gas-liquid chromatographic determinative step, **29.018;** continue study to evaluate other methods for determining residues of these pesticides in foods with a high lipid content.

(4) *Dicofol:* Appoint an Associate Referee; continue study to evaluate methods for the determination of dicofol residues in crops.

(5) *Gel Permeation Chromatography (GPC) Cleanup for Organochlorine Residues:* Initiate topic to evaluate gel permeation chromatography as a cleanup technique for determining organochlorine residues in foods.

(6) *Kepone:* Continue study of analytical methods for determining residues of Kepone in fish and shellfish.

(7) *Low Moisture-High Fat Samples, Extraction Procedure:* Initiate collaborative study of a method combining the Associate Referee's sample preparation/extraction procedure (*J. Assoc. Off. Anal. Chem.* (1982) **65,** 1122–1128) with acetonitrile partitioning, **29.014,** Florisil column cleanup, **29.015** or **29.029–29.034,** and electron capture gas-liquid chromatography, **29.018,** for determining organochlorine residues in oil seeds and other low moisture-high fat products.

*(8) *Multiresidue Methodology, Miniaturization:* Adopt as official first action the miniaturized multiresidue method collaboratively studied by the Associate Referee, for determining *p,p'*-DDE, *p,p'*-DDT, *p,p'*-TDE, dieldrin, heptachlor epoxide, and polychlorinated biphenyls in fish.

(9) *Photochemical Derivatization for Confirmation of Residue Identity:* Prepare a report for publication of the information presented by the Associate Referee in 1982 on the effect of coextractives from samples prepared by the multiresidue methods **29.001–29.018** and **29.029–29.034** on the photoderivatization of organochlorine pesticides in the Associate Referee's method for confirming residue identity by gas-liquid chromatography of their UV photolysis products (*J. Assoc. Off. Anal. Chem.* (1977) **60,** 673–678); continue study to identify the photoderivatives of selected compounds, such as endosulfan I, *cis*- and *trans*-nonachlor, and octachlor epoxide, whose conversion to useful photoderivatives was least affected by the presence of sample co-extractives.

(10) *Polychlorinated Biphenyls (PCBs):* Continue study of analytical methods for determining residues of PCBs in foods and for isolating PCBs from the organochlorine pesticide residues that are not separated from them in the multiresidue method, **29.001–29.018.**

(11) *Polychlorinated Biphenyls (PCBs) in Blood.* Initiate topic to evaluate and collabora-

tively study analytical methods for determining PCBs in blood and blood serum.

(12) *Root-Absorbed Residues, Extraction Procedures:* Appoint an Associate Referee; continue study to evaluate recently developed information on the extraction of root-absorbed residues and to develop and evaluate extraction procedures for root-absorbed residues, for incorporation into the multiresidue method, **29.001-29.018.**

(13) *Tetradifon, Endosulfan, and Tetrasul:* Continue study of the official final action method for determining endosulfan I, endosulfan II, endosulfan sulfate, tetradifon, and tetrasul in apples and cucumbers, **29.029-29.034,** to develop the intralaboratory data for the 11 nonfatty foods that remain to be tested to determine if the method is applicable to all Group I and Group II nonfatty foods (Table **29.02**).

(14) *Toxaphene:* Continue study to develop and evaluate quantitation procedures for the terminal residues of toxaphene, for use in conjunction with the multiresidue methodology, **29.001-29.018;** continue study of the approach combining electron capture capillary column gas-liquid chromatography with thin layer chromatography to identify which components of toxaphene are likely to persist and occur as residues in foods.

ORGANOPHOSPHORUS PESTICIDES

(1) *Azinphos-methyl:* Initiate topic to evaluate and collaboratively study analytical methods for determining residues of azinphos-methyl in foods; appoint an Associate Referee.

(2) *Confirmation Procedures:* Develop protocol for collaborative study of the Associate Referee's improved version of the pentafluorobenzyl bromide derivative gas-liquid chromatographic method of Coburn and Chau (*J. Assoc. Off. Anal. Chem.* (1974) **57,** 1272–1278; and *Environ. Lett.* (1975) **10,** 225–236) for review and comment by the General Referee; if approved by the General Referee, initiate collaborative study of this method for confirming the identity of organophosphorus pesticide residues recovered from water by a method such as that of Ripley et al. (*J. Assoc. Off. Anal. Chem.* (1974) **57,** 1033–1042).

(3) *Disulfoton:* Initiate topic to evaluate and collaboratively study analytical methods for determining disulfoton and its metabolites in foods; appoint an Associate Referee.

(4) *Extraction Procedures:* Appoint an Associate Referee; extend the extraction efficiency studies of Watts (*J. Assoc. Off. Anal. Chem.* (1971) **54,** 953–958) to additional organophosphorus pesticide residues and additional crops.

(5) *General Method for Organochlorine and Organophosphorus Pesticides:* Appoint an Associate Referee; continue study to evaluate the applicability of the AOAC multiresidue method, **29.001-29.018,** for determining additional organophosphorus pesticide residues in fatty and nonfatty foods; initiate study on the use of the selective N/P (nitrogen/phosphorus) detector as an alternative to the alkali flame detector used in the gas-liquid chromatographic determinative step of this method.

(6) *High Fat Samples:* Continue study to evaluate gel permeation chromatography as a cleanup technique for the determination of organophosphorus pesticides and their metabolites in fatty foods and to assess the utility of the N/P detector as an alternative to the alkali flame and flame photometric detectors used in the AOAC official methods for determining organophosphorus pesticide residues by gas-liquid chromatography.

(7) *Methamidophos:* Initiate topic and appoint an Associate Referee to evaluate and collaboratively study analytical methods for determining methamidophos and its metabolites in foods.

(8) *Monocrotophos:* Initiate topic to evaluate and collaboratively study analytical methods for determining residues of monocrotophos in foods; appoint an Associate Referee.

(9) *Phorate:* Initiate topic to evaluate and collaboratively study analytical methods for determining phorate and its metabolites in foods; appoint an Associate Referee.

(10) *Soils:* Appoint an Associate Referee; continue study to develop multiresidue extraction and cleanup methods for de-

termining organophosphorus pesticides and their metabolites in soils.

(11) *Sweep Codistillation:* Continue study to extend the official final action sweep codistillation method for determining organophosphorus pesticide residues in crops, **29.044-29.049,** to the analysis of fatty foods; evaluate the commercial sweep codistillation apparatus reported to have been developed in Australia and/or other commercial units that become available and appear to be designed for sweep codistillation cleanup of fatty food extracts.

(12) *Thin Layer Chromatography:* Conduct interlaboratory trial to evaluate the quantitative thin layer chromatographic approach for determining organophosphorus pesticides and their metabolites in foods; continue study to compare optical-scanning thin-layer chromatography with gas-liquid chromatography and high performance liquid chromatography for quantitation of organophosphorus pesticide residues.

RADIOACTIVITY

*(1) *Barium-140:* Adopt as official final action the official first action extension (1982) of the official final action method for cesium-137 in milk, **48.025-48.029,** to include barium-140 and iodine-131; discontinue topic.

(2) *Carbon-14:* Assess need to establish AOAC official methods for carbon-14 in foods, other biological materials, or water to determine whether further study in this topic is warranted; if needed, appoint Associate Referee and arrange for evaluation of data from the collaborative study conducted by former Associate Referee A.A. Mohgissi of the liquid scintillation method, based on the absorption of carbon dioxide in aqueous sodium hydroxide, for determining carbon-14 in biological samples, as outlined by the former General Referee (*J. Assoc. Off. Anal. Chem.* (1974) **57,** 308-309).

(3) *Cesium-137:* Evaluate and report results from the General Referee's collaborative study on extending the gamma-ray spectroscopic method for determining cesium-137 in milk, **48.025-48.029** (1982 revision), to the determination of cesium-137 in other foods; after submission of the collaborative study report, appoint an Associate Referee and continue study to evaluate and collaboratively test methods for determining cesium-137 in foods, biological materials and environmental samples.

*(4) *Iodine-131:* Adopt as official final action the official first action extension (1982) of the official final action method for cesium-137 in milk, **48.025-48.029,** to include barium-140 and iodine-131 (*see* rec. 1); conduct ruggedness test and interlaboratory study of the more sensitive method outlined by the former General Referee (*J. Assoc. Off. Anal. Chem.* (1979) **62,** 387-389) and recommended by the Nuclear Regulatory Commission for determining iodine-131 in milk; if warranted by the results of the interlaboratory study of the latter method, prepare collaborative study protocol for review by General Referee.

(5) *Neutron Activation Analysis:* Complete the collaborative study of the Associate Referee's method for determining sodium in neutron-irradiated biological materials; perform statistical analysis of data from the collaborative study conducted by former Associate Referee J. T. Tanner of the method outlined by the former General Referee (*J. Assoc. Off. Anal. Chem.* (1976) **59,** 350-351), for determining chlorine and bromine in neutron-irradiated biological samples; continue study to assess the feasibility of conducting collaborative studies of methods for multimetal determinations in neutron-irradiated samples and, if feasible, design collaborative study for review and comment by the General Referee.

(6) *Plutonium:* Appoint an Associate Referee; continue study of the Energy Research and Development Administration method for determining plutonium in urine, feces, and water (*ERDA HASL-300 (1976) E-Pu-01-01,* as outlined by the former General Referee in *J. Assoc. Off. Anal. Chem.* (1977) **60,** 378-379) for possible application to foods and environmental samples.

(7) *Radium-228:* Conduct ruggedness test and interlaboratory trial of the method reported by Baratta and Lumsden (*J. Assoc. Off. Anal. Chem.* (1982) **65,** 1424-1428) for the determination of radium-228 in foods and water; if method is suc-

cessful in interlaboratory trial, prepare collaborative study protocol for review and comment by the General Referee and the Statistical Consultant to Committee E.

(8) *Strontium-89 and -90:* Appoint an Associate Referee; initiate collaborative study of the method described by Baratta and Reavey (*J. Agric. Food Chem.* (1969) **17,** 1337–1339) for determining strontium-89 and -90 in foods.

(9) *Tritium:* Assess need for additional AOAC official methods for tritium to determine whether further study in this topic is warranted; if needed, appoint an Associate Referee and arrange for re-evaluation of data and revision of report on the collaborative study conducted by former Associate Referee A. A. Moghissi of the liquid scintillation method for determining tritium in urine (*J. Health Phys. Soc.* (1969) **17,** 727–729) using the modified scintillation mixture described by the former General Referee (*J. Assoc. Off. Anal. Chem.* (1979) **62,** 387–389).

WATER

(1) *Chemical Pollutants in Aquatic Biota:* Discontinue topic, with further work in this area to be carried out by the Associate Referees whose topics encompass the analytes of interest, such as the Associate Referee for Pollutant Phenols in Fish under the General Referee for Multiresidue Methods (Interlaboratory Studies) and the Associate Referees for Chlorinated Dioxins and Toxaphene under the General Referee for Organohalogen Pesticides.

(2) *Chemical Pollutants in Water and Wastewater:* Continue review of analytical methods collaboratively studied by ASTM, EPA, and others for possible adoption by AOAC as official methods; continue study.

(3) *Chlorinated Solvents in Water:* Appoint an Associate Referee; continue study to evaluate and collaboratively study methods for determining chlorinated solvents in water and wastewater.

(4) *Chlorophenoxy Alkyl Acids in Water and Wastewater:* Discontinue topic with further work in this area to be carried out by the Associate Referee for Herbicides in Water and Sediment.

(5) *Herbicides in Water and Sediment:* Initiate topic to evaluate, select, and test through collaborative study analytical methods for the determination of herbicides in water and sediment.

(6) *Major Ions and Nutrients in Water:* Initiate topic to evaluate and collaboratively study analytical methods for determining sodium, potassium, calcium, aluminum, phosphate, sulfate, and other major ions and nutrients in water.

(7) *Organophosphorus Pesticides in Water:* Appoint a new Associate Referee; continue study to evaluate and collaboratively test the method proposed by the U.S. Environmental Protection Agency or other methods for determining organophosphorus pesticides in water and wastewater.

(8) *Triazine Herbicides in Water:* Appoint an Associate Referee; continue study to evaluate and collaboratively test methods for determining triazine herbicides in water and wastewater.

(9) *Other Topic:* Continue official first action status of the atomic absorption spectrophotometric method for determining cadmium, chromium, copper, iron, lead, manganese, silver, and zinc in water, **33.089–33.094.**

Report of Committee F on Recommendations for Official Methods

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ANALYTICAL MYCOLOGY OF FOODS AND DRUGS

(1) *Baseline Mold Counts by Blending:* Continue study.

(2) *Geotrichum Mold in Canned Fruits, Vegetables, and Juices:* Continue study.

(3) *Geotrichum Mold in Frozen Fruits and Vegetables:* Continue study.

(4) *Mold and Yeast in Beverages:* Continue study.

*(5) *Standardization of Plant Tissue Concentrations for Mold Counting:* (a) In method **44.082**, Mold Count, Citrus and Pineapple Juices, Canned, Single Strength, expand description to include tube shield, trunion ring, cushion, and maximum centrifugal force. Change the third sentence to read: "Centrf. 10 min at 2200 rpm, using International type EXD centrf. (Damon/IEC Div., 300 Second Ave, Needham Hts, MA 02194) with 8-place No. 240 head, No. 320 shield, No. 325 trunion ring, and No. 571 cushion, or other centrf. giving equiv. max. relative centrifugal force of 1062.4768 × *g* as computed by following formula: $RCF_{max.} = 1.118 \times 10^{-5} N^2 r = \times g$, where N = rpm and r = radius of centrf. arm in cm (distance from center of centrf.-head to bottom of horizontal centrf. tube). The following formula may be used to det. equiv. centrf.: $N_1^2 r_1 = N_2^2 r_2$, where N_1 = 2200 rpm and r_1 = 19.6 cm." (b) In method **44.C09**, add the following: "Before calcg % mold counts for fruits dild 1 + 1, divide number of positive fields by 2."

(6) *Tomato Products, Chemical Methods for Detecting Mold:* Continue study.

(7) *Tomato Rot Fragment Count:* Continue study.

DISINFECTANTS

No report submitted by the General Referee.

(1) *Antimicrobial Agents Used by Laundries on Fabrics and Materials:* Continue study.

(2) *Sporicidal Tests:* Continue study.

(3) *Textile Antibacterial Preservatives:* Continue study.

(4) *Tuberculocidal Tests:* Continue study.

(5) *Use-Dilution Test, Variation and Amendments:* Continue study.

(6) *Virucide Tests:* Continue study.

EXTRANEOUS MATERIALS IN FOODS AND DRUGS

(1) *Asbestos Measurements in Foods, Drugs, and Cosmetics:* Discontinue topic.

(2) *Automated Filth Analysis:* Discontinue topic.

(3) *Botanical Drugs, Adulteration by Foreign Plant Materials:* Continue study.

(4) *Botanicals:* Appoint a new Co-Associate Referee.

(5) *Brine Extraction Techniques:* Continue study.

(6) *Cereals, Breakfast, Ready-to-Eat:* Discontinue topic.

(7) *Chocolate Products:* Continue study.

(8) *Cocoa Powder and Press Cake:* Continue study.

(9) *Fecal Sterols:* Continue study.

(10) *Fish, Canned:* Discontinue topic.

(11) *Food Supplement Tablets:* Continue study.

(12) *Grains, Whole, Cracking Flotation Methods:* Continue study.

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* An asterisk before a recommendation indicates that official action on that recommendation resulted in its appearance in "Changes in Methods."

The recommendations submitted by Committee F were adopted by the Association.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition; and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **63**, 374–423 (1980); **64**, 501–540 (1981); **65**, 450–521 (1982).

(13) *Insect Excreta in Flour:* Continue study.
(14) *Mammalian Excreta Fragments in Milled Food Products:* Discontinue topic.
(15) *Meats, Processed:* Continue study.
*(16) *Methods for Urine Detection:* (a) Editorially revise method **44.A04-44.A07** (TLC method for urine stains) by adding the following instructions for pre-cleaning Analtech MN 300 cellulose TLC plates before use: "**44.A06(d)** *Pre-cleaning Analtech MN 300 cellulose plates.*—Develop unused plate in either std developing tank or sandwich chamber using developing solv. **44.A05(f)**. Develop to 15 cm above lower edge of plate. Thoroly dry plate using either hair dryer, forced draft oven at ≤80° for ca 15 min, or overnight in fume hood. Plate must be room temp. and completely free of solv. odor before use." Pre-cleaning is necessary with these commercial plates because a colored impurity has been observed to travel with the solvent front and occasionally interfere with visualization of the urinary indican spot.
(b) Editorially revise the TLC method for uric acid from bird and insect excreta (**44.183-44.185**) by modifying the marker dye mixture as follows: "**44.183(e)** Dissolve 16 mg FD&C Red No. 2 and 32 mg FD&C Yellow No. 6 in 50 mL H_2O, and mix well. These dyes serve as visual markers during development, with R_f for Red No. 2 at 0.38-0.40; uric acid, 0.41-0.43; and Yellow No. 6, 0.65; using Analtech plate and sandwich chamber. Merck plates have lower R_f values and do not sep. Red No. 2 and uric acid when overspotted." The dye mixture, which is overspotted on the sample unknown, brackets the uric acid spot after TLC development. The present dye mixture results in the upper marker dye sometimes masking in the uric acid spot. The proposed dye mixture results in compact and adequately intense marker spots that do not interfere with visualization of the uric acid spot.
(17) *Mite Contamination Profiles and Characterization of Damage to Foods:* Continue study.
(18) *Mites in Stored Food:* Continue study.
(19) *Mole:* Continue topic; Associate Referee needed.
(20) *Mushroom Products, Dried:* Continue study.
(21) *Mushrooms, Canned:* Continue topic; appoint an Associate Referee.
(22) *Particulates in Large Volume Parenterals:* Continue study.
(23) *Peanut Butter, Water-Insoluble Inorganic Residues:* Discontinue topic.
(24) *Rye Bread:* Continue study.
(25) *Shrimp, Filth in:* Establish topic; appoint an Associate Referee.
(26) *Soluble Insect and Other Animal Filth:* Continue study.
(27) *Soups, Canned and Dehydrated:* Discontinue topic.
(28) *Spices:* Continue study.
(29) *Vegetable Products, Dehydrated, Isolation of Extraneous Filth:* Continue study.
*(30) *Vertebrate Excreta, Chemical Identification Test:* Editorially revise the chemical test for mammalian feces, **44.B08-44.B11**: In **44.B09**, add "(d) *Spatula.*—Curved on one end, knob on the other end (Arthur H. Thomas Co. No. 8340-H10, or equiv.)." In **44.B10(c)**, change the last paragraph to read "Long term storage: Add ca 1 mL portions to cups. Gelled plugs, in cups, may be stored for up to 4 months if sealed in a plastic bag, held at room temp., and protected from direct sunlight. Discard any gels showing pink color and/or vol. loss." In **44.B11**, revise the first paragraph to read "Transfer suspect feces . . . to cup contg 1 mL gelled WTM. Cover with addnl 1 mL cool (40-41°) WTM or, alternatively, with plug of gelled WTM. (Use clean spatula, **44.B09(d)**, to manipulate covering plug of WTM and to press plug into close contact with sample.) Place cup in 40-41° H_2O bath. Check for development of red color near particles." In **44.B11**, add as a new third paragraph "*Positive control preparation.*—Using calf intestine alkaline phosphatase (AKP) (Calbiochem No. 52457, or equiv.), prep. 1 mg/mL soln in borate buffer (stock test reagent **44.B10(b)**, without phthln diphosphate). Add 20 μL AKP soln to 1 mm diam. filter paper disks (Whatman No. 1, or equiv.). Use positive control disks with either liq. WTM or alternative gelled plug WTM. Note: Positive control disks may be stored up to 4 months if held at room temp. and protected from light." The paragraph beginning "*Test response.*—" will be the fourth paragraph in **44.B11**. The changes described will

provide a test media alternative that will save analytical time and reduce false negatives.

MICROBIOLOGICAL METHODS

(1) *Automated Methods for Foods and Cosmetics:* Continue study.

(2) *Automated Methods for Fungi:* Combine with topic Yeasts, Molds, and Actinomycetes.

*(3) *Bacillus cereus, Isolation and Enumeration:* Revise the official first action method for enumeration and confirmation of *Bacillus cereus* in foods (**46.A10-46.A15**) to incorporate additional tests for differentiating *B. cereus* from other *Bacillus* species.

(4) *Bacillus cereus Enterotoxin:* Continue study.

(5) *Bacillus cereus Toxin:* Combine under *Bacillus cereus* Enterotoxin.

(6) *Campylobacter species:* Continue study.

(7) *Canned Foods:* Continue study.

(8) *Cereal Products:* Discontinue topic.

(9) *Clostridium botulinum and Its Toxin, Detection:* Continue study.

(10) *Clostridium perfringens, Isolation and Enumeration:* Continue study.

*(11) *Coliform Bacteriology:* Adopt as official first action the hydrophobic grid membrane filter method for the enumeration of total coliforms in nonfat dry milk and canned custard. Combine topic under *Escherichia coli* and Other Coliforms.

(12) *Cosmetic Microbiology:* Transfer to General Referee for Drug and Device Related Microbiology.

(13) *Endotoxins by Limulus Amebocyte Lysate:* Continue study.

(14) *Enteropathogenic Escherichia coli:* Combine under *Escherichia coli* and Other Coliforms.

(15) *Enteropathogenic Escherichia coli, Direct Fluorescent Antibody Procedures:* Continue study.

(16) *Escherichia coli and Coliform Bacteria:* Change title to *Escherichia coli* and Other Coliforms.

(17) *Genetic Methods for Detecting Bacterial Pathogens:* Initiate topic.

(18) *Helium Leaks, Detection in Canned Foods:* Continue study.

(19) *Identification of Microorganisms by Biochemical Kits:* Initiate topic.

(20) *Microbe Identification by Capillary GC:* Discontinue topic.

(21) *Parasitology:* Continue study.

(22) *Pathogenic Yeasts, Molds, and Actinomycetes:* Retitle Yeasts, Molds, and Actinomycetes; continue study.

(23) *Salmonella:* Continue study.

(24) *Salmonella, Fluorescent Antibody Technique:* Continue study.

(25) *Somatic Cell, Automatic Optical Counting Method:* Continue study.

(26) *Somatic Cell, Fossomatic Counting Method:* Continue study.

(27) *Somatic Cell, Millipore-DNA Assay:* Discontinue topic.

(28) *Somatic Cell, Rolling Ball Viscometer Procedure:* Discontinue topic.

(29) *Staphylococcal Toxins:* Continue study.

(30) *Staphylococcus:* Appoint an Associate Referee and continue study.

(31) *Staphylococcus aureus:* Combine with *Staphylococcus.*

(32) *Sterility Testing of Medical Devices:* Transfer topic to General Referee for Drug and Device Related Microbiology.

(33) *Testing Biological Sterility Indicators:* Transfer topic to General Referee for Drug and Device Related Microbiology.

(34) *Vibrio cholerae and Detection of Its Toxin:* Continue study.

(35) *Vibrio parahaemolyticus:* Continue study.

(36) *Virology and Animal Oncology:* Continue study.

(37) *Yeast and Mold Counts by Spiral Plate Method:* Combine with Yeasts, Molds, and Actinomycetes.

(38) *Yersinia enterocolitica:* Appoint an Associate Referee and continue study.

(39) *Other Topics:* Continue official first action status of the following methods: examination of frozen, chilled, precooked, or prepared foods (**46.013-46.016**); thermophilic bacterial spores in sugars (**46.026-46.030**); virus in ground beef (**46.120-46.122**); detection of invasiveness of *E. coli* (**46.C01-46.C10**).

Report of Committee G on Recommendations for Official Methods

RICHARD L. BRUNELLE (Bureau of Alcohol, Tobacco and Firearms, Rockville, MD 20850), *Chairman;* VALVA C. MIDKIFF (University of Kentucky, Lexington, KY 40506); GLENN M. GEORGE (Salsbury Laboratories, Charles City, IA 50616); HAROLD THOMPSON (National Center for Toxicological Research, Jefferson, AR 72079); ALEXANDER MACDONALD (Hoffmann-LaRoche Corp., Nutley, NJ 07110); PATRICIA BULHACK (Food and Drug Administration, Division of Color Technology, Washington, DC 20204), *Secretary;* and RUEY K. CHI (Food and Drug Administration, Division of Mathematics, Washington, DC 20204), *Statistical Consultant*

ANTIBIOTICS

(1) *Affinity Quantitative Determination of Penicillin in Milk:* Continue official first action status of method **16.C01-16.C05;** continue study.

(2) *Bacitracin in Feeds:* Continue official first action status of method **42.C06-42.C12;** continue study.

(3) *Bacitracin in Feeds and Premixes (Chemical Determination):* Continue official first action status of method **42.C13-42.C17;** continue study.

(4) *Bambermycins:* Continue study.

(5) *Chloramphenicol in Animal Tissues:* Continue study.

(6) *Chlortetracycline in Feeds:* Continue official first action status of method **42.215-42.220;** continue study.

(7) *Erythromycins:* Continue official first action status of method **42.221-42.225;** continue study.

(8) *Lasalocid Sodium (Microbiological Assay):* Continue study.

(9) *Lincomycin in Feeds:* Continue official first action status of method **42.237-42.240;** continue study.

(10) *Monensin:* Continue official first action status of methods **42.245-42.249** and **42.250-42.255;** continue study.

(11) *Oxytetracycline:* Continue study.

*(12) *Qualitative Determination of β-Lactam Residues in Milk:* Adopt as official final action the ampule and multi-test systems for residues in milk and milk products at levels ≥0.005 IU penicillin G/mL, **16.C17-16.C22,** excluding chocolate products in the multi-test system. Continue official first action status of qualitative disc method II, **16.C06-16.C11.**

(13) *Quantitative Determination of β-Lactam Antibiotic Residues in Milk:* Continue official first action status of method **16.C12-16.C16;** continue study.

(14) *Screening Procedures for Antibiotics in Feeds:* Continue study.

(15) *Statistics of Microbiological Assay:* Continue study.

(16) *Tetracyclines in Tissues (Chromatographic Assay):* Continue study.

(17) *Tetracyclines in Tissues (Microbiological Assay):* Continue study.

(18) *Tylosin:* Continue study.

(19) *Virginiamycin, Turbidimetric Assay:* Continue study.

BIOCHEMICAL METHODS

(1) *Aminoglycosides in Animal Tissue:* Continue study.

(2) *17β-Estradiol and Diethylstilbestrol in Tissues (Immunochemical Methods):* Continue study.

(3) *Hormones in Tissues (Immunospecific Affinity Chromatography):* Continue study.

(4) *Immunochemical Methods for Staphylococcal Enterotoxin: Development of Monoclonal Antibodies:* Continue study.

(5) *Immunochemical Species Identification of Meat:* Continue study.

(6) *Performance Evaluation Methods for Non-RIA Procedures Measuring Human Chorionicgonadotropin:* Continue study.

(7) *Performance Evaluation Protocols for Clinical Chemical and Immunochemical Diagnostic Products:* Continue study.

(8) *Steroid Quantitation (Enzymatic Methods):* Continue study.

(9) *Sulfa Drugs in Animal Tissues (Immunoassay):* Continue study.

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* An asterisk before a recommendation indicates that official action on that recommendation resulted in its appearance in "Changes in Methods."

** No reports were submitted by the General Referees in the areas of Forensic Sciences, Microbial Mutagenicity, or Toxicological Tests. The recommendations for continued study were initiated by members of Committee G.

The recommendations submitted by Committee G were adopted by the Association.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition; and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **63,** 374-423 (1980); **64,** 501-540 (1981); **65,** 450-521 (1982).

COLOR ADDITIVES

(1) *Arsenic and Heavy Metals:* Continue study.
(2) *Atomic Absorption:* Continue study.
(3) *Color in Candy and Beverages:* Continue study.
(4) *Color in Cosmetics:* Continue study.
(5) *Color in Drugs:* Continue study.
(6) *Color in Other Foods:* Continue study.
(7) *FD&C Red No. 4 in Maraschino Cherries:* Continue study.
*(8) *High Pressure Liquid Chromatography:* (a) Editorially revise **34.B06** to read: "Weigh 0.250 g sample, add 10 mL 0.1M $Na_2B_4O_7$ first, then add ca 50 mL H_2O, and, when dissolved, dil. to 100 mL with H_2O." (b) Adopt as official final action the official first action method for the HPLC determination of intermediates and reaction by-products in FD&C Yellow No. 5, **34.C01-34.C06.**
(9) *Inorganic Salts:* Continue study.
(10) *Intermediates, Uncombined, in Certifiable Triphenylmethane Colors:* Continue study.
(11) *Intermediates, Uncombined, in Certifiable Water-Soluble Azo Colors:* Continue official first action status of methods **34.059-34.062, 34.053-34.058,** and **34.063-34.068.**
(12) *Subsidiary Colors in Certifiable Color Additives:* Continue study.
(13) *X-Ray Fluorescence Spectroscopy:* Continue study.

COSMETICS

(1) *Deodorants, Aluminum and Zirconium in:* Continue official first action status of method **35.019-35.023** and method **35.001-35.006.**
(2) *Essential Oils and Fragrance Materials, Components:* Continue study.
(3) *Nitrosamines:* Continue study.
(4) *Preservatives:* Continue study.

DRUG RESIDUES IN ANIMAL TISSUES

(1) *Carbadox:* Continue study.
(2) *Diethylstilbestrol:* Continue study.
(3) *Dimetridazole:* Continue study.
(4) *3,5-Dinitrobenzamide:* Continue study.
(5) *Nitrofurans:* Continue study.
*(6) *Screening Methods:* Adopt as official first action the interim first action method for the determination of multi-sulfonamides by TLC in swine, turkey, and duck tissues.
(7) *Steroids:* Continue study.
(8) *Sulfa Drugs:* Continue study.
(9) *Sulfonamide Drugs:* Continue as official first action the methods for determination of sulfamethazine in swine tissues, **41.C01-41.C08** and **41.C09-41.C12;** continue study.

DRUGS IN FEEDS

(1) *Amprolium:* Continue study.
(2) *Arprinocid:* Continue as official first action the spectrophotometric method for determination of arprinocid in feed premixes, **42.C01-42.C05;** continue study.
(3) *Arsanilic Acid:* Continue study.
(4) *Carbadox:* Continue study.
(5) *2-Chloro-1-(2,4,5-trichlorophenyl) Vinyl Dimethyl Phosphate (Rabon®):* Continue study.
(6) *Dibutyltin Dilaurate:* Continue study.
(7) *1,2-Dimethyl-5-nitroimidazole (Dimetridazole):* Continue official first action status of method **42.063-42.068;** continue study.
(8) *Ethopabate:* Continue study.
(9) *Ethylenediamine Dihydroiodide:* Continue study.
(10) *Furazolidone and Nitrofurazone:* Continue study.
(11) *Ipronidazole:* Continue study.
(12) *Larvadex:* Continue study.
(13) *Melengestrol Acetate:* Continue study.
(14) *Microscopy:* Continue study.
(15) *Nifursol:* Continue official first action status of method **42.098-42.104;** continue study.
(16) *Phenothiazine:* Continue study.
(17) *Pyrantel Tartrate:* Continue study.
(18) *Roxarsone:* Continue study.
(19) *Sulfa Drug Residues:* Continue study.
(20) *Sulfadimethoxine-Ormetoprin Mixtures:* Continue study.
(21) *Sulfamethazine and Sulfathiazole (Premix and Finished Feed Levels):* Continue study.
(22) *Sulfaquinoxaline:* Continue study.

FORENSIC SCIENCES**

(1) *ABO Blood Typing:* Continue study.
(2) *Biological Fluids (Immunoelectrophoresis):* Continue study.
(3) *Blood:* Continue study.
(4) *Bloodstains, ABH Typing:* Initiate topic.
(5) *Bloodstains, Species Determination of Dried:* Initiate topic.
(6) *Bomb Residues:* Continue study.
(7) *Documents:* Continue study.
(8) *Fingerprints:* Continue official first action status of methods **45.001, 45.002-45.004.**

(9) *Firearms:* Continue study.
(10) *Flammable Fluids:* Continue study.
(11) *Gunshot Residues:* Continue study.
(12) *Gunshot Residues by AAS:* Continue study.
(13) *Hair Examination:* Continue study.
(14) *Infrared Spectroscopy:* Continue study.
(15) *Microscopic Methods and Glass Products:* Continue study.
(16) *Paints, Pyrolysis-Gas Chromatographic Methods:* Continue study.
(17) *Safe Insulation:* Continue study.
(18) *Serial Number Restoration (Chemical Etching Techniques):* Initiate topic.
(19) *Soil Analysis:* Continue study.
(20) *Voice Print Identification:* Continue study.

MICROBIAL MUTAGENICITY TESTING**

Prophage Induction: Continue study.

TOXICOLOGICAL TESTS**

(1) *Ames Test:* Continue study.
(2) *Aspiration Tests:* Continue study.
(3) *Cell Culture–Enzyme Induction Bioassay:* Continue study.
(4) *In Vitro Mutagenic Assay:* Continue study.
(5) LD_{50} *Test:* Continue study.
(6) *Rabbit Eye Irritation Test:* Continue study.
(7) *Skin Irritation Tests:* Continue study.

VETERINARY ANALYTICAL TOXICOLOGY

(1) *Cholinesterase:* Continue study.
*(2) *Copper in Animal Tissues:* Adopt as official first action the method for atomic absorption determination of copper in serum as described by the Associate Referee.
(3) *Lead in Animal Tissues:* Continue study.
(4) *Multiple Anticoagulant Screening:* Continue study.
(5) *Multielement Analysis by ICP:* Continue study.
(6) *Nitrate/Nitrite:* Continue study.
(7) *Selenium in Animal Tissues:* Continue study.
(8) *Poisonous Plants:* Continue study.
(9) *Arsenic in Animal Tissues:* Continue study.
(10) *Molybdenum:* Continue study.
(11) *Rumensin:* Continue study.

Report of the Executive Director

DAVID B. MacLEAN
AOAC, 1111 N 19th St, Suite 210, Arlington, VA 22209

The previous report of the Executive Director was approved by the Board of Directors and the Association as published (*J. Assoc. Off. Anal. Chem.* **65**, 392–396 (1982)).

The first meeting of the Board of Directors was held January 20, 1982, in Arlington, VA. J. P. Minyard, Jr, President, presided. Other members present were W. R. Bontoyan, D. E. Coffin, C. W. Gehrke, J. B. Kottemann, B. Larsen, and H. L. Reynolds. Others present were D. B. MacLean, R. Blakely, and F. Van Dyke.

The Board of Directors took the following actions at this meeting:

(1) Directed the Executive Director to compile a Policy Manual;

(2) Ratified mail ballots for editorial arrangements for the *Journal*;

(3) Accepted with thanks the reports of the Standing Committees of AOAC;

(4) Directed the Executive Director to review and begin implementation of the Long-Range Planning Committee's recommendations on improving methods output;

(5) Increased the price of the *Journal* subscription to $75;

(6) Authorized the staff to hire an advertising consultant;

(7) Instructed the Executive Director to decide on use of budgeted travel funds for committee members to travel to committee meetings;

(8) Endorsed the idea of discussion sessions on statistics at Annual Meetings, Spring Workshops, and Regional Section Meetings;

(9) Accepted the Terms of Reference of the Editorial Board;

(10) Accepted for information the Terms of Reference of the Committee on Statistics and recommended modifications;

(11) Met briefly with Frederic Senti of the Federation of American Societies for Experi-

mental Biology;

(12) Engaged the services of Perce McKinley and Fred Baur as Liaison Representatives of AOAC;

(13) Authorized the staff to hire a professional artist to design a suitable Centennial logo based on AOAC's present logo;

(14) Approved and directed the staff to implement the Member Information Survey;

(15) Requested Howard Moore, Chairman of the State and Provincial Participation Committee, to present the Board with a proposal for the Committee's reorganized structure based on regional representatives;

(16) Referred draft Regional Section Bylaws to the Committee on the Constitution;

(17) Accepted for information the Report on the Marketing Survey for Feed Drug Reference Standards;

(18) Accepted for information the status report on the Chemical Specialty Manufacturers Association's proposal to reproduce and publish AOAC disinfectant methods in their *Compendium of Microbiological Test Methods*;

(19) Referred the question of Annual Meeting registration fees for members vs nonmembers to the Long-Range Planning Ad Hoc Committee on Membership;

(20) Authorized the Executive Director, in consultation with the Symposia and Special Programs Committee, to offer meeting space to other organizations at the Annual Meeting provided that no AOAC session be deprived of space, additional costs to AOAC are minimal, the other group's interests are related to AOAC interests, and AOAC would gain by furthering cooperation with that group;

(21) Approved the AOAC Annual Meeting henceforth be named the Annual International Meeting;

(22) Directed that the Long-Range Planning Ad Hoc Membership Committee exclude from its proposals concerning membership rights the question of voting.

The second meeting of the Board of Directors was held April 15–16, 1982, in New Orleans, LA. J. P. Minyard, Jr, President, presided. Other members present were W. R. Bontoyan, D. E. Coffin, C. W. Gehrke, B. Larsen, H. L. Reynolds. Others present were D. B. MacLean, R. Blakely, and M. Bernard.

The Board of Directors took the following actions at this meeting:

(1) Ratified the vote taken by mail ballots for *Journal* co-editors;

(2) Accepted for information the status report of the Member Information Survey;

(3) Accepted for information the contest name chosen by the judges, and declared Michael Uptmor winner of the prize;

(4) Authorized the Executive Director to pursue negotiations with Harold Egan to engage him as a Liaison Representative of AOAC;

(5) Directed the Executive Director to explore with John Munn whether he might wish to offer his services to AOAC with reference to initiatives toward U.S.-based toxicological organizations;

(6) Resolved that AOAC does not wish, at this time, to move into the business of storing and selling reference standards;

(7) Accepted the proposal by Molly Ready, Ginger Gibson, and Alan Hanks that the 1985 Spring Training Workshop be held in Dallas/Ft. Worth;

(8) Accepted for information the report from the Federation of American Societies for Experimental Biology;

(9) Charged the Ways and Means Committee with developing a modest fund-raising program, with the aid of a consultant, for the purpose of increasing the Wiley Award stipend to a level appropriate to its importance and to provide support for graduate fellowships or scholarships;

(10) Directed the Symposia and Special Programs Committee and the Centennial Committee to work together to develop technical programs for the Centennial meeting;

(11) Directed the Centennial Committee to appoint a person or subcommittee to work closely with the editorial staff to develop a firm plan for a special Centennial publication;

(12) Directed the Centennial Committee and the Symposia and Special Programs Committee to work together to make contacts for support and for sponsorship of social functions at the Centennial meeting;

(13) Referred suggestions for cooperation with national and international organizations for the Centennial to the Centennial Committee and the Symposia and Special Programs Committee for implementation;

(14) Directed that the Centennial logo be designed using only the acronym instead of the full name, and that final decision for choosing the new logo be made by the Centennial Committee;

(15) Decided that AOAC not make plans to purchase office space at this time;

(16) Increased the Wiley Award to $2500;

(17) Accepted for information the report on host banks;

(18) Decided that AOAC Regional Sections not be permitted to incorporate separately but remain a part of the parent organization;

(19) Decided that Regional Sections be encouraged, advised, and aided by AOAC staff following existing interim guidelines until finalization and publication of guidelines as AOAC policy;

(20) Authorized the Northeast Regional Section to open a checking and/or savings account;

(21) Referred the outline for the Quality Assurance Manual to the Editorial Board;

(22) Accepted for information the first draft of the Policy Manual;

(23) Accepted for information the statement regarding the reorganization of the State and Provincial Participation Committee;

(24) Accepted for information the preliminary report of the Ad Hoc Membership Committee;

(25) Accepted for information the report on the Intersociety Committee on Air Sampling and Analysis;

(26) Accepted for information the statement from the Committee on Collaborative Studies regarding truncated collaborative studies and requested further clarification from the Committee;

(27) Decided not to adopt a permanent numbering system for *Official Methods of Analysis;*

(28) Accepted the proposal for AOAC to operate the Reporting Secretariat for Pesticides and Toxic Substances for the International Organization for Legal Metrology (OIML) and that the Committee on Performance of Instrumental Methods and Data Handling be charged to take the lead in this effort;

(29) Instructed the Executive Director to prepare an outline for a laboratory certification program proposal;

(30) Accepted a recommendation for the Executive Director to offer complimentary registration to representatives of international organizations;

(31) Accepted the proposal of the Chemical Specialties Manufacturers Association (CSMA) to reprint Chapter 4, Disinfectants, of *Official Methods of Analysis* in a CSMA manual;

(32) Accepted as policy the recommendation regarding release and publication of information on members, associate members, and sustaining members of AOAC.

The third meeting of the Board was held September 8–9, 1982, in Arlington, VA. J. P. Minyard, Jr, President, presided. Other members present were: D. E. Coffin, C. W. Gehrke, J. B. Kottemann, B. Larsen, H. L. Reynolds. Others present were D. B. MacLean, R. Blakely, G. Schwartzman, and F. Van Dyke.

The Board of Directors took the following actions at this meeting:

(1) Ratified the vote taken by mail ballot for Fellows of the AOAC for 1982;

(2) Established a subcommittee to refine the point system used to determine candidates for Fellows;

(3) Approved the proposed 1983 budget;

(4) Approved the recommended bad debt write-off;

(5) Instructed the Executive Director to obtain legal opinion regarding financial liability of AOAC (reference: the Hydrolevel Case);

(6) Accepted for information the report regarding sustaining and private sustaining members and instructed the Executive Director to query these members to obtain their opinion regarding benefits/support;

(7) Recommended a vote be taken at the 1982 Annual International Meeting to change the Bylaws as follows: add to Article IV, Section 1, "No member of the Board of Directors may be elected for more than six years"; change Article X to Article XI; and add a new Article X, Subsidiary Organizations;

(8) Agreed that Regional Sections may establish their own dues systems and that full voting members of Regional Sections must be members of AOAC;

(9) Agreed that, pending affirmative vote to add a new Article for Subsidiary Organizations to the Bylaws, each Regional Section may petition the Board for a charter, and that such charter be granted upon confirmation by the Committee on the Constitution that the Regional Bylaws conform to AOAC Bylaws;

(10) Accepted for information the report of the Ad Hoc Membership Committee;

(11) Recommended a vote be conducted at the 1982 Annual International Meeting to increase the annual membership dues to $25;

(12) Charged the Ways and Means Committee with preparing a plan for a fund-raising campaign in connection with the Centennial to increase the Wiley Fund;

(13) Instructed the Executive Director to contact committee chairs to ascertain secretarial support needed at Committee meetings, especially during the Annual International Meeting;

(14) Approved certificates of appreciation for chairpersons of the 1982 Spring Meeting;

(15) Directed that staff of *Official Methods of*

Analysis publish in "Changes in Methods" and in the 14th Edition of *Official Methods of Analysis*, statistical parameters of methods, beginning with those adopted in 1982, as recommended in the Joint Committee Report (March 1980 *Journal*, page 341);

(16) Increased the limit for capital item purchases which do not require Board approval to $3000;

(17) Approved the general organization and arrangement of the Policy Manual;

(18) Accepted the Terms of Reference of Committee A, Committee D, the Official Methods Board, and the Finance Committee;

(19) Amended the Finance Committee Terms of Reference to read: "The Finance Committee shall consist of two to four members, not including the annually elected Treasurer. Their duties require proximity to AOAC headquarters."

(20) Accepted for information the proposed laboratory accreditation program.

The fourth meeting of the Board of Directors was held October 24, 1982, at the Shoreham Hotel, Washington, DC. J. P. Minyard, Jr, President, presided. Other members present were W. R. Bontoyan, D. E. Coffin, C. W. Gehrke, J. B. Kottemann, B. Larsen, H. L. Reynolds. Others present were D. B. MacLean, R. Blakely, and F. Van Dyke.

The Board of Directors took the following actions at this meeting:

(1) Recommended a vote be conducted at the 1982 Annual International Meeting to change the title of Treasurer to Secretary/Treasurer;

(2) Accepted for information the Auditor's Report and Financial Statement for the Year Ending September 30, 1982;

(3) Accepted for information the status report of contracts and support;

(4) Authorized funds for the Executive Director to rewrite and analyze, as necessary, methods and data of several EPA methods on priority pollutants in water;

(5) Directed the staff to publish the summary and conclusion of the Federation of American Societies for Experimental Biology report in the *Journal*; announce publication in *The Referee*; and obtain comment and input from the membership;

(6) Recommended that AOAC's vacant office space not be rented at this time;

(7) Emphasized that the Symposia and Special Programs Committee should work on the technical program for the Centennial Annual International Meeting, and that the Centennial Committee should work on the non-technical program;

(8) Charged the Ways and Means Committee with preparing a plan for soliciting funds for the Centennial and the Wiley Fund, and to present these plans to the Board in January 1983;

(9) Directed Helen Reynolds to be the liaison with Kenneth Helrich regarding the publishing of the history of AOAC for the Centennial;

(10) Accepted for information the minutes of the Centennial Committee Meeting and endorsed in general the work of the Committee;

(11) Accepted the modified format for statistical parameters of methods proposed by the Editorial Board, and re-endorsed the Board's action in September 1982 to include these parameters in official methods.

(12) Agreed to grant provisional charters to the Northwest and Midwest Regional Sections with full charter to be granted on approval of the Regional Sections' Bylaws by the Committee on the Constitution.

Important events affecting AOAC during 1982 included:

(1) Helen L. Reynolds resigned as Editor of the *Journal* on January 1, 1982.

(2) The 7th Annual Spring Training Workshop & Exhibition was held in New Orleans, LA, April 13–15, 1982. Approximately 355 persons attended. Technical Sessions included Mycotoxins, Pesticide Residues, Toxicological Substances, Drugs and Antibiotics in Feeds and Tissues, Food Toxicology, Pesticide Formulations, Environmental Contamination and Monitoring, Fertilizers, Pesticide Enforcement, Veterinary Toxicology, Laboratory Automation, Quality Assurance, and Workshop on Technical Presentations.

(3) Midwest Regional Section Meeting was held in Ames, IA, on June 2–3, 1982. Approximately 145 people attended sessions covering the following topics: food, feeds, forensic analysis, regulatory problems in pesticides, mycotoxins, mass spectrometry, laboratory safety, microbial analysis, and water.

(4) Northwest Regional Section Meeting was held in Olympia, WA, on June 16–17, 1982. Approximately 113 people attended sessions covering the following topics: pesticides, microbiology, the environment, food chemistry, feeds/fertilizers, and forensic sciences.

(5) Northeast Regional Section Meeting was held in Syracuse, NY, on June 22–23, 1982. Approximately 82 people attended sessions covering the following topics: pesticides, food chemistry, laboratory safety, mycotoxins, and

instrumentation.

(6) Rodney J. Noel, Malcolm C. Bowman, Joseph Sherma, James F. Lawrence and Evelyn Sarnoff were chosen as the new Co-Editors for the *Journal of the AOAC*. Their appointments began March 1 and will continue through October 1983.

(7) Odette L. Shotwell, Research Leader of the U. S. Department of Agriculture's Northern Regional Research Center in Peoria, IL, was named the 1982 winner of the Harvey W. Wiley Award.

(8) The following scientists were named 1982 Fellows of the AOAC: Wallace S. Brammell, Food and Drug Administration, Washington, DC; D. Earle Coffin, Health and Welfare Canada, Ottawa, Ontario, Canada; Thomas Fazio, Food and Drug Administration, Washington, DC; Elmer George, Jr, New York Department of Agriculture and Markets, Albany, NY; Stanley E. Katz, Rutgers University—Cook College, New Brunswick, NJ; John W. Sherbon, Cornell University, Ithaca, NY; Edward Smith, Food and Drug Administration, Washington, DC; and Arthur H. Hofberg, Ciba-Geigy Corp, Greensboro, NC.

(9) Carol Lynn Lasko, a chemistry student at the College of the Siskiyous, was the winner of the 1982–1984 Scholarship Award, the seventeenth given by AOAC.

(10) The 5th Edition of the *Handbook for AOAC Members* was published in August 1982. This new edition combines material previously published as the *Handbook* and the *AOAC Style Manual*.

(11) Bylaw amendments (a) allowing the establishment of regional sections, (b) establishing a limit of six consecutive years of service for members of the Board of Directors, (c) changing the title of Treasurer to Secretary/Treasurer, and (d) changing Article X: Amendments to Bylaws, to Article XI were approved by the membership on October 28, 1982.

(12) On October 1, 1982, AOAC signed a contract with the Health Protection Branch, Health and Welfare Canada, to develop, test, and modify, as needed, protocols to accredit laboratories engaged in the chemical and microbiological analysis of food and food products, to be completed by December 15, 1983.

(13) Sources of financial support up to October 1982 were:

Government

Consumer Product Safety Commission
Environmental Protection Agency (Office of Pesticide Formulations)
Fish and Wildlife Service
Food and Drug Administration
Health and Welfare Canada (Health Protection Branch)
Laboratory of the Government Chemist
Food Division, Ministry of Agriculture, Fisheries, and Food
National Marine Fisheries Service
US Department of Agriculture
Agricultural Research Service
Food Safety and Inspection Service

Alabama Department of Agriculture and Industries
Alberta Agriculture
Arizona Agricultural Experiment Station
Arkansas State Plant Board
California Department of Food and Agriculture
Delaware Department of Agriculture
Florida Department of Agriculture and Consumer Services
Georgia Department of Agriculture
Hawaii Department of Health
Illinois Department of Agriculture
Indiana Office of the State Chemist
Indiana State Board of Health
Iowa Department of Agriculture
Iowa State Veterinary Diagnostic Laboratory
Kentucky Agricultural Experiment Station Division of Regulatory Services
Kentucky Department of Agriculture
Maryland Department of Agriculture
Michigan Department of Agriculture
Minnesota Department of Agriculture
Mississippi State Chemical Laboratory
Missouri Experiment Station Chemical Laboratory
Montana Department of Agriculture
Nebraska State Department of Agriculture
New Jersey Department of Agriculture
New Mexico Department of Agriculture
New York Department of Agriculture and Markets
New York State Agricultural Experiment Station
North Carolina Department of Agriculture
North Dakota State Laboratories Department
Oklahoma State Department of Agriculture
Ontario Ministry of Agriculture and Food
Oregon Department of Agriculture
Pennsylvania Department of Agriculture
South Carolina Department of Agriculture
Tennessee Department of Agriculture
Texas Agricultural Experiment Station

Utah State Department of Agriculture
Vermont Agricultural Experiment Station
Virginia Division of Consolidated Laboratory Services
Wisconsin Department of Agriculture, Trade and Consumer Affairs
Wyoming Department of Agriculture

Industry
Agrico Chemical Co.
Agway, Inc.
Alcon Laboratories, Inc.
Allergan Pharmaceuticals, Inc.
American Cyanamid Co.
The Andersons
Bacardi Corp.
Boehringer Mannheim Biochemicals
Campbell Institute for Research and Technology
Cargill, Inc.
Chevron Chemical Co.
Ciba-Geigy Corp.
Coca Cola Co.
CPC North America—CPC International, Inc.
Duphar BV
Du Pont Co.
E. & J. Gallo Winery
Eastman Chemical Products, Inc.
Eli Lilly and Co.
Endo Laboratories, Inc.
FBC Ltd.
FMC Corp.
The Fertilizer Institute
GB Fermentation Industries, Inc.
General Foods Corp.
General Mills, Inc.
Gerber Products Co.
Hazleton Raltech
Heinz U.S.A.
Hershey Foods Corp.
Hoechst-Roussel Pharmaceuticals, Inc.
Hoffmann-La Roche, Inc.
ICI Americas, Inc.
International Minerals and Chemicals (IMC) Corp.
ITT Continental Baking Co.
Kraft, Inc.
The Kroger Co.
Leatherhead Food R. A.
Lehn & Fink Products Co.
McKee Baking Co.
Mead Johnson & Co.
Monsanto Agricultural Products Co.
Nabisco Brands, Inc.
National Food Processors Association
Norwich Eaton Pharmaceuticals, Inc.
O. M. Scott & Sons Co.
Ortho Pharmaceutical Corp.
Pennwalt Corp.
Pfizer, Inc.
The Pillsbury Co.
The Procter & Gamble Co.
Ralston Purina Co.
Rhone-Poulenc Chemical Co.
Salsbury Laboratories, Inc.
Schenley Distillers, Inc.
Joseph E. Seagram & Sons, Inc.
Shaklee Corp.
Smith Kline Corp.
Sunkist Growers, Inc.
Swift & Co.
Technicon Industrial Systems
The Upjohn Co.
Velsicol Chemical Corp.

(14) The Technical Advisory Groups (TAG) to the American National Standards Institute (ANSI) for the International Organization for Standardization (ISO) Technical Committee (TC) 34 on Agricultural Food Products and Technical Committee 134 on Fertilizers are actively reviewing, commenting, and voting on methods which have been submitted for consideration as International Standards.

(15) The 96th Annual International Meeting was held October 25–28, 1982, at the Shoreham Hotel, Washington, DC. Approximately 1100 persons attended. At the General Session, the 12th Award for the Best Associate Referee Report of the Year was presented to Peter F. Kane, Office of Indiana State Chemist and Seed Commissioner, Purdue University, for his report, "Collaborative Study of the Flame Photometric Determination of K_2O in Fertilizers." Special awards were presented to Nicole Hardin and Hershel F. Morris, Jr, for organizing the 1982 Spring Training Workshop in New Orleans. Other awards were given to Marjorie D. Fuller, in recognition of and appreciation for fifteen years of service as the Assistant Business Manager with the Association, and to Bernhard Larsen, in recognition and appreciation for eight years of devoted service as Treasurer of AOAC. James P. Minyard, Jr, presented his Presidential Address, "From Introspection to New Horizons" and Odette Shotwell presented her Wiley Award Address, "Successful Interagency Cooperation: The Diehlstadt Story."

The Annual Meeting featured five symposia: Advances in Nitrosamine Analysis; Impact and

Directions of Good Laboratory Practices; Detection of Deliberate Adulteration of Foods; Chemical Analysis of Drinking Water—Trace Contaminants; and Water Activity (a_W) Methods of Analysis of Foods. James P. Minyard presided over the banquet on Monday, October 25th, and Eugene Holeman served as Toastmaster. Odette Shotwell was presented the 26th Harvey W. Wiley Award, consisting of $2500 and a plaque. Entertainment for the evening was provided by the Big Band of Vic Simas. The 3rd Annual Collaborative Studies Luncheon was held on Wednesday, October 27, 1982.

On October 28, Warren R. Bontoyan took office as President and Charles W. Gehrke as President-Elect. Prince G. Harrill was elected Secretary/Treasurer. Frank J. Johnson was elected new Board Member. James B. Kottemann and D. Earle Coffin were re-elected Board members. James P. Minyard, Jr., immediate Past-President, remained on the Board.

Accepted

Report of the Treasurer and the Finance Committee

BERNHARD LARSEN
Treasurer of AOAC, Chairman of the Finance Committee
U.S. Department of Agriculture, Food Safety and Inspection Service, Washington, DC 20250

Other members: T. G. Alexander, Jr, P. G. Harrill

The Treasurer and the Finance Committee have confirmed in Fiscal Year 1982 (*1*) the actuality of the Association's claimed assets, in the form of cash and securities, and (*2*) by means of selective checks, the reliability of the Association's financial reports, a summary of which is published below.

It is a distinct pleasure to be able to report that, again for Fiscal Year 1982, the year-end Financial Statement had been completed and professionally audited before the Association's Annual Meeting, October 25–28, 1982, and reviewed by the Finance Committee prior to the Business Meeting that closes that annual meeting. This prompt closing of the books for Fiscal Year 1982 is attributable in large measure to the internal accounting practices put into place by the Association's Comptroller, Richard Blakely.

When the position of Treasurer of the AOAC was first activated eight years ago, the Treasurer, with the enthusiastic cooperation of the Finance Committee and of AOAC staff, set three priority goals. These were; (*1*) to engage a consulting firm to examine the internal accounting practices of the Association and to institute promptly such changes as the consultant's report would show to be desirable and needful, (*2*) to bring onto the Association's staff a highly qualified Comptroller, and (*3*) under the leadership of that Comptroller, to institute and refine procedures that would match the Association's growing and changing needs, using the most up-to-date business methods and tools available. The Finance Committee is happy to report that these goals have all been met, and that in their opinion the Association's bookkeeping and accounting practices are now fully attuned to the rapid evolution of the Association itself.

It remains the intention of the Treasurer (henceforth the Secretary/Treasurer by action of the membership on October 25, 1982), the Finance Committee, and the Comptroller, to maintain the momentum, and to keep the fiscal practices of the Association always fully attuned to the Association's ever evolving objectives and needs.

The Finance Committee has recommended to the Board of Directors that the Association's present accounting firm be retained for Fiscal Year 1984.

Accepted

STATE OF FINANCIAL CONDITION – SEPTEMBER 30, 1982

Assets

Current Assets:		
Cash, Bank of Virginia*		$129,211.65
Cash, Bank of Virginia, payroll		1,201.30
Cash, office fund		500.00
Cash, Mid-West fund		2,890.41
Accounts receivable, books and publications		21,939.43
Accounts receivable, contracts and grants		95,777.05
Accounts receivable, Private Sustaining Members		6,000.00
Accounts receivable, other		58.05
Accrued interest receivable		16,986.66
Inventory, books and publications—at cost		124,551.28
Prepaid expenses		3,754.11
Advances		2,973.89
Total Current Assets		$405,843.83
Investments:		
Securities	$89,783.01	
Certificates of deposit	925,697.90	
Savings	1,813.68	1,017,294.59
Fixed Assets:		
Office furniture, fixtures and equipment	$126,687.08	
Less: Accumulated depreciation	50,125.06	76,562.02
Deferred Costs:		
Methods of Analysis, 14th Edition	$46,285.95	
Spring Workshop, 1983	2,337.56	
Spring Workshop, 1984	112.39	
Annual Meeting, 1982	35,415.74	
Quality Assurance Manual	2,121.87	
EPA Manual	4,256.77	90,530.28
Total Assets		$1,590,230.73

* Interest-bearing account

Liabilities and Fund Balance

Current Liabilities:		
Accounts payable		$38,204.73
Accrued and withheld payroll taxes		1,660.19
Total Current Liabilities		$39,864.92
Deferred Income:		
Journal subscriptions	$154,849.71	
Annual Meeting—1982	38,025.00	
Contracts	7,500.00	
Private Sustaining Members	10,000.00	
Total Deferred Income		210,374.71
Reserve For Publications		200,000.00
Restricted Reserve for the 14th Edition		318,311.58
Restricted Fund—Harvey Wiley		45,767.24
Restricted Fund—Centennial		100.00

Fund Balance:		
Balance, October 1, 1981	$726,918.05	
Less: Restricted Reserve for the 14th Edition	9,427.58	
	717,490.47	
Add: Excess of income over expenses for the twelve months ended September 30, 1982	58,321.80	
Balance, September 30, 1982		775,812.27
Total Liabilities and Fund Balance		$1,590,230.73

Report of the Editorial Board

ROBERT C. RUND, *Chairman*
Office of the Indiana State Chemist, Purdue University, Department of Biochemistry, West Lafayette, IN 47907

Other members: C. W. Gehrke, A. R. Hanks, K. R. Hill, M. Ihnat, C. F. Jelinek, J. P. Minyard, Jr, I. Pomerantz, H. L. Reynolds, C. H. van Middelem

The Editorial Board convened for a half-day session on October 24, 1982, at the Shoreham Hotel, Washington, DC.

A review of the current status of past actions revealed the following:

(1) A permanent numbering system recommended last year for use with official methods was rejected by the Board of Directors because of the difficulty and expense of implementation as well as the waning of apparent need.

(2) The 5th edition of the AOAC Handbook was published. This new edition combines both aspects of the old Handbook and the Style Manual. It includes the updated Bylaws and guides for all entities of the AOAC structure. In all, 2488 copies of the 4th edition were published in 1977 and distributed. The 5th edition has an initial printing of 1659 copies.

(3) The Editorial Board requested approval of the Board of Directors to proceed with an agreement with W. C. Spendley of the United Kingdom to rearrange and edit the Wernimont statistical manuscript now owned by the Association. An advisory group will be appointed by the Editorial Board chairman to advise Mr. Spendley on matters of philosophical nature and questions of statistical precepts. The rewritten version is to be submitted to the Association in June 1983.

(4) Under "Terms of Reference" governing the Board, 5 classes of members have been established, requiring replacement or reappointment of 2 members each year with the provision that no member may serve more than 10 years in succession. As a consequence of this action, James P. Minyard, Jr, and Helen L. Reynolds will retire from the Board. The Board recognizes their long and faithful service and wishes to record grateful appreciation to each of them for many excellent contributions.

(5) A three-member committee within the Board has been established to provide guidelines and to serve as an advisory body to the managing editor of the *Journal* in the matter of advertising.

(6) A study of advertising potential continues, with the services of a consultant.

(7) The AOAC statistical manual authored by Youden and Steiner has been reprinted with the addition of some footnotes for clarification, offered by William Horwitz and James Winbush and reviewed by Edwin Glocker and Mary Natrella. This manual continues as one of our best-selling publications.

(8) Quality Assurance Principles proceedings was reprinted in the past year. Of 1110 copies reprinted, half had been sold by the end of the year.

(9) Sales of the 13th edition of the *Official Methods of Analysis* continue at a good rate. As of the end of September, 13,747 copies had been sold. This is 876 additional copies over the same time frame for the previous edition. At the current rate, AOAC may deplete the inventory

of the 13th edition before the publication of the 14th edition.

(10) A proposal being negotiated between CIPAC and AOAC for publication of *CIPAC Handbook 1B* received support from the Editorial Board. The agreement would provide for publication, promotion, storage, sale, and order fulfillment by AOAC from a manuscript supplied by CIPAC.

(11) One of the most important achievements of this past year was the reorganization of the *Journal* editorial staff. A search committee composed of David B. MacLean, Kenneth Hill, Irwin Pomerantz, Charles Jelinek, Helen Reynolds, and headed by Alan Hanks performed magnificently and obtained the services of Rodney Noel (Purdue University), Joseph Sherma (Lafayette College), James Lawrence (Health and Welfare Canada), Malcolm Bowman (Consultant) and Evelyn Sarnoff (FDA) as co-editors. These co-editors have been functioning since March 1982, and met during the 96th Annual International Meeting with the Editorial Board and the *Journal* staff.

Actions taken by the Board—

(1) Recommended permission be granted to the Chemical Specialties Manufacturing Association to reprint Chapter 4, Disinfectants, in the CSMA manual. Furthermore, that updates of this chapter through 1984 be included. Conditions for permission include (a) a limited CSMA publication of 500 copies, (b) copyright privileges retained by AOAC, (c) AOAC will be credited as the source, and (d) AOAC will receive a royalty fee per copy sold. Justification for this action is based on minimum activity in Chapter 4 and a judgment that sales of the CSMA manual will not appreciably affect sales of the 13th or 14th edition of *Official Methods of Analysis*.

(2) Moved to encourage Fred Garfield and George Schwartzman to complete work on a Laboratory Quality Assurance Handbook as proposed by the Committee on Laboratory Quality Assurance. Upon submission of a first draft, the Editorial Board will determine acceptance as an AOAC publication and, if so, financial arrangements will be negotiated with the authors. A committee of two, Irwin Pomerantz and Charles Jelinek, were requested to discuss Editorial Board concerns with the authors.

(3) Upon invitation, suggested an author to the Centennial Committee to write on the subject of the Impact of Land Grant Universities on Method Development for publication in the *Journal*.

(4) Endorsed publication by AOAC of the proceedings of the Joint IUPAC/AOAC meeting on Harmonization of Collaborative Studies, to be held during the AOAC Centennial Meeting.

(5) Recommended, with regret, that the AOAC reject publication of the Harris-Gentry *MicroAnalytical Entomology for Food Sanitation Control*.

(6) Moved to explore publication of a food salvage booklet for the Association of Food and Drug Officials of the United States on a contract basis.

(7) Returned to the Board of Directors referral concerning precautionary signal words from the Safety Committee, with a suggestion that the Safety Committee implement this practice with Associate Referees.

(8) Recommended that the Executive Director pursue publication of the third update to the *EPA Manual for Pesticides and Devices*.

The Editorial Board Chairman is appreciative of the support and professional cooperation provided him by the AOAC staff and, in particular, the editorial staff.

Accepted

Report of the Centennial Committee

WILLIAM HORWITZ, *Chairman*
Food and Drug Administration, Bureau of Foods, Washington, DC 20204

Other members: T. G. Alexander, W. R. Bontoyan, K. W. Boyer, C. A. Brunner, P. Bulhack, E. Elkins, F. M. Garfield, J. J. Karr, J. Levine, H. Miller, H. L. Reynolds

This Committee met four times during 1982 and several times with the chairman of the Symposia and Special Programs Committee. The Committee outlined a program for the centennial year which will begin with the spring 1984 meeting in Philadelphia, April 29-May 2,

for an initial review of the protocols from the Committee on Interlaboratory Studies.

Several other international organizations are expected to meet consecutively before and after the 1984 fall meeting, particularly the Collaborative International Pesticide Analytical Committee (CIPAC), probably October 22–26, and the International Symposium on Harmonisation of Collaborative Studies sponsored jointly by AOAC and the International Union for Pure and Applied Chemistry (IUPAC) under the chairmanship of Harold Egan. This meeting is intended to be a workshop with attendance by invitation to the major national and international organizations performing interlaboratory testing. Dr. Egan is exploring the availability of facilities for the symposium, which is scheduled for October 25–27, 1984.

The Centennial meeting itself will be opened by an evening reception for an anticipated approximately 350 people. The technical program will begin on Monday, the 29th, with a plenary session addressed by two speakers eminent in the scientific aspects of analytical science (suggestions are encouraged) and with special awards and addresses. There will also be a formal opening of the exhibits with refreshments and a social affair in the evening. The Harvey Wiley Award banquet will be held on Tuesday, October 30 with a program to be developed by Warren Bontoyan and the 1983–84 President.

The Associate Referee luncheon will be held on Wednesday, exhibitors' workshops that evening, and the Board of Directors luncheon on Thursday. Guests at this latter luncheon will include the officials of CIPAC and IUPAC attending the other meetings, and other distinguished visitors. It is suggested that the business meeting can be held Friday morning, November 2nd.

The centennial year will close at the Dallas spring meeting in 1985 with a review and discussion of the proposals that result from the harmonization symposium.

Four major technical topics for the fall meeting have been proposed: quality assurance; education for modern analytical chemistry (Lockhart B. Rogers of the University of Georgia has accepted this assignment), toxicology (quantitative toxicological and microbiological topics), and topics resulting from the Committee on Instrumental Methods and Data Handling.

Special exhibit themes are also intended to be developed: personal computers for non-instrumental use, such as laboratory calculations, statistical calculations, bibliography storage and searching, reference accumulation, manuscript preparation, recordkeeping, alerting, etc. (Dr. Wagstaff of FDA is considering this topic as a workshop); displays, exhibits, or pictures of analytical equipment and chemical regulatory analysis of 50–100 years ago in the area of fertilizers, drugs, foods, etc. A possible AOAC permanent exhibit of journals and books developed by the staff, such as Bulletin 107, early volumes of the *Journal*, first edition AOAC books, etc., is under consideration. Federal, state, and provincial government agencies such as FDA, USDA, the agricultural experiment stations, and corresponding organizations from Canada will be contacted to determine if they are interested in setting up exhibits at this Centennial meeting. Mr. Garfield will coordinate Centennial celebration activities with other domestic organizations.

Helen Reynolds is the chairman of the Subcommittee on Publications and will coordinate the publication of the 100 year history of AOAC, being prepared by Kenneth Helrich of Rutgers University on his sabbatical.

With regard to publicity, stories will appear in almost every issue of *The Referee*, the AOAC office has obtained pressure-sensitive stickers to place on outgoing correspondence and invoices, an insert for the postal meter is in use, and stationery with the Centennial logo is on order. A news release should be prepared and distributed for trade and professional societies.

The Ways and Means Committee has been charged by the Board of Directors to obtain the necessary financing for the Centennial. The Committee intends to develop its budget at its next meeting.

To obtain the time for the technical aspects of the program, the Committee urges that all Associate Referee and General Referee reports be placed in poster sessions for 1984. A number of commodity committees have already experimented with this type of meeting format.

At the suggestion of Lars-Ake Appelqvist of the Nordic Analytical Committee, John Lupien, FDA International Staff, will investigate the possibility of obtaining funds from international organizations to implement furnishing 5-year subscriptions to the AOAC *Journal* to laboratories of developing countries working in the area of food control.

Accepted

Report of the Committee on Gas and Liquid Chromatography and Column Specifications

RICHARD S. WAYNE, Chairman
American Cyanamid Co., Agricultural Research Division, PO Box 400, Princeton, NJ 08540

Other members: T. Gale, L. Gelber, W. Y. Ja, P. Jung, G. MacEachern, M. Margosis, H. M. McNair, G. Myrdal, W. Trujillo, M. Walters

The AOAC Committee on Gas and Liquid Chromatography was formed in January 1973 to develop standardized practices and guidelines in the use of gas and liquid chromatography for regulatory purposes in the pesticide area. The recommendations regarding operational guidelines in the use of gas chromatography were issued in 1976 (1). The Committee has since devoted its efforts to developing guidelines in the use of liquid chromatography. It became a subcommittee of the Committee on Instrumental Methods and Data Handling in 1981. The following recommendations have been approved at the 96th Annual International Meeting of the AOAC. Persons with questions or suggestions concerning the recommended guidelines presented in this report, should contact the members of the subcommittee.

High performance liquid chromatography (HPLC) has developed rapidly during the seventies and is now used routinely in many laboratories for quantitative analysis in the regulatory area. It is complementary to gas-liquid chromatography as a separation tool to achieve specificity in an analysis by virtue of its ability to analyze nonvolatile and thermally unstable compounds which are not amenable to the gas chromatographic approach. The instrumental factors that contribute to the overall performance of an analysis are numerous, as studied by Scott and Reese (2) and to a large extent can be controlled from instrument to instrument by use of procedural tools such as incorporation of an internal standard and frequent calibration during the examination. The compromise sought by the Committee was a balance of the costs of HPLC instrumentation and the costs incurred in terms of loss of productivity by requiring an excessive degree of replication to achieve the desired precision in an analysis. Consideration was also given to instrumental characteristics that could result in the introduction of systematic errors in the analysis.

Liquid Pumping Systems

A. General Considerations

In high performance liquid chromatography (HPLC), the pumping system is a vital part of the instrumentation. Selection of a pumping system presents a problem when commercial equipment is purchased because of the wide variety of designs with a corresponding wide price range. The designs of these systems basically involve 2 pump types: pumps which deliver solvent under constant pressure and pumps which deliver solvent by constant volume. The constant pressure pumps will exhibit changes in flow rate as a function of changes in column pressures occurring during the course of an analysis. The constant volume pumps maintain flow rates relatively constant; however, they do contribute to detector noise which will influence peak recognition criteria for integrating systems. Because none of these pumping systems is truly universal, the choice should depend on the aims of the analyst, and the compromises to be made in price, performance, throughput, flexibility, and reliability.

The following features of pumps were considered by the Committee: constant long-term flow, constant short-term flow (to minimize pump contributions to detector noise), operation at high pressures, flow rate flexibility, and convenience of operation and maintenance. Separation of analytes has not been a major problem in the regulatory area. Hence, a gradient elution capability was not considered as a major requirement. The more important requirement is reproducibility between injections; consequently, the simpler systems were preferred. Motor-driven reciprocating pumps having a small displacement volume (60–400 μL) and 1 to 3 pumping chambers satisfy most of the criteria.

B. Recommendations

The Committee recommends the use of a constant volume displacement pump having the following characteristics: that the pumping system be capable of a flow rate consistency over 12 h with the same solvent system under controlled temperature, measured at 3.0 mL/min, having a relative standard deviation of 0.5%; that the short-term fluctuations (pulsing) be such that, under the operating conditions, there is no significant contribution to the detector baseline attributable to the pulsing (<10% of the noise without flow); that the pump be capable of operating at a pressure of at least 4500 psi (310 bars); and that the pumping system be capable of flow rates up to 10 mL/min.

Detectors

A. General Consideration

There are many commercially available detectors in general use from which the analyst can choose for a given analysis. Some examples of these are the refractive index, conductivity, UV, visible, infrared, fluorescence, photoconductivity, dielectric constant, and electro-chemical detectors.

The fixed wavelength (254 nm) UV photometer is the most widely used for HPLC. When properly designed, this detector has high sensitivity for general detection purposes, a wide linear dynamic range, and sufficient versatility to permit its use with a reasonable wide range of solvents. It is a relatively inexpensive detector. The light source used is a low or medium pressure mercury vapor lamp, which provides better linearity and improved quantitative analysis compared with continuum sources which must pass through a monochromator. The mercury vapor lamp emits light at many discrete wavelengths, the predominant line being at 253.7 nm. A filter interposed between the lamp and the cell to remove weaker lines provides virtually monochromatic light having a finite band width. Compounds which do not have a useful end absorption at 254 nm fall in classes such as lipids, polyethers, hydrocarbons, polymers, carbohydrates, and fatty acids. In most cases, even variable wavelength detectors would have difficulty in finding a useful absorbance. It has been estimated that 70% of the compounds encountered in the regulatory area can be detected with the fixed 254 nm photometer.

The UV detector can be used with high performance columns provided the detector flow cell has a small cell volume (<20 μL) and an 8–10 mm path length. A smaller volume (<10 μL) is required to maintain resolution of microbore columns. The detector should also be compatible with integrating systems including large scale data systems. Matched impedances or preferably a high impedance input digital system relative to the detector is required to minimize signal distortion.

Some consideration should be given to the design features (3) required for a good HPLC-compatible UV detector; for instance, the response of the UV detector should provide a signal for the lowest expected concentration of analyte which is at least 5 times the background of noise in the detector (signal-to-noise ratio = 5). This detector noise is an important characteristic because it determines the ultimate response of the device. There are 2 types of noise, short-term and long-term, which affect the sensitivity detectability of a given component. Short-term noise usually arises from the detector or recorder electronics. Long-term noise consists of baseline deviations that have similar frequency to the eluted peaks and is frequently due to small changes in temperature and mobile phase composition.

The linear dynamic range of a detector is the range of sample concentration over which the detector will provide usable concentration-dependent output. The minimum of this range will depend on the signal-to-noise ratio and the maximum will depend on where the output of the detector fails to respond linearly to an increase in sample concentration and/or where the detector electronics saturate. A linear dynamic range of 3 to 4 orders of magnitude would be expected for detectors used in most analyses.

Use of a monolithic silicon photodiode as the detector satisfies the response and stability criteria for the majority of analyses in the regulatory area. A photomultiplier detector would be needed to achieve faster response times for narrow chromatographic peaks.

B. Recommendations

The Committee recommends the use of a single wavelength (254 nm) UV detector employing a low or medium pressure mercury source HPLC for analysis. Considering the wide bandwidths encountered in ultraviolet solution spectra, most compounds will have some absorbance at 254 nm. When feasible, the concentration of solute should be adjusted to give an operating range of 0.2–0.5 absorbance unit. The use of a filter photometer or a variable-wavelength detector is recommended when no usable absorbance is

found at 254 nm. It should be recognized that these detectors are inherently less sensitive than the mercury lamp by virtue of its emission characteristics.

Use of a photodiode-based detector should satisfy response and stability criteria for most HPLC analyses.

The Committee further recommends that no HPLC methods that use other detectors be adopted unless the fixed-wavelength (254 nm) detector has been shown to be unacceptable for the analysis.

Standard Columns and Column Packings

A. General Considerations

A serious problem exists today concerning the application of "new" columns and column packings by Associate Referees for use in the regulatory area. The consequence of this proliferation is a large inventory of columns in laboratories analyzing a variety of products. There are instances where an analysis can only be performed on one column made by one manufacturer. In fact, the performance of this column may not be duplicated as a result of ill-defined changes in production methods by the same manufacturer. This concern is more acute when worldwide implementation of a particular analysis is envisaged.

Most separations can be accomplished through liquid chromatography with the use of adsorption or partition techniques without the variables or complexities introduced through the use of traditional liquid-liquid or ion-exchange chromatography. With these considerations in mind, 2 types of separation can be used: normal phase through the use of silica; and reverse phase separations, including ion-pair, ion-suppression; and nonaqueous reverse phase chromatography, using octadecyl (C_{18}) bonded silica.

The criteria to be considered in column definition include adsorbent particle size and geometry, surface area, pore size, surface modification bonding and additional silylation (capping) processes, column dimensions, and column packing procedures. These factors will influence column performance, although the significance of the influence will vary with the types of applications. Chromatographers should be aware that a given analysis performed using C_{18} columns purchased from different manufacturers often results in considerable differences in selectivity, efficiency, and capacity factors, because of variation of bonding chemistry and capping techniques. Although a single manufacturer may consistently provide the user with uniformly packed equivalent columns, the possibility of laboratory-packed columns for routine interlaboratory use must also be considered.

There is a recognized need for providing some means other than random choices or specifying brand names, which is discouraged, to aid the analyst in the selection of columns expected to give equivalent or suitable performance. Attempts to identify equivalent column groups throughout the industry is a future task of this Committee.

B. Recommendation

The Committee recommends use of nominal 10–30 cm × 4 mm id stainless steel columns packed with silica or C_{18} bonded silica of nominal 5–14 micron uniform particle size and a minimum surface area of 200 sq. m/g. The column should exhibit at least 200 plates/cm. Changing technology demands that these recommendations not be so rigid as to exclude certain manufacturers who might use other than stainless steel for the column (e.g., polyethylene or nickel), narrower or wider bore tubing, or silica exhibiting different properties.

All methods recommended by the Associate Referees should call for the use of guard columns to extend the lifetime of the analytical column.

Since column performance will be altered with use, methods will require verification on at least one other column of the same type prior to collaborative study.

Specifying columns by brand name or manufacturer is not acceptable in the preparation of AOAC methods. As long as the given separation mode and basic mobile phase composition is maintained, the Associate Referee should allow flexibility in the use of alternative columns by providing a generic description and minimum performance standards, relative to retention time, elution order, and theoretical plates.

The Committee further recommends that no methods using other column types be adopted by the AOAC as official procedures unless silica or C_{18} bonded silica has been demonstrated to be unacceptable.

Quantitation

A. General Considerations

The performance of a liquid chromatographic system is ultimately measured by the accuracy and precision achievable in a particular analysis. The Committee assumed for this initial overview that the contribution to the accuracy of the

Table 1. Replications required for 95% confidence limits (CL) [a] vs relative precision of area measurement

Replicates (achieved)	Precision, 1σ relative					
	5%	4%	3%	2%	1%	0.5%
Sample	200	125	70	32	8	2
Standard	200	125	70	32	8	2
95% CL	1.00	1.01	1.01	1.00	1.00	1.00
Sample	50	32	18	8	2	1
Standard	50	32	18	8	2	1
95% CL	2.00	2.00	2.00	2.00	2.00	1.41
Sample	23	15	8	4	1	1
Standard	23	15	8	4	1	1
95% CL	2.95	2.92	3.00	2.83	2.83	1.41
Sample	6	4	2	1	1	1
Standard	6	4	2	1	1	1
95% CL	5.77	5.66	6.00	5.66	2.83	1.41
Sample	1	1	1	1	1	1
Standard	1	1	1	1	1	1
95% CL	14.14	11.31	8.49	5.66	2.83	1.41

[a] Derived 95% CL = $2\left(\sqrt{\frac{1}{n_1}+\frac{1}{n_2}}\right)\sigma_{rel}$; n_1 = number of replicates of sample and n_2 = number of replicates of standard.

analysis resulting from sampling and sample homogeneity considerations was minimal and that the reference standard has been adequately measured. The best accuracy and precision in the measurement would be meaningless if the sample is not representative or if the reference calibration is incorrectly assigned.

The precision, and to some extent, the accuracy of an analysis has a number of contributions from instrumental and procedural parameters to the measurement process. The best precision is obtained when the pumping system and mobile phase supply are provided such that constant flow rate and constant mobile phase composition are maintained. This consideration is due to the concentration dependency of the detector used in liquid chromatography as contrasted to the mass dependency of the detector used in gas chromatography. The best precisions are also obtained when injections are made through microvolume sample valves using sample loops. An automated sample introduction is preferred because replication is easily performed, and the productivity of liquid chromatographic systems is increased. There is also an improvement in precision when temperature control is provided for the column, injector, and detector. To a large extent these factors within the instrument as well as among instruments can be controlled by the use of an isocratic elution system and the incorporation of an internal standard in the analysis.

The Committee recognized the advantages in the use of modern electronic methods of chromatographic data acquisition and reduction because they are more convenient and productive, and give greater precision and accuracy than manual methods. However, careful judgment should be given to digital systems regarding integration algorithms used by various manufacturers. Arbitrary assumptions regarding the shape of a chromatographic peak should be avoided. This is implicit in the mathematical treatment to obtain an area measurement. Consideration should be given to baseline tracking and peak recognition criteria in terms of peak on-set and termination. The characteristics of the analog to digital converter (A/D) relative to digitization rate, word size, and dynamic range can distort peak shape and affect the accuracy of the area measurement process.

Manual methods of measurement, whether they be peak heights or peak areas, are less precise than digital electronic methods. Table 1 has been prepared to contrast the variances encountered in the use of various area measurements and the number of replicates required to achieve statistical significance at the 95% confidence level in an analysis. Most digital systems can achieve at least 0.5% as a 1σ interval in an analysis.

Peak height analysis is usually in the 1–2% range for 1σ interval. As one can see, 8–32 replicates of sample and 8–32 replicates of standard are required to achieve a 95% confidence interval of 1%, compared with 2 replicates of standard and 2 replicates of sample for the digital method. The ball and disc, triangulation, cut and weigh techniques are considerably worse and should be avoided.

Table 2. Comparison of precision vs % analyte

Analyte, %	Method Precision[a] CV, %	Tolerance, %	Instrument Contribution to Method Precision,[b] %
100	2.0	±2.0	25
10	2.8	$\pm 2.8 \times 10^{-1}$	17.9
1	4.0	$\pm 4.0 \times 10^{-2}$	12.5
0.1	5.7	$\pm 5.7 \times 10^{-3}$	8.8
0.01	8.0	$\pm 8.0 \times 10^{-4}$	6.3
0.001	11.3	$\pm 11.3 \times 10^{-5}$	4.4
0.0001	16.0	$\pm 16.0 \times 10^{-6}$	3.1

[a] CV % = $2^{(1-0.5 \log C)}$ Ref. 4, 5. Coefficient of variation (CV) and C (concentration) as powers of 10 (e.g., 1 ppm = 10^{-6}).

[b] Assumes instrument contribution to the variance is constant.

The practical considerations in measuring an analyte at a specific concentration requires a tolerance or range that is larger in most instances than the variance for the instrumental measurement process. This is due to the allowance that must be made for between-laboratory variability in interpreting the values produced by different laboratories. Horwitz et al. (4, 5) suggested some practical limits of acceptable variability in AOAC methods for regulatory agencies and regulated industries based on 150 independent AOAC interlaboratory collaborative studies. This is depicted in Table 2.

This tolerance is mainly the result of the difference which exists between identical measurements performed in different laboratories. This also includes the variance associated with obtaining a representative sample for analysis. The instrument contribution to the method precision drops off rapidly at the lower analyte levels. This assumes a contribution of 0.5% at the 100% level of analyte and that the contribution remains constant. Consequently, the instrument performance should be matched with the analysis requirement.

B. Recommendations

The Committee recommends a microvolume sampling valve with a sample loop. Precision of analysis may be further enhanced if an automatic sample injecting device is used.

The Committee recommends the use of isocratic conditions whenever possible. Problems with changing mobile phase composition can usually be traced to external influences unrelated to the pumping system. These may be solvent de-mixing in the reservoir, loss of a volatile component, changes in ambient temperature affecting solvent viscosity, etc. Under such conditions, it may be necessary to have a closed and thermostated system.

The Committee recommends that the calibration standard should have a known purity, preferably to ±0.5% or better. It also recommends the use of an internal standard for analyte measurements where optimal precision is required. The internal standard should be readily available, chemically stable, and unreactive, with no interfering impurities. It should be completely resolved and have a similar asymmetry to the component of interest.

The Committee recommends that the analog to digital converter (ADC) circuitry of integrators should have single bit resolution over a minimum linear dynamic range of 10,000 to 1, operating at 1 KHz. Digitization rates of 2 points/s for integrating ADCs and 40 points/s for voltage measuring ADCs should be adequate for components with a peak width at half-height greater than 4 s. For narrower peaks, the digitization rate should be increased accordingly. Isolation between ADCs and digital circuitry is recommended. Simple summation of data points for area measurement is encouraged.

Acknowledgment

The Committee expresses their thanks to D. Wharton for typing the manuscript.

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Report of the Committee on the Constitution

D. EARLE COFFIN, *Chairman*
Health and Welfare Canada, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario, Canada K1A OL2

Other members: R. Frank, F. J. Johnson, J. B. Kottemann, E. Martin, M. Rhodes, E. D. Schall

During the year 1981–82, the Committee on the Constitution made recommendations to the Board of Directors for Bylaw amendments to:

1. Limit terms of service on the Board of Directors
2. Provide for the establishment and governance of regional sections.

Model Bylaws for regional sections were developed by this Committee for approval by the Board of Directors. The Committee on the Constitution has initiated discussion with regional sections regarding the requirements of regional section Bylaws and is developing procedures for the review of regional section Bylaws as part of the process of chartering regional sections.

The Committee on the Constitution has initiated study to lead to a description of the AOAC voting process, the needs for action to improve voting procedures, and options for changing voting privileges and requirements of the Association.

Bylaws Changes

Article IV: Directors

Section 1. Board of Directors

The Board of Directors shall consist of the President, President-Elect, Secretary/Treasurer, Immediate Past-President, and three (3) Directors. The Directors are Members elected at the annual meeting for a one-year term and may serve successive terms. The President shall serve as Chairman of the Board.

No member of the Board of Directors may be elected for more than six (6) consecutive years.

Article X: Subsidiary Organizations

Section 1. Regional Sections

The Board of Directors shall set geographic limits and grant authority to groups of Members of the Association residing or working in the same geographical regions for the establishment of regional sections.

Section 2. Membership in Regional Sections

Membership in a regional section shall consist of all classes of Members of the Association residing or working within the geographical boundaries of the section. No person may be a Member of more than one regional section.

Section 3. Purpose of Regional Sections

The purpose of regional sections shall be to promote and sponsor the purpose of the Association.

Section 4. Bylaws of Regional Sections

Each regional section shall adopt for its own government, subject to approval of the Board of Directors, Bylaws not inconsistent with these Bylaws.

Section 5. Dissolution of Regional Sections

When any regional section shall cease to function as a section for a period of more than one year, or if its membership shall be less than 10 Members of the Association for a period of one year, the Board of Directors may terminate the existence of such regional section.

Adopted

Report of the European Representative

MARGREET TUINSTRA-LAUWAARS
Langhoven 12, 6721 SR Bennekom, The Netherlands

AOAC was represented at the following meetings:

(1) International Organization for Standardization (ISO) Technical Committee 34—Agricultural Food Products, November 28, 1981, BSI, London, UK.

(2) International Dairy Federation (IDF) Permanent Committee of Commission E—Analytical Standards, Laboratory Techniques, February 3 and 4, 1982, Brussels, Belgium. During a tripartite IDF/ISO/AOAC meeting, progress of the joint IDF/ISO/AOAC groups of experts was considered. President: Mrs. H. Werner (DK).

(3) West European Fish Technologists Association (WEFTA) Working Group on Analytical Methods, April 20, 1982, Ymuiden, The Netherlands. Chairman: H. Houwing (NL). AOAC will cooperate with this working group for the development of analytical methods for fish.

(4) IDF/ISO/AOAC tripartite meeting, April 24, 1982, Rome, Italy. Chairman: Mrs. H. Werner. This meeting was held in conjunction with the meeting of the FAO/WHO Committee of Government Experts on the Code of Principles Concerning Milk and Milk Products. The report of the IDF/ISO/AOAC meeting and methods of analysis were presented to the Committee of Government Experts.

(5) IDF/ISO/AOAC Chemical Week, May 24–29, 1982, NNI, Delft, The Netherlands. The 1982 Chemical Week was attended by 53 persons from 13 countries, with the following program:

Monday, May 24: E8—Nitrate, nitrite, and phosphorus in cheese, etc. Chairman: L. J. Poortvliet (NL). E31—Fat determination (gravimetric). Chairman: J. Eisses (NL).

Tuesday, May 25: E43—Food additives. Chairman: T. Stijve (CH). E12—Pesticide residues. Chairman: W. H. Heeschen (DE).

Wednesday, May 26: E17—Characterization of dried milk according to heat treatment and usage. Chairman: P. J. de Koning (NL). E26—Selection of samples. Chairman: D. C. Bettes (GB). E47—Antibiotics. Chairman: W. H. Heeschen (DE).

Thursday, May 27: E27—Nitrogen content of milk and milk products. Chairman: R. Grappin (F). E33—Mycotoxins. Chairman: H. Veringa (NL). E11—Analysis of caseins and caseinates. Chairman: P. de Hoog (NL).

Friday, May 29: E29—Automated methods for routine analysis of raw milk. Chairman: R. Grappin (F). E9—Lactic acid, lactates, and neutralizers in dried milk. Chairman: J. M. van der Bas (NL).

(6) Association of Public Analysts (APA), June 16, 1982, London, UK. Chairman: R. Dalley (UK). In a special session organized for AOAC, D. MacLean explained the organization and its goals. The European Representative will remain in direct contact with APA.

(7) Chrompack symposium, June 26, 1982, Vlissingen, The Netherlands. The AOAC representative was available to offer information to the delegates.

(8) International Union of Pure and Applied Chemistry (IUPAC) Oils and Fats Commission, September 6–8, 1982, CIVO-TNO, Zeist, The Netherlands. Cooperation with AOAC will continue. Chairman: D. Firestone (USA).

(9) FAO/WHO Codex Committee on Foods for Special Dietary Use, September 20–24, 1982, Bonn-Bad Godesberg, Germany.

In October 1981, the European Representative attended the 95th AOAC Annual International Meeting at Washington, DC.

Various contact visits to laboratories have been made, to investigate activities, needs, and methods development in the institutes. AOAC may be of assistance and could contribute by offering communication with other experts in related fields. Folders and brochures describing AOAC and its activities were distributed.

When AOAC Executive Director D. MacLean visited Europe in 1982, the European Representative made arrangements for the following visits: State Institute for Quality Control of Agricultural Food Products (RIKILT), Wageningen, The Netherlands; Food Inspection Service at Nijmegen (NL); Duphar B.V., Weesp (NL); Pharmaceutical Institute of the Free University of Brussels (Belgium); IDF Headquarters at Brussels (B), followed by the meeting of FAO/WHO Codex Committee on Pesticide Residues, The Hague, The Netherlands. The activities then were continued in the UK by visits of the European Representative and the Executive Di-

rector to the Laboratory of the Government Chemist, London, and to Peter Martin at the Public Analyst's Laboratory, Reading.

Contacts were made with the Association of Public Analysts and The Analytical Methods Committee of the Royal Society of Chemistry. All visits by Dr. MacLean in the UK were arranged by Harold Egan.

Again this year, the work of the European Representative was extended to exploring possibilities for more European activity in AOAC. Regular activity, particularly by the Committee on International Cooperation, would certainly contribute to that effort. The number of Associate Referees in Europe could certainly be increased, because of the amount of collaborative study data available on various methods. Coordination of the work in this field might call for local assistance. Representation at meetings, congresses and symposia, or less formal get-togethers will certainly be an important tool for increasing the interest in AOAC in Europe.

Accepted

Report of the Committee on Interlaboratory Studies

WILLIAM HORWITZ, *Chairman*
Food and Drug Administration, Bureau of Foods, Washington, DC 20204

Other members: R. Albert, B. Armbrecht, B. J. Boone, W. Caspary, P. R. Caudill, C. Dahl, T. Dols, H. Egan, R. Ellis, D. W. Fink, E. Glocker, R. Grappin, M. Ihnat, S. A. Katz, A. J. Malanoski, M. Margosis, K. McCully, A. Munson, J. O'Rangers, F. W. Quackenbush, H. S. Ragheb, S. Sherkin, E. Smith, W. Steller, E. Stoddard, L. Stoloff, B. K. Thompson, L. Williams, J. Winbush, E. S. Windham, J. Winter

The purpose of this committee and its functional structure as 8 subcommittees were given in last year's report (1982, **65**, 403–405). All subcommittee chairmen remain the same except Stanley Katz has replaced Gordon Carter (retired) as chairman of the Application to Biological Tests Subcommittee.

The primary product of the Committee's deliberation thus far has been a document entitled "Outline of Interlaboratory Study Procedure to Validate Performance of a Method of Analysis" and identified by the designations "1069A, Attachment H," the latest version of which was circulated December 13, 1982. This document was sent to all major international and national organizations known to conduct interlaboratory studies. Numerous editorial and substantive comments were received which were either incorporated into the revised version or, if substantive, were discussed in a commentary as part of previous versions of the document. The "Final First Draft" version, incorporating the recommendations of the Statistical Subcommittee as reviewed, modified, and endorsed by the Statistics Committee is attached for review by the membership. The Committee is grateful to the numerous individuals who submitted their recommendations for revisions. We are particularly pleased with respect to the substantial comments received from the reviewers obtained through the U.S. Pharmacopeial Convention, International Organization for Standardization (ISO), and the International Union for Pure and Applied Chemistry.

The Committee wishes to highlight the following policy changes that have been made in the section on the statistical treatment of the data, and which may be recommended for eventual adoption by the Association:

(1) The Youden Rank Sum test for removing entire laboratories as outliers under certain conditions of use on the basis of a systematic bias manifested by a rank sum not included within acceptable limits may result in the introduction of some bias. This test is not used by ISO, and John Mandel of the National Bureau of Standards, working with ASTM, does not recommend it. Experience indicates that this test should be applied with caution. However, Associate Referees will be definitely encouraged to apply the test to their data for the purpose of discussing the results from any laboratory indi-

cated as extreme by this test in order to attempt to discover potential sources of a laboratory's systematic bias.

(2) The Statistical Subcommittee had recommended the use of two-way analysis of variance (ANOVA) for the examination of the results of collaborative studies. The Statistics Committee, however, did not concur in this recommendation, pointing out that from a regulatory point of view it was the tolerance or specification point which was critical and that sample-by-sample examination was very important from several points of view, particularly for applicability. Furthermore, pooling of the samples may lead to violation of the assumption of homogeneity of variances which is essential for ANOVA. In addition, the sample-by-sample statistical analysis can be easily handled by the chemist when statistical and computer assistance are not available. However, there may be specific occasions where ANOVA is applicable.

(3) The document will also be reviewed from the point of view of consistency in the use of "sample" and "sampling" terms.

It was also reported to the Committee that even though many collaborative studies show the presence of statistical outliers by the Dixon test at the 95% confidence level, and by the Youden Rank Sum Test, they are often not of practical significance, i.e., not removing these indicated outliers still results in satisfactory (consistent with previous experimental results) statistical parameters (coefficients of variation). Therefore, it is very likely that if this observation continues to hold with additional commodity and concentration groups that the Committee will be asked to consider using the ISO outlier designation of "stragglers" for outliers found at the 95% confidence levels but not eliminating them from the calculations, unless they are also outliers above the 99% confidence level.

The Committee also considered the report from the Subcommittee on Definitions. It commended the Subcommittee for a remarkable piece of work in bringing together, discussing, and recommending definitions for analytical and related terms. It also encourages the separate publication of these documents as a valuable contribution to fundamental analytical chemistry. It recommends, however, that the papers be revised to present the summarized material and the final recommendations in considerably simplified form for use by Associate Referees. Perhaps much of the original material might be incorporated into a manual of "Collaborative Study Procedure" which the Committee believes will be needed.

The Committee intends to present its final recommendations at the 1984 Spring Workshop for review and discussion by the participants. It also hopes to present its final report at the Joint AOAC-IUPAC meeting of Harmonisation of Collaborative Studies which will be held immediately before the fall Centennial meeting of AOAC in 1984. The results of the Harmonisation meeting will be presented for discussion at the Spring Workshop of 1985.

Accepted.

Outline of Interlaboratory Study Procedure to Validate Performance of a Method of Analysis

Final First Draft

1. Preliminary Work

1.1 Determine Purpose of the Method

Purpose of the study (e.g., to determine attributes of a method, proficiency of analysts, comparison of methods, or reference values of a material), the type of method (empirical, screening, reference, definitive, practical), and the probable use of the method (enforcement, surveillance, monitoring, research) determines the relative importance of the various method attributes and the design of the interlaboratory study. These directions pertain primarily to determining the interlaboratory characteristics of a method, although many portions are also appropriate for other types of study.

1.2 Choose Method

1.2.1 Sometimes obvious (only method available)

1.2.2 Critical literature review (reported attributes are often optimistic)

1.2.3 Survey laboratories to obtain candidate methods

1.2.4 Within-laboratory comparison of attributes of candidate methods (sometimes choice may still not be objective)

1.2.5 Selection by expert (AOAC-preferred procedure—selection by Associate Referee with concurrence of General Referee)

1.2.6 Selection of committee (ISO-preferred procedure; often time-consuming)

1.2.7 Development of new method or modification of existing method when an appropriate method is not available (proceed as a research project). (This alternative is time-consuming and resource-intensive; use only as a last resort.)

1.3 Optimize Either New or Available Method (Within One Laboratory)

1.3.1 Practical Principles:

1.3.1.1 Do not conduct collaborative study with an unoptimized method. An unsuccessful study wastes a tremendous amount of collaborators' time and creates ill will. This also applies to methods formulated by committees which have not been tried in practice.

1.3.1.2 Conduct as much experimentation within a single laboratory as possible with respect to optimization, ruggedness, and interferences. Analysis of the same material on different days provides considerable information on potential variability that may be expected in practice.

1.3.2 Approaches to Optimization:

1.3.2.1 Conduct trials on basis of change of one variable at a time

1.3.2.2 Conduct ruggedness testing for control of critical variables: See Youden and Steiner, *Statistical Manual of the AOAC* (1975) pp. 33–36, 50–55. The actual procedure is even simpler than it appears. [This is an extremely efficient method for optimizing a method for a collaborative study.]

1.3.2.3 Use Deming simplex optimization to identify critical steps. See Dols and Armbrecht, *J. Assoc. Off. Anal. Chem.* (1976) **59**, 1204–1207

1.4 Protocol Development on Selected Method (as Applicable)

(Some items can be omitted; others can be combined)

1.4.1 Develop defined performance specifications for instruments and systems suitability tests to ensure satisfactory performance of critical steps (columns, instruments, etc.) in method.

1.4.2 Determine calibration function (response vs concentration in pure or defined solvent) to determine useful measurement range of method. (For some techniques, e.g., radioimmunoassay, linearity is not a prerequisite.) Indicate any mathematical transformations.

1.4.3 Determine analytical function (response vs concentration in matrix) to determine applicability to commodity(ies) of interest.

1.4.4 Interference testing (specificity):

1.4.4.1 Test effects of ubiquitous contaminants, flavors, additives, and other components which may be expected to be present and at expected concentrations.

1.4.4.2 Test nonspecific effects of matrices.

1.4.5 Conduct bias (systematic error) testing (not necessary when the method defines the property or component).

1.4.6 Conduct precision testing at the concentration levels of interest, including variation in experimental conditions normally expected in routine analysis (ruggedness).

1.4.7 Estimate limit of reliable measurement (if applicable).

1.4.8 If any of the characteristics are unacceptable, revise, retest, and reiterate such items as are necessary.

1.4.9 Conduct laboratory trial of method by analyst not familiar with method.

1.4.10 Revise method to handle questions raised and problems encountered.

1.5 Prepare Description of Method

NOTE: A collaborative study of a method involves practical testing of the written version of the method in its specific style and format.

1.5.1 Prepare method in format and style given in instructions in the *Handbook for AOAC Members* (1982), or other recognized manual, e.g., ISO Guide 18.

1.5.2 Clearly describe and explain every step in the analytical method so as to discourage deviations.

1.5.3 Check editorially for completeness, credibility (e.g., buffer pH consistent with specified chemicals, volumes not greater than capacity of container), continuity, and clarity.

1.5.4 Check for presence of systems suitability tests and convenient stopping points.

1.5.5 If time and resources are available, conduct pilot study involving three laboratories.

1.6 Invite Participation

1.6.1 *Selection of candidate laboratories.* Laboratories invited to participate should have personnel experienced in the procedure employed; experience with the method itself is not a prerequisite for selection. Lists of potential participants can be developed through personal contacts, technical societies, trade associations, and literature search.

1.6.2 *Letter of invitation.* Address formal letter to administrator responsible for assignment of laboratory effort. State reason for selecting, estimated number of person-hours for performance, number of materials to be sent, number of analyses to be required, expected date for sample distribution, and target date for completion of study. Enclose copy of the method, and a return form or card (with postage affixed, if domestic), requiring only check mark for acceptance or refusal of invitation, signature, and date.

The letter should suggest that with large studies, involving several analysts per laboratory, several familiarization materials, receipt of items at different times, and similar recurrent situations, that a study coordinator be appointed in each laboratory. The study coordinator should be responsible for receiving and storing the materials, assigning the work, dispensing materials and information related to the study, seeing that the method is followed as written, accumulating the data, assuring that the data are correctly reported, and submitting the report within the deadline.

(A file of letters that have been used previously will be available for review and adaptation to the specific project at hand.)

1.7 Instructions and Report Forms

1.7.1 Carefully design instructions and forms and scrutinize them before distribution. A pilot study (1.5.5) is also useful to uncover obscurities in these documents.

1.7.2 Send instructions and report forms immediately on receipt of acceptance, independent of materials, if selection of laboratories is not to be based on performance in pilot or training studies. The instructions should include in bold face or capital letters a statement: "THIS IS A STUDY OF THE METHOD, NOT OF THE LABORATORY. THE METHOD MUST BE FOLLOWED AS CLOSELY AS PRACTICABLE, AND ANY DEVIATIONS, NO MATTER HOW TRIVIAL THEY MAY SEEM, MUST BE NOTED ON THE REPORT FORM."

1.7.3 Include instructions regarding storage and handling, markings, and identifications to be noted, any special preparations for analysis, and criteria for use of practice materials, if included (see 1.8). Precode form for each laboratory and provide sufficient space for as much analytical detail as may be required for proper evaluation of the results, including a check of the calculations. When recorder tracings or photographs are required to evaluate method performance, request their submission both in the instructions and again as a check item on the form. Provide instructions with regard to labeling (identification with respect to item analyzed, axes, date, submittor, experimental conditions, etc.). Include a signature line for the analyst and lines for a printed or typed version of the name and address for correct acknowledgment in the report. A questionnaire may be included or sent after completion of the analyses in which the questions can be designed to reveal if modifications have been made at critical steps in the method.

1.8 Familiarization or Practice Materials

If deemed necessary, supply familiarization materials several months before actual materials are sent. When familiarization materials have been submitted, supply forms for reporting progression to satisfactory performance.

2. Design of the Collaborative Study

2.1 General Principles

2.1.1 The purpose of the collaborative study is to provide a realistic indication of the attributes of a method (particularly systematic and random deviations) as it will be performed in practice. It usually provides information on the best performance to be expected.

2.1.2 The design should attempt to identify and measure the potential sources of significant variability that may occur in actual practice, including between days, if this is a significant factor. (Within-laboratory performance on different days may provide a clue with respect to between-laboratory performance and is required information for quality control.) The best measure of within-laboratory variability is through the use of blind replicates or through the use of Youden pairs. The design must take into account how the data will later be analyzed statistically.

2.1.3 Materials sent for analysis must be presented as unknowns (blind), or at most with only an indication of concentration, and in a random pattern. [Any relationship supplied with regard to concentration (e.g., utilizing factorial aliquots or serial dilutions of the same spiking solutions) or replication is likely to diminish variability.]

2.1.4 The study must be extensive enough to assure sufficient data surviving in the face of possible loss of materials during shipment, inability of collaborators to participate after acceptance, and a maximum outlier rate of 20% outliers (1 laboratory failure out of 6, when a minimum Youden design is used).

2.2 Laboratories

Laboratories must be impressed with the importance of the study. A large investment is being made in testing the method and it will probably be the only such study that will be performed. It is important to give a fair and thorough evaluation of the method.

2.1.1 *Type:* Best types are laboratories with a responsibility related to the analytical problem. Other types may be representative (selection of laboratories that will be using the method in practice), reference (assumed to be the "best"), or the entire population of laboratories (usually certified or accredited) that will be using the method. Final selection of participants should be based on a review with the General Referee and other Associate Referees (or their counterparts in other organizations) of each laboratory's capabilities and past performance in collaborative studies, followed up, if possible, by telephone conversations or by personal visit. Selection may also be based on performance on familiarization materials. Sometimes only laboratories with dedicated or very specialized instruments must be used.

2.2.2 *Number:* Minimum of 6 (to avoid unduly large confidence bands). Fewer laboratories will widen the confidence limits of the mean and of the variance components (see design considerations 2.4.1 and 2.4.2). Optimum number of laboratories, balancing logistics and costs vs information obtained, is 8–10.

2.2.3 *Analysts:* Most designs require only one analyst per laboratory. If analyst-within-laboratory variability is a desired variance component, multiple analysts should be requested from all participating laboratories. Ordinarily two analysts from the same laboratory cannot substitute for different laboratories, unless different instruments are available, standard solutions and reagents are prepared independently, and no consultation is permitted during the work.
Different laboratories from the same organization may be used as separate laboratories if they are organizationally and/or geographically separate and operate independently in a different environment with their own instruments, standards, and reagents.

2.3 Materials

2.3.1 *Materials must be homogeneous. This is critical.* Establish by testing a representative number of laboratory samples taken at random before shipment to provide sound evidence of this point. (A collaborator who reports an outlying value will almost always initially claim receipt of a defective laboratory sample.)

2.3.2 *Label randomly* so that there is no preselection from order of presentation.

2.3.3 *Concentration range:* Choose materials to cover concentration range of interest. If concentration of interest is a tolerance limit or a specification level, bracket it.

2.3.4 *Number of materials:* Minimum number of materials is 5 to utilize the Youden rule of (laboratories × materials = 30). This anticipates no unsatisfactory materials and Youden pairs are not used (which requires an even number of materials).

2.3.5 *Nature of materials:* Materials should consist of a representative set of commodities usually analyzed, with customary and extreme values for the analyte and potential interfering substances, unless interference has been ruled out by interference testing (see 2.3.7).

2.3.6 *Size of laboratory samples:* Furnish only enough laboratory sample to provide the number of test portions specified in the instructions. If additional test portions are required, the collaborator must request them.

2.3.7 *Interferences:* If pertinent, some samples, but not all, should contain contaminants and interferences in concentrations likely to be encountered, unless they have been shown to be unimportant through within-laboratory testing (see 1.4.4). The success of the method in handling interference on an intralaboratory basis will be demonstrated by passing systems suitability tests.

2.4 Replication

When within-laboratory variability is also of interest, independent replication must be performed by supplying at least one of the following:

2.4.1 *Blind duplicate laboratory samples, randomly coded.*

(NOTE: Triplicate and higher replication is relatively inefficient compared to additional pairs of different laboratory samples, for two reasons:

(1) Triplicates of the same laboratory sample provide additional information only on within-laboratory variability, which is usually the less important component of error.

(2) Four independent analyses (two laboratory samples, each in duplicate) give two data points with narrower confidence limits by $2\sqrt{2}$ as compared with $\sqrt{3}$ for triplicates (ratio of 1.6) for an additional work input ratio of 4:3 (1.3). [The ratios are only approximate because other factors are also involved.]

PRACTICAL PRINCIPLE: With respect to replication, the highest net marginal gain is always obtained in going from 1 to 2 as compared to going from 2 to 3, 3 to 4, etc.

2.4.2 *Youden pairs:* An efficient design (maximum information with minimum work) is an even number of materials arranged as Youden pairs—each pair consisting of a set of 2 materials of slightly different composition obtained either naturally or by diluting (or by fortifying) one of the materials with a small amount of diluent (or of analyte). Each laboratory sample should be analyzed only once: replication defeats the purpose of the design.

2.4.3 *Independent materials* (NOTE: unrelated independent materials may be used as a Youden pair in the calculations, but the more they differ, the less information they provide on within-laboratory variability.)

2.5 Familiarization materials

With new, complex, or unfamiliar techniques, provide known material(s) of stated composition for practice, on different days, if practical (see 1.8). The valuable collaborative materials should not be used until the analyst can reproduce the stated value within a given range. However, it should be pointed out that one of the assumptions of the analysis of variance is that the results are independent of time (i.e., there is no drift). The Associate Referee must be satisfied that this assumption is met.

2.6 Other Design Considerations

2.6.1 The design may be reduced in the direction of less work or less cost, but at the sacrifice of less confidence in the reliability of the developed information.

2.6.2 More work is required if more confidence is needed; e.g., greater confidence is required to enforce a tolerance at 1.00 ppm than at 1.0 ppm. (The distinction is a precision requirement of the order of 1% or 10%.)

2.6.3 Thirty data points will only permit estimation of the standard deviation *of an individual reading* to within about ±25% with 95% confidence; 200 data points are required to estimate that standard deviation to within 10%, with 95% confidence. The distribution of standard deviations is actually asymmetric for small numbers of results, e.g., the relative distribution about 1.00 for 30 values (95% confidence limits) is 0.80–1.34; for 200, 0.89–1.14.

2.6.4 The validity of extrapolating the use of a method beyond concentrations and components tested can only be estimated on the basis of the amount of signal change observed as a function of the nature and concentration of the matrix and contaminant components. If the signal is more or less independent of these variables, a reasonable amount of extrapolation may be allowed. Naturally, the extrapolator assumes the burden of proof as to what is reasonable.

3. Preparation of Materials for Interlaboratory Studies

3.1 General Principles

3.1.1 Unit-to-unit variability within a series of laboratory samples must be negligible compared to analytical variability, as measured within the Associate Referee's laboratory. See also 2.3.1.

3.1.2 The containers must not contribute extraneous analytes to the determination and they must not absorb analytes, or other components of the matrix, e.g., water.

3.1.3 The materials must be stabilized, if necessary, preferably by physical means (freezing, dehydrating), or by chemical means (preservatives, antioxidants) which do not affect the performance of the method.

3.1.4 Moisture changes must be avoided, where necessary, by the use of vapor-tight containers.

3.2 Suitable Materials for Collaborative Studies

3.2.1 *A single batch of homogeneous, commercial, stable product* such as milk powder, peanut butter, vegetable oil, starch, etc., is the best type of material.

3.2.2 *Reference materials* such as supplied by standards organizations such as the National Bureau of Standards are excellent. However, they are of limited availability and, if available, they are expensive. Sometimes, the standards organization is interested in making reference material available for the analyte under study, in which case they may provide the material for the study.

3.2.3 *Synthetic materials* may be especially formulated for the study with known amounts of analyte by actual preparation. Best used for macro constituents such as drugs or pesticide formulations.

3.2.4 *Spiked materials* consisting of normal or blank materials to which a known amount of analyte has been added may be used. The amount of analyte added should not be excessive in relation to the amount present, and the analyte should be in the same chemical form as present in the material to be analyzed.

3.2.4.1 *Preparation in bulk:* Requires thorough and uniform incorporation of the analyte, often by serial dilution of solids. The danger of segregation due to differences in densities always exists. The preparation of liquid materials susceptible to segregation should be done under constant agitation. Uniformity must be checked by direct analysis, with an internal standard, or by a marker compound (dye or radioactive label).

3.2.4.2 *Individually prepared laboratory samples:* A known amount of analyte is either weighed directly or added as an aliquot of a prepared solution to preweighed portions of the matrix in individual bottles. Each collaborator is instructed to use the entire portion for the analysis, transferring the contents quantitatively. This is the preferred alternative to spiked solid materials at trace (ppm) levels, at the expense of considerably more work.

3.2.4.3 *Concentrated unknown solution for direct addition or dilution by collaborators to their own commodity:* Should be used only as a last resort, because it introduces an indeterminable (material × laboratory) interaction component. This procedure also permits compromising the design of the study by direct analysis of the spiking solution.

3.2.5 *Materials analyzed by another, presumably accurate method,* if available, in the Associate Referee's laboratory or by some or all collaborators.

3.2.6 *Only as an absolutely last resort (usually with unstable materials and preparation of material studies) should the collaborators be permitted to prepare their own materials* for analysis. It is almost impossible to avoid the personal bias introduced by knowledge of the composition of the material.

3.3 Blanks

When the absence of a component is as important as its presence, when determinations must be corrected for the presence of the component or background in the matrix, or when recovery data are required, provision must be made for the inclusion of blank materials containing "none" of the analyte. It is also important to know the variability of the blank and the tendency of the method to produce false positives. It should also be kept in mind that there are two types of blanks: commodity blanks and reagent blanks. If the reagent blank is shown to be negligible, it may be included in the commodity blank. The number of blanks to be included in a collaborative study is approximated by the relative relationship of the standard deviations of the material (s_M) and of the blank (s_B). The total variability (s) of a blank corrected value will be $s = \sqrt{s_M^2 + s_B^2}$.

3.4 Limit of Determination

If the limit of determination is important, it is necessary to provide a special design, with special attention being given to the number of blanks, and to the necessity for interpreting false positives and false negatives.

3.5 Controls

When the presence of interfering materials is critical, appropriate samples must be included.

3.6 Practical Principle

Always allow for contingencies and prepare more sets (e.g., 25%) of laboratory samples than there are collaborators. Some packages may never arrive, some materials may be spoiled, and some will be lost. New laboratories may have to be substituted for those who are unable to complete the promised work. Some may have to be analyzed at a later date for different purposes such as to verify stability on storage.

4. Submission of Laboratory Samples

4.1 *Label laboratory samples legibly and unambiguously.* If they are in breakable containers, pack well to minimize possibility of breakage. If perishable, ship frozen with solid carbon dioxide, sufficient to last several days longer than anticipated transportation time. Notify collaborators of anticipated shipping arrangements (including waybill numbers, etc.), arrival time, and required storage conditions. Use special transportation services, if necessary. For international delivery, mark as "Laboratory samples—no commercial value" or other designation as required by customs regulations of the country to which it is being sent. Hazardous materials should be packed, labeled, and conveyed as required by transportation regulations. Animal products may require special certification from health authorities.

4.2 *A return slip, to confirm safe receipt, should be included* with each package. If not sent previously, include copy of method, instructions, and report forms.

4.3 *Provide instructions for proper storage* of laboratory samples between unpacking and analysis.

4.4 *Where it is important to have all instruments calibrated with the same reference material,* supply the standard reference material to all collaborators.

5. Obligations of Collaborators

5.1 *Analyze samples at times indicated, according to submitted protocol.* With unstable materials (e.g., for microbial or decomposition problems), analyses must be started at a specified time.

5.2 *FOLLOW METHOD EXACTLY (THIS IS CRITICAL).* Any deviations because of the necessity to substitute reagents, apparatus, instruments, etc., must be recorded at the time and subsequently reported. If collaborator has no intention of following submitted method, he or she should not participate in the study.

5.3 *Conduct stated number of determinations.* More or less determinations will complicate the statistical analysis.

5.4 *Report individual values, including blanks.* Do not average or do other data manipulations unless required by the instructions. Undisclosed averaging distorts statistical measures. If blank is larger than determination, report the negative value; do not equate negative values to zero. Follow or request instructions with regard to reporting "traces" or "less than."

5.5 *Supply raw data, graphs, recorder tracings, photographs, or other documentation* as requested in the instructions.

5.6 *If apparent outliers appear spontaneously, investigate cause immediately,* by reanalysis if permitted by the protocol. Call administrator (Associate Referee) to discuss suspicious values. If coordinator indicates a value may be an outlier, also review the determination promptly to the extent possible, by reanalysis, recalculation, or preparation of new standards. If time and laboratory samples are available, obtain new laboratory samples from the administrator for repeat analysis.

Since collaborators may not necessarily know if a value is an outlier, a useful suggestion is to indicate that the results should be communicated to the administrator as soon as the protocol is complete and before time and equipment are reassigned so that repeat assays may be performed at once, if necessary.

5.7 *NOTE:* Most frequent causes of true outliers are:

5.7.1 Incorrect calculations and arithmetic errors

5.7.2 Incorrect standards due to weighing or volumetric errors (check physical constants or compare against freshly prepared standard solutions)

5.7.3 Editing errors, such as transposition of numbers

5.7.4 Adventitious contamination.

6. Statistical Analysis (This section is temporary. It will be revised with respect to nomenclature and content. It is given only for completeness. Note revisions with respect to outliers in this revision.)

6.1 ***Common Sense Test***

The first thing that should be done by the administrator with collaborative data is to plot it, material by material (or as Youden pairs), readings vs laboratory, preferably in ascending or descending order of reported concentration. Usually discrepancies will be immediately apparent: displaced means, spread replicates, outlying values, differences between methods (if used), consistent laboratory rankings, etc. Anything which is noticed by "eye-balling" will probably be statistically significant, but the converse is not always true.

6.2 ***Statistical Approach***

Perform calculations on each material individually. Only if the variances for each material are not significantly different from each other can the results for all materials be pooled for analysis of variance. If the coefficients of variation of the different materials are not significantly different, they may be averaged over the range tested.

6.3 ***Outliers***

(NOTE: Collaborative studies seem to have an inherent level of 5–15% outliers, depending, of course, on the definition of outliers. Above 20%, without an explanation (e.g., failure to follow method) is ordinarily excessive. The sooner an apparent outlier is investigated, the greater the likelihood of finding an explanation.)

Determine presence of outliers by following tests:

6.3.1 *Dixon test* for removal of isolated individual (or pairs) of values or laboratory averages at either end of the set. Ordinarily do not iterate more than twice.

6.3.2 *Cochran test* for removal of laboratories showing significantly greater variability than the others.

6.3.3 *Rank sum test* for determining if a laboratory is reporting values which are consistently low or high over all materials. This test will only be used by the Associate Referee as a basis for requesting the indicated laboratories to investigate potential sources of the systematic deviations.

6.4 Bias (Systematic Deviations) of Individual Results

Bias = amount found − amount added (or known or assigned value)

% Recovery = (measured concentration in fortified material − measured concentration in unfortified material) × 100)/(known increment in concentration)

The amount added must be a substantial fraction or more than the amount present in the unfortified material.

The "true" value is known only in cases of spiked or fortified materials, reference materials, or analysis by another (presumably unbiased) method. Concentration in the unfortified material is obtained by direct analysis or by the method of additions. In other cases, there is no direct measure of bias, and consensus values derived from the collaborative study itself must be used for the reference point.

6.4.1 NOTES:

6.4.1.1 Youden equates "true" between-laboratory variability (not including the within-laboratory variability) to bias or systematic error.

6.4.1.2 The presence of random error limits the ability to estimate the systematic error. To estimate a systematic error of magnitude comparable to the standard deviation of a single value (random error), at least 15 values are needed.

6.5 Precision (Random Error)

The relative basis (i.e., coefficient of variation, CV) is usually the useful measure of precision because it is often independent of concentration or amount of analyte. Moreover, its use facilitates comparison of variabilities at different concentrations. When the CV begins to increase rapidly with lower concentration or amount, it often delineates the limit of usefulness of the method. The most important types of precisions are:

6.5.1 *Reproducibility*—overall between-laboratory variability, including within-laboratory variability, designated as R

NOTE: This component is not obtained merely by calculating the standard deviation of all of the data (except when there are no replicates) since this term must be corrected by a replication factor (Youden, page 19). It must be extracted by the analysis of variance technique (Steiner, pages 78–81). However, this crude, overall calculation of the standard deviation may serve as a check on the arithmetic, since the two values are usually fairly close.

6.5.2 *Repeatability*—within-laboratory random error, designated as r. This is best represented by the "between-days" variability component.

6.5.3 *"True" between-laboratory variability* (without within-laboratory variability)—Youden's systematic error, designated here as L.

6.5.4 NOTES:

6.5.4.1 The relationship among these three values is:

$$R^2 = L^2 + r^2$$

From the equation, r cannot be greater than R, but occasionally the computed value of L^2 is negative. When this occurs, the discrepancy is ascribed to a sampling phenomenon and L is set equal to zero. Then $R = r$, arbitrarily. This frequency occurs in practice when r is so large that it swamps out L (poor replication). A negative square term arises from the fact that the term L^2 is calculated from the difference of two terms, each of which is calculated independently. If the second term is larger than the first, the difference is negative. But by definition, it must not be negative; therefore, the second term is set equal to zero.

6.5.4.2 When only single determinations are performed on each sample (except in the case of Youden pairs), there is no rigorous basis for calculating r, and within-laboratory variability cannot be estimated. In this case R must be assumed to be equal to L.

6.5.4.3 The ISO definitions (ISO 5725-1981) for repeatability and reproducibility appear to be considerably different, although no less useful. They are shown to be expressible in Steiner terms below. The ISO definitions use a prediction interval statement: the value below which the absolute difference between single test results of identical test material may be expected to lie with a specified probability

(usually 95%); in other words, assuming normal distribution, 95% of the absolute differences from the first result of any subsequent test result from the same laboratory or from a different laboratory on the same material will be expected to lie within the calculated interval from the first reading. The relationship between the two definitions are:

Repeatability (r) = $2\sqrt{2}\,\sigma_b$ = 2.83 m $CV_r/100$

Reproducibility (R) = $2\sqrt{2}\,\sigma_w$ = 2.83 m $CV_R/100$

where σ_b and σ_w are within- and between-standard deviations, respectively; CV_r and CV_R are the corresponding coefficients of variation; and m is the mean.

6.5.5 *Confidence limits for precision terms.* Standard deviations and coefficients of variation are estimates of "true values." The "confidence intervals" (bounded by the confidence limits) are the ranges within which the true value is expected to lie with a stated degree of confidence (customarily 95%). The confidence interval of precision terms is rarely quoted because 200 values are required to estimate the standard deviation to within approximately 10%; for the Youden minimum criterion of 30 values (laboratories × samples), the standard deviation can only be estimated to about 25%. The coefficient of variation of the CV, where the CVs are expressed as a fraction, is:

$$= \sqrt{(1 + (CV)^2)/2n},$$

where n is the number of values entering into the calculation of the CV. Approximately 50 values are required to estimate a CV = 10% to within 10% (relative).

6.6 Incorrect, Improper, or Illusionary Values (False Positive and False Negative Values)

These are not necessarily outliers (no *a priori* basis for a decision), since there is a basis for determining their incorrectness (a positive value on a blank sample or a zero or negative value on a spiked sample). There is a statistical basis for their presence: In a series of materials of decreasing concentration, as the CV increases, the percent false negatives increases from an expected 2% at a CV = 50% to 17% at a CV = 100%, merely from normal distribution statistics alone.

When false positives and/or false negatives exceed about 10%, analyses become uninterpretable from lack of confidence in the presence or absence of the analyte, unless all positive laboratory samples are reanalyzed by a more reliable (confirmatory) method. When the distributions of zeros (not necessarily false negatives) becomes greater than approximately 30%, the distribution empirically becomes bimodal and uninterpretable (is the material positive or negative?).

7. Final Report

7.1 The final report should contain a description of the materials used and their preparation, any unusual features in their distribution, and a table of all data, including outliers. Values not used for specified reasons (decomposition, failure to follow method, contamination, destroyed, etc.) should not be included in the table since they may be erroneously included in subsequent recalculations. The report should include the statistical parameters with and without specified outliers excluded. Report both the standard deviations (and corresponding means) and coefficients of variation. Proofread tables very carefully since many errors are of typographical origin. Give the names of the participants and their laboratories.

7.2 The final report should be published in an accessible publication or its availability from the organization sponsoring the method should be indicated in the published method. Without this public documentation, the usefulness of the method is very limited.

7.3 The final report should be sent to all participants, preferably at the manuscript stage.

8. References

8.1 W. J. Youden & E. H. Steiner (1975) *Statistical Manual of the AOAC.* Association of Official Analytical Chemists, 1111 North 19th Street, Suite 210, Arlington, VA 22209 USA. The third printing (1982) contains several explanatory footnotes.

8.2 *Handbook for AOAC Members* (1982). Availability as in 8.1.

Comments are still solicited on this version of the document which may be referred to as the "FINAL FIRST DRAFT." Please submit them as early as possible, but before August 1, 1983, in order to be considered by the Committee at their meeting on October 1, 1983. The document finalized at this meeting will be the basis for AOAC's presentation at the Joint AOAC/IUPAC Symposium on Harmonization of Collaborative Analytical Studies, scheduled for October 25–27, 1984, in Washington, DC. Submit comments to:

Dr. William Horwitz (202) 245-1057 (FTS 8-245-1057)
Bureau of Foods HFF-7
Food and Drug Administration
Washington, DC 20204 USA

Report of the Committee on International Cooperation

BARRY SMITH, *Chairman*
Health and Welfare Canada, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2

Other members: L. Appleqvist, W. R. Bontoyan, B. Borsje, H. Egan, E. R. Elkins, I. Hoffman, W. Horwitz, B. Larsen, P. Martin, D. L. Park, M. Rogers, R. C. Rund, D. C. Smith, R. W. Weik

The Committee met on Monday, October 25 with 16 members and guests in attendance from the United States, Canada, the United Kingdom, Sweden, Denmark, Egypt, and Japan. The Committee was particularly pleased to welcome Dr. Khayria Naguib of the National Research Centre, Food and Dairy Laboratory, Cairo, and Dr. Kageaki Aibara, Director, Department of Biomedical Research on Food, National Institute of Health, Tokyo.

The Committee heard reports on the status of joint actions between AOAC and the following bodies: Analytical Division of the Royal Society of Chemistry; International Standard Organization (ISO); IUPAC (International Union of Pure and Applied Chemistry); CIPAC (Collaborative International Pesticide Analysis Commission); Codex Committee on Methods of Analysis and Sampling; Association of Public Analysts; Nordic Committee on Food Analysis.

The Committee reviewed, in some detail, the role of AOAC as the administrator of ANSI Technical Advisory Groups which provide the vehicle for AOAC participation in ISO Technical Committees 34 and 134. While questioning the financial costs associated with participation in ISO through the ANSI link, the Committee agreed that progress had indeed been made in having ISO recognize the necessity of collaboratively studying methods of analysis. The Committee felt that more information should be obtained before making any decision or recommendation on a possible change of status with respect to liaison with ISO. In particular, the Committee felt that an attempt would be made to obtain an inventory of ISO methods which have been collaboratively studied.

Furthermore, an attempt should also be made to clarify both the regulatory and nonregulatory status of ISO methods in member countries participating in TC/34 and TC/134.

The Committee also recommended that the Executive Director of AOAC visit the ISO Central Secretariat in Geneva as part of a planned trip to Europe in 1983.

The Committee also considered, briefly, the development of guidelines concerning financial support for overseas delegates to the AOAC annual meeting. It was agreed that a small working group would deal with this by correspondence and make a recommendation to the Board before its April 1983 meeting.

The Committee also accepted, in principle, a Centennial project suggestion put forward by Lars Appleqvist aimed at providing free subscriptions of the AOAC *Journal* to scientists working in food control laboratories in developing countries. Several Committee members agreed to approach the international aid organizations in their respective countries as a possible

source of funding for this project.

Finally, the Committee listened, with interest, to a report from Michael Wehr who had recently visited a number of laboratories and government organizations in the Far East. Dr. Wehr noted that exciting opportunities exist for liaison and interaction with laboratories and government agencies in Japan, Taiwan, and the Philippines and stressed that appropriate followup action should take place.

Accepted

Report of Intersociety Committee (ISC) on Methods of Air Sampling and Analysis

BERNARD E. SALTZMAN, *Representative*
University of Cincinnati, Kettering Laboratory, Cincinnati, OH 45267

During the past year, the committee held one meeting on June 23, 1982, in New Orleans, LA. The secretary stated that book sales had improved, but as of May 1, 1982, 2100 books remained as inventory. The chairman noted that APHA has failed to respond to repeated requests for book sales promotion. Adequate funds were available to complete the manuscript of the next edition. Since this was far from ready, the final decision on selection of a publisher was deferred. However, there was almost unanimous support for the terms offered by Macmillan. The secretary will inform Macmillan and the AOAC of these sentiments.

The editor reported that no manuscripts have been completed. The subcommittee chairmen reported on their meetings with members and activities. Recommendations are being prepared to delete some methods, revise others, and add new ones. Cross-references to similar methods by ASTM, EPA, and NIOSH will be added. New sections planned include discussions of olefactory methods, x-ray diffraction, x-ray fluorescence, ion chromatography, and anodic stripping voltammetry.

A nominating committee comprised of the past three chairmen, R. F. Toro, B. E. Saltzman, and E. R. Hendrickson, was appointed to propose a nominee for chairman to take office at the end of W. T. Ingram's term in June 1983. According to the Articles of Agreement, representatives of four societies, APWA, HPS, ACS, and ISA, were eligible. Richard J. Thompson, representing ACS, was later unanimously chosen. The committee also proposed that an Assistant Editor be appointed by June 1983.

Accepted

Report of the Committee on Laboratory Quality Assurance

KEITH A. McCULLY, *Chairman*
Health and Welfare Canada, Field Operations Directorate, Tunney's Pasture, Ottawa, Ontario, Canada K1A 1B7

Other members: R. Alvarez, R. D. Fishbeck, F. M. Garfield, E. E. Martin, J. E. McNeal, S. Sherken, K. Smith, J. Winter

The Committee met October 25, 1982, in Washington, DC. Fred Garfield reported to the Committee on his progress in preparing a quality assurance manual for analytical laboratories. He is presently writing the final chapter which should be finished in approximately 1 month. Following printing, draft copies of the manual will be provided to each committee member for

in depth content and technical review. These reviews are to be completed early in 1983. The manual will then be available for review by the Editorial Board and outside expert reviewers. The committee discussed some comments received from the Editorial Board on the preliminary draft of the manual. A copy of these comments will be provided to each committee member for consideration when reviewing the draft manual. The committee recommends that the review process be completed and the manual published as soon as possible.

An announcement was published in the August 1982 Referee requesting that outside organizations provide AOAC either their quality assurance manuals or information about quality assurance programs for analytical laboratories. No responses have been received to date.

The Committee believes there is a lack of standardization among analytical laboratories concerning minimal quality control procedures. The AOAC provides a mechanism for standardization of methods by developing and approving collaboratively studied methods. However, not all laboratories follow these methods exactly as written. Collaboratively studied methods do not exist for all applications that a laboratory may encounter. In addition, analytical personnel using the methods may not be adequately trained or have adequate quality control programs in place. The committee recommends that laboratory standards of quality assurance/quality control be made more uniform. The committee urges that AOAC become the leader in analytical laboratory quality assurance by developing acceptable minimum standards of laboratory quality assurance/quality control guidelines. This encompasses the whole range of good laboratory practices that are included in quality assurance/quality control programs. The first step is the completion and publication of the AOAC Quality Assurance Manual. Subsequently, the Committee urges AOAC to sponsor the development of quality assurance workshops and training sessions for both laboratory managers and analysts.

The Committee recommends that the Committee on Collaborative Studies develop guidelines for identifying critical control points for methods identified for collaborative study. The committee recommends that these critical control guidelines be reviewed by the Official Methods Board and all Methods Committees (A-G) before adoption. The Committee feels that the identification of critical control points for current AOAC approved methods is desirable.

The Committee will continue to determine and compile a list of organizations and associations that are active in laboratory quality assurance activities. The Committee recommends that the Committee on Symposia and Special Programs consider organizing a symposium or workshop involving organizations and associations active in laboratory quality assurance activities. This would appear to be a logical followup to the Quality Assurance Manual in AOAC's role in promoting quality assurance activities.

Accepted

Report of the Life Sciences Research Office

FREDERIC R. SENTI
Federation of American Societies for Experimental Biology, 9650 Rockville Pike, Bethesda, MD 20814

During the period January to July, 1982, LSRO contacted three industrial associations, five scientific and professional societies, four governmental agencies, one international organization, and twenty-three commercial testing or research laboratories to obtain information regarding their approach to validated methodology and interests in cooperation with the Association of Official Analytical Chemists (AOAC) in the validation of methods.

Representatives of the industrial associations uniformly recognized the value of collaborative interlaboratory studies in establishing the reliability of analytical methodology. However, their approaches to methods development differ considerably. The American Petroleum Institute

(API) depends on the American Society for Testing and Materials (ASTM) to provide this service for API and its member companies. API member companies participate in ASTM methods development, including validation by round-robin testing. The industry interacts with the Environmental Protection Agency (EPA) methods development through joint participation of members of their respective staffs in ASTM Committees D-19, Water; and D-34, Solid Waste. API representatives were aware of AOAC methods but appeared satisfied with methods development as carried out by ASTM in cooperation with industry.

The Cosmetics, Fragrance and Toiletries Association (CFTA) is active in methods development and has recently developed analytical methods for dioxane, nitrosoamines, and *p*-toluidine which may occur as impurities in their product ingredients. Interlaboratory collaborative studies were part of some methods development, but were conducted by procedures other than AOAC standard protocols. The merits of AOAC procedures were recognized but, apparently, associated problems were considered to outweigh benefits. In view of the interest of the Food and Drug Administration (FDA) in reliable methods for analyzing products for toxic constituents, it is suggested that AOAC explore the mutual interests of FDA and CFTA in the validation of CFTA methods.

Contact with the Chemical Manufacturers Association (CMA) indicated interest in validated (reliable) methods but no direct activities in methods development. Methodology for testing for compliance with EPA regulations was the regulatory area of most concern. ASTM methods are used by most CMA members but AOAC methods, among several others, were also mentioned. Although the value of validated methods was recognized, no clearly evident interest was expressed in cooperation with AOAC in validation studies.

In contrast to API and CMA, the National Food Processors Association (NFPA) is engaged in an area where a majority of AOAC methods are applicable and have been available for many years. Reasons given for use of AOAC methods by NFPA laboratories included the following: the methods have been tested, validated, and approved by competent analysts; they have greater recognition in courts than nonvalidated methods; AOAC methods are approved by FDA, an important consideration in testing for compliance with FDA regulations.

Three governmental agencies (one international) not currently participating in AOAC validation programs, were contacted regarding their procedures and policies on the validation of test methods for regulatory compliance. Both domestic agencies, the Environmental Effects Branch, EPA, and the National Institute for Occupational Safety and Health (NIOSH), validate selected analytical or bioassay methods by contract. NIOSH indicated that the contract route enabled the participation of smaller commercial laboratories that would be likely to use NIOSH methods but could not afford the cost of voluntary participation in a validation study.

EPA has issued two types of contracts. In one, an EPA scientist serves as the project director (equivalent to an AOAC Associate Referee); in the other, a prime contractor provides this service and subcontracts to obtain participating laboratories. NIOSH follows the second procedure. It appears that AOAC might participate in such contracts as the prime contractor and it is suggested that AOAC explore these possibilities. Requests for proposals are published regularly by EPA in the *Commerce Business Daily*.

It is also suggested that AOAC review the results of past and ongoing validation studies of EPA and NIOSH to determine their suitability for inclusion in AOAC's *Official Methods of Analysis*.

The Organization for Economic Cooperation and Development (OECD) has published guidelines for testing the following properties of chemicals: physical-chemical, effects on biotic systems, degradation and accumulation, and short- and long-term toxicity. Data generated in one country in accordance with OECD test guidelines and OECD principles of good laboratory practice are accepted in other OECD countries for purposes of safety assessment. The published guidelines indicate that there was interlaboratory testing of methods for determining physical-chemical properties and perhaps some collaborative studies of biodegradability methods. However, the procedures followed are not described. Indications are that most methods were developed or selected by consensus of expert groups. Opportunities for AOAC appear to lie in the OECD Updating Program (established in 1981) for test guidelines. Among other objectives, this program will undertake interlaboratory comparison of test methods by expert groups. AOAC, through contact with Irwin Baumel, EPA, U.S. representative on the Updating Group, could offer its services in nominating experts to any panel that may be formed for the interlaboratory testing of

existing or proposed new guidelines. The AOAC nominee, if accepted, could advance the merits of AOAC procedures for collaborative testing of methods.

Contacts were made with the Society of Toxicologists, American College of Toxicologists, Environmental Mutagen Society, and Office of Toxic Substances, EPA, regarding interlaboratory collaborative testing of toxicological methods. Discussions emphasized short-term mutagenicity tests because these methods are in the developmental state and because of widespread interest in the possibility that a battery of these tests might supplement or supplant animal carcinogenicity tests at great savings of time and money. The salient activity in the development of short-term mutagenicity tests is the Gene-Tox program coordinated by the Office of Toxic Substances, EPA. Reports are being prepared, or have been published, by 23 work groups, each including a suggested protocol for conducting the respective mutagenicity test. Based on these suggested protocols, the Office of Toxic Substances has developed protocols for 17 or 18 of the tests. These are to be published by the National Technical Information Service, Department of Commerce, Springfield, VA. It is likely that these protocols will be accepted as standards, especially in regulatory compliance testing. The Office of Toxic Substances has no present plans for collaborative studies of the protocols.

Those members of the Environmental Mutagen Society (EMS) contacted in this study did not identify need for collaborative testing of any of the short-term mutagenicity tests at the present time. They noted that the Ames test, the most used mutagenicity test, has been collaboratively tested (not by AOAC), and suggested that further experience with the protocols for the other tests would reveal those which may need further development and collaborative study. The newly formed ASTM Committee E47, Biological Effects and Environmental Fate, has contacted EMS regarding its interest in standardization of methods in cooperation with ASTM, but no definitive response was given. We note that one of the critical issues in future directions of EMS listed in the spring issue of *EMS Newsletter* is "Should the Society endeavor to set testing standards or become involved in standardization of testing procedures?" We suggest that AOAC contact one or more members of the EMS Committee on Future Directions of the Society.

The Society of Toxicology indicated that it has a technical committee on methods and has conducted interlaboratory collaborative studies in the past. No opinion was expressed regarding short-term mutagenicity tests or that the Society had special interest in them. The Society president requested that information on AOAC procedures for validation of methods be sent to him so the subject could be discussed at their Council meeting.

The American College of Toxicologists has no committee on methods and has not engaged in methods standardization. However, discussions with the Executive Director and a Council member indicated their interest in a presentation on AOAC procedures for validation of methods at the meeting of the Council.

Members of the American Society for Microbiology (ASM) contacted thought that microbiologists concerned with food technology and food safety were familiar with AOAC methods. ASM has no ongoing activities in methods development but members of ASM have participated in AOAC collaborative studies and, indeed, an ASM member had recently been appointed an AOAC referee. The Chairman-elect of the ASM Division of Food Microbiology suggested that AOAC submit a proposal for cooperation in validation studies, outlining the respective roles of the two organizations. In contrast, the opinion was expressed that clinical microbiologists were not ready for standardized methods and, in view of the rate that clinical microbiology is progressing, it is too early to engage in validation of methods.

Of 10 commercial laboratories specializing in food analyses that were contacted, a majority used *Official Methods of Analysis* as the primary source of chemical methods. However, a majority also reported FDA *Bacteriological Analytical Manual* as the major source of microbiological methods. FDA *Pesticide Analytical Manual* and EPA methods were reported as the major sources of pesticide methods. Other microbiological methods used included those published by the American Public Health Association. The most important factors in the selection of methods were availability in a source recognized as standard and, in regulatory compliance testing, acceptance by the city, state, or federal agency involved. Validation by collaborative testing was a secondary consideration. No difficulties arising from disagreements of results among laboratories were reported from the use of nonvalidated methods or those developed by procedures other than AOAC protocols.

The results of this survey indicate that the AOAC *Official Methods of Analysis* is unique among standard sources of chemical methods for

food analyses as contrasted to microbiological methods, where there are several, competing sources. AOAC should recognize this advantage and continue the addition of chemical methods to maintain its pre-eminent position.

A majority of the commercial toxicology laboratories contacted favored the eventual standardization of methods for short-term mutagenicity tests. However, at least two considered guidelines allowing some flexibility in procedures to be adequate. Protocols described in the reports of the Gene-Tox program work groups tend to be followed and it appears that these protocols as modified and published by the Office of Toxic Substances, EPA, will be accepted by most commercial laboratories. Need was expressed, however, for protocols adapted to gases and complex mixtures such as food products as contrasted to pure chemicals for which present protocols were developed.

Little support was apparent in our contacts with commercial toxicology laboratories for collaborative studies of the short-term mutagenicity tests although it was suggested that further experience may indicate need for such studies for some of the tests.

The Food Safety and Inspection Service (FSIS), USDA, is conducting an extensive program in the development of new and improved analytical methods for residues in meat and poultry. They find it difficult to obtain volunteer laboratories to participate in collaborative validation studies. To meet this problem, collaborative studies are currently being conducted internally by 3 laboratories of FSIS and FDA. If the samples analyzed are stable, they propose to store them in anticipation of obtaining services of 2 additional laboratories for analysis of the samples at some future date. FSIS is also considering offering contracts to 2 additional laboratories as a means of obtaining the minimum 5 laboratories required by AOAC to qualify for an AOAC collaborative test. AOAC may wish to consider serving as a contractor on such studies for agencies such as FSIS.

A possible source of collaborators on analytical methods for tissue residues of animal drugs would appear to be the veterinary science field. It is suggested that the subject be discussed with AOAC's new referee for veterinary toxicology.

A new research area requiring the development of more reliable analytical methods that should be considered by AOAC is that on acid rain, its sources and control. This was brought to our attention by a Request for Proposal, "Natural sources of compounds that contribute to acid precipitation" NOAA 55-82, issued by the National Oceanic and Atmospheric Administration, July 19, 1982. Task 1 in the RFP is the comparison of techniques to measure natural emissions of sulfur compounds. The specific objective is to compare the sampling design and analytical approaches of different techniques to estimate sulfur gas emissions and to eliminate any systematic errors in sampling or analysis. The RFP also points out the need to develop methods to detect emissions of nitrogen compounds and assess their reliability. It would appear that development of analytical methods will be an ongoing activity in research on acid rain for several years and that there may be opportunity for the application of AOAC procedures for assessing the reliability of these methods.

The foregoing discussion has summarized information from contacts made by LSRO related to opportunities for AOAC in its traditional activities in the development and publication of validated methods of analysis. A logical extension of these activities is AOAC sponsorship of educational short courses dealing with analytical methodology. Several professional societies now sponsor short courses. The American Chemical Society, Institute of Food Technologists, and the American Oil Chemists Society, for example, schedule one or more short courses to meet on the days immediately preceding the annual meeting of the society. Duration of the course, depending on the subject matter, may be one to three days. Subject matter experts are recruited to give the lectures and may vary in number from two for a one-day course to 10 or 12 for a three-day course. Tuition may vary from about $250 to $500 or more.

Subject matter areas for short course sponsorship would be those in which AOAC has General Referees. Some timely subjects selected from these might be analytical methodology for determination of mycotoxins, nitrosamines, dietary fiber, or water contaminants. Although AOAC's participation in these subjects has been mainly that of methods development, the scope of the short courses could be much broader and, indeed, in many cases should be to educate the analytical chemists on other aspects of the subject (e.g., biochemistry, toxicology, physiology) and vice versa.

To avail itself of opportunities in new areas and to stimulate activities in traditional areas, AOAC will need to provide additional professional staff support. For example, our contacts in the toxicology field indicate that securing the

interest and cooperation of scientists and societies in methods development and validation will require effective liaison on the part of AOAC in order to keep abreast of methods research and to compete with other organizations whose principal interest is standardization of methods. Entering into contracts or cooperative agreements with government agencies for the supervision of collaborative studies of analytical methods also will require considerable work on the part of the AOAC staff. A successful program of short courses will require selecting timely subjects, obtaining speakers, and making other necessary arrangements. Possible sources of professional support are the services of AOAC members spending their sabbatical period at AOAC headquarters, and the part-time employment of retired AOAC members. However, an adequate number of full-time employees would need to be maintained to give continuity to each of the programs.

Report of the Long-Range Planning Committee

H. MICHAEL WEHR, *Chairman*
Oregon Department of Agriculture, Salem, OR 97310

Other members: C. Andres, F. J. Baur, G. H. Boone, W. Cobb, W. Furman, F. M. Garfield, I. Hoffman, J. J. Karr, K. Kissler, A. Munson, H. L. Reynolds

The Committee met three times during the past year: March 4-5, 1982, July 8-9, 1982, October 25-26, 1982.

The Committee is charged with providing recommendations to the Board of Directors regarding future directions of the Association and ways to improve the functioning of AOAC. During the past year, the Committee concentrated its efforts in two broad areas: (1) a comprehensive review of the membership area, carried out in response to a request from AOAC President J. P. Minyard; and (2) discussions with representatives of several federal agencies and industry as to how best to serve their respective needs over the next 2-5 years.

Membership Review

The Committee was asked to provide recommendations in the following areas: (1) What kind of an association should the AOAC be? (2) Who should be members of the association? (3) What types of categories of memberships should there be? (4) Should there be qualifications for membership and, if so, what? (5) What should benefits of membership be? (6) What goals should be set for AOAC membership; how should members be recruited, and what means should be used to obtain information on member resources?

Recommendations of the Committee to the Board of Directors included the following:

(1) AOAC should be an open scientific society serving regulatory, research, academia, industry, private laboratories, etc., in the broad areas of agriculture, public health, and the environment. AOAC should provide official scientific methods of analysis, method validation procedures, exchange of scientific ideas and knowledge, and other related activities on an international basis.

(2) Apart from honorary, emeritus, and student memberships, there should be only one general active individual membership category applicable to all individuals, irrespective of employment. The only criterion for membership should be an interest in method development. There should be no educational degree requirements for membership in AOAC.

(3) Membership types should include individual memberships, as noted in (2) above, and institutional membership. Such institutional membership should provide for membership of all organizational types—governmental, educational, and private.

(4) Benefits of individual membership should include receipt of *The Referee*, mailings of announcements, lower fee for the annual meeting on a preregistration basis, discounts on several workshops and training courses, membership directory.

(5) Individual membership dues should be set

at $25 per year, an approximate break-even point for the Association; Associate and General Referees may have their dues waived by written request.

(6) Private sustaining members should receive the following benefits: certificate of membership, two individual memberships without charge, receipt of *The Referee*, membership directory, problem-solving service, annual meeting preregistration at reduced price.

(7) Recommendations on benefits of state, federal, and nonprofit institutional benefits will be forthcoming.

Future Directions

The Committee met with representatives of four federal agencies to discuss ways in which AOAC can provide assistance over the intermediate- to long-term future. The Committee met with representatives of the following organizations:

(1) Food and Drug Administration, including representatives from the Bureau of Foods, EDRO, Medical Devices and Diagnostic Products Group, Bureau of Veterinary Medicine, Biologics Group, and Drug Group.

(2) U.S. Department of Agriculture, including representatives from the Food Safety Inspection Service—Science (also including representatives of USDA—FSIS Poultry Grading Branch) and Agricultural Research Service.

(3) Representatives of the Environmental Protection Agency, including representatives from the Office of Pesticide Programs, Office of Toxic Substances, Drinking Water Program, and Solid Waste Program.

The committee additionally held one meeting with representatives of several private firms.

Specific points of discussion included the following:

(1) How are analytical methods developed and validated by your organization? What is your organization's attitude toward method validation to interlaboratory variability?

(2) What is the current relationship and involvement of your organization with AOAC? How do you and your scientific staff feel about participating in AOAC? How can participation in AOAC be strengthened?

(3) Are there analytical areas not now represented within AOAC that should be a part of its official methods area that would benefit your organization?

(4) Are there areas of service of benefit to your organization other than methods validation that AOAC is not currently involved in but which could be of benefit to you? What areas other than methods validation are you now in that AOAC can assist with?

(5) What is your attitude towards increasing participation by industry?

(6) What are changes that should be made in todays general operation of the Association?

(7) What specific methods needs do you now have? What areas can you work in now?

(8) What future services or demands will be placed on your organization in which AOAC can assist in responding?

(9) How can commitment by your agency to participation in AOAC activities be increased? On what levels does decision making for participation in AOAC occur? How do we get into your planning cycle?

Details of these various meetings are available on request.

Other Items

Liaison Activities: Following approval by the Board of Directors of a Long-Range Planning Committee proposal to strengthen and develop liaison activities, the Committee has pursued discussion with other technical societies, trade associations, and other appropriate organizations to identify those organizations who may have an interest in developing a liaison with the Association. Several specific organizations have been contacted with the liaisons identified. Efforts will continue in this area during the upcoming year.

Accepted

Report of the Joint AOAC-AOCS-AACC-IUPAC Mycotoxin Committee

PETER SCOTT, *Chairman*
Food Research Division, Health Protection Branch, Ottawa, Ontario, Canada K1A OL2

Other members: D. L. Park, J. Wessel (AOAC); T. Romer, O. Shotwell, L. Stoloff (AACC); L. Goldblatt, R. Stubblefield, A. Waltking (AOCS); A. Pohland (IUPAC)

The Joint Mycotoxin Committee met October 28, 1982, in Washington, DC, to discuss the current situation with respect to mycotoxins and to encourage and support future activities. The deliberations were initiated with a discussion of the activities of counterpart panels within AOCS, AACC, and IUPAC and the report by the AOAC General Referee for Mycotoxins, Leonard Stoloff. The panel then turned its attention to other matters relating to mycotoxins occurring during the past year. Of special note were the FDA decision to raise the action level for aflatoxin in cottonseed meal used in animal feed (beef cattle, swine, and poultry) from 20 to 300 ppb (*Fed. Regist.* **47**(147), 33007-33008 (July 30, 1982)); and the issuance of an advisory opinion by FDA setting a level of concern for vomitoxin at 2 ppm for wheat entering the milling process, 1 ppm for finished wheat products for human consumption, and 4 ppm for wheat and wheat-milling by-products intended for use in animal feeds (beef cattle, swine, poultry, pet foods).

The major area of interest was vomitoxin (deoxynivalenol, DON) and special presentations were made by G. Bennett (USDA/NRRC), W. Davidson (SCIEX, Canada), T. Romer (Romer Labs), R. Eppley (FDA), J. Routh (Hazleton Raltech), P. Scott (Health and Welfare, Canada), O. Shotwell (USDA/NRRC), and F. S. Chu (Food Research Inst.). All facets of vomitoxin methodology were covered, as well as the result of a variety of surveys conducted during the past year.

Also discussed were a number of collaborative studies; anyone wishing to participate in these studies should contact the project directors or AOAC. The following studies are planned: Aflatoxin Check Sample Program (M. Friesen, IARC); Aflatoxin Confirmation by MS (D. Park, FDA); Ochratoxin A Residues in Tissues (S. Nesheim, FDA); DON by GLC/EC and DON Screening Procedure (R. Eppley, FDA); Penicillic Acid by HPLC (C. Thorpe, FDA); Zearalenone (G. Bennett, USDA/NRRC); and Aflatoxin M_1 (R. Stubblefield, USDA/NRRC).

Finally, information was received on the following mycotoxin symposia planned for 1983: International Mycotoxin Conference I, Cairo, Egypt, 3/19-24/83; AOAC Spring Training Workshop, Indianapolis, IN, 4/19-21/83; Gordon Research Conference: Trichothecenes, Plymouth, NH, 6/13-17/83; AOAC Midwest Regional Meeting: Mycotoxin Symposium, Ames, IA, 6/14-15/83; American Phytopathological Society: Mycotoxicology Symposium, Ames, IA, 6/26-30/83; Third International Mycological Congress and UJNR: Mycotoxin-Producing Fungi and Their Toxins, Tokyo, Japan, 8/28-9/3/83.

Report of the Committee on Safety

EDWARD H. LOSIEWICZ, *Chairman*
Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857

Other members: D. Arnold, R. Bianchi, R. Hall, J. C. Kissinger, F. Lundgren, R. J. Noel, and T. Tomczak

The Committee reviewed the request submitted to the AOAC regarding a safety problem encountered with method **7.115**, "Analysis of Iodine in Animal Feed," and recommended that the General Referees for Feeds; Fertilizers; Plants; Tobacco; Reference Materials and Standard So-

lutions modify method **7.115** to include the additional step of drying the sample on the steam bath to remove the bulk of the organic solvent before placing it in the 100° oven prior to ignition in the furnace.

The Committee discussed the use of precautionary statements associated with methods and recommended that the General Referees inform the Associate Referees to include cautionary words when writing the analytical procedure, e.g., . . . place in explosion-proof blender . . . instead of blender when appropriate to eliminate the use of too many cautionary references with a method.

The Committee is in the process of updating the precautions and references in Chapter 51.

Accepted

Report of the Committee on Symposia and Special Programs

WARREN R. BONTOYAN, *Chairman*
Environmental Protection Agency, Office of Pesticide Programs, Beltsville, MD 20705

Other members: F. J. Carleton, J. B. Cherry, D. E. Coffin, N. Hardin, C. Gehrke, B. M. McMahon, H. Morris, A. Pohland, D. Stalling

The Committee had its first meeting in October 1981. Attending were Warren R. Bontoyan, Frederick J. Carleton, Bernadette McMahon, Albert Pohland, David B. MacLean, and George Schwartzman. MacLean and Schwartzman were present as advisors. Warren Bontoyan decided during the course of the year to enlarge the Committee to accommodate additional responsibility with respect to the 1984 Centennial International Meeting.

The Committee recommended a number of symposia topics for the October 1982 International Meeting. The topics accepted were the results of a questionnaire sent to state, federal, and industry participants who are active in or aware of the AOAC program. The 96th meeting included five symposia:

Topic	*Chairperson*
Advances in Nitrosamine Analysis	Nisu P. Sen Health and Welfare Canada
Impact and Directions of Good Laboratory Practices	Carl Schulz Clement Associates
Chemical Analysis of Drinking Water Trace Contaminants	Irwin H. Pomerantz Environmental Protection Agency
Detection of Deliberate Adulteration in Foods	Martha Rhodes Florida Dept of Agriculture and Consumer Services
Water Activity Methods of Analysis of Foods	Melvin Johnston Food and Drug Administration Theodore Labuza University of Minnesota
Mycotoxin Symposium	Leonard Stoloff Food and Drug Administration

W. R. Bontoyan is also a member of the Centennial Committee and is liaison for the two committees. He has met four times with the Centennial Committee which has charged the Committee on Symposia and Special Projects to arrange the 1984 Centennial Symposia. Suggested topics are: new types of biological tests for predicting human hazards; correlation of biological tests with potency and efficacy of drugs and nutrients; correlation of in vivo and in vitro tests to predict human hazards and potency and efficacy; quality assurance topics such as initiating program improvements in operation, and quality control charts; contribution of

the biological sciences to AOAC methodology; nondestruction testing methods; direct analysis method (elimination of lengthy and complicated cleanups).

The Committee will announce the use of a poster session for all Associate Referee reports through a story in *The Referee*.

The Committee will investigate whether the industry series (Technicon, Kontes, etc.) and the evening programs (state meeting, referee topics, etc.) can be held during the day with industry functions conducted as strictly social affairs in the evening as an alternative to the hospitality suites. The Committee will also consider reinstituting the wine-tasting session, or scheduling a party sponsored by industry as an alternative to the individual hospitality suites.

A subcommittee will arrange program aspects with the banquet and help organize an elegant Sunday reception for the international groups (IUPAC, CIPAC, etc.) whose meetings will be part of the 1984 Centennial International Meeting.

On October 27, 1982, the Committee met and decided the following with respect to the 1984 fall Centennial meeting:

(1) It will endeavor to obtain eminent scientists in the area of analytical science to give the principal addresses at an afternoon plenary session.
(2) All collaborative studies and Referee reports shall be presented as posters.
(3) Beginning in 1983, all presentation abstracts (Referee reports, contributed papers, collaborative studies, etc.) shall be submitted to the Association by June 1.
(4) For the 1984 October meeting, contributed papers will be reviewed and selected for presentation by the General Referee or the appropriate committee concerned with the topic.
(5) In 1983, the Committee will meet on the days preceding the Board of Directors' meetings.
(6) The Committee will submit jointly with the Centennial Committee a proposed joint budget to the Board of Directors in January 1983 for planning and implementing the necessary arrangements for the 1984 fall Centennial Meeting.

Accepted

Report of the Ways and Means Committee

STANLEY KATZ, *Chairman*
Rutgers University—Cook College, New Brunswick, NJ 08903

Other members: R. Blinn, J. Bourke, W. P. Cochrane, C. Gehrke, J. Goleb, M. Malina, L. Perlman, W. Phillips, M. Ready

The Committee reviewed the charge of the Board of Directors concerning planning for a fund raising effort to increase the principal of the Wiley Fund to a level of $50,000–$200,000 and offers the following strategies to accomplish the charge. The recommendations have a connection both with the forthcoming Centennial and with the overall financial base of AOAC.

(1) The Committee suggests the appointment of a full-time AOAC employee whose function would be fund raising. This individual would be charged with the task of presenting, to a select, relatively small number of industrial organizations, the AOAC story, the concept of the Wiley Award, and the concept of an exclusive endowment bearing the contributor(s) name(s). The concept is similar to the endowment of a titled chair at major universities. As with an endowed chair, the funders' name(s) will be tied, inexorably, to the Wiley Fund. It is believed that the exclusiveness of the contributor(s) could be a strong factor in the success of the solicitation.

(2) The aforementioned individual also would be charged with expanding the rolls of sustaining members through an organized and concerted effort. It is suggested that increased contributions be solicited from each sustaining member. To prevent the erosion of smaller contributions, a tier of contributions should be developed based on the level of funding. The Committee believes that the concept of using the Centennial as a focal point for increased future

funding would be a viable approach. Increased funding from an expanded base could be used for Centennial activities as well as the future.

(3) The members of the Ways and Means Committee can act as a resource to the individual charged with fund raising but the prime responsibility must rest in that individual's hands.

Other areas that can be explored as sources of continuing revenue are through the offering of professional advancement courses/seminars in areas such as: (1) expert witness training; (2) statistics; (3) quality assurance/quality control; (4) laboratory safety; (5) disposal of hazardous wastes; (6) advanced instrumental techniques; (7) toxicology. In addition to the revenue from the courses, additional revenue could be earned from the sale of video tape of the courses. It is envisioned that such courses could be given in conjunction with the Annual International Meeting and the Spring Workshop. Courses would be given and tapes would be sold over a three-year cycle, allowing for state of the art/science changes.

Although no consensus was achieved, the Committee felt that a review of the relationship between dues and *Journal* subscriptions should be made to see if a suitable package could be developed which could expand the sale of the *Journal*. Similarly, the concept of an expanded *Referee* with timely scientific articles, news items, and advertising to help defray costs, should be explored.

Accepted

OFFICERS AND STANDING COMMITTEES OF THE ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS FOR THE YEAR ENDING OCTOBER 1983

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LIAISON REPRESENTATIVES

American Academy of Forensic Sciences: Richard L. Brunelle (Department of the Treasury, Bureau of Alcohol, Tobacco, and Firearms, 1401 Research Blvd, Rockville, MD 20850)

American Society of Brewing Chemists: Anthony J. Cutaia (Stroh Brewing Co., One Stroh Dr, Detroit, MI 48226)

American Society of Enologists: Arthur Caputi, Jr (E. & J. Gallo Winery, PO Box 1130, Modesto, CA 95353)

American Society for Testing and Materials:

C-7 Subcommittee XI, Agricultural Liming Materials: Robert C. Rund (Purdue University, Department of Biochemistry, West Lafayette, IN 47907)

C-21.03: Ceramic White Wares-Related Products: Benjamin Krinitz (Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232); Edward A. Steele (Food and Drug Administration, Washington, DC 20204)

D-19: Water: Theodore O. Meiggs (Environmental Protection Agency, National Field Investigations Center, Office of Enforcement, Denver, CO 80225)

E-15: Analysis and Testing of Industrial Chemicals: Edward Dellamonica (U.S. Department of Agriculture, Eastern Marketing and Nutrition Research Division, 600 E Mermaid Lane, Philadelphia, PA 19118)

E-19: Chromatography: Michel Margosis (Food and Drug Administration, Washington, DC 20204)

E-30: Forensic Sciences: Richard L. Brunelle (Department of the Treasury, Bureau of Alcohol, Tobacco and Firearms, 1401 Research Blvd, Rockville, MD 20850); Anthony Romano, Jr (Drug Enforcement Administration, Southeastern Laboratory, 5205 NW 84th Ave, Miami, FL 33166)

F-2: Flexible Barrier Materials: Subcommittee III, Test Methods: Charles V. Breder (Food and Drug Administration, Washington, DC 20204)

F-10: Meat and Meat Products: Anthony J. Malanoski (U.S. Department of Agriculture, Washington, DC 20250)

American Spice Trade Association: Damon Larry (Food and Drug Administration, Washington, DC 20204)

Codex Committee on Fish and Fishery Products: R. V. Cano (Food and Drug Administration, Bureau of Foods, Washington, DC 20204)

Collaborative International Pesticides Analytical Council: Warren R. Bontoyan (Environmental Protection Agency, Office of Pesticide Programs, Beltsville, MD 20705), *AOAC Representative;* James E. Launer (Oregon Department of Agriculture, Salem, OR 97310), *Correspondent;* Jack Plimmer (U.S. Department of Agriculture, Beltsville, MD 20705), *Correspondent*

Council on Soil Testing and Plant Analysis

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Federation of Oils, Seeds, and Fats Association, Ltd: David Firestone (Food and Drug Administration, Washington, DC 20204)

Flavor and Extract Manufacturers Association of the U.S.: Roger Middlekauf (900 17th St, NW, Washington, DC 20006)

Health and Welfare Canada: D. Earle Coffin (Health and Welfare Canada, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2)

Institute of Food Technologists: Michael Wehr (State Department of Agriculture, 635 Capital St, NE, Salem, OR 93710)

International Association for Cereal Chemistry: Helmet Glattes (International Association for Cereal Chemistry, Schmidgasse 3-7, A-2320 Schwechat, Austria)

Cereal Foods: Doris A. Baker (U.S. Department of Agriculture, Beltsville, MD 20705)

Determination of Vitamins: Mike J. Deutsch (Food and Drug Administration, Washington, DC 20204)

International Committee on Microbiological Specifications

International Dairy Federation: Robert W. Weik (Food and Drug Administration, Washington, DC 20204)

International Organization for Standardization (ISO): William Horwitz (Food and Drug Administration, Washington, DC 20204), *Liaison Coordinator;* Kathleen M. Fominaya (AOAC, 1111 N 19th St, Suite 210, Arlington, VA 22209), *Correspondence Coordinator*

Animal and Vegetable Fats and Oils (ISO/TC 34/SC 11): Robert G. Manning (SCM, Glidden Durkee Division, 16651 Sprague Rd, Strongsville, OH 44136)

Animal Feeding Stuffs (ISO/TC 34/SC 10): Donald Burdick (U.S. Department of Agriculture, Field Crop Utilization and Marketing, Box 5677, Athens, GA 30604)

Cereals and Pulses (ISO/TC 34/SC 4): Raymond Tarleton (American Association of Cereal Chemists, 3340 Pilot Knob Rd, St. Paul, MN 55121)

Cocoa (ISO/TC 34/WG 4): Robert A. Martin (Hershey Food Corp., 1025 Reese Ave, Hershey, PA 17033)

Coffee (ISO/TC 34/SC 15): George E. Boecklin (National Coffee Association of USA, 120 Wall St, New York, NY 10005)

Crude Fiber (ISO/TC 34/WG 3): David O. Holst (University of Missouri, Food Science and Nutrition, Columbia, MO 65211)

Derived Products of Fruits and Vegetables (ISO/TC 34/SC 3): Edgar R. Elkins (National Food Processors Association, Chemistry Division, 1133 20th St, NW, Washington, DC 20036)

Dried Fruits and Vegetables (ISO/TC 34/SC 13): Frank Mosehard (DFA of California, Box 270-A, Santa Clara, CA 95052)

Fertilizers and Soil Conditioners (ISO/TC 134): Robert C. Rund (Purdue University, West Lafayette, IN 47907); Frank J. Johnson (Tennessee Valley Authority, Muscle Shoals, AL 35660), *Alternate*

Fresh Fruits and Vegetables (ISO/TC 34/SC 14): Bernard J. Imming (United Fresh Fruit and Vegetable Assn, 727 N Washington St, Alexandria, VA 22317)

Meat and Meat Products (ISO/TC 34/SC 6):

Microbiology (ISO/TC 34/SC 9): R. B. Read (Food and Drug Administration, Washington, DC 20204)

Milk and Milk Products (ISO/TC 34/SC 5): Robert W. Weik (Food and Drug Administration, Washington, DC 20204)

Oleaginous Seeds and Fruits (ISO/TC 34/SC 2): Gary R. List (U.S. Department of Agriculture, Science and Education Administration, Northern Regional Research Center, Peoria, IL 61606)

Sensory Analysis (ISO/TC 34/SC 12): Patricia Prell (U.S. Army Natick R&D Command, Natick, MA 01760)

Spices and Condiments (ISO/TC 34/SC 7): Thomas F. Barnes (American Spice Trade Association, Englewood Cliffs, NJ 07632)

Tea (ISO/TC 34/SC 8): Theresa K. Kukla (Tea Association of the USA, 230 Park Ave, New York, NY 10017)

Water Quality (ISO/TC 147): Theodore O. Meiggs (Environmental Protection Agency, Denver Federal Center, Denver, CO 80225)

International Union of Pure and Applied Chemistry: Philip C. Kearney (U.S. Department of Agriculture, Beltsville, MD 20705)

Office International du Cacao et du Chocolat: Emile Toebosch (OICC, 172 Ave de Cortenberg, B1040, Brussels, Belgium)

Intersociety Committee on Manual of Methods for Air Sampling and Analysis: Bernard E. Saltzman (Kettering Laboratory, University of Cincinnati, Eden and Bethesda Aves, Cincinnati, OH 45267)

Subcommittee 1, Sulfur: F. P. Scaringelli (Environmental Protection Agency, Division of Atmospheric Surveillance, Research Triangle Park, NC 27709)

Subcommittee 2, Halogens

Subcommittee 3, Oxidants and Nitrogen: E. L. Kothny (California Air and Industrial Hygiene Laboratory, California State Department of Health, 2151 Berkeley Way, Berkeley, CA 94704)

Subcommittee 4, Carbon: M. Feldstein (Bay Area Pollution Control District, 939 Ellis St, San Francisco, CA 94109)

Subcommittee 5, Hydrocarbons: J. L. Monkman (2275 Georgina Dr, Ottawa, Ontario, Canada K2B 7M2)

Subcommittee 6, Metals: R. J. Thompson (Environmental Protection Agency, Technical Services, Research Triangle Park, NC 27711)

Subcommittee 8, Radioactivity

Subcommittee 9, Laboratory Techniques and Precautions: J. N. Pattison (University of Cincinnati, Environmental Engineering, Cincinnati, OH 45221)

Subcommittee 10, Particulates: Howard E. Ayer (University of Cincinnati, Kettering Laboratory, Eden and Bethesda Aves, Cincinnati, OH 45267)

Subcommittee 11, Source Sampling Techniques

Subcommittee 12, Standardization Coordination

Pesticides Analysis Committee of the Ministry of Agriculture in the United Kingdom:

Dithiocarbamates Panel:

Emulsifiability Panel: Keith G. Seymour (Dow Chemical Co., Agricultural Research Department, Midland, MI 48640)

Gas Chromatography Panel: Warren R. Bontoyan

Joint Dimethoate Residues Panel: Robert W. Storherr (Environmental Protection Agency, Beltsville, MD 20705)

Monuron and Diuron Panel: Howard Hammond (State Laboratories Department, North Dakota State Department of Agriculture, Bismarck, ND 58505)

Pharmaceutical Manufacturers Association Quality Control Vitamin E Committee: Alan J. Sheppard (Food and Drug Administration, Washington, DC 20204)

United States Pharmacopeial Convention: James B. Kottemann (Food and Drug Administration, Washington, DC 20204), *Delegate*

COMMITTEE A

Alan R. Hanks (Purdue University, Office of the State Chemist, West Lafayette, IN 47907), *Chairman*; A. Aner Carlstrom (Chevron Chemical Co., 940 Hensley St, Richmond, CA 94804); Warren R. Bontoyan (Environmental Protection Agency, Office of Pesticide Programs, Beltsville, MD 20705); Frank J. Johnson (TVA/National Fertilizer Development Center, Muscle Shoals, AL 35660); Louis W. Ferarra (IMC Corp., 1401 S 3rd St, Terre Haute, IN 47808); Mary Maud Sharpe (State Department of Agriculture and Consumer Services, Feed Laboratory, Tallahassee, FL 32301); Richard H. Collier (Purdue University, Department of Biochemistry, West Lafayette, IN 47907), *Secretary*; Edwin M. Glocker (14647 Roxbury Rd, Glenelg, MD 21737), *Statistical Consultant*

FEEDS

Referee: Clyde E. Jones, State Department of Agriculture, 2331 W 31st Ave, Denver, CO 80211

Amino Acid Analysis in Mixed Feeds
Wayne Stockland, Supersweet Research Farm, Box 117, Courtland, MN 56021

Fat, Crude, in Pet Foods

Fiber, Crude
David O. Holst, University of Missouri, Food Science and Nutrition, Columbia, MO 65211

Fiber, Crude, in Milk Replacers
J. G. Pierce, Pierce Consulting Service, 713 NW Westwood, Ankeny, IA 50021

Infrared Reflectance Techniques in Mixed Foods

Iodine
Stuart Meridian, West Agro-Chemical, Inc., PO Box 1386, Shawnee Mission, KS 66222

Minerals
Dianne Gehrke, Manchester Labs, PO Box 65, Manchester, IA 52057

Non-Nutritive Residues
Peter J. Van Soest, Cornell University, Department of Animal Science, Ithaca, NY 14850

Protein, Crude
Peter F. Kane, Purdue University, Department of Biochemistry, West Lafayette, IN 47907

Sampling and Sample Preparation
Valva C. Midkiff, University of Kentucky, Kentucky Experimental Station, Lexington, KY 40506

Water by Karl Fischer Method
David Wallace, Department of Agriculture, Weights and Measurements, 3125 Wyandot, Denver, CO 80211

FERTILIZERS

Referee: Robert C. Rund, Purdue University, Department of Biochemistry, West Lafayette, IN 47907

Biuret in Urea and Mixed Fertilizers
Luis F. Corominas, Fertilizantes Mexicanos SA, Piso 06760, Mexico 7 DF, Mexico

Boron
James R. Melton, Texas A&M University, Agricultural Analytical Services, College Station, TX 77843

Free and Total Water
Russell D. Duncan, Tennessee Valley Authority, National Fertilizer Development Center, Muscle Shoals, AL 35660

Iron
James Silkey, Oregon Department of Agriculture, Laboratory Services Division, Salem, OR 97310

Molybdenum

Nitrogen
Paul R. Rexroad, University of Missouri, Experi-

ment Station Chemical Laboratories, Columbia, MO 65201

Phosphorus
Frank J. Johnson, Tennessee Valley Authority, National Fertilizer Development Center, Muscle Shoals, AL 35660

Potash
Peter F. Kane, Purdue University, Department of Biochemistry, West Lafayette, IN 47907

Sampling and Preparation of Sample
Douglas Caine, Estech General Chemicals Corp., 30 N LaSalle St, Chicago, IL 60602

Slow-release Mixed Fertilizers
Stanley E. Katz, Rutgers University, Cook College, Department of Biochemistry and Microbiology, New Brunswick, NJ 08903

Sodium
Luis F. Corominas

Soil and Plant Amendment Ingredients
Clyde E. Jones, Colorado Department of Agriculture, 2331 W 31st Ave, Denver, CO 80211

Sulfur
Virginia A. Thorpe, Michigan Department of Agriculture, Laboratory Division, 1615 S Harrison Rd, East Lansing, MI 48823

Water-Soluble Methylene Ureas
Allan Davidson, OM Scott & Sons, Co., Marysville, OH 43041

Zinc
Mary L. Hasselberger, Department of Agriculture, Laboratory Division, 3703 S 14th St, Lincoln, NE 68502

HAZARDOUS SUBSTANCES

Referee: Dean Hill, Environmental Protection Agency, NEIC, Box 27225, Denver Federal Center, Denver, CO 80225

Ammonia as a Product Ingredient

Benzene in Consumer Products
Wayne G. Warner, Food and Drug Administration, Division of Toxicology, Washington, DC 20204

Carbolic Acid (Phenolic) Compounds

Chlorinated Hydrocarbons

Diethylene Glycol and Ethylene Glycol

Flammable Substances in Pressurized Containers

Flash Point of Solids and Semisolids

Formaldehyde

Hazardous Components in Resin Systems

Pentachlorophenol in Toy Paints
Hans E. A. M. Van Langeveld, Food Inspection Services, Florijrnuwe 111, Maastricht, The Netherlands

Petroleum Distillates in Mixtures

Selenium

Toxic Metals in Paints
Warren K. Porter, Jr, Consumer Product Safety Commission, 200 C St, SW, Washington, DC 20204

Turpentine

Viscosity of Liquids

PESTICIDE FORMULATIONS: CARBAMATE AND SUBSTITUTED UREA INSECTICIDES

Referee: Paul Jung, Environmental Protection Agency, Chemical Laboratory, Beltsville, MD 20705

Aldicarb
William H. McDermott, Union Carbide Corp., Agricultural Products Division, Box 12014, Research Triangle Park, NC 27709

Aminocarb
Steven C. Slahck, Mobay Chemical Corp., Agricultural Chemicals Division, Box 4913, Kansas City, MO 64120

Carbaryl
William H. McDermott

Carbofuran and Carbosulfan
E. J. Kikta, FMC Corp., Niagara Chemical Division, 100 Niagara St, Middleport, NY 14105

2,2-Dimethyl-1,3-benzodioxol-4-yl Methylcarbamate (Bendiocarb®)
Peter L. Carter, Fisons, Ltd, Agrochemical Division, Hauxton, Cambridge, CB2 5HU, UK

***o*-Isopropoxyphenyl Methylcarbamate (Propoxur®)**
Steven C. Slahck

4-(Methylthio)-3,5-xylyl Methylcarbamate (Methiocarb)
Steven C. Slahck

Methomyl
James E. Conaway, Jr, E. I. du Pont de Nemours & Co., Biochemicals Division, Wilmington, DE 19898

Oxamyl
Glenn A. Sherwood, Jr, E. I. du Pont de Nemours & Co., Biochemicals Department, Experiment Station, Wilmington, DE 19898

Pirimicarb
Peter D. Bland, ICI Americas, Inc., Biological Research Center, Box 208, Goldsboro, NC 27530

PESTICIDE FORMULATIONS: FUNGICIDES AND DISINFECTANTS

Referee: Thomas Jensen, State Department of Agriculture, 3703 S 14th St, Lincoln, NE 68502

Benomyl
Wyatt Teubert, Purdue University, Department of Biochemistry, West Lafayette, IN 47907

Captan
A. Aner Carlstrom, Chevron Chemical Co., 940 Hensley St, Richmond, CA 94804

Carboxin and Oxycarboxin
Milton Parkins, Sr, Uniroyal Chemical Co., Crop Protection Analytical Division, Naugatuck, CT 06770

Chlorothalonil
Brian H. Korsch, Diamond Shamrock Co., PO Box 348, Painesville, OH 44079

Copper Naphthenate

Dinocap

Dithiocarbamate Fungicides

Folpet

Pentachloronitrobenzene
Alan R. Hanks, Purdue University, Office of the State Chemist, West Lafayette, IN 47907

o-Phenylphenol

Triphenyltin

PESTICIDE FORMULATIONS: GENERAL METHODS

Referee: Paul D. Jung, Environmental Protection Agency, Office of Pesticide Programs, Beltsville, MD 20705

Atomic Absorption Spectroscopy
Paul D. Jung

Contaminants in Pesticide Formulations
Warren R. Bontoyan, Environmental Protection Agency, Office of Pesticide Programs, Beltsville, MD 20705

Dioxins (2,3,7,8-Tetrachlorodibenzo-*p*-dioxin in 2,4,5-T)
Ronald Thomas, Environmental Protection Agency, Office of Pesticide Programs, Beltsville, MD 20705

Nitrosamines
Dallas Wright, Jr, Environmental Protection Agency. Chemistry Laboratory, Beltsville, MD 20705

Pesticides in Spray Tank Dispersions
Robert Speth, Department of Agriculture, 350 Capitol Hill Ave, Box 11100, Reno, NV 89510

Physical Properties of Pesticides
Keith G. Seymour, Dow Chemical Co., Agricultural Research Department, Box 1706, Midland, MI 48640

Sampling
Lee C. Heinrichs, Ciba-Geigy Corp., Agricultural Division, PO Box 11422, Greensboro, NC 27409

Sampling of Pressurized Cans (Aerosols)

Volatility of Hormone-Type Herbicides
Spencer Duffy, Environmental Protection Agency, Beltsville, MD 20705

Water-Soluble Copper in Water-Insoluble Copper Fungicides

PESTICIDE FORMULATIONS: HALOGENATED INSECTICIDES

Referee: James Launer, State Department of Agriculture, Laboratory Services, 635 Capitol St, NE, Salem, OR 97310

Benzene Hexachloride and Lindane
Abram Davis, Hooker Chemical Co., Research and Development, Grand Island, NY 14072

Chlordane
John E. Forrette, Velsicol Chemical Corp., 341 E Ohio St, Chicago, IL 60611

Chlordimeform
Arthur H. Hofberg, Ciba-Geigy Corp., Analytical Chemistry Division, 410 Swing Rd, Greensboro, NC 27409

Dicofol
Alan M. Rothman, Rohm and Haas Co., Research Laboratories, 727 Norristown Rd, Spring House, PA 19477

Diflubenzuron
A. Van Rossum, Duphar BV, Box 4, 1243 ZG'S-Groveland, The Netherlands

Endosulfan
Robert W. Watson, FMC Corp., Agricultural Chemical Division, 2501 Sunland Ave, Fresno, CA 93717

Fenvalerate
R. D. Collins, Shell Development Co., PO Box 4248, Modesto, CA 95352

Heptachlor
John E. Forrette

Methoxychlor
George E. Walser, E. I. du Pont de Nemours & Co., Biochemicals Department, Wilmington, DE 19898

Perthane
Michael Sabbann, Department of Agriculture, Division of Laboratory Services, St Paul, MN 55107

Toxaphene
William H. Clark, Hercules, Inc., Analytical Division, Research Center, Wilmington, DE 19898

Trichlorfon (Dylox®)
Michael Sabbann

PESTICIDE FORMULATIONS: HERBICIDES I

Referee: Marshall Gentry, Florida Department of Agriculture and Consumer Services, Mayo Building, Tallahassee, FL 32301

Chlorophenoxy Herbicides
Robert B. Grorud, North Dakota State Laboratories, Lock Box 937, Bismarck, ND 58501

Dicamba
John Forrette, Velsicol Chemical Corp., 341 E Ohio St, Chicago, IL 60611

Pentachlorophenol
Elmer H. Hayes, Environmental Protection Agency, Chemistry Laboratory, Beltsville, MD 20705

Plant Growth Regulators
Richard K. Gard, Office of Indiana State Chemist, Purdue University, West Lafayette, IN 47907

2,3,6-Trichlorobenzoic Acid
——

PESTICIDE FORMULATIONS: HERBICIDES II

Referee: Laszlo Torma, State Department of Agriculture, Montana State University, Bozeman, MT 59715

Alanap
Pat Parkins, Uniroyal Chemical, Crop Protection Chemical Branch, Naugatuck, CT 06770

Barban
John Forrette, Velsicol Chemical Co., 341 E Ohio St, Chicago, IL 60611

Bensulide
William Y. Ja, Stauffer Chemical Co., Richmond Research Center, 1200 S 47th St, Richmond, CA 94804

Benzoylprop-Ethyl
——

Bromacil and Lenacil
Paul K. Tseng, E. I. du Pont de Nemours & Co., Biochemicals Dept., Wilmington, DE 19898

Chloroxuron
Arthur H. Hofberg, Ciba-Geigy Corp., Analytical Chemistry Division, 410 Swing Rd, Greensboro, NC 27409

Dimethyl Tetrachloroterephthalate
Brian Korsch, Diamond Shamrock Corp., PO Box 348, Painesville, OH 44077

Dinoseb
——

Diuron
Glenn A. Sherwood, E. I. Dupont de Nemours, Biochemicals Department, Wilmington DE 19898

***S*-Ethyl Dipropylthiocarbamate**
——

Fluchloralin, Profluralin, Benefin, Trifluralin, and Penoxalin
Roger Stringham, Purdue University, Department of Biochemistry, West Lafayette, IN 47907

Fluometuron
Arthur H. Hofberg

Linuron
Glenn A. Sherwood

Methazole
John Forrette

Monuron
——

Oryzalin
——

Paraquat, HPLC Analysis
Lynn Hageman, Montana Dept. of Agriculture, Montana State University, Bozeman, MT 59717

Siduron
Glenn A. Sherwood

Thiocarbamate Herbicides
William Y. Ja

PESTICIDE FORMULATIONS: HERBICIDES III

Referee: Thomas L. Jensen, State Department of Agriculture, 3703 S 14th St, Lincoln, NE 68502

Alachlor, Butachlor, and Propachlor
L. A. Furrer, Monsanto Agricultural Products Co., 800 N Lindburgh Blvd, St. Louis, MO 63166

Amitrol

Bentazone

Bromoxynil
Laurence J. Helfant, Amchem Products Inc., Agricultural Chemicals Laboratory, Ambler, PA 19002

Cacodylic Acid

Cyanazine (Bladex®)

Dalapon
Timothy S. Stevens, Dow Chemical Co., Analytical Laboratories, Midland, MI 48640

Dichlobenil
Edward E. Chapman, Thompson-Hayward Co., Box 2383, Kansas City, KS 66106

Disodium Methane Arsenate

Fluazifop-butyl
Peter D. Bland, ICI Americas, Inc., Biological Research Center, Box 208, Goldsboro, NC 27530

Glyphosate (Isopropylamine Salt *N*-(Phosphoromethyl) Glycine)
Arnold J. Burns, Monsanto Agricultural Products Co., PO Box 174, Luling, LA 70070

Metolachlor
Arthur H. Hofberg, Ciba-Geigy Corp., Analytical Section, Box 18300, Greensboro, NC 27419

Metribuzin
William Betker, Mobay Chemical Co., Agricultural Chemicals Division, Box 4913, Kansas City, MO 64120

Monosodium Methane Arsenate

Propanil
Delmas Pennington, Rohm and Haas, PO Box 591, Knoxville, TN 37901

Triazine Herbicides
Arthur H. Hofberg

PESTICIDE FORMULATIONS: INORGANIC PESTICIDES

Referee: Paul D. Jung, Environmental Protection Agency, Office of Pesticide Programs, Beltsville, MD 20705

Aluminum Phosphide
Donald Shaheen, Degesch America, Inc., Box 116, Weyers Cave, VA 24486

Sodium Chlorate

PESTICIDE FORMULATIONS: OTHER INSECTICIDES, SYNERGISTS, AND INSECT REPELLANTS

Referee: James Launer, State Department of Agriculture, Laboratory Services, Salem, OR 97310

Allethrin
Dean Kassera, McLaughlin Gormley King Co., 8810 Tenth Ave, Minneapolis, MN 55427

2,3:4,5-Bis(2-butylene)tetrahydro-2-furaldehyde (MGK 11®)
Vernon Meinen, McLaughlin Gormley King Co., 8810 Tenth Ave, Minneapolis, MN 55427

Cypromethrin
Peter D. Bland, ICI Americas, Inc., Biological Research Center, Box 208, Goldsboro, NC 27530

Dipropyl Isocinchomeronate (MGK 326®)
Dave Carlson, McLaughlin Gormley King Co., 8810 Tenth Ave, Minneapolis, MN 55427

Fumigants
Dean Yeaman, Dow Chemical Co., Analytical Laboratories, Pittsburg, CA 94565

Methyl Bromide

Nicotine
Spencer Carrigan, Department of Agriculture, University of Maryland, College Park, MD 20742

Permethrin
Hershel F. Morris, Louisiana Dept. of Agriculture, Box 16390-A, University Station, Baton Rouge, LA 70893

Piperonyl Butoxide and Pyrethrins
Dean Kassera

Resmethrin
Mark Law, Environmental Protection Agency, TSD-Chemical & Biological Investigation, Beltsville, MD 20705

Rotenone and Other Rotenoids
Rodney J. Bushway, University of Maine, Agricultural Experiment Station, Orono, ME 04469

PESTICIDE FORMULATIONS: OTHER ORGANOPHOSPHORUS INSECTICIDES

Referee: Marshall Gentry, Florida Department of Agriculture and Consumer Services, Division of Chemistry, Tallahassee, FL 32301

Crotoxyphos
Wendy King, Florida Department of Agriculture & Consumer Services, Tallahassee, FL 32304

Crufomate (Ruelene)
——

Dichlorvos
Norman A. Epstein, Diamond Shamrock Corp., Box 813, Princeton, NJ 08540

Mevinphos
Harry O. Holly, Asgrow Florida, Drawer D, Plant City, FL 33565

Monocrotophos
George Winstead, State Department of Agriculture, Pesticide Laboratory, Raleigh, NC 27611

Naled
A. Aner Carlstrom, Chevron Chemical Co., 940 Hensley St, Richmond, CA 94804

Tetrachlorvinphos
Norman A. Epstein

PESTICIDE FORMULATIONS: RODENTICIDES AND MISCELLANEOUS PESTICIDES

Referee: Marshall Gentry, Florida Department of Agriculture and Consumer Services, Division of Chemistry, Tallahassee, FL 32301

Brodifacoum (Talon®)
Peter D. Bland, ICI Americas Inc., Biological Research Center, Box 208, Goldsboro, NC 27530

Chlorophacinone
——

Diphacinone
Violet M. Stephens, State Department of Agriculture, Division of Laboratory Services, 510 State Office Building, St. Paul, MN 55155

α-Naphthylthiourea
——

***N*-3-Pyridyl-*N'*-*p*-Nitrophenyl Urea (Vacor®)**
——

Strychnine
——

Warfarin
Elmer Hayes, Environmental Protection Agency, Chemistry Laboratory, Beltsville, MD 20705

PESTICIDE FORMULATIONS: ORGANOTHIOPHOSPHATE INSECTICIDES

Referee: Edwin R. Jackson, Mississippi State Chemical Laboratory, Box CR, Mississippi State, MS 39762

Acephate
A. Aner Carlstrom, Chevron Chemical Co., 940 Hensley St, Richmond, CA 94804

Azinphosmethyl
Charles J. Cohen, Mobay Chemical Corp., Box 4913, Kansas City, MO 64120

Chlorpyriphos
Norman E. Skelly, Dow Chemical Co., Building 574,Midland, MI 48640

Coumaphos
Linda Ruiz, Bayvet Division of Cutter Labs, Box 390, Shawnee, KS 66201

Demeton
——

Demeton-*S*-Methyl
——

Diazinon
Arthur H. Hofberg, Ciba-Geigy Corp., Analytical Chemistry Division, 410 Swing Rd, Greensboro, NC 27409

Dimethoate
Richard S. Wayne, American Cyanamid Co., Agriculture Division, Box 400, Princeton, NJ 08540

Dioxathion
William H. Clark, Hercules, Inc., Analytical Division, Wilmington, DE 19898

Disulfoton
Thomas L. Jensen, State Department of Agriculture, 3703 S 14th St, Lincoln, NE 68502

Encapsulated Organophosphorus Pesticides
James J. Karr, Pennwalt Technological Center, 900 First Ave, Box C, King of Prussia, PA 19406

EPN
John Forrette, Velsicol Chemical Corp., 341 E Ohio St, Chicago, IL 60611

Ethion
James Launer, State Department of Agriculture, Laboratory Services, 635 Capitol St, NE, Salem, OR 97310

Ethoprop
Wallace Embry, Rhone-Poulenc, Inc., Box 352, Mt Pleasant, TN 38474

***O*-Ethyl *O*-(4-Methylthio) Phenyl *S*-Propyl Phosphorothioate**
Willard G. Boyd, Jr, State Chemical Laboratory, Box CR, Mississippi State, MS 39762

Fensulfothion
Margie Owen, State Chemical Laboratory, Box 329, Auburn, AL 36830

Fenthion
Willard G. Boyd, Jr

Fonophos
——

Malathion
Richard S. Wayne

Methidathion
Thomas Gale, Ciba-Geigy Corp., Box 18300, Greensboro, NC 27409

Oxydemeton-Methyl

Parathion and Methyl Parathion
Edwin R. Jackson

Phorate (*O,O*-Diethyl *S*-[(Ethylthio)methyl] Phosphorodithioate)
Roman Grypa, Agway Inc., Fertilizer Division, 978 Loucks Hill Rd, York, PA 17402

Temephos

PLANTS

Referee: J. Benton Jones, Jr, University of Georgia, Soil Testing and Plant Laboratory, Athens, GA 30602

Ashing Methods
J. Benton Jones, Jr

Atomic Absorption Methods
Robert A. Isaac, University of Georgia, College of Agriculture, Athens, GA 30602

Boron
James R. Melton, Texas A&M University, Agricultural Analytical Services, College Station, TX 77843

Chromium
Earle E. Cary, U.S. Department of Agriculture, Plant, Soil, and Nutritional Laboratory, Tower Rd, Ithaca, NY 14853

Copper and Cobalt
Duane Boline, Radian Corp., Box 9948, Austin, TX 78766

Emission Spectroscopy
Robert A. Isaac

Fluoride
Jay S. Jacobson, Boyce Thompson Institute, Tower Rd, Ithaca, NY 14853

Nitrogen, Nonprotein

Selenium
Oscar E. Olson, South Dakota State University, Experiment Station, Biochemistry Department, Brookings, SD 57006

Starch
T. Powell Gaines, University of Georgia, College of Agriculture, Department of Agronomy, Tifton, GA 31797

Sulfa in Plants
Charles W. Gehrke, University of Missouri-Columbia, Columbia, MO 65211
Larry W. Wall, University of Missouri-Columbia, Columbia, MO 65211

Sulfur

Zinc
Duane Boline

REFERENCE MATERIALS AND STANDARD SOLUTIONS

Referee: Robert Alvarez, U.S. Department of Commerce, National Bureau of Standards, Office of Standard Reference Materials, Washington, DC 20234

Stability of Organophosphorus Pesticide Standards
Gregory Doose, Food and Drug Administration, 1521 W Pico Blvd, Los Angeles, CA 90015

TOBACCO

Referee: John F. Benner, 1344 Royalty Ct, Lexington, KY 40504

Differentiation of Cigar and Cigarette Tobaccos (Sequential Differential Solvent Extraction)
John A. Steele, Department of the Treasury, Bureau of Alcohol, Tobacco and Firearms, 1401 Research Blvd, Rockville, MD 20850

Humectants in Cased Cigarettes

Nicotine, Gas Chromatography
John R. Wagner, Lorillard Corp., 426 English St, Greensboro, NC 27420

Tar and Nicotine in Cigarette Smoke
Harold C. Pillsbury, Federal Trade Commission, 6th and Pennsylvania Ave, NW, Washington, DC 20580

COMMITTEE B

Evelyn Sarnoff (Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232), *Chairman;* William W. Wright (U.S. Pharmacopeial Convention, 12601 Twinbrook Pkwy, Rockville, MD 20852); Anthony Romano, Jr (Drug Enforcement Administration, Southeast Regional Laboratory, 5205 NW 84th Ave, Miami, FL 33166); John Zarembo (Revlon Health Care, 1 Scarsdale Rd, Tuckahoe, NY 10707); Thomas Layloff (Food and Drug Administration, 1114 Market St, St. Louis, MO 63101); Eric Sheinen (Food and Drug Administration, Division of Drug Chemistry, Washington, DC 20204), *Secretary;* Roswitha E. Kelly (Food and Drug Administration, Bureau of Medical Devices, 8757 Georgia Ave, Silver Spring, MD 20853), *Statistical Consultant*

DRUGS, ACIDIC

Referee: James W. Fitzgerald, Food and Drug Administration, Winchester Engineering and Analytical Center, 109 Holton St, Winchester, MA 01890

Acetaminophen in Drug Mixtures
David J. Krieger, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Amitryptyline HCl in Dosage Forms (HPLC)
Samuel Walker, Food and Drug Administration, 850 Third Ave, Brooklyn. NY 11232

Aspirin, Phenacetin, and Caffeine with Other Drugs
Douglas D. Don, Food and Drug Administration, 1521 W Pico Blvd, Los Angeles, CA 90015

Aspirin and Salicylic Acid in Aspirin Products (Semiautomated Analysis)
William E. Juhl, Food and Drug Administration, National Center for Drug Analysis, 1114 Market St, St. Louis, MO 63101

Barbiturates
Loren Gelber, 1804 Beacon St, Brookline, MA 02146

Benzothiazine Derivatives

Benzthiazide by HPLC
Stephen Hauser, Food and Drug Administration, Winchester Engineering and Analytical Center, 109 Holton St, Winchester, MA 01890

Methyldopa
Susan Ting, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Primidone
Stanley E. Roberts, Food and Drug Administration, Winchester Engineering and Analytical Center, 109 Holton St, Winchester, MA 01890

Probenecid
Alexander G. Korzun, Food and Drug Administration, Winchester Engineering and Analytical Center, 109 Holton St, Winchester, MA 01890

Sulfamethoxazole in Tablets (HPLC)
John W. Robinson, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Sulfisoxazole in Dosage Forms (HPLC)
Robert W. Roos, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Sulfonamides (Thin Layer Chromatography)
Charlotte A. Brunner, Food and Drug Administration, Division of Drug Chemistry, Washington, DC 20204

Thiazide Diuretics, Semiautomated Individual Dosage Unit Analysis
Terry W. Moore, Food and Drug Administration, National Center for Drug Analysis, 1114 Market St, St Louis, MO 63101

DRUGS, ALKALOIDS AND RELATED BASES

Referee: Edward Smith, Food and Drug Administration, Division of Drug Chemistry, Washington, DC 20204

Aminacrine
Elaine A. Bunch, Food and Drug Administration, 909 First Ave, Seattle, WA 98174

Atropine in Morphine, Atropine Tablets, and Injections

Belladonna Alkaloids

Chlorpromazine
Donald J. Smith, Food and Drug Administration, Division of Drug Chemistry, Washington, DC 20204

Colchicine in Tablets
Richard D. Thompson, Food and Drug Administration, 240 Hennepin Ave, Minneapolis, MN 55401

Curare Alkaloids
John R. Hohmann, Food and Drug Administration, Division of Drug Biology, Washington, DC 20204

Dicyclomine Capsules
Charles L. Brownell, Food and Drug Administration, 433 W Van Buren St, Chicago, IL 60607

Epinephrine Lidocaine Combinations
Donald J. Smith

Epinephrine and Related Compounds by HPLC-Electrochemical Detectors
John M. Newton, Food and Drug Administration, 50 Fulton St, San Francisco, CA 94102

Ergot Alkaloids
Thomas C. Knott, Food and Drug Administration, 900 Madison Ave, Baltimore, MD 21201

Homatropine Methyl Bromide in Tablets
Duane Hughes, Food and Drug Administration, 1009 Cherry St, Kansas City, MO 64016

Neostigmine
Rita E. Kling, Food and Drug Administration, 2nd and Chestnut Sts, Philadelphia, PA 19106

Phenethylamine Drugs, Semiautomated Individual Unit Analysis
Percy A. McCullen, Food and Drug Administration, National Center For Drug Analysis, 1114 Market St, St. Louis, MO 63101

Pheniramine with Pyrilamine, Phenylpropanolamine, and Phenylephrine
Henry S. Scroggins, Food and Drug Administra-

tion, 4298 Elysian Fields Ave, New Orleans, LA 70122

Phenothiazines in Drugs
Edward G. Lovering, Health Protection Branch, Drug Research Lab., Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2

Physostigmine and Its Salts
Norlin W. Tymes, Food and Drug Administration, 900 Madison Ave, Baltimore, MD 21201

Pilocarpine
Irving Wainer, Food and Drug Administration, Division of Drug Chemistry, Washington, DC 20204

Rauwolfia Alkaloids
Susan Barkan, Food and Drug Administration, Division of Drug Chemistry, Washington, DC 20204

Rauwolfia serpentina
Ugo R. Cieri, Food and Drug Administration, 2nd and Chestnut Sts, Philadelphia, PA 19106

TLC Identification of Phenothiazine-type Drugs
Kurt Steinbrecher, Food and Drug Administration, 909 First Ave, Seattle, WA 98174

DRUGS, GENERAL

Referee: Ted M. Hopes, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Ampicillin and Amoxicillan
Michel Margosis, Food and Drug Administration, Washington, DC 20204

Disulfiram
Edward J. Wojtowicz, Food and Drug Administration, 599 Delaware Ave, Buffalo, NY 14202

Ethylene Oxide
Richard J. Muzeni, Food and Drug Administration, 850 Third Ave., Brooklyn, NY 11232

Fluoride
John R. Marzilli, Food and Drug Administration, 585 Commercial St, Boston, MA 02109

Medicinal Gases
Martin Woodhouse, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Menadiol Sodium Diphosphate Injection
Maurice Y. Alpert, Food and Drug Administration, Winchester Engineering and Analytical Center, 109 Holton St, Winchester, MA 01890

Mercurial Diuretics
Eddie J. Rigsby, Food and Drug Administration, 4298 Elysian Fields Ave, New Orleans, LA 70122

Mercury-Containing Drugs
Walter Holak, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Metals in Drug Bulk Powders
Walter Holak

Microcrystalline Tests

Protein Nitrogen Units in Allergenic Extracts
Joan May, Food and Drug Administration, Bureau of Biologics, 8800 Rockville Pike, Bethesda, MD 20014

Thyroid and Thyroxine Related Compounds
Donald J. Smith, Food and Drug Administration, Division of Drug Chemistry, Washington, DC 20204

Thyroid by Differential Pulse Polarography
Walter Holak

DRUGS, ILLICIT

Referee: Charles C. Clark, Drug Enforcement Administration, 5205 NW 84th Ave, Miami, FL 33166

Amphetamines in Mixtures

Benzodiazepines
Eileen Bargo, Food and Drug Administration, 900 Madison Ave, Baltimore, MD 21201

Chemical Microscopy
Richard Ruybal, Drug Enforcement Administration, 1114 Commerce St, Dallas, TX 75202

Cocaine
Charles C. Clark

Dimethyltryptamine (DMT), Diethyltryptamine (DET), and Dipropyltryptamine (DPT)

Heroin
Harold F. Hanel, Drug Enforcement Administration, 5205 NW 84th Ave, Miami, FL 33166

Lysergic Acid Diethylamide (LSD)

Marihuana and Synthetic Tetrahydrocannabinol (THC)

Methadone

Methamphetamine

Methaqualone Hydrochloride
Harold F. Hanel

Methylphenidate Phenidine Hydrochloride

Optical Crystallographic Properties of Drugs
Robert S. Ferrera, Drug Enforcement Administration, 7704 Old Springhouse Rd, McLean, VA 22101

Phencyclidine (PCP)
Charles C. Clark

DRUGS, NEUTRAL

Referee: Thomas G. Alexander, Food and Drug Administration, Division of Drug Chemistry, Washington, DC 20204

Automated Corticosteroid Methods
James F. Brower, Food and Drug Administration, National Center for Drug Analysis, 1114 Market St, St. Louis, MO 63101

Automated Methods for Progestins in Tablets
Larry K. Thornton, Food and Drug Administration, National Center for Drug Analysis, 1114 Market St, St. Louis, MO 63101

Digitoxin, Automated Individual Tablet Analysis
Benjamin Westenberger, Food and Drug Administration, National Center for Drug Analysis, 1114 Market St, St. Louis MO 63101

Estrogens

Estrogens (Fluorometric Method)
Robert W. Roos, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Ethinyl Estradiol, Automated Individual Tablet Analysis
Rudolph F. Kulousek, Food and Drug Administration, National Center for Drug Analysis, 1114 Market St, St. Louis, MO 63101

Steroid Acetates
Halver C. Van Dame, Merck Sharpe & Dohme Research Laboratories, West Point, PA 19486

Steroid Phosphates
Richard M. Venable, Food and Drug Administration, Division of Drug Chemistry, Washington, DC 20204

COMMITTEE C

D. Earle Coffin (Health and Welfare Canada, Nutritional Services, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2), *Chairman*; Arthur E. Waltking (CPC International, Inc., 1120 Commerce Ave, Union, NJ 07083); Raymond Ashworth (U.S. Department of Agriculture, Food Safety and Inspection Service, Beltsville, MD 20705); Odette L. Shotwell (U.S. Department of Agriculture, Northern Regional Research Center, Peoria, IL 61604); Betsy Woodward (State Department of Agriculture and Consumer Services, Tallahassee, FL 32304); H. B. S. Conacher (Health and Welfare Canada, Food Research Division, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2); Arthur R. Johnson (Food and Drug Administration, Division of Food Technology, Washington, DC 20204), *Secretary;* Michael O'Donnell (Food and Drug Administration, Division of Mathematics, Washington, DC 20204), *Statistical Consultant*

COFFEE AND TEA

Referee: Robert H. Dick, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Ash in Instant Tea
Francis J. Farrell, Thomas J. Lipton, Inc., 800 Sylvan Ave, Englewood Cliffs, NJ 07632

Caffeine
John M. Newton, Food and Drug Administration, 50 Fulton St, San Francisco, CA 94102

Crude Fiber in Tea

Moisture in Coffee and Tea
William P. Clinton, General Foods Corp., White Plains, NY 10625

Solvent Residues in Decaffeinated Coffee and Tea
B. Denis Page, Health and Welfare Canada, Health Protection Branch, Ottawa, Ontario, Canada K1A 0L2

Theophylline in Tea
John M. Newton

Water Extract in Tea
Elpidio de la Teja, Thomas J. Lipton, Inc., Analytical Section, 800 Sylvan Ave, Englewood Cliffs, NJ 07632

DAIRY PRODUCTS

Referee: Robert W. Weik, Food and Drug Administration, Bureau of Foods, Washington, DC 20204

Adulteration of Dairy Products with Vegetable Fat
Graham MacEachern, Agriculture Canada, Plant Products, Ottawa, Ontario, Canada K1A 0C5

Casein and Caseinates
Charles Pynes, Stauffer Chemical Co., Technical Sales and Development Department, Westport, CT 06880

Chocolate Milk, Fat Test
James T. Marshall, Kansas State University, Department of Animal Science, Manhattan, KS 66506

Cryoscopy of Milk
Robert W. Henningson, Clemson University, Office of University Research, Clemson, SC 29631

Fat, Automated Methods
W. Frank Shipe, Cornell University, Department of Dairy and Food Science, Ithaca, NY 14853

Fat in Milk (AutoAnalyzer)
Raymond L. King, University of Maryland, Department of Dairy Science, College Park, MD 20742

Infrared Milk Analyzer (IRMA)
D. A. Biggs, University of Guelph, Department of Food Science, Guelph, Ontario, Canada N1G 2W1

Lactose in Dairy Products (Chromatographic Determination)
Leslie G. West, Kraft Co., 801 Waukegan Rd, Glenview, IL 60025

Lactose in Dairy Products (Enzymatic Determination)
Dick H. Kleyn, Rutgers University, Department of Food Science, New Brunswick, NJ 08903
John W. Sherbon, Cornell University, Department of Dairy and Food Science, Ithaca, NY 14853

Moisture in Cheese
Ronald Case, Kraft Foods, 500 Peshtigo Ct, Chicago, IL 60690

Moisture in Cheese (Karl Fischer Method)
Gary H. Richardson, Utah State University, Department of Nutrition and Food Science, Logan, UT 84322

Nitrates in Cheese
James E. Hamilton, Food and Drug Administration, Division of Drug Labeling—Compliance, 5600 Fishers Lane, Rockville, MD 20857

Phosphatase, Rapid Method
Dick H. Kleyn

Phosphatase, Reactivated
Gopala K. Murthy, Food and Drug Administration, Division of Microbiology, 1090 Tusculum Ave, Cincinnati, OH 45226

Phosphorus
Wallace S. Brammell, Food and Drug Administration, Division of Color Technology, Washington, DC 20204

Protein Constituents in Processed Dairy Products
Frederick W. Douglas, Jr, U.S. Department of Agriculture, Eastern Regional Research Center, Philadelphia, PA 11918

Protein in Milk, Rapid Tests
John W. Sherbon

Protein Reducing Substance Tests
Joseph T. Cardwell, Mississippi State University, Dairy Science Department, Mississippi State, MS 39762

Solids-Not-Fat
John W. Sherbon

Vapor Pressure Osmometry
Gary H. Richardson

DECOMPOSITION AND FILTH IN FOODS (CHEMICAL METHODS)

Referee: Walter F. Staruszkiewicz, Jr, Food and Drug Administration, Division of Food Technology, Washington, DC 20204

Ammonia in Dogfish
Beverly Smith, National Marine Fisheries Service, PO Drawer 1207, Pascagoula, MS 39567

Coprostanol
James G. Stewart, Food and Drug Administration, 3032 Bryan St, Dallas, TX 75204

Crabmeat
Kurt Steinbrecher, Food and Drug Administration, 909 First Ave, Seattle, WA 98104

Diacetyl in Citrus Products
W. S. Hatcher, The Coca-Cola Co., Plymouth, FL 32768

Ethanol in Seafoods
Harold R. Throm, Food and Drug Administration, 909 First Ave, Seattle, WA 98104

Gas and Liquid Chromatography
Walter F. Staruszkiewicz, Jr.

GLC Determination of Volatile Amines—TMA and DMA
Ronald C. Lundstrom, National Marine Fisheries Service, Northeast Fisheries Center, Gloucester, MA 01930

Shellfish
Theodore L. R. Chambers, Food and Drug Administration, Division of Food Technology, Washington, DC 20204

TLC Determination of Amines in Fishery Products
Thomas R. Weber, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Tomatoes
Albert Y. Taira, Food and Drug Administration, 433 W Van Buren St, Chicago, IL 60607

FISH AND OTHER MARINE PRODUCTS

Referee: Louis L. Gershman, Food and Drug Administration, 585 Commercial St, Boston, MA 02109

Crabmeat, Identification
Judith Krzynowek, National Marine Fisheries Service, Northeast Fisheries Center, Gloucester, MA 01930

Drained Weight of Block Frozen Raw, Peeled Shrimp
Frederick J. King, National Marine Fisheries Service, Northeast Fisheries Center, Gloucester, MA 01930

Drip Fluid in Fish Fillets and Fish Fillet Blocks—Quantitation
Frederick J. King

Determination of Fish Content in Coated Products (Breaded or in Batter)
Fredrick J. King
H. Houwing, TNO, Division for Nutrition and Food Research, Box 183, 1970 AD, Ijmuiden, The Netherlands

Fish Species Identification (Thin Layer Isoelectric Focusing)
Ronald C. Lundstrom, National Marine Fisheries Service, Northeast Fisheries Center, Gloucester, MA 01930

Nitrites in Smoked Fish
Charles Cardile, Food and Drug Administration, 850 Third Ave., Brooklyn, NY 11232

Organometallics in Fish
Walter Holak, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

FOOD ADDITIVES

Referee: Thomas Fazio, Food and Drug Administration, Division of Chemistry and Physics, Washington, DC 20204

Anticaking Agents

Antioxidants
B. Denis Page, Health and Welfare Canada, Food Research Division, Tunney's Pasture, Ottawa, Ontario, Canada K1A OL2

Brominated Oils
James F. Lawrence, Health and Welfare Canada, Food Research Division, Tunney's Pasture, Ottawa, Ontario, Canada K1A OL2

Chloride Titrator
Alfred H. Free, Ames Co., Technical Services, Elkhart, IN 46514

Chlorobutanol in Milk

Dichlorodifluoromethane in Frozen Foods

Dilauryl Thiodipropionate

Dimethylpolysiloxane

Dressings
Charles R. Warner, Food and Drug Administration, Division of Chemistry and Physics, Washington, DC 20204

EDTA in Food Products
Gracia A. Perfetti, Food and Drug Administration, Division of Chemistry and Physics, Washington, DC 20204

Ethoxyquin in Meats and Eggs

Gums

Indirect Additives from Food Packages
Charles V. Breder, Food and Drug Administration, Division of Chemistry and Physics, Washington, DC 20204

Mineral Oil in Raisins
W. H. Bousfield, Australian Government Analytical Laboratory, GPO Box 2809 AA, Melbourne, Victoria 3001, Australia

Nitrates and Nitrites
Jay Fox, U.S. Department of Agriculture, Eastern Regional Research Center, Philadelphia, PA 19118

Nitrates (Selective Ion Electrode Titration)
Sandra L. Pfeiffer, Gerber Products Co., Central Research Division, Freemont, MI 49412

Nitrosamines
Nisu P. Sen, Health and Welfare Canada, Food Directorate, Tunney's Pasture, Ottawa, Ontario, Canada, K1A 0L2

Polycyclic Aromatic Hydrocarbons in Foods
Frank L. Joe, Food and Drug Administration, Division of Chemistry and Physics, Washington, DC 20204

Polysorbates
Charles F. Smullin, ICI United States Inc., Chemical Research Department, Wilmington, DE 19897

Propylene Chlorohydrin
Roberta M. Beebe, Food and Drug Administration, 50 United Nations Plaza, San Francisco, CA 94102

Sodium Lauryl Sulfate

MEATS, POULTRY, AND MEAT AND POULTRY PRODUCTS

Referee: Richard L. Ellis, U.S. Department of Agriculture, Scientific Services, Food Safety and Inspection Service, Washington, DC 20250

Automated Methods
Jon L. Schermerhorn, Department of Agriculture and Markets, New York State Food Laboratory, Albany, NY 12235

Bioassay Methods for Meat and Poultry Products

Bone Content
Paul Corrao, U.S. Department of Agriculture, Food Safety and Inspection Service, Beltsville, MD 20705

Chemical Antibiotic Methods

Fat in Meat Products
Jon E. McNeal, U.S. Department of Agriculture, Food Science and Inspection Service, Washington, DC 20250

Fat and Moisture Analysis, Rapid Methods
Julio D. Pettinati, U.S. Department of Agriculture, Eastern Regional Research Center, 600 E Mermaid Lane, Philadelphia, PA 19118

HPLC Methods for Meat and Poultry Products

Histologic Identification Methods
Albert M. Carey, U.S. Department of Agriculture, Food Safety and Inspection Service, Beltsville, MD 20705

Identification of Meats, Serological Tests

Nitrates and Nitrites
Francis B. Suhre, U.S. Department of Agriculture, Food Safety and Inspection Service, Beltsville, MD 20705

Nitrosamines in Bacon
Earl L. Greenfield, U.S. Department of Agriculture, Food Safety and Inspection Service, Beltsville, MD 20705

Non-Meat Proteins in Meat
Julio D. Pettinati
Khee C. Rhee, Texas A & M University, Food Protein Research and Development Center, College Station, TX 77843
Marion Greaser, University of Wisconsin, Muscle Biology Laboratory, Madison, WI 53706

Protein in Meat
Francis B. Suhre

Specific Ion Electrode Applications
Randy Simpson, U.S. Department of Agriculture, Food Safety and Inspection Service, Beltsville, MD 20705

Steroid Analysis

Sugars and Sugar Alcohol

Temperature, Minimum Processing
James Eye, U.S. Department of Agriculture, Food Safety and Inspection Service, Beltsville, MD 20705

MYCOTOXINS

Referee: Peter M. Scott, Health and Welfare Canada, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2

Aflatoxin M
Robert D. Stubblefield, U.S. Department of Agriculture, Northern Regional Research Center, Peoria, IL 61604

Aflatoxin Methods
Douglas L. Park, Food and Drug Administration, Office of Science, Washington, DC 20204

Alternaria Toxins
Douglas King, U.S. Department of Agriculture, Western Regional Research Center, 800 Buchanan St, Albany, CA 94710

Citrinin
David Wilson, University of Georgia, Department of Plant Pathology, Tifton, GA 31794

Ergot Alkaloids

Grains
Odette L. Shotwell, U.S. Department of Agriculture, Northern Regional Research Center, Peoria, IL 61604

Ochratoxins
Stanley Nesheim, Food and Drug Administration, Division of Chemistry and Physics, Washington, DC 20204

Penicillic Acid
Charles W. Thorpe, Food and Drug Administration, Division of Chemistry and Physics, Washington, DC 20204

Sterigmatocystin
Octave J. Francis, Jr, Food and Drug Administration, 4293 Elysian Fields Ave, New Orleans, LA 70122

Tree Nuts
Vincent P. DiProssimo, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Trichothecenes
Robert M. Eppley, Food and Drug Administration, Division of Chemistry and Physics, Washington, DC 20204

Zearalenone
Glenn A. Bennett, U.S. Department of Agriculture, Science and Education Administration, Northern Regional Research Center, Peoria, IL 61604

OILS AND FATS

Referee: David Firestone, Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Antioxidants
B. Denis Page, Health and Welfare Canada, Food Research Division, Health Protection Branch, Ottawa, Ontario, Canada K1A 0L2

Chromatographic Methods
William G. Doeden, Swift and Co., 1919 Swift Dr, Oak Brook, IL 60521

Cyclopropene Fatty Acids
Gordon Fisher, U.S. Department of Agriculture, Box 19687, 1100 Robert E. Lee Blvd, New Orleans, LA 70179

Emulsifiers
H. Bruschweiler, Laboratoire Federal d'Essai des Materiaux, Industrie, Genie Civil Arts et Metiers, 9001 St. Gallen, Unterstrasse II, Switzerland

Karl Fischer Method for Determination of Water
Raffaele Bernetti, CPC International, Moffett Technical Center, PO Box 345, Argo, IL 60501

Lower Fatty Acids
Giovanni Bigalli, Hershey Foods Corp., 1025 Reese Ave, Hershey, PA 17033

Marine Oils
Robert G. Ackman, Nova Scotia Technical College, Box 1000, Halifax, Nova Scotia, Canada B3J 2X4

Olive Oil Adulteration
Enzo Fedeli, Experimental Station for Oils and Fats, via Giuseppe Colompo 79, 20133 Milano, Italy

Oxidized Fats

Pork Fat in Other Fats
Laila El-Sayed, Cairo University, Faculty of Pharmacy, Cairo, Egypt

Spectrophotometric Methods
Alan J. Sheppard, Food and Drug Administration, Division of Nutrition, Washington, DC 20204

Sterols and Tocopherols
Hal T. Slover, U.S. Department of Agriculture, Nutrition Institute, Beltsville, MD 20705

PLANT TOXINS

Referee: Samuel W. Page, Food and Drug Administration, Division of Chemistry and Physics, Washington, DC 20204

Pyrrolizidine Alkaloids

Solanaceous Alkaloids

PROCESSED VEGETABLE PRODUCTS

Referee: Thomas R. Mulvaney, Food and Drug Administration, Division of Food Technology, Washington, DC 20204

Fibrous Material in Frozen Green Beans
George W. Varseveld, Oregon State University, Department of Food Science and Technology, Corvallis, OR 97331

pH Determination
Frederick E. Boland, Food and Drug Administration, Division of Food Technology, Washington, DC 20204

Sodium Chloride
Wallace S. Brammell, Food and Drug Administration, Division of Color Technology, Washington, DC 20204

Volume of Entrapped Air in Flexible Retort Pouches

Water Activity in Foods
William H. Stroup, Food and Drug Administration, Food Engineering Branch, 1090 Tusculum Ave, Cincinnati, OH 45226

SEAFOOD TOXINS

Referee: Edward P. Ragelis, Food and Drug Administration, Division of Chemistry and Physics, Washington, DC 20204

Ciguatoxins, Biochemical Methods
Yoshitsugi Hokama, University of Hawaii at Manoa, School of Medicine, Honolulu, HI 96844

Paralytic Shellfish Poisoning (Immunoassay Method)
Edward P. Ragelis

Shellfish Poisons
William L. Childress, Food and Drug Administration, 585 Commercial St, Boston, MA 02109

Tetradotoxins
Yururu Shimizu, University of Rhode Island, College of Pharmacy, Kingston, RI 02881

COMMITTEE D

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ALCOHOLIC BEVERAGES

Referee: Randolph H. Dyer, Bureau of Alcohol, Tobacco and Firearms, 1401 Research Blvd, Rockville, MD 20850

Acetate in Wines and Fruit Juice (Enzymatic Assay)
Leo P. McCloskey, 126 National St, Santa Cruz, CA 95060

Alcohol Content by Oscillating U-Tube Density Meter
Duane H. Strunk, Joseph E. Seagram & Sons, Inc., Research and Development Department, Box 240, Louisville, KY 40201

Alcohol Content of High Solids Distilled Spirits
Duane H. Strunk

β-Asarone
Randolph H. Dyer

Bromide Ion in Wine

Carbon Dioxide in Wine
Arthur Caputi, Jr, E. & J. Gallo Winery, PO Box 1130, Modesto, CA 95353

Citric Acid in Wine
Leonard Mascaro, Boehringer Mannheim, Biochemicals Co., Box 50816, Indianapolis, IN 46250

Color Intensity for Distilled Alcoholic Products
Duane H. Strunk

Coumarin in Wine
Randolph H. Dyer

Diethylpyrocarbonate in Beverages
Heinrich Wunderlich, Farbenfabriken Bayer A.G., Analytisches Laboratorium, 415 Krefeld-Uerdingen, Germany

Ethanol in Wine by GLC
Arthur Caputi, Jr

Flavor Compounds in Malt Beverages
George Charalambous, Anheuser-Busch Inc., Technical Center, St. Louis, MO 63118

Glycerol in Wine
Eric N. Christensen, E. & J. Gallo Winery, Box 1130, Modesto, CA 95353

Hydrogen Cyanide

Malic Acid in Wine
Robert Dowrie, Almaden Vineyards, 1530 Blossom Hill Rd, San Jose, CA 95118

Malt Beverages and Brewing Materials
Anthony J. Cutaia, Stroh Brewing Co., One Stroh Dr, Detroit, MI 48226

Sorbic Acid in Wine
Arthur Caputi, Jr

Sugars in Wine
Guenther Henniger, Boehringer Mannheim GmbH, Bahnhofstrasse 5, D-8132, Tutsing/Obb. Postfach 120, GFR

Sulfur Dioxide in Wine (Ripper Method)
James M. Vahl, Paul Masson Vineyards, PO Box 97, Saratoga, CA 95070

Tartrates in Wine
Masao Ueda, E. & J. Gallo Winery, PO Box 1130, Modesto, CA 95353

Vanillin and Ethyl Vanillin
Felipe Alfonso, Bureau of Alcohol, Tobacco and Firearms, 1401 Research Blvd, Rockville, MD 20850

Volatile Acidity in Wine
Gordon J. Pilone, The Christian Brothers, Mont La Salle Vineyards, PO Box 420, Napa, CA 94558

Volatile Congeners in Alcoholic Beverages
Duane H. Strunk

CACAO PRODUCTS

Referee: ——

Caffeine and Theobromine
Wesley R. Kreiser, Hershey Foods Corp., Hershey, PA 17033

Carbohydrates in Chocolate Products
William J. Hurst, Hershey Foods Corp., Hershey, PA 17033

Moisture in Cacao Products
Robert A. Martin, Hershey Foods Corp., Hershey, PA 17033

Shell in Cacao Products, Micro Methods
Wesley R. Kreiser

CEREAL FOODS

Referee: Doris Baker, U.S. Department of Agriculture, Nutrition Institute, Beltsville, MD 20705

Iron
James Martin, Food and Drug Administration, 1182 Peachtree St, NW, Atlanta, GA 30309

Phytates
Barbara F. Harland, Food and Drug Administration, Division of Nutrition, Washington, DC 20204

Starch in Raw and Cooked Cereals
Bert D'Appolonia, North Dakota State University, Department of Chemistry and Technology, Fargo, ND 58102

FLAVORS

Referee: ——

Additives in Vanilla Flavorings
Sidney Kahan, Fitelson Laboratories, 350 W 31st St, New York, NY 10001

Citral
——

Essential Oils
——

Glycyrrhizic Acid and Glycyrrhizic Acid Salts
Peter S. Vora, McAndrews and Forbes Co., Third St and Jefferson Ave, Camden, NJ 08104

Imitation Maple Flavors, Identification and Characterization
——

Organic Solvent Residues in Flavorings
——

Vanillin and Ethyl Vanillin in Food
Sidney Kahan

FRUITS AND FRUIT PRODUCTS

Referee: Frederick E. Boland, Food and Drug Administration, Division of Food Technology, Washington, DC 20204

Adulteration of Orange Juice by Pulpwash and Dilution
Donald R. Petrus, Florida Department of Citrus, Box 1088 AREC, Lake Alfred, FL 33850

Fruit Acids
Elia D. Coppola, Ocean Spray Cranberries, Inc., Research and Development, Bridge St, Middleboro, MA 02346

Fruit Juices, Identification and Characterization
——

Isoascorbic Acid (Erythorbic Acid-Antioxidant) in Fruit Purees
——

Orange Juice Content
Carl Vandercook, U.S. Department of Agriculture, Agricultural Research Service, Fruit and Vegetable Chemistry Laboratory, Pasadena, CA 91106

NONALCOHOLIC BEVERAGES

Referee: John M. Newton, Food and Drug Administration, 50 Fulton St, San Francisco, CA 94102

Caffeine and Methyl Xanthanes in Nonalcoholic Beverages
John M. Newton

Lasiocarpine and Pyrrolizidines in Herbal Beverages
——

PRESERVATIVES AND ARTIFICIAL SWEETENERS

Referee: William S. Adams, Food and Drug Administration, 585 Commercial St, Boston, MA 02109

Benzoates and Hydroxybenzoates in Food
——

Benzoates, Saccharin, and Caffeine, High Pressure Liquid Chromatography
Betsy Woodward, Florida Department of Agriculture and Consumer Services, Mayo Bldg, Tallahassee, FL 32304

Formaldehyde
Robert J. Reina, Food and Drug Administration, 585 Commercial St, Boston, MA 02109

Meats, Ground, Screening Methods for Chemical Preservatives
John J. Maxstadt, Department of Agriculture and Markets, New York State Food Laboratory, 120 Washington Ave, Albany, NY 12235

Organic Preservatives (Thin Layer Chromatography)
Colette P. Levi, General Foods Corp., White Plains, NY 10602

Preservatives (Quantitative Methods)

———

Saccharin and Its Salts
Walter Holak, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

SPICES AND OTHER CONDIMENTS

Referee: Raymond Way, Crescent Manufacturing Co., Box 3985, Seattle, WA 98124

Ash and Pungent Principles in Mustard

———

Extractable Color in Capsicum Spices and Oleoresins
James E. Woodbury, Cal-Compak Food, Inc., Quality Control, PO Box 265, Santa Ana, CA 92702

Moisture in Dried Spices
Louis A. Sanna, Santa Maria Chili, Inc., Box 1028, Santa Maria, CA 93456

Monosodium Glutamate in Foods

———

Pungency of Capsicums and Oleoresins

———

Vinegar

———

SUGARS AND SUGAR PRODUCTS

Referee: Arthur R. Johnson, Food and Drug Administration, Division of Food Technology, Washington, DC 20204

Chromatographic Methods
Michael Gray, Bio-Rad Laboratories, 32nd & Griffin Ave, Richmond, CA 94804

Color, Turbidity, and Reflectance-Visual Appearance
Frank G. Carpenter, U.S. Department of Agriculture, Southern Regional Research Laboratory, Box 19687, New Orleans, LA 70179

Corn Syrup and Corn Sugar
Raffaele Bernetti, CPC International, Box 345, Argo, IL 60501

Dry Substance
Joseph F. Dowling, Refined Syrups and Sugars Inc., 1 Federal St, Yonkers, NY 10702

Enzymatic Methods
Marc Mason, Yellow Springs Instrument Co., Box 279, Yellow Springs, OH 45387

HPLC Determination of Lactose at High Purity Levels

———

Honey
Jonathan W. White, Jr, 217 Hillside Dr, Navasota, TX 77868

Lactose Purity Testing
Janice R. Saucerman, Mead Johnson & Co., Environmental Analytical Services, Evansville, IN 47721

Maple Sap and Syrups
Maria Franca Morselli, University of Vermont, Botany Department, Burlington, VT 05405

Stable Carbon Isotope Ratio Analysis
Landis Doner, U.S. Department of Agriculture, Eastern Regional Research Center, 600 E Mermaid Lane, Philadelphia, PA 19118

Standardization of Sugar Methods of Analysis
Margaret A. Clarke, Sugar Processing Research, Inc., Box 19687, New Orleans, LA 70179

Sugar in Cereal
L. Zygmunt, Quaker Oats Co., 617 W Main St, Barrington, Il 60010

Sugar in Sugar Cane
Luis Vidaurreta, Louisiana State University, Chemistry Dept, 211 Choppin Hall, Baton Rouge, LA 70803

Weighing, Taring, and Sampling
Melvin Lerner, Department of the Treasury, Bureau of Customs, Washington, DC 20226

VITAMINS AND OTHER NUTRIENTS

Referee: Mike J. Deutsch, Food and Drug Administration, Division of Nutrition, Washington, DC 20204

Amino Acids
John P. Cherry, U.S. Department of Agriculture, Eastern Regional Research Center, 600 E Mermaid Ln, Philadelphia, PA 19118

Automated Nutrient Analysis
Jonathan De Vries, General Mills Inc., 9000 Plymouth Ave, Minneapolis, MN 55427

Biotin
Jacob M. Scheiner, Hoffmann-La Roche, Nutley, NJ 07110

Carotenoids
Forrest W. Quackenbush, Purdue University, Department of Biochemistry, West Lafayette, IN 47907

Choline in Feeds
Paul Anderson, Raltech, Box 7545, Madison, WI 53707

Dietary Fiber
Leon Prosky, Food and Drug Administration, Division of Nutrition, Washington, DC 20204

Energy Value of Foods (Biological)
Eugene Robiadek, Raltech, Box 7545, Madison, WI 53707

Fat in Food by Chloroform Methanol Extraction
Chester E. Daugherty, Campbell Soups, Campbell Place, Camden, NJ 08151

Folic Acid
Lynn Hoepfinger, Mead Johnson and Co., Nutritional Quality Control, Evansville, IN 47721

HPLC Assay for Total A, D, and E Content in Foods, Feeds, and Pharmaceuticals
James V. Bruno, Waters Associates, 34 Maple St, Milford, MA 01757

Iodine in Foods
Robert A. Moffitt, Carnation Co., 8015 Van Nuys Blvd, Van Nuys, CA 91412

Niacinamide (Polarography)
Albert Y. Taira, Food and Drug Administration, 433 W Van Buren St, Chicago, IL 60607

Pantothenic Acid, Total Activity in Foods
Raymond Cooke, Laboratory of the Government Chemist, Food Composition and Nutrition, Cornwall House, Stamford St, London, UK SE1 9NQ

Protein Quality, Evaluation in Foods
Philip H. Derse, DS Associates, 979 Jonathan Dr, Madison, WI 53713

Sodium
Edgar R. Elkins, National Food Processors Association, 1133 20th St, NW, Washington, DC 20036

Thiamine Assay, Enzyme and Column Packing Reagents
Wayne Ellefson, Raltech Scientific Services, Box 7545, Madison, WI 53707

Vitamin A in Foods and Feeds
Donald B. Parrish, Kansas State University, Department of Biochemistry, Manhattan, KS 66506

Vitamin C in Milk-Based Foods
Cora E. Weeks, Food and Drug Administration, National Center for Nutrient Analysis, Washington, DC 20204

Vitamin D
Ellen J. de Vries, Duphar B.V., Research Department 30, PO Box 2, Weesp, The Netherlands

Vitamin E in Foods and Feeds
James P. Clark, Henkel Corp., 2010 E Hennepin Ave, Minneapolis, MN 55413

Vitamin E in Pharmaceuticals (Gas Chromatography)
Alan J. Sheppard, Food and Drug Administration, Division of Nutrition, Washington, DC 20204

Vitamin K
S. A. Barnett, Mead Johnson & Co., 2404 Pennsylvania Ave, Evansville, IN 47721

COMMITTEE E

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INDUSTRIAL PROCESS WASTE

Referee: David Friedman, Environmental Protection Agency, Office of Solid Waste, Washington, DC 20460

Organics
James Poppiti, Environmental Protection Agency, Office of Solid Waste, Washington, DC 20460

Reactivity Testing
Florence Richardson, Environmental Protection Agency, Office of Solid Waste, Washington, DC 20460

METALS AND OTHER ELEMENTS

Referee: Kenneth Boyer, Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Atomic Absorption
Milan Ihnat, Agriculture Canada, Chemistry and Biology Research Institute, Ottawa, Ontario, Canada, K1A 0C5

Cadmium and Lead in Earthenware
Benjamin Krinitz, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Carbon Rod Atomization Techniques
Robert W. Dabeka, Health and Welfare Canada, Health Protection Branch, Ottawa, Ontario, Canada K1A 0L2

Emission Spectrochemical Methods
Fred L. Fricke, Food and Drug Administration, 1141 Central Pkwy, Cincinnati, OH 45202

Fluorine
Robert W. Dabeka

Hydride Generating Techniques
Stephen G. Capar, Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Mercury
Walter Holak, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Methyl Mercury in Fish and Shellfish
Susan Hight, Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Multielement Analysis of Infant Food Formulas by ICP
Ronald F. Suddendorf, Food and Drug Administration, Division of Nutrition, Washington, DC 20204

Multimetal Residues by Resin Column Separations
Richard A. Baetz, Food and Drug Administration, 3032 Bryan St, Dallas, TX 75204

Multielement Determination after Closed System Digestion
Walter Holak

Organometallics
John Jones, Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Polarography
Raymond J. Gajan, Sr, Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Tin
Edgar R. Elkins, Jr, National Food Processors Association, Chemistry Division, 1133 20th St NW, Washington, DC 20036

Voltammetric Methods
Eric Zink, Environmental Sciences Associates, 45 Wiggins Ave, Bedford, MA 01730

MULTIRESIDUE METHODS (INTERLABORATORY STUDIES)

Referee: Paul E. Corneliussen, Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Comprehensive Multiresidue Methodology
Jerry E. Froberg, Food and Drug Administration, 1521 W Pico Blvd, Los Angeles, CA 90015

Gas-Liquid Chromatography (Alkaline Precolumn)
George A. Miller, Food and Drug Administration, 5003 Federal Office Bldg., Seattle, WA 98174

Organophosphorus Pesticide Residues
Ronald R. Laski, Food and Drug Administration, 599 Delaware Ave, Buffalo, NY 14202

Pesticides in Meat and Meat Products

Pollutant Phenols in Fish
Larry Smith, Fish and Wildlife Service, Columbia Natural Fisheries, Columbia, MO 65201

Whole Blood
Henry M. Stahr, Iowa State University, College of Veterinary Medicine, Ames, IA 50010

ORGANOHALOGEN PESTICIDES

Referee: Bernadette McMahon, Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Chlordane
Wilber Saxton, Food and Drug Administration, 5003 Federal Office Building, Seattle, WA 98174

Chlorinated Dioxins
David Firestone, Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Chlorinated Hydrocarbons in Poultry
James Ault, ABC Laboratories, 7200 E ABC Lane, Box 1097, Columbia, MO 65205

Chlorobenzilate, Chloropropylate, and Bromopropylate
Roy S. Brosdal, Food and Drug Administration, 240 Hennepin Ave, Minneapolis, MN 55401

Chlorophenoxy Alkyl Acids
Allan E. Smith, Agriculture Canada, Research Branch, Box 440, Regina, Saskatchewan, Canada S4P 3A2

Dicofol

Ethylene Oxide and Its Chlorohydrin
A. R. Stemp, Kraft Co., 801 Waukegan Rd, Glenview, IL 60025

Fenvalerate

Fumigants

Gel Permeation Chromatography

Inorganic Bromides in Grains
King T. Zee, Environmental Protection Agency. Benefits and Field Studies Division, Beltsville, MD 20705

Kepone
Francis D. Griffith, Jr, Division of Consolidated Laboratory Services, Richmond, VA 23219

Low Moisture-High Fat Samples (Extraction Procedure)
Leon D. Sawyer. Food and Drug Administration, 240 Hennepin Ave, Minneapolis, MN 55401

Miniaturization of Multiresidue Methods
D. Ronald Erney, Food and Drug Administration, 1560 E Jefferson Ave, Detroit, MI 48207

Pentachlorophenol
Arnold P. Borsetti, Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Pentachlorophenol in Animal and Poultry Tissue
Douglas Gillard, U.S. Department of Agriculture, Food Safety and Inspection Service, Beltsville, MD 20705

Permethrin

Photochemical Derivatization for Confirmation of Residue Identity
Paul M. Ward, Food and Drug Administration, 60 Eighth St NE, Atlanta, GA 30309

Polychlorinated Biphenyls (PCBs)
Leon D. Sawyer

Resmethrin
Calvin Corbey, Environmental Protection Agency, Benefits and Field Studies Division, Beltsville, MD 20705

Root Absorbed Residues (Extraction Procedure)

Tetradifon, Endosulfan, and Tetrasul
Lawrence R. Mitchell, Food and Drug Administration, 60 Eighth St NE, Atlanta, GA 30309

Toxaphene
Larry G. Lane, Mississippi State Chemical Laboratory, Box CR, Mississippi State, MS 39762

ORGANONITROGEN PESTICIDES

Referee: W. H. Newsome, Health and Welfare Canada, Food Research Division, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2

Anilazine

Benzimidazole-Type Fungicides
Mikio Chiba, Agriculture Canada, Vineland Station, Ontario, Canada L0R 2E0

Captan and Related Fungicides
Dalia Gilvydis, Food and Drug Administration, 1560 E Jefferson Ave, Detroit, MI 48207

Carbamate Insecticides (Gas-Liquid Chromatography)
Roderick W. Young, Virginia Polytechnic Institute, Department of Biochemistry and Nutrition, Blacksburg, VA 24061

Carbamate Insecticides (Liquid Chromatography)
Richard Krause, Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Carbofuran
Sujit Witkontin, FMC Corp., 100 Niagara St, Middleport, NY 14105

Carbamate Herbicides

Chlorothalonil

Daminozide

Dinitro Compounds

Diquat and Paraquat
Brian Worobey, Health and Welfare Canada, Food Research Division, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2

Dithiocarbamates, General Residue Methods

Maleic Hydrazide

1-Naphthaleneacetic Acid and 1-Naphthaleneacetamide

Organotin in Fungicides
Richard D. Cannizzaro, Thompson-Hayward Chemical Co., 5200 Speaker Rd, Kansas City, KS 66110

Sodium o-Phenylphenate

Substituted Urea

Thiocarbamate Herbicides

s-Triazines

Trifluralin

ORGANOPHOSPHORUS PESTICIDES

Referee: Keith A. McCully, Health and Welfare Canada, Field Operations Directorate, Ottawa, Ontario, Canada K1A 1B7

Azinphos-methyl

Confirmation Procedures
Bill Lee, Inland Waters Directorate, Water Quality Branch, 867 Lakeshore Rd, PO Box 5050, Burlington, Ontario, Canada L7R 4A6

Disulfoton

Extraction Procedures

General Method for Organochlorine and Organophosphorus Pesticides

High Fat Samples
Ronald Scharfe, Agriculture Canada, Pesticide Laboratory, Ottawa, Ontario, Canada K1A 0C5

Methamidophos

Monocrotophos

Phorate

Phosphine
T. Dumas, Research Institute, University Sub Post Office, London, Ontario, Canada N6A 3K0

Sweep Codistillation
Randall R. Watts, Environmental Protection Agency, Mail Drop 69, Research Triangle Park, NC 27711

Thin Layer Chromatography
Melvin E. Getz, U.S. Department of Agriculture, Agricultural Environmental Quality Institute, Beltsville, MD 20705

RADIOACTIVITY

Referee: Edmond J. Baratta, Food and Drug Administration, Northeast Radiological Health Laboratory, Winchester, MA 01890

Carbon-14

Cesium-137
Edmond J. Baratta

Iodine-131
Eugene Easterly, Environmental Protection Agency, PO Box 15027, Las Vegas, NV 89114

Neutron Activation Analysis
William Stroube, National Bureau of Standards, Reactor Building 235, Washington, DC 20234

Plutonium

Radium-228
Jacqueline Michel, Research Planning Institute, 925 Gervais St, Columbia, SC 29201

Tritium

WATER

Referee: Alfred S. Y. Chau, Canada Centre for Inland Waters, PO Box 5050, Burlington, Ontario, Canada L7R 4A6

Chemical Pollutants in Water and Wastewater
Larry B. Lobring, Environmental Protection Agency, 26 W St Clair St, Cincinnati, OH 45268

Chlorinated Solvents in Water

Chlorophenoxy Alkyl Acid Residues in Water and Wastewater

Herbicides in Water and Sediment
Bill Lee, Canada Centre for Inland Waters, Box 5050, Burlington, Ontario, Canada L7R 4A6

Major Ions and Nutrients in Water
Larry K. Bailey, Geological Survey, Denver Federal Center, Denver, CO 80225

Organohalogen Pesticides in Water
Marie Siewierski, Rutgers University, Cook College, New Brunswick, NJ 08903

Organophosphorus Pesticides in Water

Triazine Herbicides in Water

COMMITTEE F

Paris M. Brickey (Food and Drug Administration, Division of Microbiology, Washington, DC 20204), *Chairman*; Michael Wehr (Oregon Department of Agriculture, 635 Capitol St, NE, Salem, OR 97310), K. Rayman (Health and Welfare Canada, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2); Robert M. Twedt (Food and Drug Administration, Division of Microbiology, 1090 Tusculum Ave, Cincinnati, OH 45226); Donald E. Lake (American Can Co., 433 N NW Highway, Barrington, IL 60010); Donald Mastrorocco (Hershey Foods Corp., 19 E Chocolate Ave, Hershey, PA 17033), *Secretary*; Foster D. McClure (Food and Drug Administration, Division of Mathematics, Washington, DC 20204), *Statistical Consultant*

ANALYTICAL MYCOLOGY OF FOODS AND DRUGS

Referee: Stanley M. Cichowicz, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Baseline Mold Counts by Blending
Ruth Bandler, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Chemical Methods for Detecting Mold
Ruth Bandler

Geotrichum candidum Morphology
Sylvia Y. Yetts, Food and Drug Administration, 3032 Bryan St, Dallas, TX 75204

Geotrichum Mold in Canned Fruits, Vegetables, and Fruit Juices
Stanley M. Cichowicz

Geotrichum Mold in Frozen Fruits and Vegetables
Jane Kaminski, Food and Drug Administration, United Nations Plaza, San Francisco, CA 94102

Howard Mold Counting, Use of Widefield Eyepiece
Roseamond J. Scott, Superior Laboratory Services, Route 4, Box 245, Portland, IN 47371

Howard to Viable Mold Counts of Frozen Fruits and Vegetables, Comparison
Maria P. Chaput, Food and Drug Administration, 585 Commercial St, Boston, MA 02109

Microscopic Appearance of Mold Hyphae, Effect of Freezing
Charles N. Roderick, Food and Drug Administration, 3032 Bryan St, Dallas, TX 75204

Microscopic Mold Count Methods, Use of Compound Microscope
Don Vail, Jr, Food and Drug Administration, 1182 W Peachtree St, NW, Atlanta, GA 30309

Microscopic Mold Counts, Effects of Interfering Plant Material
Deborah M. Floyd, Food and Drug Administration, 3032 Bryan St, Dallas, TX 75204

Mold in Spices
Karan L. Repsher, Food and Drug Administration, 900 Madison Ave, Baltimore, MD 21201

Molds and Yeasts in Beverages
Stanley M. Cichowicz

Standardization of Plant Tissue Concentrations for Mold Counting
Stanley M. Cichowicz

Tomato Products, Refractive Index
Jerome La Greca, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Tomato Rot Fragment Count
Gerald E. Russell, Food and Drug Administration, 1560 Jefferson Ave, Detroit, MI 48207

DISINFECTANTS

Referee: Reto Engler, Environmental Protection Agency, Office of Pesticide Programs, Registration Division, Washington, DC 20460

Antimicrobial Agents Used by Laundries on Fabrics and Materials
Luther B. Arnold, Vikon Chemical Co., PO Box 1520, Burlington, NC 27215
Jamie McGee, Dow Corning Corp., Midland, MI 48640

Sporicidal Tests
Ted Wendt, Surgikos, 2500 Arbrook Dr, Arlington, TX 76010

Textile Antibacterial Preservatives
Luther B. Arnold

Tuberculocidal Tests
Joseph Ascenzi, Arbrook Inc., Arlington, TX 76010

Use-Dilution Test, Variation and Amendments
Gayle Mulberry, Hill Top Research, Inc., Box 42501, Cincinnati, OH 45242

Virucide Tests
Dale Fredell, Economics Laboratory, Osborne Bldg, St. Paul, MN 55102

EXTRANEOUS MATERIALS IN FOODS AND DRUGS

Referee: John S. Gecan, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Asbestos Measurements in Foods, Drugs, and Cosmetics

Botanical Drugs, Adulteration by Foreign Plant Materials
Frank D'Amelio, Bio Botanica, 2 Willow Park Center, Farmingdale, NY 11735

Botanicals
Arnold E. Schulze and Marvin Nakashima, Food and Drug Administration, Division of Microbiology, Washington, DC 20204
Joseph A. McDonnell, Food and Drug Administration, 1521 W Pico Blvd, Los Angeles, CA 90015
Harriett R. Gerber

Brine Extractions, Techniques
Clarence C. Freeman, Food and Drug Administration, 4298 Elysian Fields Ave, New Orleans, LA 70122

Chocolate Products
Donald A. Mastrorocco, Jr, Hershey Chocolate Co., Hershey, PA 17033

Cocoa Powder and Press Cake
C. Robert Graham, Lancaster Laboratories, Inc., 2425 New Holland Pike, Lancaster, PA 17601

Fecal Sterols
Ruth Bandler, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Food Supplement Tablets
Charles E. Highfield, Health and Welfare Canada, Health Protection Branch, 2301 Midland Ave, Toronto, Ontario, Canada M1P 4R7

Grains, Whole, Cracking Flotation Methods
Richard Trauba, Food and Drug Administration, 240 E Hennepin Ave, Minneapolis, MN 55401

Insect Excreta in Flour
Raymond Galacci, Food and Drug Administration, 900 Madison Ave, Baltimore, MD 21201

Isolation of Extraneous Filth from Dehydrated Vegetable Products
Francis J. Farrell, Thomas J. Lipton, Inc., 800 Sylvan Ave, Englewood Cliffs, NJ 07632

Meats, Processed
Phillip Alioto, Wisconsin Department of Agriculture, 4702 University Ave, Madison, WI 53705

Methods for Urine Detection
Robert S. Ferrera, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Mite Contamination Profiles and Characterization of Damage to Foods
Diane Peace, Health and Welfare Canada, Bureau of Microbiological Hazards, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2

Mites in Stored Foods
Jack L. Boese, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Mole, Filth in

Mushroom Products, Dried
Jack L. Boese
Alan R. Olsen, Food and Drug Administration, 1521 W Pico Blvd, Los Angeles, CA 90015

Mushrooms, Canned
Russell G. Dent, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Particulates in Large-Volume Parenterals
Gordon Oxborrow, Food and Drug Administration, Minneapolis Center for Microbiological Investigations, 240 Hennepin Ave, Minneapolis, MN 55401

Performance Evaluation of Methods for Filth
Jack L. Boese
Russell G. Dent
James Carpus and Alan Whiteman, Food and Drug Administration, 433 W Van Buren St, Chicago, IL 60607

Rye Bread
Richard R. Haynos, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Shrimp
Alan R. Olsen

Soluble Insect and Other Animal Filth
George P. Hoskin and Harriett R. Gerber, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Soups, Canned and Dehydrated

Spices
Susan M. Brown, McCormick & Co., Inc., Hunt Valley, MD 21031

Spirulina
John S. Gecan
John Quintero, Food and Drug Administration, 1521 W Pico Blvd, Los Angeles, CA 90015

Urine Detection
Robert S. Ferrera

Vertebrate Excreta, Chemical Identification Tests
Harriet R. Gerber, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

FOOD MICROBIOLOGY

Referee: Wallace H. Andrews, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Automated Methods for Food and Cosmetics
James E. Gilchrist, Food and Drug Administration, Division of Microbiology, 1090 Tusculum Ave, Cincinnati, OH 43226

***Bacillus cereus*, Isolation and Enumeration**
Stanley M. Harmon, Food and Drug Administration, Division of Microbiology, Washington, DC 20204
Gayle Lancette, Food and Drug Administration, Minneapolis Center for Microbiological Investigations, 240 Hennepin Ave, Minneapolis, MN 55401

***Bacillus cereus* Enterotoxin**
Reginald W. Bennett and Stanley M. Harmon, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Campylobacter Species
Chong Park, Health and Welfare Canada, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2

Canned Foods
Cleve B. Denny, National Food Processors Association, 1133 20th St, NW, Washington, DC 20036

***Clostridium botulinum* and Its Toxin, Detection**
Donald A. Kautter, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

***Clostridium perfringens*, Isolation and Enumeration**
Stanley M. Harmon

Endotoxins by Limulus Amebocyte Lysate
Christine Twohy, Food and Drug Administration, 240 Hennepin Ave, Minneapolis, MN 55401

Enteropathogenic *Escherichia coli*, Direct Fluorescent Antibody Procedure for Detection
James F. Yager, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

***Escherichia coli* and Other Coliforms**
Ira J. Mehlman, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Genetic Methods for Detection of Bacterial Pathogens
Walter Hill, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Helium Leaks, Canned Foods
James E. Gilchrist and Ulysses S. Rhea, Food and Drug Administration, Minneapolis Center for Microbiological Investigations, 240 Hennepin Ave, Minneapolis, MN 55401

Hydrophobic Grid Membrane Filter Methods
Phyllis Entis, QA Laboratories, Ltd, 135 The West Mall, Toronto, Ontario, Canada M9C 1C2

Identification of Microorganisms by Biochemical Kits
Nelson Cox, U.S. Department of Agriculture, Southern Regional Research Center, Box 5677, Athens, GA 30613

Parasitology
Richard A. Rude, Food and Drug Administration, Minneapolis Center for Microbiological Investigations, 240 Hennepin Ave, Minneapolis, MN 55401
Robert Barnard, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Salmonella
Paul L. Poelma, Food and Drug Administration, Division of Microbiology, Washington, DC 20204
Dean Wagner, Food and Drug Administration, Minneapolis Center for Microbiological Investigations, 240 Hennepin Ave, Minneapolis, MN 55401

***Salmonella*, Fluorescent Antibody Technique**
John P. Schrade, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Somatic Cell, Automated Optical Counting Method
Wesley N. Kelley, University of South Dakota, State Chemical Laboratory, Vermillion, SD 57609

Somatic Cell, Fossomatic Counting Method
R. D. Mochrie, North Carolina State University, Animal Science Department, Raleigh, NC 27650

Spore-Formers and Non-Spore-Formers in Low Acid Foods
Mary L. Schafer, Food and Drug Administration, 1090 Tusculum Ave, Cincinnati, OH 45226

Staphylococcus
Gayle Lancette

***Staphylococcus* Toxin**
Reginald W. Bennett

Sugars
Cleve B. Denny

***Vibrio cholerae* and Detection of Its Toxins**

Vibrio parahaemolyticus
Eugene H. Peterson, Food and Drug Administration, Minneapolis Center for Microbiological Investigations, 240 Hennepin Ave, Minneapolis, MN 55401

Virology and Animal Oncology
Edward P. Larkin, Food and Drug Administration, Division of Microbiology, 1090 Tusculum Ave, Cincinnati, OH 45226

Yeast, Molds, and Actinomycetes
Philip B. Mislivec, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Yersinia enterocolitica

DRUG AND DEVICE RELATED MICROBIOLOGY

Referee: Gordon Oxborrow, Food and Drug Administration, Minneapolis Center for Microbiological Investigations, 240 Hennepin Ave, Minneapolis, MN 55401

Testing Biological Sterility Indicators
Gordon Oxborrow

Sterility Testing of Medical Devices
Daniel A. Quagliaro, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

COMMITTEE G

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ANTIBIOTICS

Referee: Stanley E. Katz, Rutgers University, Department of Biochemistry and Microbiology, New Brunswick NJ 08903

Affinity Quantitative Determination of Penicillin in Milk
Stanley E. Charm, Tufts Medical School, Enzyme Center, 136 Harrison Ave, Boston, MA 02111

Bacitracin in Feeds
Carol Harpster, AL Laboratories, 185 LeGrand Ave, Northvale, NJ 07647
John B. Gallagher, International Minerals & Chemicals Corp., 1331 S First St, Terre Haute, IN 47808

Bacitracin in Premixes and Foods (Chemical Method)
John B. Gallagher

Bambermycins
Jean Olsen, Hoechst Pharmaceuticals, Inc., Rte 202-206 N, Somerville, NJ 08876

Chloramphenicol in Animal Tissues
Edward H. Allen, Food and Drug Administration, Bureau of Veterinary Medicine, Beltsville, MD 20705

Chlortetracycline in Feeds

Cup Plate System for Antibiotic Analysis
Virginia A. Thorpe, State Department of Agriculture, 1615 S Harrison Rd, East Lansing, MI 48823

Design and Computerizatioin of Microbiological Assays
Peter Kahn, Rutgers University, Cook College, Department of Biochemistry and Microbiology, New Brunswick, NJ 08903

Erythromycins

Lasalocid Sodium in Feeds (Microbiological Assay)
Jacob M. Scheiner, Hoffmann-La Roche Inc, Food and Agricultural Products, 340 Kingsland St, Nutley, NJ 07110

Lincomycin in Feeds
A. William Neff, The Upjohn Co., Agricultural Division, Kalamazoo, MI 49001

Monensin
Robert E. Scroggs, Elanco Products Co., Box 1750, Indianapolis, IN 46206

Oxytetracycline
Dorothy M. Brennecke, 3981 Dover Pl, St Louis, MO 63116

Qualitative Delvo-test for β-Lactam Residues in Milk
Wesley N. Kelley, State Chemical Laboratory, University of South Dakota, Vermillion, SD 57609

Qualitative Determination of β-Lactam Antibiotic Residues in Milk
James Messer, Food and Drug Administration, 1090 Tusculum Ave, Cincinnati, OH 45226

Quantitative Determination of β-Lactam Antibiotic Residues in Milk
Ronald Case, Kraft Foods, Kraft Ct, Glenview, IL 600225
Roy Ginn, Dairy Quality Control Institute, Inc., 2353 N Rice St, St. Paul, MN 55113

Screening Procedures for Antibiotics in Feeds
Mary L. Hasselberger, Department of Agriculture, Laboratory Division, 3703 S 14th St, Lincoln, NE 68502

Statistics of Microbiological Assay
John R. Murphy, Elanco Products Co., PO Box 1750, Indianapolis, IN 46206

Tetracyclines in Tissues (Chromatographic Assay)
Ray B. Ashworth, U.S. Department of Agriculture, Food Safety and Inspection Service, Beltsville, MD 20705

Tetracyclines in Tissues (Microbiological Assay)
Stanley E. Katz

Turbidimetric Virginiamycin Assay
Dorothy M. Brennecke

Tylosin
Paul Handy, Eli Lilly & Co., Box 708, Greenfield, IN 46140

BIOCHEMICAL METHODS

Referee: John O'Rangers, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857

Aminoglycosides in Animal Tissue

17β-Estradiol and Diethylstilbestrol in Tissues (Immunochemical Methods)

Hormones in Tissues (Immunospecific Affinity Chromatography)
Alonza R. Hayden, U.S. Department of Agriculture, Meat Science Research Laboratory, Beltsville, MD 20705

Hybridoma-Monoclonal Antibodies
Richard Meyer, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Immunochemical Species Identification of Meat
David Berkowitz, U.S. Department of Agriculture, Serology Branch, Beltsville, MD 20705

Performance Evaluation Methods for Non-RIA Procedures Measuring Human Chorionicgonadatropin
Lillian Gill, Food and Drug Administration, 900 Madison Ave, Baltimore, MD 21201

Performance Evaluation Protocols for Clinical, Chemical, and Immunochemical Diagnostic Products

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Steroid Quantitation (Enzymatic Methods)

Sulfa Drugs in Animal Tissues (Immunoassay Procedures)

COLOR ADDITIVES

Referee: Keith S. Heine, Food and Drug Administration, Division of Color Technology, Washington, DC 20204

Arsenic and Heavy Metals
Catherine Bailey, Food and Drug Administration, Division of Color Technology, Washington, DC 20204

Atomic Absorption in Color Analysis
Lueangier Moten, Food and Drug Administration, Division of Color Technology, Washington, DC 20204

Cosmetics
Sandra Bell, Food and Drug Administration, Division of Color Technology, Washington, DC 20204

Color in Candy and Beverages
Mary Young, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Color in Drugs
Edward Woznicki, Colorcon Inc., Moyer Blvd, West Point, PA 19486

Color in Other Foods
Nicholas Adamo, Food and Drug Administration, Division of Color Technology, Washington, DC 20204

FD&C Red No. 4 in Maraschino Cherries
Ronald E. Draper, Food and Drug Administration, 50 United Nations Plaza, San Francisco, CA 94102

High Pressure Liquid Chromatography
Elizabeth A. Cox, Food and Drug Administration, Division of Color Technology, Washington, DC 20204

Inorganic Salts
Wallace S. Brammell, Food and Drug Administration, Division of Color Technology, Washington, DC 20204

Intermediates, Uncombined, in Certifiable Triphenylmethane Colors
Alan Scher, Food and Drug Administration, Division of Color Technology, Washington, DC 20204

Intermediates, Uncombined, in Certifiable Water-Soluble Azo Colors
Daniel M. Marmion, Allied Chemical Corp., 1051 S Park Ave, Buffalo, NY 14240

Subsidiary Colors in Certifiable Color Additives
John E. Bailey, Food and Drug Administration, Division of Color Technology, Washington, DC 20204

X-Ray Fluorescence Spectroscopy
Catherine Bailey

COSMETICS

Referee: Ronald L. Yates, Food and Drug Administration, Division of Cosmetics Technology, Washington, DC 20204

Deodorants, Aluminum and Zirconium in
Paul Beavin, Jr, Food and Drug Administration, 900 Madison Ave, Baltimore, MD 21201

Essential Oils and Fragrance Materials, Components
Harris H. Wisneski, Food and Drug Administration, Division of Cosmetics Technology, Washington, DC 20204

Nitrosamines

Preservatives

DRUG RESIDUES IN ANIMAL TISSUES

Referee: Charlie J. Barnes, Food and Drug Administration, Division of Chemistry and Physics, Washington, DC 20204

Benzimidazole
Leon LeVan, Hazleton Raltech, 3301 Kinsman Blvd, Madison, WI 53704

Carbadox
Jose E. Roybal, Food and Drug Administration, U.S. Customhouse, Denver, CO 80202

Diethylstilbestrol
Robert K. Munns, Food and Drug Administration, 20th and California Sts, Denver, CO 80202

Dimetridazole
Maritza C. Pullano, Food and Drug Administration, U.S. Courthouse, Denver, CO 80202

3,5-Dinitrobenzamide
Raymond B. Ashworth, U.S. Department of Agriculture, Food Safety and Inspection Service, Beltsville, MD 20852

Nitrofurans
—

Screening Methods
Henry R. Cook, U.S. Department of Agriculture, Food Safety and Inspection Service, Beltsville, MD 20705

Steroids
—

Sulfa Drugs
—

Sulfonamide
—

DRUGS IN FEEDS

Referee: Rodney J. Noel, Purdue University, Department of Biochemistry, West Lafayette, IN 47907

Amprolium
Kathleen Eaves, Texas A&M University, Agriculture Analytical Services, College Station, TX 77843

Arprinocid
David W. Fink, Merck, Sharpe & Dohme, Inc., Analytical Research Department, E Scott Ave, Rahway, NJ 07065

Arsanilic Acid
—

Carbadox
Mark A. Litchman, Pfizer Inc., Agricultural Division, 1107 S Missouri St, Lee's Summit, MO 64063

2-Chloro-1-(2,4,5-trichlorophenyl) Vinyl Dimethyl Phosphate (Tetrachlorvinphos) (Rabon)
—

Dibutyltin Dilaurate (Butynorate)
Glenn M. George, Salsbury Laboratories, Research Division, Charles City, IA 50616

1,2-Dimethyl-5-nitroimidazole (Dimetridazole)
Larry J. Frahm, Salsbury Laboratories, Research Division, Charles City, IA 50616

Ethopabate
Kathleen Eaves

Ethylenediamine Dihydroiodide
Gary Ross, North Dakota State Laboratories, 2635 E Main St, Bismarck, ND 58501

Furazolidone and Nitrofurazone
Robert E. Smallidge, Purdue University, Department of Biochemistry, West Lafayette, IN 47907

Ipronidazole
Edward Waysek, Hoffmann-La Roche Inc., Food and Agricultural Products, 340 Kingsland St, Nutley, NJ 07110

Larvadex
Arthur Hofberg, Ciba-Geigy Corp., 41 Swing Rd, Greensboro, NC 27409

Melengestrol Acetate
Raymond Davis, The Upjohn Co., Henrietta St Labs, Kalamazoo, MI 49001

Microscopy
Patrick Cox, Land O'Lakes, Inc., 2827 8th Ave S, Ft Dodge, IA 50501

Nifursol
Glenn M. George

Phenothiazine
—

Pyrantel Tartrate
James A. Braswell, Pfizer, Inc., Agriculture Division, 1107 S Missouri St, Lee's Summit, MO 64063

Roxarsone
Glenn M. George

Sulfa Drug Residues
Robert K. Munns, Food and Drug Administration, 20th and California Sts, Denver, CO 80202

Sulfadimethoxine-Ormetoprim Mixtures
Edward Waysek

Sulfamethazine and Sulfathiazole (Premix and Finished Feed Levels)
Dwight M. Lowie, State Department of Agriculture, 4000 Reedy Creek Rd, Raleigh, NC 27607

Sulfaquinoxaline
David W. Fink

FORENSIC SCIENCES

Referee: Jew-Ming Chao, Burlington County Forensic Science Laboratory, Woodland Rd, Mt. Holly, NJ 08060

ABO Blood Typing
Henry C. Lee, State Police Forensic Science Laboratory, Box A-D, Amity Station, New Haven, CT 06516

Biological Fluids (Immunoelectrophoresis)
James D. Hauncher, Michigan State Police, Scientific Laboratory, 42145 W Seven Mile Rd, Northville, MI 48167

Blood
Ralph Plackenhorn, Pennsylvania State Police, Laboratory Division, PO Box 38, Greensburg, PA 15601

Blood Stains, ABH Typing

Blood Stains, Species Determination

Bomb Residues
William Kinard, Department of the Treasury, Bureau of Alcohol, Tobacco and Firearms, 1401 Research Blvd, Rockville, MD 20850

Documents

Fingerprints
Charles M. Conner, Department of the Treasury, Bureau of Alcohol, Tobacco and Firearms, 550 Main St, Cincinnati, OH 45202

Firearms
James Booker, State Crime Laboratory, 591 Hathaway Bldg, PO Box 1895, Cheyenne, WY 82001

Flammable Fluids
Phillip Wineman, Department of the Treasury, Bureau of Alcohol, Tobacco and Firearms, 1401 Research Blvd, Rockville, MD 20850

Gunshot Residue
William Kinard

Gunshot Residue by Atomic Absorption Spectroscopy
Kent A. Oakes, State Regional Crime Laboratory, 15725 W Ryerson Rd, New Berlin, WI 53151

Hair Examination
Walter C. McCrone, Walter C. McCrone Associates, 2820 S Michigan Ave, Chicago, IL 60616

Infrared Spectroscopy
Kent A. Oakes

Microscopic Methods and Glass Products
Walter C. McCrone

Paints, Pyrolysis-Gas Chromatographic Methods

Safe Insulation

Serial Number Restoration (Chemical Etching Techniques)

Soils, Geological Analysis
R. C. Murray, University of Montana, Office of the Associate Vice-President for Research and Dean of the Graduate School, Missoula, MT 59801

Voice Print Identification
Lonnie L. Smrkovski, Michigan State Police, 714 S Harrison Rd, East Lansing, MI 48823

MICROBIAL MUTAGENICITY TESTING

Referee: Frederick Deserres, National Institute of Environmental Protection, Box 12233, Research Triangle Park, NC 27709

Prophage Induction
John H. S. Chen, Environmental Protection Agency, Beltsville, MD 20706

TOXICOLOGICAL TESTS

Referee: Samuel I. Shibko, Food and Drug Administration, Division of Toxicology, Washington, DC 20204

Ames Test
Virginia C. Dunkel, Food and Drug Administration, Division of Toxicology, Washington, DC 20204

Aspiration Tests
Robert E. Osterberg, Food and Drug Administration, Division of Toxicology, Washington, DC 20204

Cell Culture-Enzyme Induction Bioassay
June A. Bradlaw, Food and Drug Administration, Division of Toxicology, Washington, DC 20204

***In Vitro* Mutagenic Assay Utilizing the Thymidine Kinase Heterozygous Locus of L5178Y Mouse Lymphoma Cells**
Kenneth Palmer, Food and Drug Administration, Division of Toxicology, Washington, DC 20204

LD_{50} Test
Frederick Sperling, Howard University Medical School, Department of Pharmacology, Washington, DC 20001

Rabbit Eye Irritation Test
Francis N. Marzulli, Food and Drug Administration, Division of Toxicology, Washington, DC 20204

Skin Irritation Tests
Robert M. Herir, Consumer Product Safety Commission, Bureau of Biomedical Science, Bethesda, MD 20207

VETERINARY ANALYTICAL TOXICOLOGY

Referee: P. Frank Ross, U.S. Department of Agriculture, National Veterinary Services Laboratory, Ames, IA 50010

Arsenic in Animal Tissue
Tracy Hunter, Division of Consolidated Laboratory Services, 1 N 14th St, Richmond, VA 23219

Cholinesterase
Paula Martin, Iowa State University, Veterinary Diagnostic Laboratory, Ames, IA 50010

Copper in Animal Tissue
David Osheim, U.S. Department of Agriculture, National Veterinary Services Laboratory, Ames, IA 50010

Lead in Animal Tissue
R. J. Everson, Purdue University, School of Veterinary Medicine, West Lafayette, IN 47907

Molybdenum
Howard Casper, North Dakota State University, Veterinary Diagnostic Laboratory, Fargo, ND 58102

Monensin
Ronda A. Moore, Iowa State University, College of Veterinary Medicine, Ames, IA 50010

Multiple Anticoagulant Screening
John D. Reynolds, Animal Disease Laboratory, 235 N Walnut St, Centralia, IL 62801

Multielement Analysis by ICP
Emmett Beazelton, Michigan State University, Department of Pharmacology and Toxicology, East Lansing, MI 48824

Nitrates and Nitrites
Norman R. Schneider and Michael P. Carlson, Veterinary Diagnostic Center, Department of Veterinary Science, Lincoln, NE 68583

Poisonous Plants
George Rottinghaus, University of Missouri, College of Veterinary Medicine, Columbia, MO 65211

Selenium in Animal Tissue
James E. Roof, State Veterinary Diagnostic Laboratory, PO Box 1430, Harrisburg, PA 17105

Sodium Monofluoroacetate
Henry M. Stahr, Iowa State University, College of Veterinary Medicine. Ames, IA 50010

CHANGES IN OFFICIAL METHODS OF ANALYSIS

The following changes in the Methods of the Association become effective, as provided in Article VII, Section 6 of the Bylaws, on the thirtieth day after publication of this *Journal* issue. Section numbers refer to the 13th edition, 1980, unless otherwise specified.

Newly adopted methods are numbered in the style of the 13th edition. The first section of the first new method in each chapter is numbered with the chapter number plus **.D01.** Subsequent sections are numbered **.D02, .D03, .D04,** et seq. The **D** signifies that the method was adopted at the 1982 Annual Meeting. Methods adopted at the 1979, 1980, and 1981 Meetings are designated by the chapter number plus **.A01, .B01, .C01,** etc., respectively. Revisions of the 13th edition sections are given the same number(s) they replace.

"Changes in Methods" is accompanied by an index, which is cumulative for actions on methods between editions of *Official Methods of Analysis.*

1. AGRICULTURAL LIMING MATERIALS

No additions, deletions, or changes.

2. FERTILIZERS

(1) The official first action modified comprehensive nitrogen method, **2.061-2.062,** was adopted official final action.

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(2) The following liquid chromatographic method for the determination of urea and water-soluble methyleneureas in fertilizers was adopted official first action:

Urea and Water-Soluble Methyleneureas in Fertilizers

Liquid Chromatographic Method

Official First Action

2.D01 ***Principle***

Sample is ground to pass 40 mesh sieve, extd with H_2O, and filtered. Urea, methylenediurea (MDU), and dimethylenetriurea (DMTU) are detd by liq. chromatogy using external stds and refractive index detection.

2.D02 ***Apparatus***

(**a**) *Liquid chromatograph.*—With refractive index detector and pump capable of delivering mobile phase at 2 mL/min at pressures up to 2000 psig. Operating conditions: flow rate 1.0 mL/min (1500 psi); attenuator 8X; ambient temp.; injection vol. 10 μL. Sample injector with fixed sample loop preferred.

(**b**) *Chromatographic column.*—Partisil 5 ODS-3, 4.6 mm id × 25 cm (Whatman, Inc., 9 Bridewell Pl, Clifton, NJ 07014; other manufacturers' small particle reverse phase columns may be substituted with adjustments in operating conditions).

(**c**) *Strip chart recorder.*—Range to match output of detector.

2.D03 ***Reagents***

(**a**) *Mobile phase.*—HPLC grade H_2O.

(**b**) *Purified methylenediurea (MDU) and dimethylenetriurea (DMTU).*—Ext 50 g N-only ureaformaldehyde (UF) fertilizer with acetone 8 h on soxhlet extractor. Select UF fertilizer with high MDU/DMTU-to-urea ratio. Remove thimble from extractor, let air-dry, and collect residue. Mix 30 g acetone-washed residue in 300 mL H_2O and filter or centrf. Inject 100 mL supernate onto Waters Associates PrepPak 500 C-18 cartridge (5.7 × 30 cm) in preparative liq. chromatograph (Waters Associates Inc. Prep-500, or equiv.) at ambient temp. and with H_2O mobile phase at 150 mL/min. Collect top third of MDU and DMTU peaks. Evap. collected fractions to dryness in hood, using heat lamps. Dry using vac. over P_2O_5. Confirm identity using anal. liq. chromatogy and elemental analysis: mp of purified material, detd in pyrex, should be 205–207°d for MDU and 231–232°d for DMTU.

(**c**) *External std solns.*—(*A*) Accurately weigh ca 1.0 g each of urea (Baker Analyzed Reagent) and purified MDU, transfer both weighed compds to same 100 mL vol. flask, and dil. to vol. with H_2O. (*B*) Accurately weigh 0.0125, 0.025, 0.050, and 0.10 g purified DMTU into sep. 50 mL vol. flasks. (*C*) Pipet 2, 5, 10, and 15 mL of mixed urea/MDU stds (*A*) into the vol. flasks from (*B*), resp. Dil. to ca 40 mL with H_2O and warm as necessary to dissolve DMTU. Cool to room temp. and dil. to vol. Approx. std contents = (*1*) 0.25 mg DMTU + 0.4 mg urea/MDU per mL; (*2*) 0.50 mg DMTU + 1.0 mg urea/MDU per mL; (*3*) 1.00 mg DMTU + 2.0 mg urea/MDU per mL; (*4*) 2.00 mg DMTU + 3.0 mg urea/MDU per mL.

2.D04 ***Preparation of Sample***

Grind sample to pass 40 mesh sieve. Accurately weigh 2.000 g well mixed ground sample into 200 mL vol. flask. Add 150 mL distd or deionized H_2O, place on wrist-action shaker 20 min, and dil. to vol. with H_2O. Using glass fiber paper, filter portion into 4 mL vial. Filter again thru 0.45 μm filter before injection.

2.D05 ***Determination and Calculations***

Inject 10 μL of each mixed std until peak hts agree ±2%. Inject 10 μL sample. Repeat stds after all samples have been injected. Std peak hts should agree within 3% of initial std peak hts. Average peak hts for each component and plot mg/mL vs peak hts.

$$\% \text{ Urea N} = \text{mg/mL (from graph)} \times 9.33/\text{g sample}$$

$$\% \text{ MDU N} = \text{mg/mL (from graph)} \times 8.484/\text{g sample}$$

$$\% \text{ DMTU N} = \text{mg/mL (from graph)} \times 8.236/\text{g sample}$$

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(3) The official final action flame photometric method, **2.091–2.096**, and the official final action automated flame photometric method, **2.097–2.101,** for the determination of potash in fertilizers have been expressed as a single method, using instrument parameters to judge instrument acceptability. The method was adopted official first action:

K_2O in Fertilizers

Flame Photometric Method

(Manual or Automated)

Official First Action

(*Caution:* See **51.007**)

2.D06 ***Method Parameters***

Any flame photometer, manual or automated, capable of detecting K, using Li as internal std, and meeting method performance characteristics described below, is satisfactory. Samples are extd with ammonium oxalate soln or ammonium citrate soln. Appropriate dilns of ext are mixed with $LiNO_3$ internal std soln and aspirated or pumped into flame photometer. La_2O_3 is added to $LiNO_3$ soln to eliminate the phosphate effect. Final soln to be introduced to flame should have the following composition: (a) concn of K_2O in range such that std curve response is linear over that range, (b) const amt of Li in range 5 to 40 ppm, (c) selected concn of La <1400 ppm, and (d) 0.2N HNO_3. Exact concn of $LiNO_3$ and La_2O_3 are optimized for particular instrumentation as described in performance specifications below. Ratio of K intensity at 768 nm to Li intensity at 761 nm is detd, and compared with similar ratios from std set of ≥6 stds, prepd from NBS or primary std KH_2PO_4. Stds are arranged in ascending order and evenly distributed thru chosen range.

2.D07 ***Preparation of Sample***

(**a**) *Ammonium oxalate extraction.*—Weigh 1 g sample into 500 mL vol. flask, add 50 mL 4% $(NH_4)_2C_2O_4$ and 125 mL H_2O, boil 30 min, and cool. Dil. to vol. with H_2O, mix, and filter or let stand until clear.

(**b**) *Ammonium citrate extraction from direct available phosphorus extract.*—Prep. as in **2.050.** (If solns must be held overnight, add 3–4 drops of $CHCl_3$.)

2.D08 ***Performance Specifications***

System performance criteria.—Detailed example of specific instrumental system capable of meeting specified performance criteria follows this performance section. It is necessary to verify that this or any other particular system meets all of the following performance criteria before samples are analyzed. Levels specified are to be considered min. acceptable levels. Various criteria are written for automated instrument, but should also apply to manual instrument systems.

(**a**) *$LiNO_3$ concentration level.*—Amt of $LiNO_3$ in final soln aspirated into flame is adjusted partly for convenience of instrument parameters, but should be such that Li and K channels give roughly equal responses. This can be detd either by displaying each channel's output sep., or by displaying ratio of K to Li response and then interchanging Li and K filters and displaying ratio again.

Using either procedure, sample midrange K^+ stds under analysis conditions while varying concn of Li^+ until acceptable concn of Li^+ is found.

(**b**) *Noise.*—Adjust detector output to 90% full scale with high std sampled continuously. Noise must be <2% full scale peak to peak. Note that some instruments, flow injection analysis systems, for example, are not designed to pump samples continuously. In this case, substitute repeated sampling for continuous sampling, and consider noise to be difference between adjacent peak maxima. (Optimum performance on ex-

ample instrument system described in this method is ca ½% peak to peak. To reduce noise on example system, stabilize flame, stabilize pumping rate, stabilize back-pressure, change pump tubes, clean manifold, and/or rework manifold to ensure adequate mixing. To det. min. noise limit of instrument, collect system waste soln, connect short length of tubing directly to photometer aspirator, and aspirate waste soln directly into flame.)

(c) *Carryover.*—Adjust detector output to give ca 10% and 90% full scale response for low and high stds, resp. Sample 3 high stds followed by 5 low stds on system under analysis conditions. Carryover, defined as difference between first low std and mean of other low stds, may not be >1% full scale. (Optimum performance on example system is negligible carryover. To reduce carryover on example system, clean manifold and aspirator, check manifold connections for dead space, redesign manifold shortening hydraulic system wherever possible, decrease sampling rate, and/or reduce std range.)

(**d**) *Drift.*—Adjust instrument to give detector response ca 50% full scale with middle std sampled continuously. Sample middle std continuously for the time it would take to analyze 30 samples. For instruments not designed to sample continuously, draw smooth line thru 30 middle std peaks. Drift may not exceed 1% full scale per any 10 sample segment. (Optimum performance on example system is zero drift. To reduce drift on example system, stabilize room and soln temps, adjust manifold to maintain const back-pressure, and/or stabilize flame.) As long as drift does not exceed 1% per 10 peak level, routine data may be further improved by inserting a middle std periodically between groups of samples. This allows mathematical peak ht correction, assuming linear drift.

(e) *Precision.*—With instrument calibrated for 10% and 90% full scale for low and high stds, resp., sample 30 middle stds under analysis conditions. Range of instrument response may not vary >2% full scale. (Optimum performance on example system is 0.7% full scale. To improve precision on example system, reduce noise, check sampler timing, and/or decrease sampling rate.)

(**f**) *Std curve.*—Std curve consists of ≥6 different stds, evenly distributed thru std concn range. Prep. solns from NBS or primary std KH_2PO_4, dried 2 h at 105°. Include factor for actual purity of std material in calcns of std concn.

With instrument calibrated for ca 10% and 90% response for low and high stds, resp., run stds in order of ascending concn under analysis conditions. Response should be linear. Mathematically perform first degree least squares fit to std curve data. Alternatively, use calculator capable of least squares fits. First order least squares fit may be performed as follows: Assume that points to be fitted are $(X_1, Y_1), (X_2, Y_2), \cdots (X_n, Y_n)$. Calc. means by:

$$\overline{X} = \frac{1}{n}\Sigma X_i \quad \overline{Y} = \frac{1}{n}\Sigma Y_i$$

Slope of least square fitted line is given by:

$$b_1 = \frac{\Sigma(X_i - \overline{X})(Y_i - \overline{Y})}{\Sigma (X_j - \overline{X})^2} = \frac{(\Sigma X_i Y_i) - n\overline{XY}}{(\Sigma X_j^2) - 1/n(\Sigma X_j)^2}$$

Intercept for line is given by:

$$b_0 = \overline{Y} - b_1\overline{X}$$

Equation of resulting line is:

$$Y = b_0 + b_1 X$$

Using derived equation and individual std responses, calc. concn for each std. Compare calcd and known concns for each std. Calcd value may not differ from known value by >±2% in any one instance. Also, av. of absolute values of those % differences may not be >1%. (Optimum performance on example system is 0.75% and 0.37%, resp. To improve std curve fit, optimize parameters (**b**) thru (e) above and/or reduce std range.)

(g) *Phosphate effect.*—For example system, amt of La_2O_3 in $LiNO_3$ reagent is sufficient to eliminate phosphate effect (depression of instrument response to K by phosphate ion). If other than example automated system is used, elimination of phosphate effect must be verified. Using KNO_3, prep. 200 mL soln of K_2O with concn equal to twice that of highest std. Pipet 50 mL of that soln into each of two 100 mL vol. flasks. Dil. one to vol. and mix. Add sufficient $NH_4H_2PO_4$ soln to the other flask such that concn of P_2O_5 will be as high as highest concn of P_2O_5 anticipated in any sample ext. Dil. to vol. and mix. Sample 10 portions of each soln, alternating, under analysis conditions. Average 10 responses for each soln. Av. responses of the 2 solns must not differ from each other by >1%. Select min. amt of La_2O_3 which will eliminate phosphate effect. (Optimum performance of example system is <0.5%. To improve performance, adjust amount of La_2O_3.)

(**h**) *Overall performance of system.*—Performance characteristics mentioned above are worst case examples. A system functioning marginally

in many categories would probably fail the following overall performance check.

Verify overall performance as follows: Ext and analyze once each 20 different Magruder samples, or other similar performance check samples previously detd by interlaboratory study. Also ext and analyze 5 independent 1 g portions of NBS or primary std KH_2PO_4. Randomize Magruder and KH_2PO_4 sample order. Calc. % K_2O. Av. bias of Magruder results, Σ (Magruder grand av. − calcd % K_2O)/20, must be $<\pm 0.1$. Av. of absolute value of differences must be <0.4. (Optimum values on example system are ca ±0.02 and ±0.2, resp.)

For 5 analyses of KH_2PO_4, difference between mean of calcd % K_2O and known % K_2O must not be $>\pm 0.2$, and std deviation must not be >0.25. (Optimum values for example system are ±0.1 and 0.15, resp.)

(**i**) *Ongoing performance checks.*—(*1*) Conduct daily performance check by analyzing same performance check sample at least once in every 60 regular samples, and at least once in each run. (*2*) Repeat (**h**) above at least twice per year, and whenever system has not been used for prolonged periods.

Example Automated Instrument System

2.D09 *Apparatus*

Automatic analyzer.—AutoAnalyzer with following modules (available from Technicon Instruments Corp.): sampler II or IV, pump III, flame photometer IV, and recorder. Computer or calculator capable of least square fits is desirable.

2.D10 *Reagents*

(**a**) *Ammonium oxalate soln.*—Dissolve 40 g $(NH_4)_2C_2O_4$ in 1 L H_2O.

(**b**) *Ammonium citrate soln.*—Should have sp. gr. of 1.09 at 20° and pH of 7.0 as detd potentiometrically.

Dissolve 370 g cryst. citric acid in 1.5 L H_2O and nearly neutze by adding 345 mL NH_4OH (28–29% NH_3). If concn of NH_3 is <28%, add correspondingly larger vol. and dissolve citric acid in correspondingly smaller vol. H_2O. Cool, and check pH. Adjust with NH_4OH (1+7) or citric acid soln to pH 7. Dil. soln, if necessary, to sp. gr. of 1.09 at 20°. (Vol. will be ca 2 L.) Keep in tightly stoppered bottles and check pH from time to time. If pH has changed from 7.0, readjust.

(**c**) *Lithium nitrate soln.*—Dissolve 1.642 g La_2O_3 in 30 mL HNO_3, add 0.9935 g dried (2 h at 105°) $LiNO_3$ and 1 mL Flaminox 1% soln (Fisher Scientific Co.), and dil. to 1 L with H_2O.

(**d**) *Sampler wash and dilution water soln.*—Dil. 1 mL Flaminox 1% soln to 1 L with H_2O.

(**e**) *Potassium std solns.*—(*1*) *Stock std soln.*—1 mg K_2O/mL. Dissolve 2.889 g dried (2 h at 105°) KH_2PO_4 (NBS SRM 200) in H_2O, and dil. to 1 L. (*2*) *Working std solns.*—10, 20, 30, 40, 50, and 55 μg K_2O/mL. Accurately measure by buret 10, 20, and 30 mL stock std soln into 1 L vol. flasks, and 20, 25, and 27.5 mL into 500 mL vol. flasks. Add 0.2 g $(NH_4)_2C_2O_4$ per 500 mL final vol. if samples are prepd by ammonium oxalate extn, or add 12 mL ammonium citrate soln per 500 mL final vol. if samples are prepd by ammonium citrate extn. Dil. to vol. with H_2O and mix. (Add 3 mL $CHCl_3$ to preserve citrate std solns for long periods.)

2.D11 *Analytical System*

Assemble manifold as in Fig. **2:D1**. Use 1.6–2.0 mm id glass transmission tubing for all reagent flow upstream from D1 fitting. Use clear std pump tubes for air and soln stream flow.

Air and H_2O are combined thru injection fitting (116-0492-01). Hard thin-wall polyethylene tubing (ca 0.30 in. id) connects air bar tubing to injection fitting. Sample is introduced immediately downstream thru second injection fitting (194-G012-01), designed to eliminate double peaks in recorder output. Mixing of sample and H_2O occurs in double 10-turn coil with insert (157-B089). $LiNO_3$ reagent is introduced thru insert. Another 10-turn coil (157-0251) further mixes solns.

Portion of soln is aspirated to flame photometer thru A4 fitting (116-0200-04). Hard, thin-wall polyethylene tubing (ca 0.045 in. id) connected to photometer is inserted and glued to tee arm of A4 fitting. Remaining unaspirated soln is drawn thru double 10-turn mixing coil (157-0248-01) and thru D1 fitting (116-0203-01). Large diam. branch of D1 fitting leads to pump and waste. Small diam. branch of D1 fitting is connected to 6 ft (1.83 m) of Tygon tubing (0.030 in. id) to waste. D1 fitting is oriented with small diam. branch low, so that only soln, and no air, enters 0.030 in. tubing. This establishes const back pressure and therefore stable aspiration conditions at flame photometer.

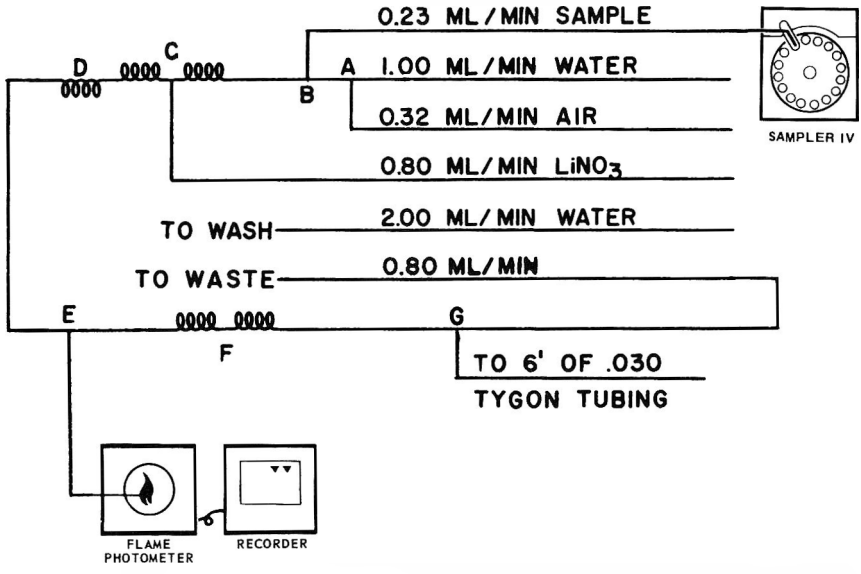

Figure 2:D1. Manifold for K_2O in fertilizers. A, injection fitting 116-0492-01; B, injection fitting 194-G012-01; C, double 10-turn coil with insert 157-B089; D, 10-turn coil 157-0251; E, A4 fitting 116-0200-04; F, double 10-turn coil 157-0248-01; G, D1 fitting 116-0203-01.

2.D12 *Startup and Shutdown Procedures*

Start system and place reagent lines in proper solns. Let equilibrate 30 min before beginning calibration. Adjust flame photometer as follows: (*1*) damping control to damp 3 position; (*2*) flame ht of main cone ca 4 cm; (*3*) atomizer adjust control set to give atomization rate of ca 1.3–1.4 mL/min. Rate of atomization is detd by subtracting rate of flow to waste from rate of flow upstream from (disconnected) A4 fitting. Use 0.3 and 0.6 neut. density filters for Li and K detectors, resp.

Initially it may be necessary to manually fill system downstream from A4 fitting with H_2O, making certain that 6 ft of 0.030 tubing is filled. System is shut down after pumping H_2O thru reagent lines ≥15 min.

2.D13 *Checkout and Calibration*

After equilibration, pump 10 μg K_2O/mL std thru system and adjust baseline control of photometer to read 10% full scale. Pump 55 μg K_2O/mL std thru system and adjust std calibration control to read 90% full scale. If noisy conditions exist, check for aspiration of air at A4 fitting, or check for air entering lower arm of D1 fitting. If drift exists, check room and solns for temp. stability. Std curves should be virtually linear.

2.D14 *Determination*

Pipet aliquots of sample solns, Table **2:D1,** into 250 mL vol. flask, dil. to vol. with H_2O, and mix 15 times. For 10 mL aliquots of citrate extns, add 4 mL ammonium citrate soln to aliquots before dilg to vol. Run samples in groups of 10. Place 10 thru 55 μg K_2O/mL stds in order in sampler, preceded by extra 10 μg/mL std. Place 30 μg/mL std after every 10th sample, to be used for drift correction. End series with two 30 μg/mL stds. Sample at rate of 40/h, 2:1 sample-to-wash ratio.

2.D15 *Calculations*

Correct sample peak hts for drift. Correct peak hts of first 10 samples as follows:

$$H_c = H - [(D_1 - D_0)/14][L + 3]$$

where H_c = corrected peak ht; H = uncorrected peak ht; D_1 = ht of first drift correction std; D_0 = ht of 30 μg std in initial std sequence; and L =

Table 2:D1. K_2O aliquots

% K_2O expected	Aliquot, mL
<2	250 (no diln)
2–6.49	100
6.50–19.99	30
≥20	10

position No. of sample peak to be corrected. Correct subsequent sample peak hts as follows:

$$H_c = H - [D_x - D_0] - [(D_y - D_x)/11][P]$$

where D_x = ht of drift std preceding sample to be corrected; D_y = ht of drift std following sample to be corrected; and P = position No. of sample within group of 10.

Calc. least squares fitted curve of emission against K_2O concn. Calc. μg K_2O/mL of corrected peak hts from equation:

$$\% K_2O = (\mu g\ K_2O/mL \times 12.5)/(\text{aliquot} \times \text{g sample})$$

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(4) The following atomic absorption spectrophotometric method for the determination of chelated iron in iron chelate concentrates was adopted official first action:

Chelated Iron in Iron Chelate Concentrates

Atomic Absorption Spectrophotometric Method

Official First Action

(Applicable to Fe ethylenediaminetetraacetate (EDTA), Fe hydroxyethylethylenediaminetriacetate (HEDTA), Fe diethylenetriaminepentaacetate (DTPA), Fe ethylenediaminedi-*o*-hydroxyphenylacetate (EDDHA), Fe *N,N*-bis(2-hydroxy-5-sulfobenzyl)glycine (DPS), and Fe citrate. Not applicable to mixed fertilizers, or to samples contg non-chelated metals other than Fe.)

2.D16 ***Principle***

Sample is dissolved in H_2O, and non-chelated Fe is pptd as $FeOH_3$ at pH 8.5 and removed. Chelated Fe is detd by AAS, using std solns contg Na_2H_2EDTA.

2.D17 ***Apparatus and Reagents***

(a) *Sodium hydroxide soln.*—0.5N. Dissolve 20 g NaOH in H_2O and dil. to 1 L.

(b) *Disodium EDTA soln.*—0.66%. Dissolve 0.73 g $Na_2H_2EDTA.2H_2O$ in H_2O) and dil. to 100 mL.

(c) *Iron std solns.*—(*1*) *Stock soln.*—1000 μg Fe/mL. Dissolve 1.000 g pure Fe wire in ca 30 mL 6N HCl with boiling. Dil. to 1 L. (*2*) *Intermediate soln.*—100 μg Fe/mL. Pipet 10 mL Fe stock soln and 10 mL Na_2H_2EDTA soln into 100 mL vol. flask and dil. to vol. with H_2O. (*3*) *Working solns.*—Dil. aliquots of intermediate soln with 0.5N HCl to make ≥4 std solns within range of detn (2–20 μg Fe/mL).

(d) *Atomic absorption spectrophotometer.*—With air-C_2H_2 flame. See **2.109(a).**

2.D18 ***Determination***

Weigh sample contg ca 40 mg Fe into 200 mL tall-form beaker. Wet with 2–3 drops of alcohol and dissolve in 100 mL H_2O. Add 4 drops of 30% H_2O_2, mix and adjust pH of soln to 8.5 with 0.5N NaOH. If pH drifts above 8.8, discard soln and repeat analysis. Transfer soln to 200 mL vol. flask, dil. to vol. with H_2O, and mix. Filter soln thru quant. paper. Pipet 10 mL filtrate into 200 mL vol. flask and dil. to vol. with 0.5N HCl. Measure A of solns, using lean air-C_2H_2 flame as in **2.112** and det. concn of Fe in sample (μg Fe/mL) from either calibration curve or digital concn readout. In same manner, det. Fe blank on all reagents used.

$$\% \text{ Chelated iron} = (\mu g\ Fe/mL \text{ in sample} - \mu g\ Fe/mL \text{ in blank}) \times 0.4/\text{g sample}$$

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(5) The following atomic absorption spectrophotometric method for the determination of sodium in fertilizers was adopted official first action:

Sodium in Fertilizers

Atomic Absorption Spectrophotometric Method

Official First Action

2.D19 ***Reagents and Apparatus***

(a) *Ammonium oxalate soln.*—Dissolve 40 g $(NH_4)_2C_2O_4$ in 1 L H_2O.

(b) *Sodium chloride.*—Dry 2 h at 105°.

(c) *Atomic absorption spectrophotometer.*—Model AA6 (Varian Techtron Pty Ltd, 679-701 Springvale Rd, Mulgrave, Vic., Australia 3170), or equiv.

2.D20 ***Preparation of Solution***

Weigh 2.5 g (<4% Na) or 1.25 g (4–20% Na) sample into 250 mL vol. flask, add 125 mL H_2O and 50 mL $(NH_4)_2C_2O_4$ soln, and boil 30 min. Cool, dil. to vol., mix, and pass thru dry filter. For samples contg <1% Na, use this soln for detn. For samples contg 1–20% Na, place 20 mL in 100 mL vol. flask, dil. to vol. with H_2O, and mix.

2.D21 ***Preparation of Standard Curve***

Dissolve 2.5421 g dried NaCl in H_2O and dil.

to 1 L (1000 ppm Na). Prep. std solns to cover range 0–200 ppm at intervals ≤20 ppm Na.

2.D22 ***Determination***

Set wavelength at 330.3 nm using air-C_2H_2 flame. Aspirate stds and samples. Plot curve from std values and det. Na content of sample solns from plot of A against ppm Na. Calc. % Na as follows:

≤1%: ppm Na × $25/M$ = %Na

1–20%: ppm Na × $125/M$ = %Na

where M = wt of sample (mg).

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(6) The applicability of the official first action atomic absorption spectrophotometric method (**8.023–8.026**) for the determination of aluminum in baking powders and chemicals to determining aluminum in aluminum sulfate-type soil acidifiers was adopted official first action:

Aluminum in Aluminum Sulfate-type Soil Acidifiers

Atomic Absorption Spectrophotometric Method

Official First Action

2.D23 ***Apparatus and Reagents***

(**a**) *Atomic absorption spectrophotometer.*—Perkin-Elmer Model 303, or equiv. See **8.023** for typical operating parameters.

(**b**) *Diluting soln.*—Add 20 mL H_2SO_4 and 2.5 g NaCl to 500 mL H_2O. Dil. to 1 L with H_2O.

(**c**) *Aluminum std solns.*—(*1*) *Stock soln.*—1 mg Al/mL (1000 ppm). Accurately weigh 1.000 g pure Al and dissolve in ca 25 mL HCl. Evap. almost to dryness, add 500 mL H_2O, 20 mL H_2SO_4, and 2.5 g NaCl, and dil. to 1 L with H_2O. (*2*) *Working solns.*—Dil. aliquots of stock soln with dilg soln, (**b**), to make 4 std solns within range 50–150 ppm.

2.D24 ***Preparation of Sample***

Accurately weigh ca 1 g sample into 500 mL screw-cap erlenmeyer, add 250 mL H_2O, and shake on wrist-action shaker ca 15 min. Quant. transfer to 500 mL vol. flask and dil. to vol. with H_2O. Filter thru Whatman No. 2 paper. Dil. aliquot as necessary (diln factor = F) with dilg soln, (b), for concn range 50–150 ppm.

2.D25 ***Determination***

Set up app. as shown in Table **8:01,** or use previously established optimum settings for app. Zero app. while aspirating dilg soln, (**b**). Det. A of std solns within 50–150 ppm range, alternating with sample soln readings. Flush burner with dilg soln, (**b**), and check zero point between readings.

Det. Al content from std curve of A against μg Al/mL.

$$\% \text{ Al} = (\mu\text{g Al/mL}) \times F \times (500/\text{g sample}) \times 10^{-4}$$

3. PLANTS

No additions, deletions, or changes.

4. DISINFECTANTS

No additions, deletions, or changes.

5. HAZARDOUS SUBSTANCES

No additions, deletions, or changes.

6. PESTICIDE FORMULATIONS

(1) The following official first action methods were adopted official final action:

(a) Terbuthylazine, AOAC-CIPAC gas-liquid chromatographic method, **6.B20–6.B27.**

(**b**) Pyrethrins and piperonyl butoxide, gas-liquid chromatographic method, **6.C22–6.C25.**

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(2) The official first action gas-liquid chromatographic method for the determination of pentachloronitrobenzene, **6.C08–6.C11,** was adopted official final action after modification as follows:

In **6.C09(c),** delete the last sentence, "Discard after 3 days."

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(3) The following liquid chromatographic method for the determination of brodifacoum in technical material and liquid and bait formulations was adopted official first action:

Brodifacoum in Technical Material and Formulations

Liquid Chromatographic Method

Official First Action

6.D01 ***Principle***

A weighed sample of tech. brodifacoum, brodifacoum conc., or bait ext is dissolved in triphenylbenzene internal std soln and detd by reverse phase liq. chromatgy and UV detection.

6.D02 ***Apparatus***

(**a**) *Liquid chromatograph.*—Flow rate 1 mL/

min; loop injection 10 μL; mobile solv. MeOH-H_2O-HOAc (94.2 + 5.0 + 0.8), filtered and degassed; UV detection at 254 nm with range to give peak hts ca 60–80% full scale. Retention times (min)—brodifacoum 6.2, internal std 11.7.

(b) *Column.*—25 cm × 4.6 mm Zorbax ODS 5 μm reverse phase column (DuPont Instruments Inc.).

(c) *Centrifuge.*—Equipped with 15 mL capped tubes.

(d) *Macerator.*—With 400 mL stainless steel cup/impeller assembly, such as Sorvall Omnimixer (DuPont Instruments Inc.).

(e) *Rotary evaporator.*—Fitted with vac. and cold H_2O supplies.

(f) *Filter.*—Spread 10 g Celite 545 on 9 cm No. 5 filter paper wetted with MeOH in buchner. Press filter and prewash with 30 mL MeOH.

6.D03 ***Reagents***

(a) *1,3,5-Triphenylbenzene internal std soln.*—Accurately weigh ca 100 mg pure 1,3,5-triphenylbenzene into 500 mL vol. flask, dissolve in 200 mL CH_2Cl_2, and dil. to vol. with MeOH.

(b) *Brodifacoum std soln.*—Accurately weigh ca 100 mg brodifacoum std of known purity (ICI Americas Inc., PO Box 208, Goldsboro, NC 27530) into 100 mL vol. flask. Dissolve in 40 mL CH_2Cl_2. Dil. to vol. with MeOH. Transfer 10.0 mL each of brodifacoum std soln and internal std soln to 50 mL vol. flask and dil. to vol. with dilg soln.

(c) *Diluting soln.*—CH_2Cl_2-MeOH (2 + 3).

(d) *Extracting soln.*—CH_2Cl_2-formic acid (50 + 1).

Store reagents in tightly capped dark bottles to avoid evapn and decomposition. Check internal std soln for interfering components by injecting 10 μL into liq. chromatograph.

6.D04 ***Determination***

(a) *Technical material.*—Accurately weigh ca 100 mg sample into 100 mL vol. flask. Dissolve in 40 mL CH_2Cl_2. Dil. to vol. with MeOH. Transfer 10.0 mL each of sample soln and internal std soln to 50 mL vol. flask. Dil. to vol. with dilg soln.

(b) *Powder concentrate.*—Accurately weigh amt sample contg ca 5 mg brodifacoum into 250 mL capped conical flask. Add 100 mL extg soln and shake 1 min. Filter thru Celite, using two 30 mL washes of extg soln. Evap. filtrate at 60° under vac. Dissolve residue in 20.0 mL dilg soln and 5.0 mL internal std soln.

(c) *Liquid concentrate.*—Accurately weigh amt sample contg ca 5 mg brodifacoum into 25 mL vol. flask. Add 5.0 mL internal std soln, and dil. to vol. with dilg soln.

(d) *Pelleted bait.*—Grind amt sample contg ca 2 mg brodifacoum in anal. mill. Transfer to tared macerator cup and accurately weigh. Add 250 mL extg soln and homogenize 10 min. Filter thru Celite using three 50 mL washings of extg soln. Rotary-evap. filtrate at 60° under vac. Dissolve residue in 8.0 mL dilg soln and 2 mL internal std soln. Centrf. sample to remove remaining solids.

Inject 2 or more aliquots of std soln into liq. chromatograph to set integration parameters and stabilize instrument. Monitor response factor until results agree within 2%. Inject 4 aliquots of std soln and 2 aliquots of sample soln in succession. Calc. response factor, R, for each:

$$R = \text{area brodifacoum peak} / \text{area internal std peak}$$

Peak hts can be used in place of peak areas for tech. material and bait formulations, but not for liq. formulations.

$$\text{Brodifacoum, \%} = (R/R') \times (W'/W) \times P \times F$$

where R and R' = av. response factor for sample and std solns, resp.; W and W' = mg sample and std, resp.; P = purity (%) of std; F = scaling factor = 1 for technical material, 1/20 for powder and liq. concs, 1/50 for pelleted bait.

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(4) The following liquid chromatographic method for the determination of rotenone in formulations was adopted official first action:

Rotenone in Formulations
Liquid Chromatographic Method
Official First Action

6.D05 ***Principle***

Sample is extd with dioxane, and rotenone is detd by reverse phase HPLC with UV detection at 280 nm.

6.D06 ***Apparatus and Reagents***

(a) *Liquid chromatograph.*—M6000A pump, U6K injector, Model 450 variable UV detector (all Waters Associates, Inc.), and Omni-Scribe recorder (Houston Instrument, Austin, TX 78753), or equiv. system. Operating conditions: column ambient; flow rate 1.0 mL/min for Partisil column, 1.5 mL/min for Zorbax column, 1.2 mL/min for Bondapak column; injection vol. 5

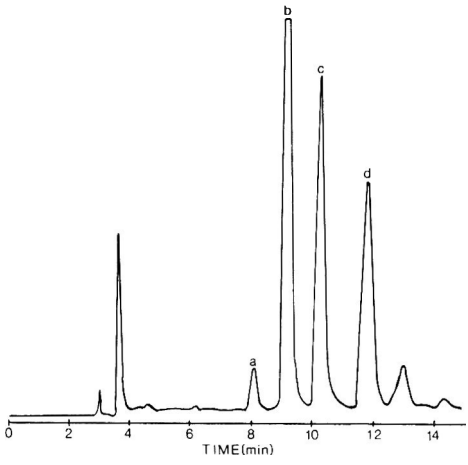

Figure 6:D1. Liq. chromatogram of rotenone sample with Whatman column: a, rotenolone; b, tephrosin and an unknown; c, rotenone; d, deguelin.

μL for Partisil column and 10 μL for others; detector wavelength 280 nm; absorbance range 0.4 AUFS; chart speed 1 cm/min.

(**b**) *Chromatographic columns.*—Partisil 5 ODS-3, 5 μm particle size, stainless steel, 25 cm × 4.6 mm id (Whatman Inc., Clifton, NJ 07014). Zorbax C_8, 10 μm particle size, stainless steel, 25 cm × 4.6 mm id (DuPont Co.). μBondapak C_{18}, 10 μm particle size, stainless steel, 30 cm × 3.9 mm id (Waters Associates, Inc.).

(**c**) *Mobile phases.*—Use LC grade org. solvs (Fisher Scientific Co.). Use glass-distd H_2O treated to remove org. compds by passing thru C_{18} column system (Millipore Corp., Bedford, MA 01730), or use HPLC grade H_2O. Use MeOH–H_2O (75 + 25), (68 + 32), and (66 + 34) for Partisil, Zorbax, and Bondapak, resp. If necessary, adjust mobile phase to give adequate sepn of tephrosin, rotenone, and deguelin in test soln (Fig. 6:D1).

(**d**) *Test soln.*—Accurately weigh portion of well mixed sample of Noxfish Fish Toxicant or powd cubé root ext (Penick Co., Lyndhurst, NJ 07071) contg ca 20 mg rotenone into 125 mL g-s erlenmeyer. Add 50 mL dioxane, and mix.

(**e**) *Std soln.*—Accurately weigh ca 20 mg 99% pure rotenone (Penick Co.) into 50 mL vol. flask and dil. to vol. with dioxane (reagent grade). Keep rotenone from light or store in actinic glassware.

(**f**) *Sample extraction solvent.*—Reagent grade dioxane.

6.D07 *Preparation of Sample*

(**a**) *Solid formulations.*—Accurately weigh portion of well mixed sample contg ca 20 mg rotenone into 125 mL g-s erlenmeyer. Pipet in 50 mL dioxane, stopper, and shake 1½ h on rotary shaker. Let settle and filter aliquot thru 0.45 μm organic filter (Millipore Corp.), or equiv.

(**b**) *Liquid formulations.*—Use same procedure as above, omitting rotary shaking and settling.

6.D08 *Determination and Calculation*

Inject std soln followed by 2 injections of sample soln and another injection of std soln. Measure peak hts, average, and calc. as follows:

$$\% \text{ Rotenone} = (PH/PH') \times (W'/W) \times \% \text{ purity of std}$$

where PH and PH' = av. peak hts of sample and std solns, resp.; W' = g rotenone std/50 mL; and W = g sample extd.

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(5) The following liquid chromatographic method for the determination of diflubenzuron in formulations was adopted official first action as a CIPAC–AOAC method:

Diflubenzuron (*N*-(((4-Chlorophenyl)amino)-carbonyl)-2,6-difluorobenzamide) in Formulations

Liquid Chromatographic Method

Official First Action

CIPAC-AOAC Method

6.D09 *Principle*

Diflubenzuron is dissolved in 1,4-dioxane, sepd by HPLC, and detd from peak areas vs linuron internal std. Identity is confirmed by retention time.

6.D10 *Apparatus*

(**a**) *Liquid chromatograph.*—Provided with const flow pump and 20 μL sample loop. Operating conditions: mobile phase flow rate, 1.3 mL/min; detector sensitivity, 128 × 10^{-3} AUFS; temp., ambient, should not fluctuate >2°; retention time of diflubenzuron relative to internal std, ca 1.36.

(**b**) *Detector.*—UV spectrophtr or fixed wavelength UV detector at 254 nm.

(**c**) *Recorder.*—Range to match output of HPLC detector.

(**d**) *Liquid chromatography column.*—Stainless steel, 250 × 4.6 mm, packed with Zorbax BP-C_8 (E.I. DuPont de Nemours & Co.), or equiv. (e.g., μBondapak C_{18}, 10 μm, Waters Associates, Inc.;

Spherisorb ODS, 5 μm, Phase Separations Ltd, Deeside Industrial Estate, Queensferry Clwyd, UK; Zorbax BP-ODS, 7 μm, E.I. DuPont de Nemours).

(e) *Filter.*—Acrodisc disposable filter assembly, 1.2 μm (Gelman Sciences, Inc., 600 S Wagner Rd, Ann Arbor, MI 48106), or equiv.

6.D11 ***Reagents***

(a) *Mobile phase.*—Acetonitrile–H_2O–1,4-dioxane (45 + 45 + 10). Mix 450 mL acetonitrile, 450 mL H_2O, and 100 mL 1,4-dioxane and degas.

(b) *Solvent mixture.*—Acetonitrile–H_2O (45 + 55).

(c) *Internal std soln.*—Accurately weigh 25 mg linuron (National Physical Laboratory, Div. of Chemical Standards, Teddington, Middlesex, TW11 OLW, UK) into 100 mL vol. flask, dil. to vol. with acetonitrile, and mix.

(d) *Diflubenzuron std soln.*—Accurately weigh ca 50 mg pure diflubenzuron (Duphar B.V., PO Box 2, 1380 AA Weesp, The Netherlands) into 100 mL vol. flask. Add 50 mL dioxane and dissolve by heating 30 min in 80° H_2O bath. Swirl occasionally. Add 40 mL dioxane, cool, and dil. to vol. with dioxane. Pipet 5 mL into 50 mL vol. flask, add 5.00 mL internal std soln, (c), dil. to vol. with solv. mixt., (b), and mix.

(e) *1,3-Di(4-chlorophenyl)urea.*—Duphar B.V.

6.D12 ***System Performance Check***

Dissolve, in 100 mL vol. flask, 4.2 mg diflubenzuron and 1.8 mg 1,3-di(4-chlorophenyl)urea in 20 mL dioxane, dil. to vol. with solv. mixt., (b), and mix. Filter thru 1.2 μm filter before injection.

Inject 20 μL onto column and det. resolution (R) by following formula:

$$R = 2\,d/(W_1 + W_2)$$

where R = resolution; d = distance between peak maxima; W_1 and W_2 = peak width at baseline of diflubenzuron and 1,3-di(4-chlorophenyl)urea, resp. Resolution should be >1. If necessary, resolution can be improved by slightly increasing H_2O content of mobile phase.

6.D13 ***Preparation of Sample***

(a) *Diflubenzuron pre-concentrate.*—Accurately weigh sample contg 1.0 g diflubenzuron into 200 mL vol. flask. Add 150 mL dioxane and heat 30 min in 80° H_2O bath. Swirl occasionally. Add 40 mL dioxane, cool, and dil. to vol. with dioxane. Pipet 10 mL into 100 mL vol. flask and dil. to vol. with dioxane. Pipet 5 mL dild soln into 50 mL vol. flask, add 5.00 mL internal std soln, (c), dil. to vol. with solv. mixture (b), and mix. Filter thru 1.2 μm filter.

(b) *Water dispersible powder.*—Accurately weigh sample contg 0.5 g diflubenzuron into 200 mL vol. flask. Add 150 mL dioxane and heat 30 min in 80° H_2O bath. Swirl occasionally. Add 40 mL dioxane, cool, and dil. to vol. with dioxane. Immediately pipet 20 mL homogeneous suspension into 100 mL vol. flask and dil. to vol. with dioxane. Pipet 5 mL dild soln into 50 mL vol. flask, add 5.00 mL internal std soln, (c), dil. to vol. with solv. mixture, (b), and mix. Filter thru 1.2 μm filter.

6.D14 ***Determination***

Inject 20 μL diflubenzuron std soln, (d). Det. peak areas (or peak hts × retention times) of diflubenzuron and internal std. Repeat injections until response ratio (area diflubenzuron peak/area internal std peak) varies <1% for successive injections. Inject 20 μL sample soln.

6.D15 ***Calculation***

$$\%\ \text{Diflubenzuron} = RR \times W' \times V \times 100/(RR' \times W)$$

where RR and RR' = ratio of area of diflubenzuron peak to area of internal std peak for sample and std, resp.; W and W' = g sample and std, resp.; V = diln factor (= 20 and 10 for preconc. and H_2O-dispersible powder, resp.).

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(6) The following gas-liquid chromatographic method for the determination of endosulfan in formulations was adopted official first action as a CIPAC–AOAC method:

Endosulfan (Hexachlorohexahydromethano-2,4,3-benzodioxathiepin 3-Oxide) in Formulations

Gas-Liquid Chromatographic Method

Official First Action

CIPAC-AOAC Method

6.D16 ***Principle***

Sample is extd with toluene and α- and β-endosulfan isomers are detd sep. by thermal conductivity or flame ionization GLC, using di(2-ethylhexyl)phthalate as internal std.

6.D17 ***Apparatus***

(a) *Gas-liquid chromatograph.*—Suitable for on-column injection; equipped with thermal

conductivity detector. Flame ionization detector may be used with proper diln of samples and stds.

(b) *Chromatographic column.*—3 mm id × 1.5 m (5 ft) glass column packed with 10% OV-210 on 80–100 mesh Chromosorb W-HP. Condition column ≥16 h, at 250°, using carrier gas at ca 25 mL/min. Operating conditions: injector 300°, detector 250°, column 230°, He carrier gas flow ca 60 mL/min.

6.D18 ***Reagents***

(a) *Toluene.*—GLC quality.

(b) *Internal std soln.*—15 mg/mL. Dissolve 15 g di(2-ethylhexyl)phthalate, 99%+, in toluene and dil. to 1 L with toluene.

(c) *Endosulfan std soln.*—Accurately weigh 0.300 g endosulfan of known α- and β-isomer content (Riedel de Haen Co., D-3016 Hannover-Seelze 1, GFR) into 25 mL g-s flask. Pipet in 10 mL internal std soln, (b), mix, and store ≥20 min at 60° to ensure dissolution.

6.D19 ***Preparation of Sample***

(a) *Technical endosulfan.*—Accurately weigh 0.300 g sample into 25 mL g-s flask, pipet in 10 mL internal std soln, (b), mix, and store ≥20 min at 60°.

(b) *Emulsifiable concentrates.*—Prep. as in (a), using wt equiv. to 0.300 g endosulfan.

(c) *Wettable powder.*—Accurately weigh sample contg 0.300 g endosulfan into 25 mL g-s flask, pipet in 10 mL internal std soln, (b) mix, store ≥20 min at 60°, and centrf.

6.D20 ***Determination***

Inject 2 μL portions of std soln, (c), until response factor for each component varies <1% for successive injections. Inject duplicate 2 μL portions of sample soln followed by 2 μL portions of std soln. For each injection, calc. response ratio of α-isomer peak to internal std peak, and ratio of β-isomer peak to internal std peak, either as peak ht or digital integrator count for area. Retention time for each isomer and internal std should be the same for sample and std solns.

6.D21 ***Calculation***

$$\% \ \alpha\text{-isomer} = C_\alpha = R_\alpha \times W' \times P_\alpha/(R'_\alpha \times W)$$

$$\% \ \beta\text{-isomer} = C_\beta = R_\beta \times W' \times P_\beta/(R'_\beta \times W)$$

where R_α and R'_α = response ratios of α-isomer peaks to internal std peaks for sample and std soln, resp.; R_β and R'_β = response ratios of β-isomer peaks to internal std peaks for sample and std soln, resp.; W and W' = wt (g) of sample and endosulfan std, resp.; P_α and P_β = % α- and β-isomers in std, resp.

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(7) The following liquid chromatographic method for the determination of glyphosate technical and formulations was adopted official first action:

Glyphosate (*N*-Phosphonomethyl Glycine) Technical and Formulations

Liquid Chromatographic Method

Official First Action

6.D22 ***Principle***

Samples are dissolved in phosphate buffer mobile phase and injected directly into ion exchange chromatogc system using fixed vol. loop. Peak area response as measured by UV detector is quantitated by external std technic.

6.D23 ***Apparatus and Reagents***

(a) *Liquid chromatograph.*—Able to generate over 1000 psi and measure *A* at 195 nm.

(b) *Loop injector.*—Rheodyne Model 7120 syringe loading (Supelco, Inc.), or equiv.

(c) *Strip chart recorder.*—Houston Instrument 10 mV full scale (Industrial Scientific, PO Box 60002, Houston, TX 77060), or equiv.

(d) *Electronic integrator.*—Capable of handling detector output.

(e) *Chromatographic column.*—25 cm × 4.6 mm id, ¼ in. od, strong anion exchange, e.g., Partisil 10 SAX (available from Whatman, Inc., 9 Bridewell Pl, Clifton, NJ 07014).

(f) *Methanol.*—HPLC grade (available from Burdick & Jackson Laboratories, Inc.).

(g) *Water.*—HPLC grade (available from Burdick & Jackson Laboratories, Inc.). Use thruout.

(h) *Potassium dihydrogen phosphate.*—Primary std grade (available from Fisher Scientific Co.).

(i) *Phosphoric acid.*—85%, reagent grade (available from Fisher Scientific Co.).

(j) *Glyphosate std.*—Monsanto Co.

(k) *Mobile phase.*—Dissolve 0.8437 g KH_2PO_4 in 960 mL H_2O. Add 40 mL MeOH and mix well. Using pH meter buffered at pH 2.0, adjust pH to 1.9 with 85% H_3PO_4. Filter and degas before use.

6.D24 ***Preparation of Standard***

Accurately weigh ca 400 ± 10 mg glyphosate std (dried 2 h at 105°) into 100 mL vol. flask. Dil.

to vol. with mobile phase and stir to dissolve (30 min may be required to dissolve std). Soln is stable ≥1 week.

6.D25 ***Preparation of Sample***

Accurately weigh sample contg ca 400 mg glyphosate into 100 mL vol. flask contg ca 50 mL mobile phase. Dil. to vol. with mobile phase and mix well.

6.D26 ***Determination***

Adjust operating parameters so that glyphosate elutes at 2.5–4.0 min. Maintain all parameters consistent thruout std and sample analysis. Typical values are as follows: flow rate 2.3 mL/min; pressure ca 1200 psi; chart speed 0.5 cm/min; *A* range 0.2 AUFS; column temp. ambient; injector vol. 50 μL.

Let mobile phase flow thru system until steady baseline is obtained; 1 h may be required for new column. When new columns are installed or instrument has not been used for 24 h, make at least 6 rapid injections of std soln; then inject std soln until peak areas for successive injections agree ±1%. Then inject sample soln until peak areas for successive injections agree ±1%. Let all components from samples elute (ca 10–12 min) before making next injection.

6.D27 ***Calculation***

Average peak areas from 2 successive injections that agreed ±1% from both std and sample solns.

$$\% \text{ Glyphosate} = (R/R') \times (W'/W) \times P$$

where R = av. peak area of sample; R' = av. peak area of std; W = mg sample; W' = mg std; and P = % purity of std. To convert % glyphosate to isopropylamine salt, multiply by 1.3496.

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(8) The following liquid chromatographic method for the determination of fensulfothion in formulations was adopted official first action:

Fensulfothion (*O,O*-Diethyl *O*-[*p*-(Methylsulfinyl)phenyl] Phosphorothioate) in Formulations

Liquid Chromatographic Method

Official First Action

6.D28 ***Principle***

Sample is dissolved in or extd with MeOH, benzophenone is added as internal std, and fensulfothion is detd by liq. chromatgy and UV detection at 230 nm.

6.D29 ***Apparatus***

(**a**) *Liquid chromatograph.*—Able to generate >1000 psi and equipped with detector able to measure *A* at 230 nm. Typical operating conditions: temp., ambient; flow rate, 0.8 mL/min; wavelength, 230 nm; chart speed, 2 mm/min; sample size, 10 μL. Conditions may be varied to accommodate instrument and column differences.

(**b**) *Column.*—Whatman Partisil PXS 10/25 ODS-2, stainless steel 25 cm × 4.6 mm id (Whatman, Inc., Clifton, NJ 07014), or equiv.

(**c**) *Filter.*—10 μm Teflon, or similar type.

6.D30 ***Reagents***

(**a**) *Methanol.*—Distd in glass (Burdick & Jackson Laboratories, Inc., or equiv.).

(**b**) *Phosphoric acid.*—85% (Fisher Scientific Co.).

(**c**) *Internal std soln.*—0.25 mg benzophenone/mL. Accurately weigh ca 250 mg benzophenone (Eastman Kodak Co.) into small flask. Transfer to 1 L vol. flask and dil. to vol. with same MeOH to be used in mobile phase. Concn may be varied so that when std soln (**d**) is injected, peak ht of benzophenone matches peak ht of fensulfothion within 20%.

(**d**) *Std soln.*—0.3 mg fensulfothion/mL, within optimum linearity range. Accurately weigh ca 150 mg fensulfothion (Mobay Chemical Co., Box 4913, Kansas City, MO 64120) into 125 mL flask. Pipet in 100 mL MeOH, shake to mix. Pipet 10 mL aliquot of soln into 125 mL flask with screw cap, add exactly 40 mL internal std soln, and shake to mix. Prep. fresh std daily. Keep ref. std in freezer.

(**e**) *Mobile phase.*—MeOH-H_2O (80 + 20) buffered to 0.0025M with H_3PO_4. Mix 800 mL MeOH + 200 mL H_2O + 156 μL H_3PO_4, and degas. If using column other than ODS-2, adjust MeOH-H_2O ratio as necessary.

6.D31 ***Preparation of Sample***

(**a**) *Spray concentrate.*—Accurately weigh sample contg ca 150 mg fensulfothion into 125 mL flask. Pipet in 100 mL MeOH and shake to mix. Pipet 10 mL aliquot into 125 mL flask with screw cap, add exactly 40 mL internal std soln, and shake to mix.

(**b**) *Granular formulations.*—Pour sample into 400 mL beaker and thoroly mix, turning granules over ≥10 strokes with wide spatula. Take weighed amt from beaker before sample is

poured back into sample container. Accurately weigh sample contg ca 150 mg fensulfothion into 125 mL flask. Pipet in 100 mL MeOH and place on mech. shaker 15–30 min. Filter thru 10 μm Teflon or similar type filter. Place 10 mL aliquot of filtrate in 125 mL flask with screw cap, add exactly 40 mL internal std soln, and shake to mix.

6.D32 ***Determination***

Adjust liq. chromatgc operating parameters to elute fensulfothion in 4–7 min. Maintain all parameters const thruout analysis. Benzophenone will elute 2–4 min after fensulfothion.

Adjust injection size and attenuation to give 60–80% on-scale peaks. Make repetitive injections of std until response is stable, and ratios of fensulfothion peak ht to benzophenone peak ht for successive injections vary ≤1%. Then make duplicate injections of sample followed by injection of std. Calc. av. ratio of fensulfothion peak ht to benzophenone peak ht for each set of duplicate injections and calc. % fensulfothion.

$$\text{Fensulfothion, \%} = R \times W' \times P/(R' \times W)$$

where R and R' = av. peak ht ratios for sample and std, resp.; W = mg sample; W' = mg fensulfothion anal. std; and P = % purity of fensulfothion anal. std. Integrator area ratios may be substituted for peak ht ratios.

7. ANIMAL FEED

No additions, deletions, or changes.

8. BAKING POWDERS AND BAKING CHEMICALS

No additions, deletions, or changes.

9. BEVERAGES: DISTILLED LIQUORS

The following densitometric method for the determination of alcohol in liqueur-type and alcoholic dairy products was adopted official first action:

Alcohol in Liqueur-type and Alcoholic Dairy Products

Densitometric Method

Official First Action

(Applicable to products contg dissolved solids)

9.D01 ***Principle***

Sample is distd in special all-glass still and % alcohol in distillate is detd by density meter.

9.D02 ***Apparatus and Reagents***

(**a**) *Liqueur still.*—All Ace Glass, Inc., Cat. Nos: (*1*) *Boiling flask.*—300 mL with 35/20 joint (No. 6902-25).

(*2*) *Vigreaux distilling column.*—With 35/20 joints, 50 cm long (measured from lowest to highest indents) × 2.2 cm od (No. 36578-94).

(*3*) *Connecting adapter.*—With 35/20 joints (No. 35125-99).

(*4*) *Liebig condenser.*—With 35/20 female joint on top and straight 7 mm od tube as outlet (H_2O jacket length ca 38 cm) (No. 35994-96).

(*5*) *Condenser outlet connector tube.*—20 cm long × 7 mm od glass tube.

(*6*) *Clamps.*—3 pinch-type clamps, size 35 (No. 7670-10).

(*7*) *Volumetric flasks.*—50 mL (No. 7102-12).

(**b**) *Density meter.*—Mettler/Paar DMA 55D (Mettler Instrument Corp., Hightstown, NJ 08520).

(**c**) *Silicone antifoam.*—GE-66, GE-70 silicones, or equiv.

9.D03 ***Assembly of Distillation Equipment***

Mount Vigreaux column and condenser vertically on sep. ring stands. Apply film of silicone grease to glass joints and use pinch clamps to secure adapter to top of Vigreaux column and condenser. Connect condenser connector outlet tube to end of condenser with a 10 × 1/4 in. piece of Tygon tubing.

9.D04 ***Distillation Procedure***

Liqueurs.—Place 50 mL vol. flask contg >50 mL sample in const temp. bath. When sample temp. has stabilized, adjust vol. to mark. Quant. transfer sample to 300 mL boiling flask, rinsing vol. flask with three 8 mL portions of H_2O and adding rinsings to boiling flask. Add 1 or 2 glass beads to boiling flask and 1 or 2 drops of silicone antifoam, if sample foams. (Silicone spray *cannot* be used as antifoam because many sprays contain volatile solvs.) Connect boiling flask contg sample to bottom of Vigreaux column with pinch clamp. Add 2.5 mL H_2O to original vol. flask and use flask as distillate receiver. Submerge extension tube from condenser into H_2O in receiver set in ice bath.

Place screen on support clamp under boiling flask and heat flask with gas burner or elec. heater. Do not use elec. heating mantle because solids will carbonize on inside wall of flask. Lower receiver after ca 42 mL distillate has been collected to permit all condensate to drain from condenser into receiver. Permit last 6 mL dis-

tillate to drip into receiver to rinse condenser and extension tube. Total distn time should be >25 but <40 min. Remove 50 mL vol. flask, stopper, swirl to mix contents, and place in const temp. bath. When sample temp. has stabilized to same temp. as before distn, add H_2O to adjust vol. to mark, and mix.

Air bubbles in fresh distillates may be dissipated by placing vol. flask momentarily in ultrasonic bath, by tapping flask gently on the side, or by allowing it to stand for some period before measuring proof.

Alcoholic dairy products.—Treat as described above, except use total of 50 mL H_2O to transfer sample from vol. flask to boiling flask.

9.D05 ***Determination***

Det. % alcohol in distillate by **9.C03-9.C07.**

10. BEVERAGES: MALT BEVERAGES AND BREWING MATERIALS

No additions, deletions, or changes.

11. BEVERAGES: WINES

(1) The official first action volumetric method for the determination of carbon dioxide in wine, **11.063-11.065,** was declared surplus.

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(2) The following gas-liquid chromatographic method for the determination of ethanol in wine was adopted official first action:

Ethanol in Wine
Gas-Liquid Chromatographic Method
Official First Action

11.D01 ***Apparatus and Reagents***

(**a**) *Gas chromatograph.*—With flame ionization detector, integrator, heated on-column injector, and any one of the following columns and corresponding operating conditions:

(*1*) **6 ft** × 2 mm id glass, packed with 80-100 mesh Poropak QS.

(*2*) **6 ft** × 2 mm id glass, packed with 0.2% Carbowax 1500 on 80-100 mesh Carbopack C (Supelco Chromatography Supplies, Supelco Park, Bellefonte, PA 16823).

(*3*) 6 ft × 1/4 in. Cu, packed with 3% Carbowax 600 on 40-60 mesh Chromosorb T.

Conditions	Col. 1	Col. 2	Col. 3
Carrier gas:	N	N	N
Flow rate, mL/min:	30	15	55
Oven temp.	200°	105°	88°
Injector temp.	225°	175°	150°
Detector temp.	225°	175°	150°

Adjust air and H for flame detector to optimum for carrier gas flow of column used. Adjust electrometer sensitivity to provide ≥50,000 counts of integrator counts for internal std peak.

(**b**) *Diluter.*—Capable of ±0.1% precision.

(**c**) *Internal std soln.*—0.1% (v/v) *n*-butanol in H_2O.

(**d**) *Alcohol std soln.*—Prep. alcohol-H_2O soln contg approx. % alcohol expected in sample. Det. exact % alcohol by pycnometer, **9.013(a);** refractometer, **9.016;** hydrometer, **9.014;** or other appropriate AOAC method, or use Std Ref. Material 1590, Stabilized Wine (NBS).

11.D02 ***Determination***

Dil. alcohol std soln 1:100 with internal std soln. Inject at least three 1.0 μL aliquots and det. av. response ratio of area of alcohol peak to area of *n*-butanol peak (*RR'*). Dil. sample 1:100 with internal std soln, inject 1.0 μL, and det. response ratio (*RR*).

$$\% \text{ alcohol} = (RR \times \% \text{ alcohol in std})/RR'$$

12. BEVERAGES: NONALCOHOLIC AND CONCENTRATES

No additions, deletions, or changes.

13. CACAO BEAN AND ITS PRODUCTS

No additions, deletions, or changes.

14. CEREAL FOODS

The official first action liquid chromatographic method for the determination of fructose, glucose, sucrose, and maltose in presweetened cereal, **14.C01-14.C04,** was adopted official final action.

15. COFFEE AND TEA

The official first action ISO method for the determination of loss on drying of instant coffee, **15.012-15.013,** was adopted official final action.

16. DAIRY PRODUCTS

(1) The official first action qualitative color reaction tests method for the detection of penicillins in fluid milk products (*J. Assoc. Off. Anal.*

Chem. **65,** 1193 (1982)), **16.C17-16.C22,** was adopted official final action, applicable for detecting and confirming ≥0.005 IU penicillin G/mL, and excluding the use of the multi-test system for chocolate products. The following modifications are required:

(a) Add as an applicability statement before "*Principle*": "(Multi-test system not applicable to chocolate products)".

(b) In **16.C17,** fourth sentence, change "≥0.007 IU/mL" to read "≥0.005 IU/mL."

(c) In **16.C22,** delete the last sentence.

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(2) The interim official first action potentiometric method for the determination of chloride in cheese, *J. Assoc. Off. Anal. Chem.* **65,** 1350 (1982), was adopted official first action as an IDF-ISO-AOAC method to replace the present official final action IDF-ISO-AOAC method for total chlorides, **16.242-16.243,** which was repealed official first action. The official final action method for the determination of total chlorides, **16.224** (12th edition), was reinstated as an alternative method.

Chloride in Cheese

Potentiometric Method

Official First Action

IDF-ISO-AOAC Method

16.D01 ***Apparatus and Reagents***

(a) *Electrodes.*—See **32.026(b).**

(b) *$AgNO_3$ std soln.*—0.08–0.12N or 0.0856N; see **32.027(b).**

(c) *NaCl std soln.*—0.08–0.12N or 0.0856N; see **32.027(c).**

16.D02 ***Standardization***

See **32.028.**

16.D03 ***Determination***

Accurately weigh 2–5 g ground or grated cheese into small blender chamber and add 30 mL H_2O at ca 55°. Suspend cheese by blending; rinse down sides of container with ca 10 mL H_2O. Add 2–3 mL HNO_3 (1 + 3) and titr. with stdzd $AgNO_3$ soln to endpoint; see **32.030.**

16.D04 ***Calculation***

$$\% \text{ Chloride as } \% \text{ NaCl} = \text{mL } AgNO_3 \times \text{N } AgNO_3 \times 0.05844 \times 100/\text{g sample}$$

$$\% \text{ Cl} = \text{mL } AgNO_3 \times \text{N } AgNO_3 \times 0.03545 \times 100/\text{g sample}$$

17. EGGS AND EGG PRODUCTS

No additions, deletions, or changes.

18. FISH AND OTHER MARINE PRODUCTS

(1) The official first action method for the determination of ammonia in crabmeat, **18.027-18.030,** was adopted official final action.

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(2) The following interim procedure for cooking seafood products was adopted as a revision of procedure **18.003:**

18.D01 ***Cooking of Seafood Products Procedure***

Cooking procedure is based on heating product to internal temp. ≥160°F (70°C). Cooking times vary according to size of product and equipment used. To det. cooking time, cook extra sample same way using temp. measuring device with probe of known length to det. internal temp. Cooking equipment, including cooking oil for deep fat frying, shall be free from substances which interfere with sensory evaluation of cooked product.

Methods of heating product include, but are not limited to, baking, bake-in-foil, broiling, boil-in-bag, shallow pan frying, deep fat frying, oven frying, grilling, poaching, steaming, and microwave heating.

19. FLAVORS

No additions, deletions, or changes.

20. FOOD ADDITIVES: DIRECT

(1) The following interim official first action liquid chromatographic method for the determination of antioxidants in oil was adopted official first action as a IUPAC-AOAC method:

Antioxidants in Oil

Liquid Chromatographic Method

Official First Action

IUPAC-AOAC Method

Applicable to propyl gallate (PG), 2,4,5-trihydroxybutyrophenone (THBP), *tert*-butylhydroquinone (TBHQ), nordihydroguaiaretic acid (NDGA), 2- and 3-*tert*-butyl-4-hydroxyanisole (BHA), 2,6-di-*tert*-butyl-4-hydroxymethylphenol (Ionox-100), and 3,5-di-*tert*-butyl-4-hydroxytoluene (BHT).

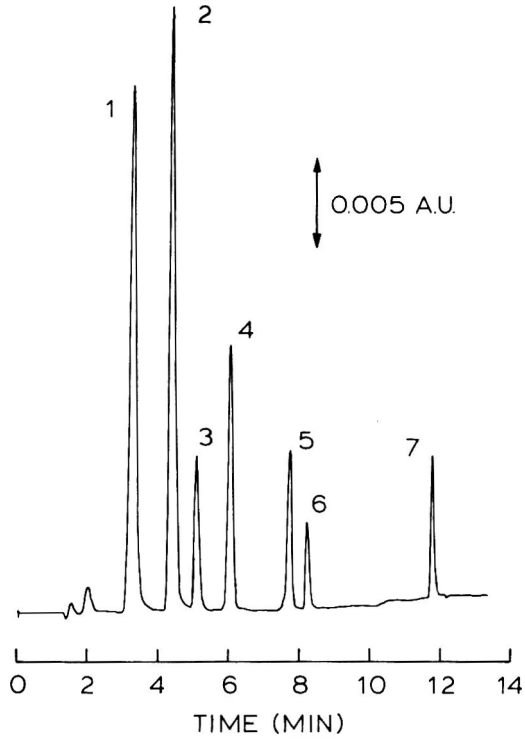

Fig. 20:D1. Chromatographic separation of antioxidant standards, ca 0.2 μg each antioxidant: 1, PG; 2, THBP; 3, TBHQ; 4, NDGA; 5, BHA; 6, Ionox-100; 7, BHT.

20.D01 ***Principle***

Sample is dissolved in hexane and antioxidants are extd into acetonitrile. Soln is concd, dild with equal vol. isopropanol, and injected into liq. chromatograph with UV detection at 280 nm.

20.D02 ***Apparatus and Reagents***

(a) *Gradient liquid chromatograph.*—Equipped with 10 mV strip chart recorder, 20 μL sample loop injection valve, and detector to measure *A* at 280 nm. Typical operating conditions: detector sensitivity, 0.05 AUFS; time const, 0; temp., ambient; flow rate, 2 mL/min.

(b) *Chromatographic column.*—Stainless steel, 250 × 4.6 mm id packed with 10 μm LiChrosorb RP-18 (E. Merck, Darmstadt, GFR), or equiv. Use guard column if desired. Baseline separation of all 7 antioxidants should be obtained as shown in Figure **20:D1.**

(c) *Glassware.*—Rinse all glassware with $CHCl_3$, acetone, and MeOH, in that order, and blow dry with N.

(d) *Solvents.*—Distd-in-glass acetonitrile, 2-propanol, and hexane.

(e) *Mobile phase.*—Use HPLC grade solvs, or equiv. *A. Distilled H_2O.*—Add 5% HOAc. *B. Acetonitrile.*—Add 5% HOAc. Use linear gradient, from 30% B in A to 100% B over 10 min with 4 min hold at 100% B at 2 mL/min. For sample only, increase flow rate to 6 mL/min at 100% B for 5 min or until nonpolar lipids are eluted. For samples and stds, return to 30% B in A over 1 min at 2 mL/min and let baseline, pressure, and mobile phase composition stabilize (ca 10 min). Run blank gradient (no injection). Peaks interfering with detn of any antioxidant should not be present. If small peaks are present which cannot be eliminated, all relevant peak heights are to be corrected for interference.

(f) *Antioxidants.*—BHA (mixt. of 2- and 3-BHA), BHT, TBHQ, Ionox-100, THBP, and PG (available from Polyscience Corp., Niles, IL 60648); NDGA (Food Chemicals Codex Ref. Std), or equiv.

(g) *Std solns.*—Refrigerate all antioxidant solns

out of direct light. Prep. all solns with 2-propanol–acetonitrile (1 + 1). (*1*) *Stock soln.*—1 mg/mL. Accurately weigh and transfer 50 mg each antioxidant into one 50 mL vol. flask, dissolve, dil. to vol., and mix. (*2*) *Std soln.*—0.01 mg/mL (10 μg/mL). Pipet 1 mL stock soln into 100 mL vol. flask, dil. to vol., and mix.

(**h**) *Extracting solvents.*—Sat. hexane and acetonitrile by shaking together 2 min, and sep. Unless otherwise specified, use these satd solvs in *Extraction* below.

20.D03 ***Determination***

(**a**) *Extraction.*—(*1*) *Liquid oils.*—Accurately weigh ca 20 g oil into 50 mL beaker and quant. transfer to 100 mL vol. flask, rinsing beaker with hexane. Dil. to vol. with hexane and mix. Pipet 25 mL aliquot into 125 mL separator and ext with three 50 mL portions of acetonitrile. If emulsions form, break by holding separator under hot tap H_2O 5–10 s. Collect exts in 250 mL separator and let combined exts slowly drain into 250 mL r-b flask to aid removal of hexane–oil droplets. Note: At this point, the 150 mL acetonitrile ext may be stored overnight under refrigeration.

Evap. to 3–4 mL, using flash evaporator with ≤40° H_2O bath and completing evapn in <10 min. Note: Losses of TBHQ may occur if evapn time is prolonged. Use efficient vac. source and ice–H_2O cooling to decrease evapn time. Using disposable pipet, transfer acetonitrile–oil droplet mixt. to 10 mL g-s graduate. Rinse flask with small portions of non-satd acetonitrile and transfer rinsings to graduate with disposable pipet until 5 mL is collected. Rinse disposable pipet and continue to rinse flask with small portions of 2-propanol, transferring all rinsings to graduate until exactly 10 mL is collected. Mix contents of graduate. Note: Avoid delay in analysis after prepg sample soln because loss of TBHQ may occur.

(*2*) *Lards or shortenings.*—Accurately weigh 10 g lard or shortening into 150 mL beaker. Add ca 30 mL hexane and dissolve sample, heating gently if necessary. Quant. transfer to 100 mL vol. flask, rinsing beaker with hexane. Dil. to vol. and mix. Continue extns as in (*1*) above, beginning "Pipet 25 mL aliquot . . .".

(**b**) *Chromatography.*—Using sample loop injection valve, inject, in duplicate, 20 μL prepd sample soln onto column, and solv. program as described. Inject 20 μL antioxidant std soln (10 μg/mL) and solv. program as described before and after each sample. For sample peaks off scale, quant. dil. sample soln with 2-propanol–acetonitrile (1 + 1). Identify peaks by comparison with retention times of std. Note: Octyl gallate (OG, available from Pfaltz and Bauer, Inc., Stamford, CT), if present, may co-elute with Ionox-100, but can be sepd with H_2O–MeOH gradient as follows: 30% B (MeOH with 5% HOAc) in A (H_2O with 5% HOAc) to 100% B over 10 min. If both Ionox-100 and OG are present, accurate quant. may not be possible.

Carry out reagent blank detn, substituting 25 mL hexane for hexane–oil. Continue extn as in (*1*) above, beginning "Pipet 25 mL aliquot . . ." Inject 20 μL reagent blank soln and solv. program as described. Peaks interfering with detn of any antioxidant should not be present. Using blank gradient chromatogram as guide to follow baseline, det. av. peak ht of antioxidant sample from duplicate injections (corrected for reagent and gradient blanks), and av. peak ht of antioxidant std from duplicate injections before and after sample (corrected for gradient blank).

20.D04 ***Calculation***

Calc. concn of antioxidant as follows:

$$\text{Antioxidant, ppm} = (C_s/R_s) \times (R_x/W_x) \times D$$

where R_x and R_s = peak hts from sample and std, resp.; C_s = concn of std in μg/mL; W_x = wt of sample in g/mL in 10 mL final ext; and D = diln factor if soln injected is dild.

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(2) The following gas-liquid chromatographic method for the determination of benzoic acid and sorbic acid in foods was adopted official first action as a NMKL–AOAC method:

Benzoic Acid and Sorbic Acid in Foods
Gas-Liquid Chromatographic Method
Official First Action
NMKL-AOAC Method

(Collaboratively tested on apple juice, almond paste, and fish homogenate (at 0.5–2 g/kg levels), representing carbohydrate-rich, pasty and rich in fat and carbohydrates, and protein-rich foods.)

20.D05 ***Principle***

Benzoic acid and sorbic acid are isolated from

food by extn with ether and successive partitionings into aq. NaOH and CH_2Cl_2. Acids are converted to trimethylsilyl (TMS) esters and detd by GLC. Phenylacetic acid and caproic acid are used as internal stds for benzoic acid and sorbic acid, resp.

20.D06 *Apparatus*

(a) *Gas chromatograph.*—With linear oven temp. programmer, flame ionization detector, and recorder. Following conditions have been found satisfactory: column, 1.8 m × 2 mm (id) coiled glass with 3% OV-1 on 100–120 mesh Varaport 30. Operating temps: oven 80–210°, 8°/min; injection port 200°, detector 280°. N carrier gas 20 mL/min. Approx. retention times 2.5, 4, 5, and 6 min for caproic acid, sorbic acid, benzoic acid, and phenylacetic acid, resp.

(b) *Centrifuge.*—With head for 30 and 200 mL centrf. flasks.

(c) *Mechanical shaker.*—Bühler SM-B, or equiv.

20.D07 *Reagents*

Use anal. reagents thruout.

(a) *Internal std soln.*—Dissolve 250 mg phenylacetic acid and 250 mg caproic acid in 100 mL 3% aq. KOH soln.

(b) *Silylating agent.*—*N*-Methyl-*N*-trimethylsilyl-trifluoracetamide (MSTFA), available from Pierce Chemical Co, Rockford, IL, or Machery-Nagel Co, D-5160 Düren, GFR.

(c) *Std solns.*—Prep. mixed std solns in $CHCl_3$ of benzoic acid, sorbic acid, phenylacetic acid, and caproic acid, resp., of following concns:

1) 200, 200, 750, and 750 μg/mL
2) 400, 400, 750, and 750 μg/mL
3) 600, 600, 750, and 750 μg/mL
4) 800, 800, 750, and 750 μg/mL
5) 1000, 1000, 750, and 750 μg/mL

20.D08 *Preparation of Sample*

Homogenize sample in mech. mixer. If consistency of sample makes mixing difficult, use any technic to ensure that sample material will be homogeneous.

20.D09 *Extraction*

(a) *General method.*—Accurately weigh 5.0 g homogenized sample into 30 mL centrf. tube with Teflon-lined screw cap. Add 3.00 mL internal std soln, 1.5 mL H_2SO_4 (1 + 5), 5 g sand, and 15 mL ether. Screw cap on tightly to avoid leakage. Mech. shake 5 min and centrf. 10 min at 1500 × *g*. Transfer ether layer with disposable pipet to 250 mL separator. Repeat extn twice with 15 mL ether each time.

Ext combined ether phases twice with 15 mL 0.5N NaOH and 10 mL satd NaCl soln each time. Collect aq. layers in 250 mL separator, add 2 drops of Me orange, and acidify to pH 1 with HCl (1 + 1). Ext with CH_2Cl_2, using successive portions of 75, 50, and 50 mL. If emulsion forms, add 10 mL satd NaCl soln. Drain CH_2Cl_2 exts thru filter contg 15 g anhydrous Na_2SO_4 into 250 mL r-b flask. Evap. CH_2Cl_2 soln in rotary evaporator at 40° just to dryness.

(b) *Cheese and food products with paste-like consistency.*—Accurately weigh 5.0 g homogenized sample into 200 mL centrf. flask. Add 15 mL H_2O and stir with glass rod until sample is suspended into aq. phase. Add 3.00 mL internal std soln, 1.5 mL H_2SO_4 (1 + 5), and 25 mL ether. Stopper flask carefully and check for leakage. Mech. shake 5 min and centrf. 10 min at 2000 × *g*. Transfer ether layer with disposable pipet to 250 mL separator. Repeat extn twice with 25 mL ether each time. Continue as in (a), beginning "Ext combined ether phases. . .".

20.D10 *Derivatization and Gas Chromatography*

Add 10.0 mL $CHCl_3$ to residue in 250 mL r-b flask. Stopper and shake manually 2 min. Transfer 1.00 mL $CHCl_3$ soln to 8 mL test tube with Teflon-lined screw cap and add 0.20 mL silylating agent. Cap and let stand 15 min in oven or H_2O bath at 60°. Inject duplicate 1 μL portions of sample soln into gas chromatograph. Start temp. program when solv. peak emerges. Measure peak hts and calc. peak ht ratios of benzoic acid/phenylacetic acid and sorbic acid/caproic acid. Use av. of duplicate ratios. Peak ht ratios for duplicate injections should differ ≤5%.

20.D11 *Preparation of Standard Curves*

Transfer 1.00 mL std solns to five 8 mL test tubes with Teflon-lined screw caps. Add 0.20 mL silylating agent to each tube, cap, and let stand 15 min in oven or H_2O bath at 60°. Inject duplicate 1 μL portions of std solns into gas chromatograph. Use same conditions as for sample soln. Measure peak hts and calc. peak ht ratios of benzoic acid/phenylacetic acid and sorbic acid/caproic acid, resp. Peak ht ratios for duplicate injections should differ ≤5%. Plot wt ratios (x) vs av. peak ht ratios (y) for each pre-

servative. Calc. slope and intercept of std curve by method of least squares.

20.D12 ***Calculation***

$$\text{Preservative, mg/kg} = [(y - a)/b] \times (W'/W) \times 1000$$

where b = slope of std curve; a = intercept; y = av. peak ht ratio of preservative/internal std; W = wt of sample in g; and W' = wt of internal std in mg.

21. FOOD ADDITIVES: INDIRECT

No additions, deletions, or changes.

22. FRUITS AND FRUIT PRODUCTS

(1) The following official first action carbon ratio mass spectrometric methods were adopted official final action:

(a) Corn syrup in apple juice, **22.B01-22.B04.**

(b) Corn syrup in orange juice, **22.C01-22.C04.**

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(2) The following interim official first action method for the determination of soluble solids in citrus fruit juices as degrees Brix, *J. Assoc. Off. Anal. Chem.* **66,** 371 (1983), was adopted official first action:

22.D01 ***Soluble Solids in Citrus Fruit Juices as Degrees Brix — Official First Action***

Proceed as in **22.024.** Correct values for sucrose by refractometer for acidity by adding $(0.012 + 0.193x - 0.0004x^2)$ to sucrose value, where x = % anhyd. citric acid in sample = 0.064 × mL 0.1N alkali/100 g as detd in **22.060** or **22.061.**

23. GELATIN, DESSERT PREPARATIONS, AND MIXES

No additions, deletions, or changes.

24. MEAT AND MEAT PRODUCTS

(1) The official first action block digestion method for the determination of crude protein in meats, **24.B01-24.B03,** was adopted official final action.

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(2) The following titrimetric method for the determination of calcium in mechanically separated poultry and beef was adopted official first action:

Calcium in Mechanically Separated Poultry and Beef

Titrimetric Method

Official First Action

24.D01 ***Reagents***

(a) *Ethylenedinitrilotetraacetic acid dihydrate (EDTA).*—0.02M. Dissolve 7.44 g EDTA (99+% purity) in H_2O in 1 L vol. flask and dil. to vol. Stdze with ACS $CaCO_3$ primary std as in **36.050(d)** (3 significant figures).

(b) *Hydroxy naphthol blue indicator.*—Mallinckrodt Chemical Works.

(c) *KOH-KCN solution.*—Dissolve 280 g KOH in 500 mL H_2O. Cool, add 66 g KCN, dissolve, and dil. to 1 L in H_2O. *Caution:* Use care in handling of KCN. HCN is evolved on contact with acid or moisture. Flush titrd soln down drain in fume hood with ample *cold* H_2O.

(d) *Calcium carbonate.*—0.02M. Weigh 2.000 g ACS primary std $CaCO_3$ (dried 2 h at 100°) and transfer to 1 L vol. flask. Add 500 mL H_2O and 10-12 mL HCl (1 + 1). Heat just to boiling to dissolve $CaCO_3$. Cool and dil. to vol. with H_2O. Pipet 25 mL aliquot of EDTA (a) into 400 mL beaker and det. ratio of EDTA to $CaCO_3$ by titration as under *Determination* beginning "... add ca 50 mL H_2O, place on mag. stirrer..." Use av. of 3 detns.

24.D02 ***Determination***

Weigh 10 g sample, transfer to 300 mL tall form beaker, add 30 mL HCl (1 + 1), several glass beads, cover with watch glass, and place on hot plate in fume hood. Bring to boil slowly, digest 20 min, cool, and filter (Whatman No. 4 paper) into 200 mL vol. flask. Wash filter paper with H_2O until 190 mL is obtained. Dil. to vol. and mix. Pipet 20 mL aliquot into 400 mL beaker, add ca 50 mL H_2O, place on mag. stirrer, and add 200-300 mg hydroxy naphthol blue indicator, stir, and adjust pH to 12.5 ± 0.2 with KOH-KCN soln. (Do not exceed pH 12.7, to prevent pptn. If pptn occurs, discard and repeat.) Add 10-25 mL 0.02M EDTA accurately measured to provide back-titrn of min. of 3 mL. Mix on mag. stirrer and titr. sample with 0.02M $CaCO_3$ to permanent purple end point. If back-titrn is <3 mL, repeat with larger aliquot of EDTA soln.

$$\text{\% Calcium (Ca)} = (A - B \times R) \times M \times 4$$

$$\text{\% Bone (for poultry)} = (\%\text{Ca} - 0.015)F$$

where A = mL EDTA added; B = mL $CaCO_3$ of back titrn; R = EDTA:$CaCO_3$ ratio; M = molarity

of EDTA soln; 4 = meq. wt Ca (0.040 g) × 100(%); 0.015 = correction for natural Ca in poultry tissue; F = Ca-to-bone conversion factor (6.25 for broilers and fryers; 4.55 for turkeys and mature fowl).

25. METALS AND OTHER ELEMENTS

(1) The following atomic absorption spectrophotometric method for the determination of copper in serum was adopted official first action:

Copper in Serum
Atomic Absorption Spectrophotometric Method
Official First Action

25.D01 ***Principle***

Samples are dild 1 + 1 with H_2O, and Cu is detd by AAS using std solns prepd in 10% glycerol.

25.D02 ***Apparatus and Reagents***

(**a**) *Atomic absorption spectrophotometer.*—Equipped with nebulizer and air-C_2H_2 burner head. Monitor performance by assuring that 4.0 mg/L std produces response ≥0.200 absorbance unit.

(**b**) *External control.*—Precilip, Cat. No. 125067 (Bio-Dynamics/bmc, 9115 Hague Rd, Indianapolis, IN 46250), or equiv. with established value for Cu. Dil. according to label.

(**c**) *Glycerol USP.*—10% (v/v) aq. soln.

(**d**) *Copper std solns.*—(*1*) *Stock std soln.*—1000 mg/L. Dissolve 1.000 g Cu metal in min. vol. of HNO_3-H_2O (1 + 1). Dil. to 1000 mL with 1% HNO_3. (*2*) *Intermediate std soln.*—100 mg/L. Dil. 10 mL stock std soln to 100 mL with H_2O. (*3*) *Working std solns.*—Dil. 0.0, 0.25, 0.5, 1.0, 2.0, and 4.0 mL intermediate std soln to 100 mL with 10% glycerol to give std solns contg 0.0, 0.25, 0.5, 1.0, 2.0, and 4.0 mg Cu/L.

25.D03 ***Sample Preparation***

Rinse all glassware used with 2N HCl. Mix samples thoroly before pipetting. Using Mohr pipet, transfer 1.0 mL serum and 1.0 mL Precilip (external control) to sep. test tubes. Add 1.0 mL H_2O to each and mix 5 s on vortex mixer or cap tubes and shake 10 s. Use 1 external control for each 10 samples or fraction thereof.

25.D04 ***Determination***

Analyze by AAS using the following conditions: wavelength 324.7 nm; slit 0.7 nm; flame air-C_2H_2 (lean-blue). Aspirate series of working std solns, external control soln, and sample dilns. Repeat analysis if Cu value in external control soln is not within accepted range. Prep. std curve of concn, mg Cu/L, vs A, and det. concn of sample. Multiply result by 200 to account for sample diln and to convert result to μg Cu/100 mL.

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(2) The following gas-liquid chromatographic method for the determination of methyl mercury in fish and shellfish was adopted official first action:

Methyl Mercury in Fish and Shellfish
Gas-Liquid Chromatographic Method
Official First Action

25.D05 ***Principle***

Org. interferences are removed from homogenized sample by acetone wash followed by benzene wash. Protein-bound Me Hg is released by addn of HCl and extd into benzene. Benzene ext is concd and analyzed for CH_3HgCl by GLC.

25.D06 ***Reagents***

(**a**) *Solvents.*—Acetone, benzene, and isopropanol are all distd in glass (Burdick & Jackson Laboratories, Inc.; MCB Manufacturing Chemists, Inc.). *Note:* Benzene is a possible carcinogen.

(**b**) *Hydrochloric acid soln (1 + 1).*—Add concd HCl to equal vol. of distd or deionized H_2O and mix. Ext HCl soln 5 times with ¼ its vol. of benzene by shaking vigorously 15 s in separator. Discard benzene exts. Soln may be mixed in advance but must be extd immediately before use.

(**c**) *Carrier gas.*—GLC quality Ar-CH_4 (95 + 5).

(**d**) *Sodium sulfate.*—Heat overnight in 600° furnace, cool, and store in capped brown bottle. Line cap with Al foil to prevent contamination from cap.

(**e**) *Methyl mercuric chloride std solns.*—Keep tightly stoppered. (*1*) *Stock std soln.*—1000 μg Hg/mL. Weigh 0.1252 g CH_3HgCl (ICN-K&K Laboratories, Inc., Plainview, NY 11803) into 100 mL vol. flask. Dil. to vol. with benzene. (*2*) *High intermediate std soln.*—40 μg Hg/mL. Dil. 10.0 mL stock soln to 250.0 mL with benzene. (*3*) *Low intermediate std soln.*—2.0 μg Hg/mL. Dil. 10.0 mL high intermediate std soln to 200.0 mL

with benzene. (4) *Working std solns.*—0.010–0.30 μg Hg/mL. Prep. monthly by dilg with benzene in vol. flasks as follows: Dil. 15 mL of 2.0 μg Hg/mL std to 100.0 mL, 10.0 mL to 100.0 mL, and 10.0 mL to 200.0 mL for 0.30, 0.20, and 0.10 μg Hg/mL, resp. Dil. 20 mL of 0.10 μg Hg/mL std to 25.0 mL, 10.0 mL to 25.0 mL, 10.0 mL to 50.0 mL, and 10.0 mL to 100.0 mL for 0.080, 0.040, 0.020, and 0.010 μg Hg/mL, resp.

(**f**) *Mercuric chloride column treatment soln.*—1000 ppm $HgCl_2$. Dissolve 0.1 g $HgCl_2$ in 100 mL benzene.

25.D07 *Apparatus*

Wash all glassware with detergent (Micro Laboratory Cleaner, International Products, Trenton, NJ 08601, or equiv.) and rinse thoroly with hot tap H_2O followed by distd or deionized H_2O.

(**a**) *Centrifuge.*—Model UV (International Equipment Co., Needham Heights, MA 02194), or equiv.

(**b**) *Centrifuge tubes.*—50 mL capacity with ground glass or Teflon-lined stoppers.

(**c**) *Kuderna-Danish (K-D) concentrators.*—250 mL flask (No. K570001, Kontes Glass Co.) and 10 mL graduated concentrator tube (No. K570050, size 1025, Kontes Glass Co.).

(**d**) *Modified Snyder distilling column.*—Modify Snyder column (No. K503000 size 121, Kontes Glass Co.) in either of 2 ways: (*i*) Shorten 3-section, 3-ball column to 2-section, 2-ball column by cutting off top at uppermost constriction. (*ii*) Insulate 3-section, 3-ball column by wrapping glass wool around top section and holding it in place with Al foil. Glass wool and foil must surround only top section above top ball.

(**e**) *Carborundum boiling chips.*—20 mesh, HCl-washed.

(**f**) *Graduated cylinders.*—Class A, 25 mL capacity, with ground-glass stopper (Kimble 20036, or equiv.).

(**g**) *Transfer pipets.*—Disposable glass, Pasteur-type 5¾ in. long (No. 13-678-6A, Fisher Scientific Co., or equiv.).

(**h**) *Dropping pipets.*—5 mL capacity (No. 13-710B, Fisher Scientific Co., or equiv.).

(**i**) *Gas chromatograph.*—Hewlett-Packard Model 5710A or equiv., equipped with linear ^{63}Ni electron capture detector and 6 ft × 2 mm id silanized glass column packed with 5% DEGS-PS on 100–120 mesh Supelcoport (Supelco, Inc.). Pack column *no closer* than 2.0 cm from injection and detector port nuts and hold packing in place with 2 cm high quality, silanized glass wool at both ends. Install oxygen scrubber and molecular sieve dryer (No. HGC-145, Analabs, North Haven, CT 06473, or equiv.) between carrier gas supply and column. Condition column according to manufacturer's instructions as follows: Flush column 0.5 h with carrier gas flowing at 30 mL/min at room temp. Then heat 1 h at 100°. Next, heat column to 200° at programmed heating rate of 4°/min and hold at 200° overnight. Do not connect column to detector during this conditioning process. Maintain 30 mL/min carrier gas flow at all times during conditioning, treatment, and use. Operating conditions: column 155°; injector 200°; detector, 300°; carrier gas flow 30 mL/min; and recorder chart speed 0.5–1.0 cm/min. Under these conditions and with $HgCl_2$ column treatment procedure described below, CH_3HgCl peak will appear 2–3 min after sample injection.

25.D08 *Mercuric Chloride Column Treatment*

5% DEGS-PS conditioned according to manufacturer's instructions can be used to det. CH_3HgCl only after treatment by $HgCl_2$ soln, (**f**). Treat column any time column has been heated to 200°. Because column performance degrades with time, also treat column periodically during use. Perform appropriate $HgCl_2$ treatment procedures described below. Procedure (**b**) produces most stable baseline and is recommended over procedure (**c**) for routine use.

(**a**) *Following 200° column conditioning.*—If column has just been conditioned overnight at 200°, use this procedure. Adjust column temp. to 160° and connect detector. When baseline is steady, treat column by injecting 20 μL $HgCl_2$ treatment soln 5 times at 5–10 min intervals. (Change in column performance may be monitored by injecting 5 μL 0.010 μg Hg/mL std soln before and between $HgCl_2$ treatment soln injections.) During treatment procedure, large broad peaks will elute. (CH_3HgCl peak retention time will decrease and peak ht will increase.) Approximately 1½–1¾ h after last $HgCl_2$ treatment soln injection, a final large peak will elute. (CH_3HgCl peak ht and retention time will be stable.) This broad peak and CH_3HgCl peak ht stability signal completion of treatment process. Adjust column temp. to 155° and wait for steady baseline; then column is ready for use.

(**b**) *On day preceding sample extract analysis.*—If column has been treated by procedure (**a**) or used at 155° to analyze sample exts, column may be treated at end of working day for next day's use as follows: Lower column temp. to 115° and inject 20 μL $HgCl_2$ treatment soln *one* time.

Broad peaks will elute between 11 and 15 h after $HgCl_2$ injection. Next working day, increase column temp. to operating temp. When baseline is steady (ca 15–30 min), column is ready for use.

(c) *During sample extract analysis at 155°.*—If column has been used at 155° for ext analysis and column performance has degraded enough to require $HgCl_2$ treatment, increase column temp. to 160°, inject *one* 20 μL aliquot of $HgCl_2$ treatment soln, and monitor baseline. Large, broad peaks will elute 1–1½ h after $HgCl_2$ injection, signaling completion of treatment process. Decrease column temp. to 155° and wait for steady baseline; then column is ready for use.

25.D09 *Extraction of Methyl Mercury Chloride*

Perform all operations, except weighing, in laboratory hood. Accurately weigh 2 g homogenized sample into 50 mL centrf. tube. Add 25 mL acetone, stopper, and shake vigorously 15 s. Remove stopper, cover with foil, and centrf. 2–5 min at 2000 rpm. Carefully decant and discard acetone. (Use dropping pipet to remove acetone, if necessary.) Repeat 25 mL acetone wash step twice more. Break up tissue with glass stirring rod before shaking, if necessary. Add 20 mL benzene, stopper, and shake vigorously 30 s. Remove stopper, cover with foil, and centrf. 2–5 min at 2000 rpm. Carefully decant (or draw off with dropping pipet) and discard benzene. Extraneous peaks in final GLC anal. chromatograms indicate that more vigorous shaking with acetone and benzene is required.

Add 10 mL HCl soln to centrf. tube contg acetone and benzene-washed sample. Break up tissue with glass stirring rod, and ext sample by adding 20 mL benzene and shaking gently but thoroly 2 min. Remove stopper, cover with foil, and centrf. 5 min at 2000 rpm. If emulsion forms, add 2 mL isopropanol and gently stir benzene layer to break emulsion, taking care not to disturb aq. phase, and recentrf. Carefully transfer benzene layer to K-D concentrator, using 5 mL dropping pipet. Rinse centrf. tube walls with 3–4 mL benzene and transfer rinse to K-D concentrator. Repeat extn step twice more, adding 20 mL benzene and shaking *1* min each time. Combine all 3 benzene exts in K-D concentrator.

Place 4–6 boiling chips in K-D concentrator, connect Snyder column, wet Snyder column bubble chambers with 3–4 drops of benzene, and immediately place tube in steam bath or vigorously boiling H_2O bath. Evap. so that 8 mL remains when cooled to room temp. Cool. Disconnect concentrator tube and quant. transfer soln to 25 mL g-s graduate using Pasteur-type transfer pipet. Dil. to 20.0 mL with benzene and mix. Add 4 g Na_2SO_4 and mix again. Na_2SO_4 must be added to 20 mL concd sample ext within 10 h of first acetone wash. Tightly stoppered exts may be held overnight at this point. Analyze by GLC.

25.D10 *Gas-Liquid Chromatography*

Verify that system is operating properly by injecting 5 μL vols. of 0.01 μg Hg/mL working std soln into chromatograph. Difference between CH_3HgCl peak hts for 2 injections should be ≤4%. Check detector linearity by chromatographing all 0.01–0.30 μg Hg/mL working std solns.

Inject duplicate 5 μL vols. (equiv. to 0.5 mg sample) of ext. Difference between CH_3HgCl peak hts for 2 injections should be ≤4%. Next, inject duplicate 5 μL vols. of std soln with CH_3HgCl concn approx. equal to or slightly greater than ext CH_3HgCl concn. Because column performance and peak ht slowly decrease with time, calc. each sample concn by comparison to std soln injected immediately after sample.

Calc. Me Hg content of homogenate in μg Hg/g (ppm Hg) by comparing av. CH_3HgCl peak ht of duplicate sample injections with av. CH_3HgCl peak ht of duplicate std injections.

$$\text{ppm Hg} = (R/R') \times (C'/C) \times 20$$

where R = av. peak ht of duplicate sample injections; R' = av. peak ht of duplicate std injections; C = g sample; C' = concn of Hg in CH_3HgCl std soln (μg Hg/mL).

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(3) The following hot leach atomic absorption method for the determination of cadmium and lead in cookware was adopted interim official first action as a WHO–AOAC method after the 1982 meeting:

Cadmium and Lead in Cookware
Hot Leach Atomic Absorption Method
Interim Official First Action
WHO-AOAC Method

25.D11 *Principle*

Enameled and ceramic cookware contg 4% acetic acid is heated by elec. hot plate (or by internal heating elements, if present, that are not

exposed to the leach solution) to produce slow boil or simmering of solv. soln for 2 h. Pb and Cd in extg solv. are detd by AAS.

25.D12 ***Apparatus***

See **25.031** plus the following:

(a) *Hot plate.*—Thermoline Model HP-A1915B (Thermoline Corp., Dubuque, IA 52001), or equiv.

(b) *Variable transformer.*—Cat. No. 09-521-100 (Fisher Scientific Co.), or equiv.

25.D13 ***Reagents***

See **25.032.** Use only deionized distd H_2O.

Plus (e) *Acetic acid.*—Glacial.

25.D14 ***Cleaning of Laboratory Glassware***

After normal cleaning, soak all glass and plastic ware used to prep., transfer, or store anal. solns in HNO_3–H_2O (4 + 6) ≥ 24 h; thoroly rinse with H_2O before use.

25.D15 ***Preparation of Standard***

See **25.033.**

Samples of ware must be free of grease or other material which could influence test. Gently wash sample with detergent soln, using pad of absorbent cotton. Rinse ware thoroly with H_2O and let drain dry.

Fill volume of ware is ⅔ of vol. required to fill ware to overflowing or to cover rest, if one is present.

25.D16 ***Extraction***

Fill ware to ⅔ total vol. with H_2O; cover with self-cover or clean sheet of opaque borosilicate glass to prevent evapn of soln. When leach soln is to be analyzed for Cd, ensure that light is excluded from test surface. Heat on hot plate adjusted by variable transformer to produce simmer or slow boil of leaching soln, or use internal heating element, if present. Also use variable transformer to prevent excessively rapid boiling in ware containing heating elements. If contained heating element is not able to produce temp. high enough to boil soln, then highest temp. reached is test temp.

When boiling or highest temp. has been reached, add sufficient glacial acetic acid to make soln 4% acetic acid, cover, and continue heating 2 h.

At end of 2 h, re-establish initial vol. of solv. with 4% acetic acid. Dip-stick (glass rod marked for depth of soln required) is useful for replacing losses. Stir thoroly and remove test sample at once.

25.D17 ***Determination***

See **25.034.**

26. NATURAL POISONS

(1) The official first action method for the determination of aflatoxins in peanuts and peanut products, Method II (BF Method), **26.032–26.036,** was revised to include the water slurry method for obtaining the analytical sample from the lot sample. The following changes are required:

(a) Change **26.034** to read:

26.034 ***Preparation of Sample***

See **26.028.** Alternatively, prep. peanut samples by H_2O slurry method: Blend 1100 g peanuts comminuted in subsampling mill with 1500 mL H_2O and 22 g NaCl 3 min at medium speed in 1 gal. blender cup.

(b) In **26.035,** add as new sentences at end of first paragraph:

If peanut sample is prepd by alternative H_2O slurry method, weigh 130 g slurry into blender jar. Add 50 mL 2.2% salt soln, 150 mL MeOH, and 100 mL hexane.

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(2) The official first action method for the determination of aflatoxins in cottonseed products, **26.A01–26.A09,** was revised to allow those concerned with the disposal of lead-containing wastes a choice of a more benign residue. The following changes are required:

(a) Change **26.A02(e)** to read:

"(e) *Lead acetate soln.* . . . and dil. to 1 L. Alternatively, use *zinc acetate–aluminum chloride soln:* Dissolve 200 g $Zn(OAc)_2$ and 5 g $AlCl_3$ in H_2O and dil. to 1 L."

(b) In **26.A04,** change the first paragraph to read:

"Measure 100 mL filtrate . . . and refilter as above, or, alternatively, replace $Pb(OAc)_2$ soln with $Zn(OAc)_2$–$AlCl_3$ soln."

27. NUTS AND NUT PRODUCTS

No additions, deletions, or changes.

28. OILS AND FATS

No additions, deletions, or changes.

29. PESTICIDE RESIDUES

The following gas-liquid chromatographic method for the determination of chlorinated pesticides and polychlorinated biphenyls in fish was adopted official first action:

Chlorinated Pesticides and Polychlorinated Biphenyls in Fish

Gas-Liquid Chromatographic Method

Official First Action

29.D01 *Principle*

Chlorinated pesticides and polychlorinated biphenyls (PCBs) are extd from prepd fish sample with pet. ether, cleaned up on Florisil column, and detd by GLC against ref. stds.

See **29.002, 29.005, 29.008-29.010** for general app., reagents, and technics.

29.D02 *Apparatus*

(a) *Gas chromatograph.*—With on-column injection system, 6 ft glass column (4 mm id), packed with 10% DC-200 on 80–100 mesh Chromosorb WHP, and electron capture detector. Other liq. phases such as 5% OV-101 on suitable supports may be substituted if known to give adequate resolution for compds present in samples.

Linearized ^{63}Ni detector capable of producing ½ scale deflection for 1 ng heptachlor epoxide is suggested; however, other equiv. electron capture detectors may be used. Operate GLC in accordance with manuf. directions, adjusting to provide necessary response and resolution.

(b) *Chromatographic tube.*—10 mm id × 300 mm column with Teflon stopcock, coarse fritted disk, ₹24/40 top joint (Kontes Glass Co. K-420550, or equiv.).

(c) *Kuderna-Danish concentrators.*—Snyder distg column (Kontes K-503000-0121); 125 mL K-D flask (Kontes K-570001-9010) (special item) ₹ 19/22 lower joint; 10 mL concentrator tube (Kontes K-570050-1025).

(d) *Micro Snyder column.*—Kontes K-569251, ₹ 19/22.

29.D03 *Reagents*

(a) *Florisil.*—PR grade, 60–80 mesh (Floridin Co.). Must meet **29.002(i)** specifications.

(b) *Solvents.*—Pet. ether, ethyl ether, hexane, and alcohol, known to be suitable for pesticide residue detn.

(c) *Glass wool (Pyrex).*—Must be free of interference with electron capture detection.

(d) *Sodium sulfate.*—Anhyd., granular, reagent grade, free of interference with electron capture detection.

29.D04 *Extraction*

Weigh 20 g thoroly ground and mixed sample into metal blender cup. Moisten 40 g granular Na_2SO_4 with pet. ether and add to sample. Mix sample, using stirring rod, let stand 20 min, and mix again. Add 100 mL pet. ether to sample and blend 1–2 min. (Lourdes blender in series with rheostat set at 40–60%, or equiv., may be used.) Centrf. balanced sample cup 1–2 min at ca 2000 rpm to obtain clear pet. ether ext. Place glass wool plug in funnel, overlay with 20 g granular Na_2SO_4, and place funnel in 250 mL vol. flask. Decant pet. ether ext thru Na_2SO_4 into vol. flask. Mix sample again with stirring rod, add 100 mL pet. ether, and ext as before. Repeat using 70 mL pet. ether. Dil. to vol. with pet. ether.

Transfer 25 mL aliquot to tared 100 mL flat bottom extn flask. Place flask on steam bath to evap. solv., leave addnl 30 min on steam bath, remove, and cool. Weigh flask and det. % fat in fish.

For fish contg <10% fat, transfer 25 mL aliquot to 125 mL K-D concentrator. For fish contg >10% fat, take aliquot contg not >200 mg fat. Add several granules of 20–30 mesh carborundum and conc. to ca 3 mL on steam bath. Let cool and remove Snyder column. Rinse concentrator with two 1 mL portions of pet. ether and, using only current of air, conc. sample to 3 mL for transfer to Florisil column.

29.D05 *Florisil Cleanup*

Use 4 g Florisil adjusted for lauric acid value (*JAOAC* **51,** 29(1968)). Add Florisil to 300 × 10 mm id chromatgc tube and add Na_2SO_4 to ht 2 cm above Florisil. Completely open stopcock, tap tube to settle adsorbent, and mark tube 1 cm above Na_2SO_4 layer.

Add 20–25 mL pet. ether wash to Florisil column; as solv. level reaches mark, place 125 mL K-D flask under column. Using disposable Pasteur pipet, transfer 3 mL sample to column, and wash tube with 1 mL pet. ether and add wash to column. Solv. level must not go below mark. Temporarily close stopcock if necessary. Add 35 mL pet. ether–ether mixt. (94 + 6) and elute PCBs and DDT, and its analogs. When solv. level reaches mark, change K-D flask, and add 35 mL pet. ether–ether (85 + 15) to elute compds such as dieldrin and endrin. Add several granules of carborundum to first concentrator, attach Snyder column, and carefully conc. on steam bath. Let concentrator cool, remove Snyder column and evap. solv. under air to appropriate vol. for GLC detn. Fractions contg mixt. of PCBs and chlorinated compounds such as DDE may require addnl sepn technics.

29.D06 ***Additional Cleanup***

Often addnl cleanup is required for second fraction (85 + 15) to prevent deterioration of GLC column. Conc. pet. ether–ether (85 + 15) fraction under current of air to 2 mL, add 1 mL 2% alc. KOH, attach micro-Snyder column, and carefully reduce to ≤1 mL on steam bath. Reflux sample 15 min, remove, and cool. Add 2 mL alcohol–H_2O (1 + 1) and 5 mL hexane, and shake 1 min. Centrf. to sep. layers. Transfer as much hexane layer as possible to second tube, using disposable Pasteur pipet, and repeat extn with 5 mL hexane. Conc. combined hexane to appropriate vol. for GLC analysis.

29.D07 ***Gas-Liquid Chromatography***

See **29.018.**

30. SPICES AND OTHER CONDIMENTS

No additions, deletions, or changes.

31. SUGARS AND SUGAR PRODUCTS

(1) The following official first action methods for honey were adopted official final action:

(a) Nitrogen, **31.115.**

(b) Proline, **31.116-31.118.**

(c) Direct polarization, **31.119(a), (b), (c).**

(d) Higher sugars or "dextrin," alternative method II, **31.133-31.137.**

(e) Higher sugars or "dextrin," HPLC method, **31.138-31.142.**

(f) High fructose corn syrup, TLC method, **31.148-31.149.**

(g) Hydroxymethylfurfural, **31.A01-31.A02.**

(h) Carbon ratio MS method for the determination of adulteration, amendment to **31.150-31.153** ("Changes in Methods," *J. Assoc. Off. Anal. Chem.* **64,** 512 (1981)).

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(2) The official first action method for direct polarization of honey at 87°, **31.119(d),** was deleted.

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(3) The following procedures for honey were deleted:

(a) Dextrin (approximate), **31.122.**

(b) Commercial invert sugar, resorcinol test, **31.146-31.147.**

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(4) The official first action liquid chromatographic method for the determination of saccharides in corn syrups, **31.228-31.236,** was extended interim official first action to include the determination of minor saccharides in corn sugar containing dextrose levels in excess of 98%, *J. Assoc. Off. Anal. Chem.* **65,** 1366 (1982). The extended method has been adopted official first action.

Minor Saccharides in Corn Sugar
Liquid Chromatographic Method
Official First Action

31.D01 ***Principle***

Polysaccharides in high (98+%) dextrose corn sugar are quantitated vs maltose std and calcd as disaccharides (DP_2) and tri- and higher saccharides (DP_{3+}).

31.D02 ***Determination***

Proceed as in **31.229-31.236,** except use only Aminex Q15-S resin column, **(31.229(g)),** or equiv., and use ca 3 g maltose.H_2O std/25 mL instead of mixed sugar std, **31.230(e).** Compute dry basis concn of maltose by multiplying by 0.94. Det. response factor for maltose dry basis by injecting 10 μL std soln and proceeding as in **31.233.** Prep. sample as in **31.234.** Complete detn as in **31.235,** but inject 50 μL dild sample.

31.D03 ***Calculations***

Results are computed automatically when using **31.229(c).** List DP_2 results and combine all tri- and higher saccharides and list sum as DP_{3+}. Subtract sum of DP_2 and DP_{3+} from 100 to obtain glucose (dextrose) (DP_1) value. Report results on ash-free, carbohydrate dry substance basis. In absence of computing integrator, list areas for DP_2 and sum for DP_{3+}, multiply each by maltose response factor and by 100 to obtain $\%DP_2$ and $\%DP_{3+}$. Subtract sum of $\%DP_2$ and $\%DP_{3+}$ from 100 to obtain $\%DP_1$.

32. VEGETABLE PRODUCTS, PROCESSED

No additions, deletions, or changes.

33. WATERS; AND SALT

No additions, deletions, or changes.

34. COLOR ADDITIVES

(1) The official first action liquid chromatographic method for the determination of intermediates and reaction by-products in FD&C Yellow No. 5, **34.C01-34.C06** (*J. Assoc. Off. Anal. Chem.* **65,** 933(1982)), was adopted official final action.

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(2) The official final action liquid chromatographic method for the determination of inter-

mediates and by-products in FD&C Red No. 40, **34.B01-34.B06,** was editorially revised to specify addition of sodium borate first in the determinative step. Failure to add the sodium borate first may result in decomposition of DMMA.

Change the first sentence of **34.B06** to read:

"Weigh 0.250 g sample, add 10 mL 0.1M $Na_2B_4O_7$ first, then add ca 50 mL H_2O, and, when dissolved, dil. to 100 mL with H_2O."

35. COSMETICS

No additions, deletions, or changes.

36. DRUGS: GENERAL

(1) The official first action colorimetric method for the determination of disulfiram in tablets, **36.B01-36.B04** (*J. Assoc. Off. Anal. Chem.* **64,** 554(1981)), was adopted official final action.

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(2) The following atomic absorption spectrophotometric method for the determination of mercury in mercury-containing drugs was adopted official first action:

Mercury in Mercury-Containing Drugs
Atomic Absorption Spectrophotometric Method
Official First Action

36.D01 ***Principle***

Samples are digested in H_2O-HCl-HNO_3, and Hg is detd by AAS using air-C_2H_2 flame or flameless technic (low Hg levels).

36.D02 ***Apparatus***

Rinse all glassware before use with HNO_3 (1 + 1) followed by H_2O. For low Hg levels, decontaminate boiling flasks before use as follows: Add 5 mL H_2O-HCl-HNO_3 (4 + 3 + 1), place on steam bath 20 min, and rinse with H_2O.

(**a**) *Atomic absorption spectrophotometer.*—Equipped with air-C_2H_2 flame, or equipped with Hg hollow cathode lamp and gas flow-thru cell (Fig. **25:02**), 25(id) × 115 mm with quartz windows cemented in place. Operating conditions: wavelength 253.7 nm, slit width 160 μm, lamp current 3 mA, and sensitivity scale 2.5.

(**b**) *Diaphragm pump.*—Neptune Dyna-Pump, or equiv. Coat diaphragm and internal parts of pump with acrylic-type plastic spray. Use 16 gage Teflon tubing for all connections.

(**c**) *Gas inlet adapter.*—24/40 ⅋ (Kontes Glass Co. No. K-181000).

(**d**) *Digestion flask.*—250 mL flat-bottom boiling flask with 24/40 ⅋ joint.

36.D03 ***Reagents***

(**a**) *Reducing soln.*—Mix 50 mL H_2SO_4 with ca 300 mL H_2O. Cool to room temp. and dissolve 15 g NaCl, 15 g hydroxylamine sulfate, and 25 g $SnCl_2$ in soln. Dil. to 500 mL.

(**b**) *Diluting soln.*—To 1 L vol. flask contg 300-500 mL H_2O, add 58 mL HNO_3 and 67 mL H_2SO_4. Dil. to vol. with H_2O.

(**c**) *Magnesium perchlorate.*—Drying agent placed in filter flask (Fig. **25:02**). Replace as needed. (*Caution:* $Mg(ClO_4)_2$ is explosive when in contact with org. substances.)

(**d**) *Mercury stock soln.*—1000 μg/mL. Dissolve 0.1354 g $HgCl_2$ in 100.0 mL H_2O.

(**e**) *Digestion soln.*—H_2O-HCl-HNO_3 (4 + 3 + 1). Prepare immediately before use.

(**f**) *$K_2Cr_2O_7$ soln.*—5%, aq.

36.D04 ***Sample Preparation***

(**a**) *Ointments.*—Mix sample thoroly and accurately weigh portion contg ca 5 mg Hg into 50 mL beaker. Add 5 mL H_2O-HCl-HNO_3 (4 + 3 + 1). Cover with watch glass and heat on steam bath 30 min. Cool to room temp., swirl beaker to coagulate fat, and decant soln and three 10 mL H_2O rinses into 50 mL vol. flask. Add 2 mL 5% $K_2Cr_2O_7$, dil. to vol., and mix. Prep. reagent blank, beginning "Add 5 mL H_2O-HCl-HNO_3 . . .".

(**b**) *Tinctures.*—Pipet aliquot contg ca 5 mg Hg into 50 mL vol. flask, place on steam bath, and evap. almost to dryness in current of air. Add 5 mL H_2O-HCl-HNO_3 (4 + 3 + 1), and heat on steam bath 30 min. Blow air into flask 2-3 min, while swirling contents, to expel N oxides. Cool to room temp., add ca 30 mL H_2O and 2 mL $K_2Cr_2O_7$ soln, dil. to vol. with H_2O, and mix. Prep. reagent blank, beginning "Add 5 mL H_2O-HCl-HNO_3 . . .".

(**c**) *Injectables.*—Pipet aliquot contg ca 5 mg Hg into 50 mL vol. flask, add 5 mL H_2O-HCl-HNO_3 (4 + 3 + 1), and proceed as in (**b**), beginning ". . . and heat on steam bath . . .".

(**d**) *Preservatives and solns (or samples contg low levels of Hg).*—Pipet duplicate aliquots contg 0.5 μg Hg (0.1 mL Eppendorf pipet, or equiv., dilg sample if necessary), into sep. decontaminated 250 mL boiling flasks, add 5 mL H_2O-HCl-HNO_3 (4 + 3 + 1) to each flask, and heat on steam bath 30 min. Cool to room temp., and add 95 mL dilg soln, (**b**). Prep. 2 reagent blanks, beginning "Add 5 mL H_2O-HCl-HNO_3 . . .".

36.D05 ***Standard Preparation***

(a) *0 and 100 μg Hg/mL std solns (for samples a, b, and c).*—Pipet 0 and 5 mL 1000 μg/mL Hg stock soln into 50 mL vol. flasks, add 5 mL H_2O-HCl-HNO_3 (4 + 3 + 1), ca 30 mL H_2O, and 2 mL $K_2Cr_2O_7$ soln, dil. to vol. with H_2O, and mix.

(b) *0.5 μg Hg std soln (for sample d).*—Dilute 1000 μg/mL Hg stock soln to 5 μg/mL. Pipet duplicate 0.1 mL aliquots of this soln (Eppendorf pipet or equiv.) into sep. decontaminated 250 mL boiling flasks, (**d**). Add 5 mL H_2O-HCl-HNO_3 (4 + 3 + 1), and heat on steam bath 30 min. Cool to room temp., add 95 mL dilg solution, (**b**), and mix.

36.D06 ***Determination***

(a) *Samples a, b, and c.*—Operate atomic absorption spectrophtr with air-C_2H_2 flame according to manufacturer's specifications. Zero instrument with 0 μg/mL Hg std soln, and measure A of 100 μg/mL Hg std soln, blank soln, and sample solns, using 4X scale expansion.

(b) *Sample d.*—Adjust output of pump to ca 2 L air/min by regulating speed of pump with variable transformer. Connect app. as in Fig. **25:02,** except for gas inlet adapter. With pump working and spectrophr zeroed, add 20 mL reducing soln to dild aliquot. Immediately connect gas inlet adapter and aerate ca 3 min. (Adjust aeration time to obtain max. A.) Record A, disconnect pressure on "out" side of pump, and open vent on filter flask to flush system. Analyze in following sequence: reagent blank, 0.5 μg Hg std soln, sample solns, and 0.5 μg/mL std soln.

36.D07 ***Calculations***

(a) Flame AAS:

$$\text{mg Hg/g or mL} = (A - A_B) \times C'/(A' \times W \times 20)$$

(b) Flameless AAS:

$$\text{mg Hg/g or mL} = (A - A_B)/(A' - A_B) \times (C'/V) \times F \times 1/1000$$

where A, A_B, and A' = absorbance of sample, blank, and std solns, resp.; C' = concn of std soln (μg/mL, flame AAS; μg, flameless AAS); W = wt (g) or vol. (mL) of sample taken; V = vol. sample (mL) added to 250 mL boiling flask; F = diln factor if sample was dild.

37. DRUGS: ACIDIC

(1) The following liquid chromatographic method for the determination of amitriptyline hydrochloride in tablets and injectables was adopted interim official first action after the 1982 meeting:

Amitriptyline in Tablets and Injectables
Liquid Chromatographic Method
Interim Official First Action

37.D01 ***Principle***

Amitriptyline content of tablets and injectables is detd by liq. chromatography, using trifluoperazine as internal std and UV detection at 239 nm.

37.D02 ***Apparatus and Reagents***

(a) *Liquid chromatograph.*—Tracor Model 950 solvent pump, Model 970A variable wavelength detector, Model 26325 recorder (Tracor Instruments Inc., Austin, TX 78721), and 20 μL Rheodyne Model 7120 loop injector (Rheodyne Inc., Berkeley, CA 94710). *Operating conditions:* column temp., ambient; solv. flow rate, 1.33 mL/min; detector wavelength, 239 nm; attenuation, 16 AUFS; recorder, 1 mV; chart speed, 1 in./4 min.

(b) *Chromatographic column.*—Stainless steel, 300 × 3.9 mm id, packed with 10 μm μBondapak CN (Waters Associates, Inc.), or equiv.

(c) *Methanol.*—AR grade (Fisher Scientific Co.).

(d) *Mobile phase.*—MeOH–0.005M ammonium acetate (90 + 10).

(e) *Culture tubes.*—95 × 25 mm with screw cap (Kimble).

(f) *Internal std soln.*—Accurately prepare ca 0.5 mg Trifluoperazine HCl Ref. Std/mL MeOH.

(g) *Std soln.*—0.04 mg/mL. Accurately weigh ca 10 mg USP Amitriptyline HCl Ref. Std and transfer to 250 mL vol. flask. Dissolve in 1 mL MeOH, add 25.0 mL internal std soln, dil. with MeOH, and mix.

37.D03 ***Sample Preparation***

(a) *Tablets.*—Weigh and finely powder ≥20 tablets. Accurately weigh and transfer amt of powd. equiv. to 10 mg amitriptyline HCl into screw-cap culture tube and add 25.0 mL internal std soln. Tumble on rotator 15 min at ca 30 rpm, and filter, if necessary. Dil. accurately measured vol. of soln with MeOH to ca 0.04 mg/mL.

(b) *Single tablet.*—Place one tablet in 95 × 25 mm screw-cap culture tube and crush to fine powd. with glass rod. Add 25.0 mL MeOH and mix. Tumble on rotator 15 min at ca 30 rpm, and

filter, if necessary. Pipet accurately measured aliquot (*A*) of this soln, equiv. to 2 mg amitriptyline HCl, into 50 mL vol. flask, add 5.0 mL internal std soln, dil. to vol. with MeOH, and mix.

(c) *Injectables.*—Accurately pipet vol. of injectable, equiv. to 10 mg amitriptyline HCl, into 50 mL vol. flask, add 25.0 mL internal std soln, dil. to vol. with MeOH, and mix. Dil. accurately measured vol. of this soln with MeOH to ca 0.04 mg/mL.

37.D04 ***Determination***

Equilibrate system with mobile phase at 1.33 mL/min, until baseline is steady. Inject measured vol. of std soln into chromatograph by microsyringe or sampling valve. Adjust injection vol. and operating parameters so amitriptyline HCl in std soln injection gives peak ht ca 60% full scale and retention time ca 7 min. Under these conditions, 5 replicate injections of std soln should give coefficient of variation of $\leq 3\%$ and resolution factor (*R*) between the 2 main peaks should be ≥ 1. Make alternate injections of equal vols. of sample and std solns. Measure peak hts for amitriptyline HCl and internal std in sample and std solns, and det. response ratios.

37.D05 ***Calculations***

Tablets: $\text{mg/tablet} = RR/RR' \times C \times T/W$

Single tablet: $\text{mg/tablet} = RR/RR' \times C \times 5/A$

Injectables: $\text{mg/mL} = RR/RR' \times C/V$

where *RR* and *RR'* = ratio of amitriptyline HCl peak ht to internal std peak ht for sample and std solns, resp.; *C* = mg amitriptyline HCl in 250 mL std soln; *T* = av. tablet wt, g; *W* = wt sample taken, g; *A* = aliquot taken, mL; *V* = vol. injectable taken, mL.

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(2) The following liquid chromatographic method for the determination of sulfisoxazole in tablets, solutions, and ointments was adopted interim official first action after the 1982 meeting:

Sulfisoxazole in Tablets, Solutions, and Ointments

Liquid Chromatographic Method

Interim Official First Action

37.D06 ***Principle***

Sulfisoxazole content of tablets, solns, and ointments is detd by reverse phase HPLC using ternary aq. mobile phase, UV detection at 254 nm, and sulfadimethoxine as internal std.

37.D07 ***Apparatus***

(a) *Liquid chromatograph.*—DuPont Model 841 solv. pump equipped with 254 nm detector (E. I. duPont de Nemours and Co.), 10 μL injection valve (Valco Instruments Inc., PO Box 55603, Houston, TX 77055), and Model 3380A integrator (Hewlett-Packard). Equiv. HPLC system and strip chart recorder may be used.

(b) *Chromatographic column.*—Stainless steel, 30 cm × 3.9 mm id, packed with 10 μm μBondapak C_{18} (Waters Associates, Inc.) or equiv. meeting appropriate HPLC system suitability requirements.

37.D08 ***Reagents***

(a) *Solvents.*—UV grade MeOH and *n*-heptane (Fisher Scientific Co., or equiv.), and acetonitrile (Burdick & Jackson Laboratories, Inc., or equiv.).

(b) *Mobile phase.*—Acetonitrile–acetic acid–H_2O (30 + 1 + 69); flow rate 2.0 mL/min. Retention times: sulfisoxazole, ca 4 min; internal std, ca 5.5 min. Vary ratio of acetonitrile to H_2O to meet HPLC system suitability requirements. Increased acetonitrile decreases retention time.

(c) *Internal std soln.*—Dissolve 80 mg USP Ref. Std Sulfadimethoxine in MeOH and dil. to 100 mL with MeOH.

(d) *Sulfisoxazole std soln (5.0 mg/100 mL).*—Transfer 100 mg accurately weighed USP Ref. Std Sulfisoxazole (previously dried 2 h at 105°) to 100 mL vol. flask and dil. to vol. with MeOH. Transfer 5.0 mL aliquot to 100 mL vol. flask contg 5.0 mL internal std soln, dil. to vol. with mobile phase, and mix.

37.D09 ***Sample Preparation***

(a) *Tablets.*—Det. av. wt and grind to pass No. 60 sieve. Transfer accurately weighed portion of powd. equiv. to 500 mg sulfisoxazole to 100 mL vol. flask, add 25 mL MeOH, stopper, mix on mech. shaker 30 min, dil. to vol. with MeOH, mix, and filter. Transfer 20.0 mL aliquot of filtrate to 100 mL vol. flask and dil. to vol. with MeOH. Transfer 5.0 mL aliquot to 100 mL vol. flask contg 5.0 mL internal std soln, dil. to vol. with mobile phase, and mix.

(b) *Liquids (injections and ophthalmic solns).*—Accurately transfer vol. of dosage form contg ca 200 mg sulfisoxazole to 200 mL vol. flask and dil. to vol. with MeOH. Transfer 5.0 mL aliquot to

100 mL vol. flask contg 5.0 mL internal std soln, dil. to vol. with mobile phase, and mix.

(c) *Ointments.*—Transfer accurately weighed amt of sample (*S*) contg ca 50 mg sulfisoxazole to 125 mL separator contg 50 mL *n*-heptane, shake to disperse ointment, and ext with three 25 mL portions of MeOH–H_2O (2 + 1), passing each ext consecutively thru second 125 mL separator contg 50 mL *n*-heptane. Collect exts in 100 mL vol. flask and dil. to vol. with MeOH. Transfer 10.0 mL aliquot to 100 mL vol. flask contg 5.0 mL internal std soln, dil. to vol. with mobile phase, and mix.

37.D10 ***Determination***

Equilibrate column with mobile phase at flow rate of 2 mL/min. Make 3 replicate injections of sulfisoxazole std soln. Using either peak area or peak ht measurements, for each injection det. response ratio of sulfisoxazole to internal std. In suitable system, capacity factor, k', for sulfisoxazole should be 1.0–3.0; resolution factor, R, for sulfisoxazole peak and internal std peak should be ≥ 1.5; and coefficient of variation of response ratio for 3 replicate injections each of sample and sulfisoxazole std working solns should be $\leq 2.0\%$. When chromatgc requirements are met, make alternate injections of std and sample solns, and det. response ratio for each. Retention times for sulfisoxazole and internal std must be same for sample and std soln injections.

37.D11 ***Calculations***

Calc. content of sulfisoxazole in dosage form as follows:

Tablets: mg/tablet

$$= (RR/RR') \times C \times (T/S) \times 100$$

Solns: $mg/mL = (RR/RR') \times (C/V) \times 40$

Ointments: $mg/g = (RR/RR') \times (C/S) \times 10$

where RR and RR' = response ratio of sample and std solns, resp.; C = amt sulfisoxazole in 100 mL final std soln, mg; T = av. tablet wt, g; S = sample wt, g; and V = vol. soln taken, mL.

38. DRUGS: ALKALOID AND RELATED BASES

(1) The official first action high pressure liquid chromatographic method for the determination of antihistamine–adrenergic combinations in syrups or tablets, **38.B01–38.B06,** was adopted official final action.

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(2) The following methods were declared surplus:

(a) Atropine in tablets, infrared method, **38.029–38.030.**

(b) Ephedrine in inhalants, Method II, **38.038.**

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(3) The official first action liquid chromatographic method for the determination of physostigmine salicylate and sulfate in solutions, **38.C01–38.C06,** was extended to include the ointment dosage form. The following changes are required:

(a) In line 2 of the title add "and Ointments."

(b) Change **38.C04** to read:

38.C04 ***Sample Preparation***

(a) *Solutions.*—Transfer aliquot of sample (*V*) contg ca 3 mg physostigmine or its salts to 100 mL vol. flask contg 5.0 mL internal std soln and dil. to vol. with CH_3CN.

(b) *Ointments.*—Transfer accurately weighed sample (*W*) contg ca 3 mg physostigmine or its salts to 60 mL separator. Add 20 mL *n*-hexane and ext with four 20 mL portions of CH_3CN. Collect exts in 100 mL vol. flask contg 5.0 mL internal std soln and dil. to vol. with CH_3CN.

(c) Change **38.C06** to read:

38.C06 ***Determination***

Make duplicate 10 μL injections each of sample soln and appropriate std soln, alternating sample and std solns. Calc. results by using response ratios (RR) rel. to internal std, based on peak areas:

For solns:

Physostigmine (or salt), mg/mL

$$= 100 \times (RR/RR') \times (C/V)$$

For ointments:

Physostigmine (or salt), mg/g

$$= 100 \times (RR/RR') \times (C/W)$$

where RR and RR' = response ratio of sample and std, resp.; C = concn of std (mg/100 mL); V = mL soln; W = g ointment. Identification is based on same retention times for samples and stds.

39. DRUGS: NEUTRAL

No additions, deletions, or changes.

40. DRUGS: ILLICIT

(1) The following official first action methods were adopted official final action:

(a) Cocaine hydrochloride, gas chromatographic method, **40.002-40.005.**

(b) Phencyclidine hydrochloride in powders, gas chromatographic method, **40.016-40.018.**

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(2) The official final action method for the determination of diacetylmorphine (heroin) in tablets, **40.006,** was repealed official first action.

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(3) The following liquid chromatographic method for the determination of oxazepam in tablets and capsules was adopted official first action:

Oxazepam in Tablets and Capsules
Liquid Chromatographic Method
Official First Action

40.D01 ***Principle***

Oxazepam is extd into MeOH and detd by liq. chromatogy with UV (254 nm) detector.

40.D02 ***Apparatus***

(Equiv. ap. may be substituted)

(a) *Liquid chromatograph.*—Model 204 equipped with 2 Model 6000A pumps, Model 660 solv. programmer, Model 440 UV (254 nm) detector, Model U6K injector (all Waters Associates, Inc.), and Model 3380A integrator (Hewlett-Packard).

(b) *HPLC column.*—Bondapak C_{18}, 3.9 mm id × 30 cm long (Waters Associates, Inc.) at ambient temp.

(c) *Filter.*—Millipore type EG (pore size 0.2 μm) (Millipore Corp., Bedford, MA 01730) or 0.45 μm MeOH compatible equiv.

40.D03 ***Reagents***

(a) *Methanol.*—UV grade (MCB Reagents), or equiv.

(b) *Mobile phase.*—MeOH-H_2O-glac. HOAc (60 + 40 + 1) at flow rate of 1.0 mL/min. MeOH concn and flow rate may be varied to give approx. retention time of 6-8 min for oxazepam.

(c) *System suitability std soln.*—Dissolve 10 mg USP Oxazepam and 15 mg USP 2-Amino-5-chlorobenzophenone in 250.0 mL MeOH.

(d) *Oxazepam std soln.*—Transfer 25 mg USP Oxazepam, accurately weighed, to 250 mL vol. flask. Add 5 mL H_2O and dil. to vol. with MeOH. Soln is stable 90 min.

40.D04 ***Sample Preparation***

(a) *Tablets.*—Det. av. wt/tablet and grind tablets to pass No. 60 mesh sieve. Transfer accurately weighed portion of powder contg 25 mg oxazepam to 250 mL vol. flask. Add 5 mL H_2O and 25 mL MeOH. Mix thoroly. Add 75 mL MeOH and place in ultrasonic bath 10 min. Dil. to vol. with MeOH. Stir 30 min. Filter portion of soln thru type EG filter into small g-s flask. Soln is stable 90 min.

(b) *Capsules.*—Det. av. wt of capsule contents. Transfer accurately weighed portion of capsule contents contg 25 mg oxazepam to 250 mL vol. flask. Proceed as in (a).

40.D05 ***System Suitability Check***

(a) *Resolution.*—Inject 10.0 μL system suitability std soln. Retention times for oxazepam and 2-amino-5-chlorobenzophenone should be ca 6 and 14 min, resp. Resolution factor, R, for the 2 peaks should be ≥5.0, using following formula:

$$R = 2(t' - t)/(PW + PW')$$

where t and t' = mm retention of oxazepam and 2-amino-5-chlorobenzophenone peaks, resp.; and PW and PW' = mm peak widths measured at baseline of oxazepam and 2-amino-5-chlorobenzophenone, resp. Adjust MeOH concn if resolution is unsatisfactory.

(b) *Reproducibility.*—Make five 10.0 μL injections of oxazepam std soln and measure peak areas. In suitable system, coefficient of variation is not >2.0%.

40.D06 ***Determination***

Make duplicate 10 μL injections each of sample soln and std soln, alternating sample and std solns. Calc. results by using peak areas:

$$\text{mg Oxazepam/tablet or capsule} = (PA/PA') \times C \times (T/S)$$

where PA and PA' = peak area for sample and std solns, resp.; C = mg oxazepam/250 mL std soln; T = av. wt, g, of tablet or capsule contents; S = sample wt, g.

41. DRUGS AND FEED ADDITIVES IN ANIMAL TISSUES

The following interim official first action thin layer chromatographic screening method for the determination of sulfonamides in animal tissues was adopted official first action:

Sulfonamides in Animal Tissues

Thin Layer Chromatographic Screening Method

Official First Action

(Applicable to swine, turkey, and duck tissues)

41.D01 ***Principle***

Sulfonamides are extd with ethyl acetate after addn of sulfapyridine as internal std. Exts are cleaned up by partitioning between org. and aq. solvs, and chromatographed on silica gel TLC plates. Developed plates are treated with fluorescamine and scanned by fluorescence densitometer.

41.D02 ***Reagents***

(a) *Ethyl acetate, hexane, methylene chloride, and methanol.*—Distd in glass (Burdick & Jackson Laboratories, Inc.).

(b) *Glycine buffer soln.*—Prep. glycine (Fisher Scientific Co.) as 0.2M aq. soln and adjust pH to 12.25 with NaOH.

(c) *Fluorescamine derivatizing soln.*—Dissolve 25 mg fluorescamine (Pierce Chemical Co.) in 250 mL acetone. Replace soln after treating 8–9 plates.

(d) *Sulfonamide stds.*—Com. sulfamethazine (SMZ), sulfadimethoxine (SDM), sulfaquinoxaline (SQX), sulfathiazole (STZ), sulfabromomethazine (SBR), and sulfapyridine (SPY).

(e) *Stock std solns.*—Dissolve 100 mg sulfonamide in 100 mL acetone. Store in refrigerator.

(f) *Fortification std solns.*—Combine sulfonamides of interest and dil. to 5.00, 2.50, and 1.25 μg/mL (equiv. to 0.2, 0.1, and 0.05 ppm in tissue), using 0.05M pH 7.5 phosphate buffer. All solns should contain 2.50 μg SPY/mL. Store fortification stds in refrigerator and prep. weekly.

(g) *Internal std soln.*—Using stock std soln (e), prep. 2.50 μg SPY/mL 0.05M pH 7.5 phosphate buffer.

41.D03 ***Apparatus***

(a) *Densitometer.*—CAMAG TLC/HPTLC scanner (Applied Analytical Industries, Wilmington, NC 28403) equipped with 400 nm interference filter on excitation source. Replace std 400 nm cutoff filter on photomultiplier by 500 nm interference filter. Slit dimensions 7.8 × 0.3 mm. Scan plates at either 1 or 2 mm/s.

(b) *TLC plates.*—20 × 20 cm channeled plates, channels 8 cm wide with 0.25 mm silica gel layer and pre-adsorbent spotting zones (Whatman LK6D, Whatman, Inc.).

(c) *TLC spotting capillaries.*—20 μL glass capillary tubes (Corning Glass Works).

(d) *Heat strip.*—Automatic spotter heat strip set at 85° (Analytical Instrument Specialties, Inc., Libertyville, IL 60048). Any equiv. heating device with temp. control can be substituted.

(e) *TLC tank.*—Std 2-trough tank (Alltech Associates, Deerfield, IL 60015), atm. fully satd by lining with satn pads.

(f) *Derivatization tank.*—Stainless steel (A. H. Thomas).

(g) *Evaporator.*—N-Evap (Organomation Associates, Inc., Northborough, MA 01532).

(h) *Homogenizer.*—Tekmar SDT Tissumizer (Tekmar Co., Cincinnati, OH 45222).

(i) *Shaker.*—Horizontal reciprocating shaker set at ca 240 cycles/min (Eberbach Corp., Ann Arbor, MI 48106).

(j) *Centrifuge.*—Set at 2500 rpm for 5 min (International Equipment Co., Needham Heights, MA 02194).

(k) *Polypropylene centrifuge tubes.*—50 mL capacity (Corning Glass Works).

41.D04 ***Sample Extraction***

Accurately weigh ca 2.5 g homogenized liver or muscle into 50 mL centrf. tube. Add 100 μL internal std soln. Prep. 3 control samples (using tissue known to be free of sulfonamides) and fortify with internal std (0.1 ppm) and each sulfonamide of interest, one control each at 0.05, 0.10, and 0.20 ppm. Wait 15 min and then add 25 mL ethyl acetate. Blend muscle samples 1 min with Tissumizer, and centrf. For liver samples, cap tube tightly, shake 20 min on horizontal shaker, and centrf. Transfer ethyl acetate to clean tube and discard tissue. Add 10 mL glycine buffer to ext, mech. shake 5 min, and centrf. Vac.-aspirate and discard org. phase. Adjust pH of aq. phase to 5.2–5.3 by adding 2 mL (1 + 1) mixt. of 2M pH 5.25 phosphate buffer and 1.7M HCl. Check pH and make final adjustments with either addnl buffer or 0.1N NaOH. Add 5 mL hexane, mech. shake 5 min, and centrf. Aspirate and discard hexane phase. Remove any solid or emulsified material remaining at interface. Add 10 mL CH_2Cl_2, shake 5 min, centrf., and aspirate and discard aq. phase. Add 10 μL diethylamine to CH_2Cl_2 ext and conc. just to dryness under stream of N at 40°. During evapn, occasionally rinse tube walls with CH_2Cl_2. Redissolve residue in 100 μL MeOH and mix 30 s on vortex mixer. Let stand 5 min before chromatgy to let insoluble oils settle.

41.D05 ***Chromatography***

Apply 20 μL portion of sample to pre-adsorbent spotting zone of TLC plate. Avoid using lane at each side of plate, and spot the 3 fortified control samples at intervals across plate to minimize effects of across-plate variation. Develop plate 1 cm in MeOH followed by 2 developments, 6 cm and 12 cm, in $CHCl_3$–*tert*-BuOH (80 + 20). Between each development, dry plate 1 min at 110°. If STZ is suspected or present, prewash $CHCl_3$–*tert*-BuOH with H_2O. Maintain development tank temp. at 30–33° for optimum resolution of multiple sulfonamides. Derivatize compds by quickly dipping plate in fluorescamine soln. Bands are visible after 15–30 min. Scan each lane and obtain its response scanning curve. For each sample and std (each lane), det. ratio of response for sulfonamide of interest to response for internal std (SPY).

41.D06 ***Calculations***

For each sulfonamide, calc. slope and intercept of std curve, concn vs response ratio, using linear regression and results for the 3 fortified samples. Plotting sulfonamide concn on *y*-axis results in std error of est. ($S_{y.x}$) having dimension of ppm, thus simplifying estn of confidence intervals. For quality assurance purposes, $S_{y.x}$ should be $\leq$0.02 ppm, and correlation coefficient, *r*, should be $\geq$0.995. Use linear regression slope and intercept to calc. concn of samples from their respective sulfonamide/internal std peak ht ratios.

42. DRUGS IN FEEDS

No additions, deletions, or changes.

43. VITAMINS AND OTHER NUTRIENTS

(1) The official first action liquid chromatographic method for the determination of vitamin D in mixed feeds, premixes, and pet foods, **43.C01-43.C09**, was adopted official final action.

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(2) The following chloroform–methanol extraction method for the determination of fat in foods was adopted official first action:

Fat in Foods
Chloroform-Methanol Extraction Method
Official First Action

(Applicable to processed and prepd foods. Not applicable to meat.)
(*Caution:* See **51.004, 51.011,** and **51.056.**)

43.D01 ***Apparatus and Reagents***

(**a**) *Blending assembly.*—Semi-micro, stainless steel blending assembly for Waring blender (American Scientific Products Co., Cat. No. S8395-1, or equiv.).

(**b**) *Enzyme soln.*—1% Clarase 40,000 in 0.5M Na acetate soln. Suspend 10 g Clarase 40,000 (Miles Laboratories, Inc.) in 200 mL 0.5M Na acetate soln in 1 L vol. flask, dil. to vol. with 0.5M Na acetate soln, and mix thoroly. (Stable at least 7 days when stored at 4°.)

43.D02 ***Determination***

Accurately weigh ca 5 g well minced sample (3 g if >10% fat) into 50 mL digestion tube, add enough enzyme soln so that total H_2O content (enzyme soln added plus original moisture in sample) is 32 mL. Shake gently until sample is thoroly mixed with enzyme soln and place tube in 45–50° H_2O bath 1 h. Mix thoroly and quant. transfer digest to blending assembly with 80.0 mL MeOH and 40.0 mL $CHCl_3$.

Cover and blend 2 min at high speed. Remove cover, add 40.0 mL $CHCl_3$, cover, and blend 30 s. Remove cover, add 40 mL H_2O, cover, and blend addnl 30 s.

Transfer ext to 250 mL polypropylene centrf. bottle and centrf. 10 min at ca 3000 rpm (when rotor diam. is 11.5 in.) to clarify bottom $CHCl_3$ layer. Pipet 20 mL $CHCl_3$ ext into tared 100 mL beaker. (Care should be taken to prevent transferring aq. phase.) Evap. to dryness on steam bath in fume hood. Dry fat residue in 101° oven to const wt (ca 30 min), cool in desiccator to const wt (ca 30 min), and weigh. Calc. % fat = (g residue/g sample) × 4 × 100.

(*Note:* Ratio of $CHCl_3$–MeOH–H_2O is critical for quant. extn of fat. This may necessitate detn of moisture content of sample to optimize amt of H_2O (as enzyme soln) in initial extn step. Reference source such as USDA Agriculture Handbook No. 8, *Composition of Foods*, or USDA Agriculture Handbook No. 456, *Nutritive Value of American Foods*, is adequate for this purpose. Also use as guide to pre-estimate fat content so that proper sample wt can be chosen for analysis.)

44. EXTRANEOUS MATERIALS: ISOLATION

(1) The official final action method for mold count of citrus and pineapple juices, canned, single strength, **44.082,** was revised to update and expand the description of the centrifuge.

In **44.082,** change the third sentence to read:

"Centrf. 10 min at 2200 rpm, using International type EXD centrf. (Damon/IEC Div., 300 Second Ave, Needham Hts, MA 02194) with 8-place No. 240 head, No. 320 shield, No. 325 trunion ring, and No. 571 cushion, or other centrf. giving equiv. max. relative centrifugal force of 1060 × *g* as computed by following formula: $RCF_{max.} = 1.118 \times 10^{-5} N^2 r \times g$, where N = rpm and r = radius of centrf. arm in cm (distance from center of centrf.-head to bottom of horizontal centrf. tube). The following formula may be used to det. equiv. centrf.: $N_1^2 r_1 = N_2^2 r_2$, where N_1 = 2200 rpm and r_1 = 19.6 cm."

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(2) The official first action thin layer chromatographic method for uric acid from bird and insect excreta, **44.183-44.185,** was editorially revised by modifying the dye marker mixture to improve visualization of the uric acid spot. This change supersedes the erratum published in "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **63,** 417 (1980).

Change **44.183(e)** to read:

"(e) *Dye mixture.*—Dissolve 16 mg FD&C Red No. 2 and 32 mg FD&C Yellow No. 6 in 50 mL H_2O, and mix well. These dyes serve as visual markers during development, with R_f for Red No. 2 at 0.38–0.40; uric acid, 0.41–0.43; and Yellow No. 6, 0.65, using Analtech plate and sandwich chamber. Merck plates have lower R_f values and do not sep. Red No. 2 and uric acid when overspotted."

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(3) The official first action thin layer chromatographic method for urine stains, **44.A04-44.A07,** was editorially revised. Instructions were added to preclean commercial plates to remove a colored impurity that occasionally interferes in visualization of the urinary indican spot.

Add the following to **44.A06:**

(d) *Pre-cleaning Analtech MN 300 cellulose plates.*—Develop unused plate in either std developing tank or sandwich chamber using developing solv. **44.A05(f).** Develop to 15 cm above lower edge of plate. Thoroly dry plate using either hair dryer, forced draft oven at ≤80° for ca 15 min, or overnight in fume hood. Plate must be room temp. and completely free of solv. odor before use.

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(4) The official first action chemical test for mammalian feces, **44.B08-44.B11,** was revised to include the use of gelled working test medium (WTM). This change provides an alternative to save analytical time and reduce false negatives.

The following changes are required:

(a) In **44.B09,** add:

"(d) *Spatula.*—Curved on one end, knob on the other end (Arthur H. Thomas Co. No. 8340-H10, or equiv.)."

(b) In **44.B10(c),** change the last paragraph to read:

"Long term storage: Add ca 1 mL portions to cups. Gelled plugs, in cups, may be stored for up to 4 months if sealed in a plastic bag, held at room temp., and protected from direct sunlight. Discard any gels showing pink color and/or vol. loss."

(c) In **44.B11,** revise the first paragraph to read:

"Transfer suspect feces . . . . to cup contg 1 mL gelled WTM. Cover with addnl 1 mL cool (40–41°) WTM or, alternatively, with plug of gelled WTM. (Use clean spatula, **44.B09(d),** to manipulate covering plug of WTM and to press plug into close contact with sample.) Place cup in 40–41° H_2O bath. Check for development of red color near particles."

(d) In **44.B11,** add as a new third paragraph:

"*Positive control preparation.*—Using calf intestine alkaline phosphatase (AKP) (Calbiochem No. 52457, or equiv.), prep. 1 mg/mL soln in borate buffer (stock test reagent **44.B10(b),** without phthln diphosphate). Add 20 μL AKP soln to 1 mm diam. filter paper disks (Whatman No. 1, or equiv.). Use positive control disks with either liq. WTM or alternative gelled plug WTM. Note: Positive control disks may be stored up to 4 months if held at room temp. and protected from light."

(e) The paragraph beginning "*Test response.*—" will be the fourth paragraph in **44.B11.**

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(5) The official first action fruit tissue standardization in the Howard mold count of fruit nectars, purees, and pastes, **44.C08-44.C09,** was revised to facilitate regulatory comparability. Add as a last sentence in **44.C09:**

"Before calcg % mold counts for fruits dild 1 + 1, divide number of positive fields by 2."

45. FORENSIC SCIENCES

No additions, deletions, or changes.

46. MICROBIOLOGICAL METHODS

(1) The following interim official first action method for the differentiation of members of the *Bacillus cereus* group, *J. Assoc. Off. Anal. Chem.* **65,** 1134 (1982), was adopted official first action to provide supplementary procedures for the identification of *B. cereus* isolated from foods by method **46.A10-46.A15:**

Differentiation of Members of *Bacillus cereus* Group

Official First Action

(Typical strains of *B. cereus* isolated from foods by **46.A10-46.A15** can be differentiated from other members of *B. cereus* group including: (1) insect pathogen *B. thuringiensis,* (2) mammalian pathogen *B. anthracis,* and (3) rhizoid strains of *B. cereus* var. *mycoides.*)

46.D01 *Apparatus*

(**a**) *Staining rack.*—Rack must be accessible from below for heating slides.

(**b**) *Inoculating loops.*—One each, 26 gage nichrome wire with loop 2 mm id and one 24 gage nichrome wire loop 3 mm id.

46.D02 *Media and Reagents*

(**a**) *Mannitol-egg yolk-polymyxin (MYP) agar.*—1.0 g beef ext, 10.0 g peptone, 10.0 g D-mannitol, 10.0 g NaCl, 0.025 g phenol red (as soln), and 15.0 g agar dild to 900 mL with H_2O. Adjust to pH 7.2 ± 0.1, heat to dissolve, and dispense 225 mL portions into 500 mL flasks. Autoclave 15 min at 121°. Cool to 50° in H_2O bath and add 12.5 mL sterile 50% egg yolk emulsion (**b**) and 2.5 mL polymyxin B soln contg 10 000 units per mL (if available) to each 225 mL medium. (Addn of polymyxin B soln is optional when medium is to be used for testing reactions of pure cultures.) Mix well and dispense 18 mL portions into 100 × 15 mm sterile petri dishes. Dry plates 24 h at room temp. before use. (Dehydrated mannitol-egg yolk-polymyxin (MYP) agar contg 50% egg yolk enrichment is satisfactory.)

(**b**) *Egg yolk emulsion.*—50%. Wash fresh eggs with stiff brush and drain. Soak 1 h in 70% alcohol. Aseptically remove yolk and mix (1 + 1) with sterile 0.85% NaCl soln. (50% egg yolk enrichment is satisfactory.)

(**c**) *Nutrient agar slants and plates.*—3.0 g beef ext, 5.0 g peptone, and 15.0 g agar dild to 1 L with H_2O (dehydrated nutrient agar is satisfactory). Heat to dissolve, and dispense 6.5 mL portions into 125 × 16 mm screw-cap tubes. Autoclave 15 min at 121° and slant tubes until medium solidifies. Final pH 6.8 ± 0.2. For plates, dispense 100–500 mL portions in bottles or flasks and autoclave 15 min at 121°. Cool to 50° in H_2O bath and dispense 18–20 mL portions in 100 × 15 mm sterile petri dishes. Dry plates 24–48 h at room temp. before use.

(**d**) *Motility medium.*—10.0 g trypticase, 2.5 g yeast ext, 5.0 g dextrose, 2.5 g Na_2HPO_4, and 3.0 g agar dild to 1 L with H_2O. Heat to dissolve. Dispense 2 mL portions into 13 × 100 mm tubes, and autoclave 10 min at 121°. Final pH 7.4 ± 0.2. Alternatively, dispense 100 mL amts in 150 mL bottles and autoclave 15 min at 121°. Cool to 50° and aseptically dispense 2 mL into sterile 13 × 100 mm tubes. For best results, store at room temp. 2–4 days before use to prevent growth along side of medium.

(**e**) *Trypticase-soy-sheep blood (TSSB) agar.*—Dil. 15.0 g trypticase, 5.0 g phytone peptone, 5.0 g NaCl, and 15.0 g agar to 1 L with H_2O. Adjust pH to 7.0 ± 0.2. Heat to boiling to dissolve, and dispense 100–500 mL portions in bottles or flasks. Autoclave 15 min at 121° and cool to 48° in H_2O bath. Add 5 mL sterile defibrinated sheep blood per 100 mL medium. Mix well, and dispense 18–20 mL portions into 100 × 15 mm petri dishes. (Trypticase-soy or tryptic-soy agar plates contg. 5% sheep blood are satisfactory.)

(**f**) *Basic fuchsin stain.*—Dissolve 0.5 g basic fuchsin in 20 mL alcohol and dil. to 100 mL with H_2O. Filter soln if necessary thru fine paper to remove excess dye particles. Store in tightly stoppered container. (TB Carbol-fuchsin ZN stain is satisfactory.)

(**g**) *Butterfield's buffered phosphate diluent.*—(*1*) *Stock soln.*—Dissolve 34.0 g KH_2PO_4 in 500 mL H_2O, adjust to pH 7.2 with ca 175 mL 1N NaOH, and dil. to 1 L with H_2O. Store in refrigerator. (*2*) *Diluent.*—Dilute 1.25 mL stock soln to 1 L with H_2O. Prep. 90 ± 1 mL diln blanks with this soln and autoclave 15 min at 121°. Dispense 0.5 mL portions sterile diluent into sterile 13 × 100 mm tubes for preparing suspension of cultures to be tested.

(**h**) *Methanol fixative.*—Dispense undild methanol in plastic squeeze bottle for use in fixing slides.

46.D03 *Differential Tests*

(**a**) *Preparing test inoculum.*—Inoculate sep. nutrient agar slants with each culture to be tested. Incubate slants 18–24 h at 30° and transfer 3 mm loopful of culture from each slant to 100 × 13 mm tube contg 0.5 mL sterile phosphate buffered diluent. Suspend culture in diluent with

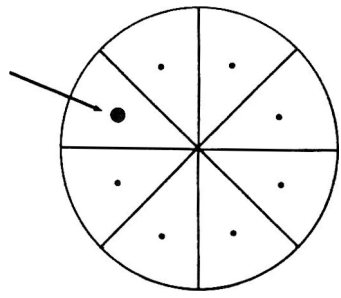

Figure 46:D1. Diagram of template for marking and inoculating *B. cereus* confirmatory plates. Each section is labeled and inoculated in the center, as indicated by arrow.

vortex mixer. Alternatively, inoculate 5 mL trypticase-soy broth and incubate tubes 18 h at 30°. Mix culture well and use for performing differential tests. Latter procedure is preferred for rhizoid strains and other strains which do not disperse well in phosphate buffer.

(b) *Reaction on MYP agar.*—Mark bottom of MYP agar plate into 6–8 equal segments with black felt pen as indicated in Fig. **46:D1** and label each section. Place plate in upright position on piece of white paper and inoculate one or more of the prelabeled sections by gently touching surface of agar with 2 mm loopful of culture. Let inoculum be absorbed and incubate plates in upright position 24–48 h at 30–35°. Check for lecithinase production as indicated by zone of ppt surrounding growth. Mannitol fermentation is neg. if growth and surrounding medium are eosin pink. These reactions should be observed with all organisms of *B. cereus* group except rare lecithinase-neg. variants.

(c) *Motility tests.*—Inoculate BC motility medium by stabbing down center with 3 mm loopful of culture. Incubate 18–20 h at 30° and examine for type of growth along stab. Motile strains produce diffuse growth into medium away from stab. Nonmotile strains except *B. cereus* var. *mycoides* grow only in and along stab. Strains of *B. cereus* var. *mycoides* often produce "fuzzy" growth in semisolid media resulting from cellular expansion but are not motile by means of flagella. Recheck doubtful results by alternative microscopic motility test as follows: Add 0.2 mL sterile H_2O to nutrient agar slant and inoculate with 3 mm loopful of culture. Incubate slant 6–8 h at 30°, and mix small loopful of liq. culture from base of slant with drop of sterile H_2O on microscope slide. Apply cover glass and examine immediately for signs of motility. *B. cereus* and *B. thuringiensis* cultures are usually actively motile by means of peritrichous flagella. *B. anthracis* and typically rhizoid strains of *B. cereus* var. *mycoides* are nonmotile.

(d) *Rhizoid growth.*—Inoculate predried nutrient agar plate by touching medium surface near center with 2 mm loopful of culture. Let inoculum be absorbed, and incubate plate in upright position 24–48 h at 30°. Check plate for rhizoid growth characterized by root or hairlike structures which may extend several cm from point of inoculation. Many *B. cereus* strains produce rough irregular colonies that should not be confused with rhizoid growth. This property is characteristic only of strains which are classified as *B. cereus* var. *mycoides.*

(e) *Hemolytic activity.*—Mark bottom of trypticase-soy-sheep blood agar plate into 6–8 equal segments (see Fig. **46:D1**) with black felt marking pen. Label each segment and inoculate one or more segments near center by gently touching agar surface with 2 mm loopful of culture. Let inoculum be absorbed, and incubate plates 24 h at 30–32°. Check plates for hemolytic activity as indicated by 2–4 mm zone of complete (beta) hemolysis surrounding growth. *B. cereus* is usually strongly hemolytic, whereas *B. thuringiensis* and *B. cereus* var. *mycoides* are often weakly hemolytic and produce complete hemolysis only underneath colonies. *B. anthracis* is usually nonhemolytic after 24 h of incubation. *Caution:* Nonmotile, nonhemolytic cultures could be *B. anthracis.* See precautions under *interpreting test results,* (g).

(f) *Detection of toxin crystals.*—Inoculate nutrient agar slant with loopful of culture. Incubate slant 24 h at 30° and hold at room temp. 2–3 days. Make smear on microscope slide with sterile H_2O. Air-dry and briefly heat-fix by passing slide slowly over burner flame; let cool, and place slide on staining rack. Flood slide with methanol, wait 30 s, and pour off methanol. Dry thoroughly by passing through burner flame. Return slide to staining rack, and flood completely with 0.5% aq. soln of basic fuchsin or TB Carbol-fuchsin ZN stain. Heat slide gently from below with micro burner until steam is seen. Wait 1–2 min and repeat this step. Let stand 30 s, pour off stain, and rinse slide thoroughly in 1 L clean tap H_2O. Dry slide without blotting and examine microscopically under oil immersion for presence of free spores and darkly stained tetragonal (diamond-shaped) toxin crystals. Free toxin crystals are usually abundant after 3 days but will not be detectable unless sporangia have lysed. Therefore, if free spores are not seen, leave cultures at room temp. for a few more days and repeat test. *B. thuringiensis*

produces protein toxin crystals that usually can be detected by staining, but are not produced by other members of *B. cereus* group.

(g) *Interpreting test results.*—On basis of test results, identify as *B. cereus* those isolates which are actively motile, strongly hemolytic, and do not produce rhizoid growth or protein toxin crystals. Nonmotile strains of *B. cereus* may be encountered and a few are weakly hemolytic. These strains can be differentiated from *B. anthracis* by their resistance to penicillin and to gamma bacteriophage. *Caution:* Nonmotile, nonhemolytic strains could be *B. anthracis*, and should be handled with special care and submitted to pathology laboratory such as Centers for Disease Control for identification or destroyed by autoclaving. Noncrystalliferous variants of *B. thuringiensis* and nonrhizoid strains derived from *B. cereus* var. *mycoides* cannot be differentiated from *B. cereus* by tests described.

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(2) The following hydrophobic grid membrane filter method for the enumeration of total coliforms in selected foods was adopted official first action for nonfat dry milk and canned custard:

Enumeration of Coliforms in Selected Foods
Hydrophobic Grid Membrane Filter Method
Official First Action

(Applicable to nonfat dry milk and canned custard)

46.D04 *Principle*

The hydrophobic grid membrane filter (HGMF) method uses a membrane filter implanted with hydrophobic material in a grid pattern. These hydrophobic lines act as barriers to the spread of colonies, thereby dividing the membrane filter surface into a number of separate compartments of equal and known size. The number of squares occupied by colonies is enumerated and converted to a most probable number value of organisms by using the formula given below.

46.D05 *Apparatus*

(a) *Hydrophobic grid membrane filter (HGMF).*—Membrane filter has pore size of 0.45 μm and is imprinted with nontoxic hydrophobic material in grid pattern. ISO-GRID (available from QA Laboratories Ltd, 135 The West Mall, Toronto, Ontario, Canada M9C 1C2) or equiv. meets these specifications.

(b) *Filtration units for HGMF.*—Equipped with 5 μm mesh prefilter to remove food particles during filtration. One unit is required for each sample. ISO-GRID (available from QA Laboratories Ltd) or equiv. meets these specifications.

(c) *Pipets.*—1.0 mL serological with 0.1 mL graduations; 1.1 mL or 2.2 mL milk pipets are satisfactory.

(d) *Blender.*—Waring Blendor, or equiv., multispeed model, with low-speed operation at 12 000–14 000 rpm, and 250 mL glass or metal blender jars with covers. One jar is required for each sample.

(e) *Vac. pump.*—Water aspirator vac. source is satisfactory.

(f) *Manifold or vac. flask.*

46.D06 *Culture Media and Reagents*

(a) *Peptone/Tween 80 diluent.*—Dissolve 1.0 g peptone (Difco 0118) and 10.0 g Tween 80 in 1 L H_2O. Dispense enough vol. into diln bottles to give 90 ± 1 mL after autoclaving 15 min at 121°.

(b) *M-FC agar.*—10.0 g tryptose, 5.0 g proteose peptone No. 3, 3.0 g yeast ext, 5.0 g NaCl, 12.5 g lactose, 1.5 g bile salts No. 3, 0.1 g aniline blue, and 15.0 g agar dild to 1 L with H_2O (M-FC Agar, Difco 0677 is satisfactory). Heat to boiling. Temper to 50–55°. Adjust pH to 7.4 ± 0.1. Dispense ca 18 mL portions into 100 × 15 mm petri dishes. Surface dry plated medium before use.

46.D07 *Sample Preparation*

Aseptically weigh 10 g sample into sterile blender jar. Add 90 mL diluent (a) and blend 2 min at low speed (12 000–14 000 rpm). Use this 1:10 diln to prep. further serial dilns if required.

46.D08 *Analysis*

(See Fig. **46:D2.**) Turn on vac. source. Place sterile filtration unit on manifold or vac. flask. Open clamp A. Pull forward on latch B, and rotate back funnel portion C. Aseptically place sterile HGMF on surface of base D. Rotate funnel forward and clamp unit shut by pressing with thumb on latch B.

Aseptically add ca 15–20 mL sterile H_2O or diluent to funnel. Pipet 1.0 mL of 1:10 sample diln into funnel. Apply free end of vac. tubing E to suction hole F to draw liq. thru prefilter mesh G. Aseptically add addnl 10–15 mL H_2O or diluent to funnel and draw thru mesh as before. Close clamp A to direct vac. to base of filtration unit and draw liq. thru HGMF.

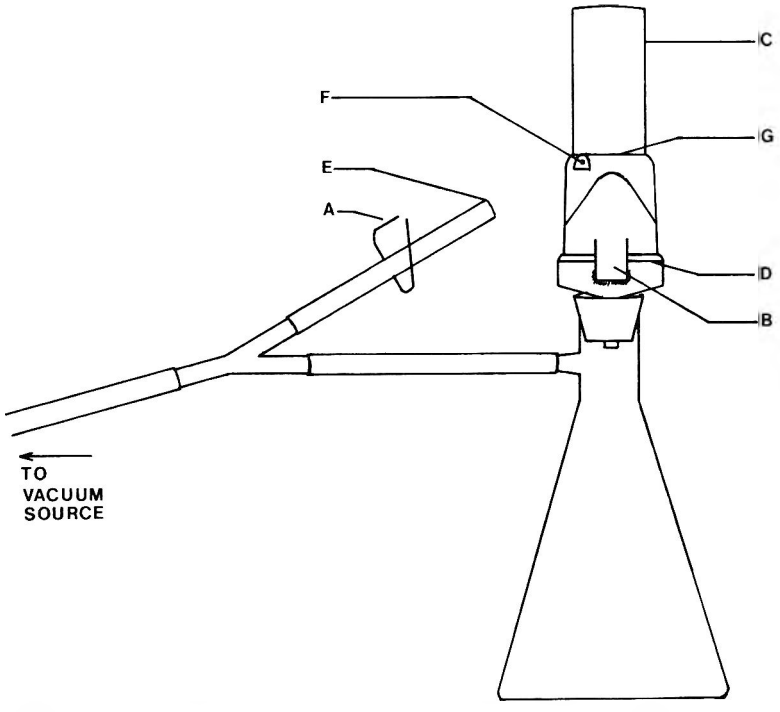

Figure 46:D2. Schematic of hydrophobic grid membrane filtration unit.

Open clamp A. Release latch B, and rotate back funnel C. Aseptically remove HGMF and place on surface of pre-dried M-FC agar (**b**). Avoid trapping air bubbles between filter and agar. Incubate 24 ± 2 h at 35°.

Count all squares contg one or more blue colonies. Include any shade of blue. Score each square as either occupied or empty. Convert occupied square count to MPN with the formula

$$\text{MPN} = [N \log_e (N/(N - x))]$$

where N = total number of squares and x = number of occupied squares. Multiply by diln factor and report as MPN of total coliform bacteria/g.

47. MICROCHEMICAL METHODS

No additions, deletions, or changes.

48. RADIOACTIVITY

The official first action extension of the official final action method, **48.025-48.029,** for the determination of cesium-137 in milk by gamma-ray spectroscopy, using simultaneous equations, to include barium-140 and iodine-131, *J. Assoc. Off. Anal. Chem.* **65,** 1039 (1982), was adopted official final action.

49. SPECTROSCOPIC METHODS

No additions, deletions, or changes.

50. STANDARD SOLUTIONS AND MATERIALS

No additions, deletions, or changes.

51. LABORATORY SAFETY

No additions, deletions, or changes.

52. REFERENCE TABLES

No additions, deletions, or changes.

ERRATA AND EMENDATIONS, OFFICIAL METHODS OF ANALYSIS, AOAC, 1983

The following changes should be made in the 13th edition:

Section	*Page*	
7.115	138	Line 3: Change to read ". . . is moist. Heat on steam bath to remove alcohol. Then dry at ca 100° . . ."
22.069(c)	367	Line 2: Change to read ". . . Dissolve 0.5 g *l*-malic acid in ca 50 mL"
28.070	450	Change equation to read: $\%\ \text{Non-elution material} = \dfrac{\left\lvert PA' \times \left(\dfrac{W' + W}{W'}\right) - PA \right\rvert \times 100}{PA' \times \left\lvert \left(\dfrac{W' + W}{W'}\right) - 1 \right\rvert}$
29.039	476	*Purity test.* Line 2: Change to read "thermionic or flame photometric detector. . ."
29.040(b)	476	Change to read "(**b**) *Gas chromatograph.*—With potassium chloride thermionic detector (see **29.045(i)** and **(k)**) or flame photometric detector (see **29.040(e)** and **(i)**)."
Table **31:01**	512	Last column, next to last line: Change to read "0.3–0.4"
36.033	594	Change last sentence to read: "Proceed as in **36.032**, first par., beginning ". . . let stand at room temp. ca 18 hr."
36.089	603	In last sentence, second par., change "**36.036(b)**" to read "**36.030(b).**"
44.121(b)	806	Change to read ". . .5 g Na_4EDTA in 150 mL H_2O, add 100 mL isopropranol, . . ."
44.123(a)	806	Line 5: Change to read ". . .50 mL Na_4EDTA, **44.121(b)**, . . ."
48.015(a)	866	Line 6: Delete final "f".
Reference	929	
Table		Change title to read "**52.010** . . ."

The following correction should be made in "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **63**, March 1980:

44.A01	409	Par. 2, lines 2–4: Change to read ". . . After stirring, add 50 mL Tween-Na_4EDTA reagent, **44.121**, (25 mL (**a**) plus 25 mL (**b**)) slowly down stirring rod. . . ."

The following corrections should be made in "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **65**, March 1982:

6.C09(a)	453	Line 2: Change to read ". . . 1.8 m × 4 mm (id)"
44.122(e)	502	Lines 1 and 2: Change to read "(e) *Alternative solvent saver (reflux) technic for ground turmeric.*—Weigh . . ."
44.122(e)	502	Par. 1: Delete last 2 lines "(Precaution: Only use isopropranol as solv. with this technic.)"
48.026(a)	506	Line 5: Change "^{207}Ba" to read "^{207}Bi"
48.029(b)	507	Line 3: Delete "e.g., ^{131}I,"

INDEX

Entries are located by section numbers. Official first actions are designated by 1 asterisk. Actions on present official methods are identified as in the following example: **6.372** (revision, 1980), which shows that a revision of **6.372,** which was adopted at the 1979 meeting, appeared in "Changes in Methods" in the March 1980 issue of *J. Assoc. Off. Anal. Chem.*

The number in brackets following each compound for which a determination is given is the registry number assigned to it by Chemical Abstracts Service. This is a unique number for that compound; it permits entry into the chemical information system of the Chemical Abstracts Service. For synonyms, the CAS registry number is given with the common name.

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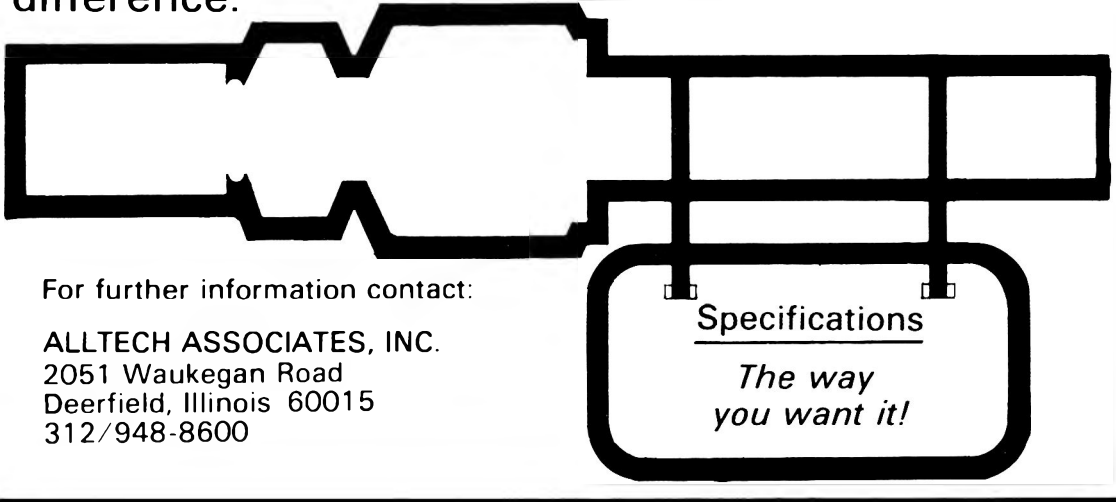

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